LIVIA LUZ SOUZA NASCIMENTO

Neurodegeneração no Envelhecimento: Lições da Síndrome de Cockayne

Tese de doutorado apresentada ao programa de Pós-graduação em Microbiologia do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do Título de Doutor em Ciências.

São Paulo

2022

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Ata de defesa de Tese do(a) Senhor(a) Livia Luz Souza Nascimento no Programa: Ciências Biológicas (Microbiologia), do(a) Instituto de Ciências Biomédicas da Universidade de São Paulo.

Aos 28 dias do mês de junho de 2022, no(a) via remota realizou-se a Defesa da Tese do(a) Senhor(a) Livia Luz Souza Nascimento, apresentada para a obtenção do título de Doutora intitulada:

"Neurodegeneração no envelhecimento: lições da Síndrome de Cockayne"

Após declarada aberta a sessão, o(a) Sr(a) Presidente passa a palavra ao candidato para exposição e a seguir aos examinadores para as devidas arguições que se desenvolvem nos termos regimentais. Em seguida, a Comissão Julgadora proclama o resultado:

Nome dos Participantes da Banca	Função	Sigla da CPG	Resultado
Carlos Frederico Martins Menck	Presidente	ICB - USP	Não Votante
Fábio Papes	Titular	UNICAMP - Externo	aprovado
Nicolas Carlos Hoch	Titular	IQ - USP	aprovado
Enrique Mario Boccardo Pierulivo	Suplente	ICB - USP	Aprovado

Resultado Final: Aprovado

Parecer da Comissão Julgadora *

Eu, Lucianna Vicente da Silva _____, lavrei a presente ata, que assino juntamente com os(as) Senhores(as). São Paulo, aos 28 dias do mês de junho de 2022.

p/ Fábio Papes

p/ Nicolas Carlos Hoch

p/ Enrique Mario Boccardo Pierulivo

Carlos Frederico Martins Menck
Presidente da Comissão Julgadora

* Obs: Se o candidato for reprovado por algum dos membros, o preenchimento do parecer é obrigatório.

A defesa foi homologada pela Comissão de Pós-Graduação em 12/07/2022 e, portanto, o(a) aluno(a) Livia jus ao título de Doutora em Ciências obtido no Programa Ciências Biológicas (Microbiologia) - Área de concentração: Microbiologia.

Presidente da Comissão de Pós-Graduação

Profa. Dra. Ana Paula Lepique
Presidente da CPG/ICB

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DECLARAÇÃO

Em adendo ao Certificado de Isenção protocolo **CEP-ICB nº 781/2015/2013**, informo que o título do projeto foi alterado para *"Indução de pluripotência em fibroblastos deficientes em XPD para estudo fenotípico"* sob a responsabilidade da aluna **Livia Luz Souza Nascimento** e orientação do Prof. Dr. **Carlos Frederico Martins Menck**, do Departamento de Microbiologia, com inclusão de linhagens celulares, o qual foi reanalisado pela CEUA - Comissão de Ética no Uso de Animais e pelo CEPSH – Comitê de Ética em Pesquisa com Seres Humanos, tendo sido deliberado que o referido projeto não utilizará animais que estejam sob a égide da Lei nº 11.794, de 8 de outubro de 2008, nem envolverá procedimentos regulados pela Resolução CONEP nº 466/2012.

São Paulo, 14 de junho de 2018.

Profa. Dra. **Luciane Valéria Sita**
Coordenadora da CEUA - ICB/USP

Profa. Dra. **Camila Squarzoni Dale**
Coordenadora do CEPSH - ICB/USP

Aos pacientes e seus familiares, que apesar da difícil progressão da síndrome de Cockayne, ainda doam tempo e amostras para pesquisas científicas.

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"We are at the very beginning of time for the human race. It is not unreasonable that we grapple with problems. But there are tens of thousands of years in the future. Our responsibility is to do what we can, learn what we can, improve the solutions, and pass them on."

- Richard P. Feynman

RESUMO

Livia Luz Souza Nascimento. Neurodegeneration in aging: Lessons from Cockayne Syndrome. Tese de Doutorado em Microbiologia, Universidade de São Paulo, São Paulo. 2022.

O envelhecimento está associado ao surgimento de diversas disfunções teciduais, como as doenças neurodegenerativas. Esse processo não é totalmente compreendido, principalmente devido à dificuldade de estudar a organização neuronal humana. Uma saída é estudar as síndromes associadas ao envelhecimento prematuro, exacerbando os processos celulares em modelos *in vitro*. A Síndrome de Cockayne (CS) é um desses distúrbios em que os indivíduos têm uma expectativa de vida não superior a 20 anos, durante os quais os indivíduos são afetados por sintomas neurológicos graves associados à atrofia cerebral. No nível celular, essa síndrome é caracterizada por alteração funcional da via de reparo do DNA através da excisão de nucleotídeos, que é responsável por reparar grandes torções de DNA, como as causadas pela luz UV. De fato, mutações nessa via também podem levar a um aumento de 10.000 vezes nos tumores de pele, mas interessantemente não em pacientes com CS – a relação dessa via com o tecido neural e a neurodegeneração não foram bem estabelecidos. Neste trabalho, usando células pluripotentes induzidas de fibroblastos dérmicos de pacientes com CS, observamos uma alta sensibilidade a drogas que oxidam o DNA, sugerindo que a instabilidade genômica nesses pacientes pode ser causada por espécies reativas produzidas pelo próprio metabolismo celular. Além disso, diferenciamos células pluripotentes em células progenitoras neurais, bem como em culturas maduras de neurônios e astrócitos. Nessas culturas, observamos um aumento no número de lesões de DNA logo após o tratamento oxidante em relação as linhagens celulares selvagens, indicando cinética de reparo mais lenta nas células CS. Também descobrimos que esse tipo de dano no DNA não resulta no bloqueio da RNA polimerase, um efeito anteriormente considerado um dos principais envolvidos nos sintomas graves da CS. Ademais mostramos que em células CS esse dano é sinalizado de maneira diferente das células selvagens, efeito que pode integrar as bases moleculares da progressão da doença. Paralelamente, desenvolvemos modelo tridimensional in vitro do córtex cerebral desses pacientes e observamos depleção maciça da zona de progenitores neurais, não observado nas células controle. Esse efeito pode estar relacionado com o fenótipo de problemas neurológicos observados em pacientes CS. Finalmente, encontramos depósitos de proteínas tóxicas relacionadas ao envelhecimento e demência neste modelo, reforçando novamente o aspecto de envelhecimento acelerado da doença. Esperamos que, além de ajudar a entender os mecanismos da CS com este novo modelo 3D, possamos fornecer uma plataforma para testes terapêuticos, mas sobretudo, promover um modelo que possa ser usado para estudar a neurodegeneração associado ao envelhecimento da população em geral.

Palavras-chave: Envelhecimento. Síndrome de Cockayne. Neurodegeneração. Reparo de DNA. Organóide.

ABSTRACT

Livia Luz Souza Nascimento. Neurodegeneration in aging: Lessons from Cockayne Syndrome. Ph.D. thesis in Microbiology, Universidade de São Paulo, São Paulo. 2022.

Aging is associated with the development of various tissue dysfunctions, such as neurodegenerative diseases. This process is not fully established, mainly due to the difficulty of accessing human neural tissue. One way forward is to study syndromes associated with premature aging that show enhanced cellular processes in *in vitro* models. Cockayne syndrome (CS) is one such disorder in which individuals have a life expectancy of no more than 20 years, during which they suffer from severe neurological symptoms associated with brain atrophy. At the cellular level, the nucleotide excision repair (NER) pathway is altered, which is known to repair bulky DNA damage, such as those caused by UV light. Mutations in this pathway can also lead to a 10,000-fold increase in skin tumors, but not in CS patients—the relationship between NER and neurodegeneration is unclear. Here, using induced pluripotent cells derived from dermal fibroblasts of CS patients, we observed a high sensitivity to drugs that oxidize DNA, suggesting that genomic instability in these patients may be caused by reactive species produced by cell metabolism. Furthermore, we differentiated pluripotent cells into neural progenitor cells and mature cultures of neurons and astrocytes. We observed an increase in the number of DNA lesions in these cultures compared to controls shortly after oxidant treatment, suggesting slower repair kinetics in these cells. We also found that this type of DNA damage does not lead to blockage of RNA polymerase, an effect previously thought to be a significant contributor to the severe symptoms of CS. We also observed that DNA damage signaled differently from control cells, an effect that may integrate the molecular basis of disease progression. Furthermore, we developed a tridimensional *in vitro* model of the cerebral cortex of these patients and observed a marked depletion of neural progenitor regions, an effect that might be associated with CS progression. Finally, in this model, we identify toxic protein deposits associated with aging and dementia, which reinforces the accelerated aging aspect of the syndrome. We hope that with this new 3D model, we can contribute to a better understanding of the mechanisms of CS and provide a platform for therapeutic research, and, more ultimately, offer a model to study age-related neurodegeneration in the general population.

Keywords: Aging. Cockayne Syndrome. Neurodegeneration. DNA Repair. Organoid.

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8-oxoG: 8-hydroxyguanine

AD: Alzheimer's disease

AT: Ataxia telangiectasia

BER: Base excision repair

BSA: Bovine serum albumin

CBO: Cortical Brain Organoids

CS: Cockayne syndrome

DAPI: 4´,6-diamidino-2-fenilindole

DDR: DNA Damage Response

DMEM: Dulbecco Modified Eagle Medium

DSB: Double-strand breaks

EB: Embryoid bodies

FPG: Formamidopyrimidine-DNA glycosylase

GG-NER: Global genome nucleotide excision repair

GH: Growth hormone

gRNA: Guide RNA

H_2O_2: Hydrogen peroxide

HGPS: Hutchinson-Gilford progeria syndrome

H2AX: histone 2A family member X

γH2AX: Phosphorylated histone 2A family member X in Serine 139

IGF1: Insulin-like growth factor 1

iPSCs: Induced Pluripotent Stem Cells

$KBrO_3$: Potassium bromate

KO: Knockout

mtOGG-1: 8-oxoguanine glycosylase 1

NAD^+: Nicotinamide adenine dinucleotide

NEAA: Non-essential amino acids

NER: Nucleotide excision repair

NFT: Neurofibrillary tangles

NM: Neuronal Media

NPC: Neural progenitor cells

PAR: NAD+ molecules to poly-ADP-ribose

PARP1: Poly-ADP-ribose polymerase 1

PGC-1a: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

RNA Pol: RNA polymerase

ROS: Reactive Oxygen Species

SASP: Senescence-Associated Secretory Phenotype

SH: A thiol or thiol derivative is any organosulfur compound

SIRT1: Sirtuin 1

TC-NER: Transcription-coupled nucleotide excision repair

TTD: Trichothiodystrophy

UV: Ultraviolet radiation

UVSS: UV-sensitive syndrome

UVSSA: UV-Stimulated Scaffold Protein A

XP: Xeroderma pigmentosum

WS: Werner syndrome

WT: Wild type

SUMMARY

1 INTRODUCTION

Aging is not a predetermined, precise, and stringent process – it might be just an absence of natural selection for maintenance (DA COSTA et al., 2016). There is no such thing as dying of old age; we all die because a vital part of us is in check - however, we added new players to this stalemate throughout human history. Case in point: Brazilians had a life expectancy of 29 years at the beginning of twentieth century, whereas today, it is at least 75 years (RILEY, 2005). While respiratory infections were the leading cause of death at that time, the development of vaccines and antibiotics significantly reduced mortality from infectious diseases over the last decades. Now, with the advent of the revolutionary mini-organ in a dish and personalized target therapy, we may experience a significant impact on the prognosis of cancer patients in the following decades (KONDO; INOUE, 2019). As we learn to use resources and develop new technologies, we prolong our life and healthspan.

What does this tell us about the future? Life expectancy is projected to exceed 120 years in the coming decades (DE BEER; BARDOUTSOS; JANSSEN, 2017), which could change the contours of our society. Can we also prevent diabetes, dementia, and neurodegeneration? Will we continue to retire at 60? Will we be cognitively and physically capable of working by age 90? Therefore, new frontiers of human aging are being explored, and efforts to delay it are emerging, which should be seen as natural as taking antibiotics. However, this is impossible without a deep understanding of how aging really works.

Aging is a leading risk factor for chronic and fatal diseases, including cancer, cardiovascular and neurodegenerative diseases (NICCOLI; PARTRIDGE, 2012). Aging has also been associated with diabetes, osteoporosis, arthritis, cataracts, chronic liver disease, and increased infections (ASPRAY; HILL, 2019; BOSKEY; COLEMAN, 2010; DE MAEYER; CHAMBERS, 2021; MAESO-DÍAZ; GRACIA-SANCHO, 2020; SHIELS; HEJTMANCIK, 2015). Many modifications are often observed in skin aging, such as tissue sagging, wrinkles, elastic fiber degeneration, skin pigmentation, decreased sebaceous gland activity, and decreased hair growth (BOLOGNIA, 1995). When it comes to the heart, aging causes stiffening of the aorta and large arteries, hypertension, diastolic dysfunction, coronary vascular insufficiency, fibrosis, and heart failure of varying degrees (SUSIC;

FROHLICH, 2008). Here, we will focus on alterations on the neuronal tissue. In fact, the leading risk factor for neurodegeneration is aging. Neurodegeneration is characterized by cerebral atrophy, reductions in both gray and white matter, associated enlargement of the cerebral ventricles, leading to decreased abilities of learning, memory, attention, decision-making speed, sensory perception (vision, hearing, touch, smell, and taste), and motor coordination (HOU et al., 2019; MATTSON; ARUMUGAM, 2018).

1.1 THE HALLMARKS OF AGING

At the cellular level, aging is characterized by several hallmarks: epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, altered intracellular communication, senescence, stem cell exhaustion, telomere attrition, and genomic instability (LÓPEZ-OTÍN et al., 2013). Each of these topics is disclosed below.

Age-related **epigenetic changes** occur at multiple levels, including reduced levels of core histones, promoter hypermethylation, post-translational histone modifications and altered DNA methylation patterns, replacement of default histones with histone variants, and altered noncoding RNA expression. It has been shown that a progressive accumulation of histone H4K16 acetylation, H4K20 trimethylation, H3K4 trimethylation, and reduced H3K9 methylation and H3K27 trimethylation is associated with aging (CARRERO; SORIA-VALLES; LO, 2016; FRAGA; ESTELLER, 2007; HAN; BRUNET, 2012; ZHANG et al., 2020). These epigenetic changes lead to aberrant gene expression, reactivation of transposable elements, genomic instability, and tumorigenesis (FRAGA; ESTELLER, 2007; PAL; TYLER, 2016). Moreover, age-related epigenetic changes have been associated with neurodegeneration (LARDENOIJE et al., 2015).

Protein homeostasis, or proteostasis, is a state of equilibrium in which the correct conformation, concentration, and localization of proteins are maintained by preventing the accumulation of inactive or toxic proteins, mainly through proteasomal degradation. Protein dysfunction can occur due to misfolding, impaired chaperone quality control, aggregation, transcriptional mutagenesis, or protein oxidation/carbonylation by reactive species (KOROVILA et al., 2017). The resulting accumulation of misfolded, mismatched complexes and misplaced proteins leads to decreased cellular function, loss of protein solubility, and protein precipitation.

The ability to maintain protein homeostasis through a broad network of molecular chaperones, proteolytic systems and their regulators is progressively declined during aging (HIPP; KASTURI; HARTL, 2019; KAUSHIK; CUERVO, 2015; LÓPEZ-OTÍN et al., 2013). To illustrate, decreased solubility and increased precipitation in the human eye lens can lead to cataracts - commonly observed among the older population (SHIELS; HEJTMANCIK, 2015). Moreover, the imbalance in protein homeostasis has been identified as a cause of neurodegeneration in the elderly and is directly associated with the emergence of proteinopathies such as Alzheimer's (AD) and Parkison disease (DOUGLAS; DILLIN, 2010; KURTISHI et al., 2018).

The **deregulated nutrient sensing** in aging is a consequence of accelerated anabolic signaling and somatotrophic axis dysregulation (BARTKE; LIST; KOPCHICK, 2016; LÓPEZ-OTÍN et al., 2013). Anabolic signaling is increased by dysregulation of the mammalian/mechanistic target of the rapamycin (mTOR) network, which orchestrates responses such as cell growth, proliferation, apoptosis, and inflammation (WEICHHART, 2018). The somatotrophic axis controls the mTOR network, coordinated by growth hormone (GH) and insulin-like growth factor (IGF-1). Mutations in these nutrient and growth signaling chains have been shown to alter the lifespan in various model organisms (MILMAN; HUFFMAN; BARZILAI, 2016). In addition, modulation of somatotrophic axis/mTOR through dietary restriction has been shown to prolong lifespan and healthspan (GRANDISON; PIPER; PARTRIDGE, 2009; KAPAHI; KAEBERLEIN; HANSEN, 2017; KENNEDY; STEFFEN; KAEBERLEIN, 2007; LÓPEZ-OTÍN et al., 2013).

Mitochondrial dysregulation occurs due to a combination of factors such as the accumulation of damage on mitochondrial DNA (mtDNA), oxidized/misfolded proteins that can lead to reactive oxygen species (ROS) leakage, ineffective clearance of damaged mitochondria, reduced efficiency of mitochondrial bioenergetics, and disrupted apoptotic signaling that can trigger inflammatory responses (BALABAN; NEMOTO; FINKEL, 2005; KAUPPILA; KAUPPILA; LARSSON, 2017; LÓPEZ-OTÍN et al., 2013). Mitochondrial dysfunction accelerates aging in mammals and is associated with neurodegenerative processes (LIMA et al., 2022; LIN; BEAL, 2006; LÓPEZ-OTÍN et al., 2013).

A striking age-related change in **intercellular communication** is inflammation (LÓPEZ-OTÍN et al., 2013). Age-related chronic diseases are associated with an inflammatory state, as evidenced by local infiltration of inflammatory cells such as macrophages and higher circulating levels of proinflammatory cytokines, complement components, and adhesion molecules (SARKAR; FISHER, 2006). The increased inflammatory status is supported by factors secreted by senescent cells, another hallmark found in age (BEKTAS et al., 2018).

Telomeres are repetitive sequences at the ends of chromosomes (TTAGGG) that protect the genome. Part of the 5' telomeric sequence is lost during DNA replication, leaving coding DNA intact. Telomerase adds nucleotides to the ending sequence in stem cells, ensuring minimal telomere shortening during cell divisions. However, telomerase activity is low or absent in somatic cells, and telomere length shortens over time with each cell division. Telomeres that have been shortened to critical lengths are recognized as DNA double-strand breaks (DSBs) and trigger mechanisms of DNA-damage responses (DDRs) that culminate in cellular senescence (HAYFLICK; MOORHEAD, 1961; KRUK; RAMPINO; BOHR, 1995).

Cellular senescence is an evolutionary cell replication mechanism with multiple implications on cellular transcription and overall secretome – known as senescence-associated secretory phenotype (SASP) (KOWALD; PASSOS; KIRKWOOD, 2020; MCHUGH; GIL, 2018). Senescence has been described as a cellular aging clock mechanism in which revolutions are determined by telomere attrition (HERBIG et al., 2006). Another theory suggests that cellular senescence may have evolved concurrently with apoptosis to limit damaged cell proliferation and tumorigenesis, but instead of cell death, a persistent G1 arrest state takes place (SAPIEHA; MALLETTE, 2018). Moreover, senescent cells have a crucial role in development, wound healing, and tissue repair – as SASP can mobilize progenitors to re-establish cell number and promotes tissue and matrix clearance. Post-mitotic cells have been reported to undergo senescence expressing an exacerbated SASP (VON ZGLINICKI; WAN; MIWA, 2021) – a pattern observed in AD neurons with TAU neurofibrillary tangles (MUSI et al., 2018).

Stem cell exhaustion is another important hallmark of aging. Stem cells have the ability to self-renew and differentiate into several types of cells, and such a feature is critical for the long-

term maintenance of the body (LÓPEZ-OTÍN et al., 2013). Along with cellular senescence, depletion of stem cells can lead to exhaustion of mitotic cells resulting in an inability to regenerate and loss of tissue function (ROCHA et al., 2013). Aged human mesenchymal stem cells, which can differentiate into bone, cartilage, muscle and fat cells, and connective tissue, show a decline in differentiation potential and proliferation rate (FEHRER; LEPPERDINGER, 2005). In satellite cells, muscle-specific stem cells, reductions in number and function are associated with aging and contribute to increased susceptibility to age-related muscle pathology (GOPINATH; RANDO, 2008). In hematopoietic stem cells of aged mice, a differential capacity to generate myeloid and lymphoid progenitors was implicated in the loss of immune function and a higher incidence of myeloid leukemia compared to younger mice (ROSSI et al., 2005). In the human brain, a marked reduction in adult neurogenesis is associated with a decline in cognitive function during aging – this is thought to be due to the inability of neural stem cells to re-enter the cell cycle (AUDESSE; WEBB, 2020b). In addition to the preciousness of nuclear DNA, stem cells also induce the degradation of mitochondria with specific mutations in mtDNA (HÄMÄLÄINEN et al., 2015). Furthermore, stem cells have lower metabolic activity and lower mitochondrial content to reduce ROS (Reactive Oxygen Species) production and the risk of mutagenesis (ROCHA et al., 2013). However, when all these powerful mechanisms are insufficient and DNA damage cannot be repaired, stem cells undergo early senescence, differentiation, or apoptosis to avoid the accumulation of DNA damage. Thus, preventing the mutations in the genome from spreading throughout the body and failing to regenerate the surrounding tissues (MANI; REDDY; PALLE, 2020; ROCHA et al., 2013).

Notably, of all the factors contributing to aging, **genomic instability** is probably the most widely accepted and studied—mainly because it likely precedes all the other aging traits mentioned here. DNA damage can lead to transcriptional and replication arrest triggering DDR. Once the DDR process is activated, it blocks cell cycle progression. It induces signaling pathways that lead to epigenetic changes, loss of protein homeostasis, and mitochondrial dysfunction leading to cellular inflammation, senescence, or cell death – depleting tissues of living and functional cells.

Unfortunately, the genome is constantly threatened by exogenous factors such as ultraviolet (UV) and ionizing radiation and endogenous threats such as DNA replication errors, spontaneous hydrolysis reactions, and ROS (Hoeijmakers, 2009). A typical example is that approximately 2,000 to 10,000 depurination events occur in a single human cell every 24 hours (LINDAHL, 1993). In a meta-analysis study involving more than 4,500 individuals, it was shown that DNA damage was positively correlated with aging (SOARES et al., 2014). This correlation is consistent with other studies showing a gradual accumulation of the DNA lesion 8-oxoguanine (8-oxodG) in skeletal muscle (MECOCCI et al., 1999), lymphocytes (HUMPHREYS et al., 2007), and leukocyte (SIOMEK et al., 2006), and it was associated with frailty in the elderly population (WU et al., 2009). The mtDNA was also shown to accumulate DNA damage over time (CORRAL-DEBRINSKI et al., 1992).

While DNA damage is well accepted as the major factor for genomic instability, it remains unsure what are the main sources of DNA damage that causes aging. DNA lesions caused by ROS have been implicated as the culprit for a long time (HARMAN, 1955) and its role on genomic instability and aging largely debated in the literature (CADENAS; DAVIES, 2000; DRÖGE, 2003; DRÖGE; SCHIPPER, 2007; GOLDEN; HINERFELD; MELOV, 2002; LIOCHEV, 2013; SALMON; RICHARDSON; PÉREZ, 2010; ZSURKA et al., 2018). ROS are the result of the excitation of O_2, mainly through mitochondrial metabolism, leading to the formation of singlet oxygen ($^1O_2$) or the transfer of one, two, or three electrons to O_2 leading to the formation of the superoxide (O_2^-), hydrogen peroxide (H_2O_2), or hydroxyl radical ($^\bullet OH$) respectively. When interacting with DNA, ROS can cause thymine (T) glycol resulting from the saturation of the 5,6 double bond of some pyrimidines (FRIEDBERG et al., 1995); deamination of cytosine (C) to form uracil (JOVANOVIC; SIMIC, 1986); abasic site due to hydrolysis of N-glycosidic bonds (BOITEUX; GUILLET, 2004); oxidation of guanine (G) 8-OxoG (this altered base may mismatch with adenine (A) and is therefore mutagenic (BRUSKOV, 2002)); single-strand breaks (SSB) due to the sequestration of hydrogen from the DNA by the hydroxyl radical (KAWANISHI; MURATA, 2006); and DSB (HOEIJMAKERS, 2001; SIDDIQI; BOTHE, 1987). In toto, whether primarily caused by ROS or not, DNA damage can accumulate throughout the lifespan and integrate into the genome as mutations.

A well-documented DNA aggressor is UV light. UV light is a type of non-ionizing electromagnetic radiation that, when absorbed by DNA, forms two main photoproducts: pyrimidine-6,4-pyrimidones (6,4PPs) and cyclobutane dimers (CPDs), which cause a bulk distortion on DNA. All photoproducts are highly toxic to cells because they block the progress of replicative and transcriptional polymerases resulting in fork collapse, DNA breaks, and, ultimately, cell death (MENCK; MUNFORD, 2014). If not repaired, UV-induced DNA damage can cause mutations leading to cellular senescence or transformation. Thus, UV-induced mutations have been associated with aged skin degeneration and skin cancer (GONZAGA, 2012).

Accumulation of mutations during life is evidenced by the increased risk of developing cancer (TAKESHIMA; USHIJIMA, 2019). Thus, cancer and aging can be seen as two different manifestations of the same underlying process: the accumulation of mutations (LÓPEZ-OTÍN et al., 2013). Nonetheless, there is substantial evidence in the literature for an association between 'healthy' aging and mutations (MOSKALEV et al., 2013; SKINNER; TURKER, 2005; ZHANG; VIJG, 2018). Chromosomal structural changes were associated with aging in the mouse brain (FAGGIOLI et al., 2012) and human blood cells (FORSBERG et al., 2012). Furthermore, with the advancement and popularity of technologies such as single-cell sequencing, it has become possible to detect mutation accumulation at the base level (RISQUES; KENNEDY, 2018). For instance, human hematopoietic stem and progenitor cells were shown to accumulate approximately 11 point mutations per division (OSORIO et al., 2018). Similar results were found in satellite cells from human skeletal muscle (FRANCO et al., 2018), liver (BRAZHNIK et al., 2020), and esophageal epithelium (MARTINCORENA et al., 2018). Prefrontal cortex and hippocampus neurons also accumulate more somatic mutations with age (LODATO et al., 2018). Interestingly, in the fetal forebrain, it was estimated that about 1.3 mutations occur per division per cell (BAE et al., 2018) – which suggests that aging is a process that begins at conception. Another study showed that adult stem cells derived from the small intestine, colon, and liver steadily accumulate mutations over time, with about 40 new mutations per year, although cancer rates vary widely between these tissues (BLOKZIJL et al., 2016). In mtDNA, a 5-fold increase in point mutations was found in human brains over 80 years old (KENNEDY et al., 2013).

Interestingly, the strongest indication that increased mutations leads to aging is that cancer survivors treated with DNA-damaging drugs exhibit accelerated aging (ARMSTRONG et al., 2014; HENDERSON; NESS; COHEN, 2014; NESS et al., 2013) - such as cardiopathies (ARMSTRONG et al., 2013; NESS et al., 2013), diabetes (BAKER et al., 2013), cognitive impairment (DIETRICH, 2010; KOPPELMANS et al., 2013), dementia (KOPPELMANS et al., 2013), neurodegeneration (DIETRICH, 2010; MCLEARY et al., 2019), white matter abnormalities (DIETRICH, 2010), hearing loss (VAN AS; VAN DEN BERG; VAN DALEN, 2016; WHELAN et al., 2011), vision loss (DE BLANK et al., 2016), cataract (CHODICK et al., 2016), stroke (FULLERTON et al., 2015), and impaired balance, coordination, and tremors (KING et al., 2017). Interestingly, the literature is conflicting about the risk of AD in cancer survivors (KESLER et al., 2017; THINNES, 2012).

Luckily, organisms have developed defense mechanisms to repair DNA damage during evolution. DSBs are usually repaired by homologous recombination repair (HR) or non-homologous end-joining (NHEJ); base excision repair (BER) removes some single base alterations; lesions that cause bulk distortions in the DNA strand are solved by the nucleotide excision repair (NER) pathway. Mismatch repair (MMR) checks the complementarity of nitrogenous bases during and after replication (WOOD et al., 2001).

Notably, all DNA repair pathways in stem cells are upregulated to prevent the spread of mutations through tissue (MAYNARD et al., 2008; ROCHA et al., 2013). For instance, it has been shown that BER glycosylases are upregulated in mouse neural progenitor cells, and their levels decrease after differentiation (HILDRESTRAND et al., 2007). Likewise, NER proteins are highly downregulated in several differentiated human cell types (FROSINA, 2010). As a consequence, it was observed that human induced pluripotent stem cells (iPSCs) have a 10-fold lower mutation rate per cell division than somatic cells (ROUHANI et al., 2016). As mentioned earlier, aging is also associated with stem cell exhaustion, suggesting that overall DNA repair body capacity decreases with age, ignoring the DNA damage that ultimately leads to genomic instability. Furthermore, in differentiated cells, the activity of BER, NER, and NHEJ pathways decreased with age, regardless of the differentiation stage. In fact, studies show that NER capacity decreases by 1% per year as humans age (NIEDERNHOFER et al., 2018). Thus, reduced NER gene expression is responsible for delayed DNA repair after UV in aging skin (TAKAHASHI et al., 2005). Besides that, decreased MMR

activity is associated with aging and the appearance of DNA microsatellites (GORBUNOVA et al., 2007). DNA damage-induced expression of DNA polymerase β and AP endonuclease is impaired in old mice—indicating that the DDR also ages (GORBUNOVA et al., 2007). Overall, DNA repair capacity decreases with aging, and the unrepaired lesions overwhelm DDR, leading to cellular senescence, apoptosis, or mutation, increasing other aging traits and genomic instability.

Perhaps the most compelling evidence that genomic instability is associated with aging is that inborn chromatin dysregulation is associated with premature aging. These rare progeria syndromes, often fatal in infancy, share a common origin: genomic instability caused by impaired DNA repair or chromatin regulation. Cockayne Syndrome (CS), Xeroderma Pigmentosum (XP), and Trichothiodystrophy (TTD) are NER-related disorders. Several other human syndromes with clinical phenotypes related to aging, such as Werner syndrome (WS), Bloom syndrome, Rothmund-Thomson syndrome, Fanconi Anemia, Seckel Syndrome, Ataxia Telangiectasia (AT), Ataxia Telangiectasia-like Disorder, Cerebro-retinal Microangiopathy with Calcifications and Cysts, Nijmegen Breakage Syndrome, Dyskeratosis Congenital, Ruijs–Aalfs syndrome Down syndrome, and Hoyeraal-Hreidarsson Syndrome, are caused by deficient DNA repair and telomerase complex impairment. Hutchinson-Gilford Progeria Syndrome (HGPS), Néstor-Guillermo Progeria Syndrome, Atypical Progeria Syndromes, Restrictive Dermopathy, and Mandibuloacral Dysplasia are caused by defects in the nuclear envelope structure, which is known to regulate chromatin (CARRERO; SORIA-VALLES; LO, 2016; NIEDERNHOFER et al., 2018; ZULEGER; ROBSON; SCHIRMER, 2011). Because of their age-accelerating nature, these diseases can give us important clues about the aging process - here, we examine neurodegeneration in Cockayne syndrome.

1.2 NUCLEOTIDE EXCISION REPAIR DISORDERS

NER pathway is the most versatile and conserved DNA repair mechanism (COSTA et al., 2003). It is divided into two sub-pathways that differ in how they detect DNA damage. In global genome repair (GG-NER), the XPC-RAD23D complex and XPE can detect bulk DNA damage, such as those induced by UV light, in any region of the genome, accounting for 99% of lesions (DIGIOVANNA; KRAEMER, 2012). In transcription-coupled repair (TC-NER), lesion-blocked RNA

polymerase provides the initial signal for the recruitment of the NER machinery, which is dependent on CSA and CSB proteins. After the recruitment of the excision complex, both GG-NER and TC-NER follow the same path. In this complex, TFIIH contains the helicases XPB and XPD that are responsible for unwinding the DNA at the damage site (approximately 30 bp), while RPA coats the single-strand DNA and the XPA protein maintains and stabilizes the repair complex near the lesion. The endonucleases XPF/ERCC1 and XPG cut the DNA flanking the lesion, removing approximately 30 nucleotides. The complementary strand is used as a template by polymerases δ, ε, and/or κ to fill the gap associated with PCNA (COSTA et al., 2003; FUSS; TAINER, 2011). Finally, DNA ligase I complete the repair. An illustrated schematic view of this DNA repair pathway is shown in Figure 1.

5 FINAL CONSIDERATIONS

In this work, we use progeria syndrome to study age-related neurodegeneration. We were able to expand our knowledge about the mechanisms of this disease and give indications of aging mechanisms, such as the limitations of DDR.

Here, we found that cellular reprogramming provides no alternative or redundant approach to repairing bulky DNA damage beyond NER and, therefore, NER deficiency in the developing body can be studied using iPSC technology. Furthermore, we found that differentiation status alters cell fate between apoptosis or persistent accumulation of DNA damage, which may lead to mutation and senescence. In addition, we observed distinct NER kinetics between mature neurons and their progenitors. Therefore, a good suggestion is that the *in vitro* models consider the cell type being studied, not just the genotype – particularly in therapeutic research.

We also show that ROS leads to apoptosis by activating caspase-3 in CS iPSCs, suggesting that their inability to cope with cellular metabolism-induced DNA damage during early development can explain the CS phenotype. Contrary to expectations, this inability in CS neural stem cells is not associated with transcriptional arrest but might be an accomplice to genomic and transcriptional mutations. Furthermore, some of this inability is also due to impaired DNA damage response, opening new routes to study the factors that drive CS progression.

This work develops an unpreceded *in vitro* cortical brain organoid to study CS. In this three-dimensional structure, we observed exhaustion of neural stem cells from tissue, corroborating with secondary microcephaly observed in patients. Using this new tool, we were able observed the, for the first time, accumulation of cytotoxic proteins found in other age-related diseases. Moreover, research therapy for CS already has a platform (brain organoids) and a starting point (drugs designed for tauopathy). In addition, we designed a CRISPR-based method to correct CSB mutation, which still needs testing and development. In toto, this model provides an alternative to monolayer culture and animal models that do not mimic the neurological deficits of patients with this syndrome.

In conclusion, we hope that these data, along with the detailed overview of aging aspects of CS in the Introduction section, will demonstrate that research into this disease can provide meaningful answers to aging. Ultimately, the CS brain organoids can be used to study aging-related neurodegeneration *in vitro*. We believe that with this thesis, we contributed to increasing our understanding of the mechanisms of CS progression, but we also hope that this novel *in vitro* CS model will help develop new therapeutic strategies for patients.

PUBLICATIONS

1. "Transcription blockage by DNA damage in nucleotide excision repair-related neurological dysfunctions"

Gustavo Satoru Kajitani, Livia Luz Souza Nascimento*, Maira Neves, Giovana Leandro, Camila Garcia, Carlos FM Menck. *This authors contribuited equally to this work*

In this paper (Appendix A), recently published in the *Seminars in Cell and Developmental Biology* Journal, we discussed the role of NER in endogenously induced DNA damage such as R-loops and the diversity of DNA damage caused by ROS. In addition, we discussed the different implications of DNA damage and repair in neuronal cells, given their quiescent cell cycle and transcriptional profiles (KAJITANI & NASCIMENTO et al., 2021).

2. "Cockayne Syndrome: The many challenges and approaches to understand a multifaceted disease."

Alexandre Teixeira Vessoni, Camila Chaves Coelho Guerra, Gustavo Satoru Kajitani, Livia Luz Souza Nascimento and Camila Carrião Machado Garcia.

In this second paper (Appendix B), published in *Genetics and Molecular Biology*, we revisited mutations in CSA and CSB genes and discussed these proteins' functions and CS study models (VESSONI et al., 2020).

3. “Different gene expression profiles in iPSC-derived motor neurons from ALS8 patients with variable clinical courses suggest mitigating pathways for neurodegeneration."

Danyllo Oliveira , David A Morales-Vicente, Murilo S Amaral, Livia Luz Souza Nascimento, Andrea L Sertié, Felipe S Leite, Claudia Navarro, Carolini Kaid, Joyce Esposito, Ernesto Goulart, Luiz Caires, Luciana M Alves, Uirá S Melo, Thalita Figueiredo, Miguel Mitne-Neto, Oswaldo K Okamoto, Sergio Verjovski-Almeida and Mayana Zatz.

In this work, published in 2020 in the *Human Molecular Genetics* journal, I collaborated using my knowledge of cell death and oxidative stress to help identify distinct signaling pathways at the cellular level in ALS8 patients (OLIVEIRA et al., 2020).

In addition, the COVID-19 pandemic started in November of 2019 but only got to Brazil in March of the following year. During this period, presential activities in the laboratory were restricted, which hindered the satisfactory conduct of experiments related to this paper. During the COVID-19 pandemic, the Brazilian federal government has compromised the transparency of epidemiological data. With Dr. Pilar Veras, we created an open-access panel that aggregates

crucial information such as COVID-19 case counts and deaths, population mortality, and virus spread projections. We manage to update our panels daily and categorize the data for different cities, states, and regions. This panel was most relevant in 2020, when the official federal COVID platform had not been updated for several weeks - when we had more than 27 thousand visualizations. The project helps to raise awareness among Brazilians about the epidemic. In fact, the platform was also announced by FAPESP (“Plataforma reúne gráficos interativos sobre a evolução da COVID-19 no Brasil | AGÊNCIA FAPESP”, 2020). In this time-consuming project, we learned valuable skills such as analyzing big data and python programming language.

REFERENCES[1]

AAMANN, M. D. et al. Cockayne syndrome group B protein promotes mitochondrial DNA stability by supporting the DNA repair association with the mitochondrial membrane. **FASEB journal : official publication of the Federation of American Societies for Experimental Biology**, v. 24, n. 7, p. 2334–46, jul. 2010.

ADAMS, J. W.; CUGOLA, F. R.; MUOTRI, A. R. Brain organoids as tools for modeling human neurodevelopmental disorders. **Physiology**, v. 34, n. 5, p. 365–375, 1 set. 2019.

ALUPEI, M. C. et al. Loss of Proteostasis Is a Pathomechanism in Cockayne Syndrome. **Cell Reports**, v. 23, n. 6, p. 1612–1619, 8 maio 2018.

ANDRADE, L. N. DE S. et al. Evidence for premature aging due to oxidative stress in iPSCs from Cockayne syndrome. **Human molecular genetics**, v. 21, n. 17, p. 3825–34, 1 set. 2012.

ANDREWS, A. D. et al. Cockayne's Syndrome Fibroblasts Have Increased Sensitivity to Ultraviolet Light But Normal Rates of Unscheduled DNA Synthesis. **Journal of Investigative Dermatology**, v. 70, n. 5, p. 237–239, 1 maio 1978.

ANDREWS, A. D.; BARRETT, S. F.; ROBBINS, J. H. Xeroderma pigmentosum neurological abnormalities correlate with colony-forming ability after ultraviolet radiation. **Proceedings of the National Academy of Sciences**, v. 75, n. 4, p. 1984–1988, 1 abr. 1978.

ANZALONE, A. V. et al. Search-and-replace genome editing without double-strand breaks or donor DNA. **Nature 2019 576:7785**, v. 576, n. 7785, p. 149–157, 21 out. 2019.

ANZALONE, A. V.; KOBLAN, L. W.; LIU, D. R. Genome editing with CRISPR–Cas nucleases, base editors, transposases and prime editors. **Nature Biotechnology 2020 38:7**, v. 38, n. 7, p. 824–844, 22 jun. 2020.

ARMSTRONG, G. T. et al. Modifiable risk factors and major cardiac events among adult survivors of childhood cancer. **Journal of clinical oncology : official journal of the American Society of Clinical Oncology**, v. 31, n. 29, p. 3673–3680, 10 out. 2013.

ARMSTRONG, G. T. et al. Aging and risk of severe, disabling, life-threatening, and fatal events in the childhood cancer survivor study. **Journal of clinical oncology : official journal of the American Society of Clinical Oncology**, v. 32, n. 12, p. 1218–1227, 20 abr. 2014.

ASPRAY, T. J.; HILL, T. R. Osteoporosis and the Ageing Skeleton. **Sub-cellular biochemistry**, v. 91, p. 453–476, 2019.

AUDESSE, A. J.; WEBB, A. E. Mechanisms of enhanced quiescence in neural stem cell aging. **Mechanisms of Ageing and Development**, v. 191, p. 111323, 1 out. 2020.

[1] *Following the guidelines: The International Committee of Medical Journal Editors (ICMJE) Uniform Requirements for Manuscripts NISO Z39.29-2005 (R2010) Available from: https://www.nlm.nih.gov/bsd/uniform_requirements.html

BAE, T. et al. Different mutational rates and mechanisms in human cells at pregastrulation and neurogenesis. **Science**, v. 359, n. 6375, p. 550–555, 2 fev. 2018.

BAKER, K. S. et al. Impact of treatment exposures on cardiovascular risk and insulin resistance in childhood cancer survivors. **Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology**, v. 22, n. 11, p. 1954–1963, nov. 2013.

BALABAN, R. S.; NEMOTO, S.; FINKEL, T. Mitochondria, oxidants, and aging. **Cell**, v. 120, n. 4, p. 483–495, 25 fev. 2005.

BALAJEE, A. S. et al. Reduced RNA polymerase II transcription in intact and permeabilized Cockayne syndrome group B cells. **Proceedings of the National Academy of Sciences of the United States of America**, v. 94, n. 9, p. 4306–4311, 29 abr. 1997.

BARTKE, A.; LIST, E. O.; KOPCHICK, J. J. The somatotropic axis and aging: Benefits of endocrine defects. **Growth Hormone & IGF Research**, v. 27, p. 41–45, 1 abr. 2016.

BATENBURG, N. L. et al. Cockayne Syndrome group B protein interacts with TRF2 and regulates telomere length and stability. **Nucleic Acids Research**, v. 40, n. 19, p. 9661–9674, 1 out. 2012.

BECK, K. D. et al. Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. **Neuron**, v. 14, n. 4, p. 717–730, 1995.

BEKTAS, A. et al. Aging, inflammation and the environment. **Experimental Gerontology**, v. 105, p. 10–18, 1 maio 2018.

BERQUIST, B. R. et al. Human Cockayne syndrome B protein reciprocally communicates with mitochondrial proteins and promotes transcriptional elongation. **Nucleic acids research**, v. 40, n. 17, p. 8392–8405, set. 2012.

BLOKZIJL, F. et al. Tissue-specific mutation accumulation in human adult stem cells during life. **Nature 2016 538:7624**, v. 538, n. 7624, p. 260–264, 3 out. 2016.

BOITEUX, S.; GUILLET, M. Abasic sites in DNA: repair and biological consequences in Saccharomyces cerevisiae. **DNA Repair**, v. 3, n. 1, p. 1–12, jan. 2004.

BOLOGNIA, J. L. Aging skin. **The American Journal of Medicine**, v. 98, n. 1, p. S99–S103, 16 jan. 1995.

BOSKEY, A. L.; COLEMAN, R. Aging and bone. **Journal of dental research**, v. 89, n. 12, p. 1333–1348, dez. 2010.

BOVÉ, J.; MARTÍNEZ-VICENTE, M.; VILA, M. Fighting neurodegeneration with rapamycin: mechanistic insights. **Nature reviews. Neuroscience**, v. 12, n. 8, p. 437–452, ago. 2011.

BRADFORD, P. T. et al. Cancer and neurologic degeneration in xeroderma pigmentosum: long term follow-up characterises the role of DNA repair. **Journal of medical genetics**, v. 48, n. 3, p. 168–76, mar. 2011.

BRAZHNIK, K. et al. Single-cell analysis reveals different age-related somatic mutation profiles between stem and differentiated cells in human liver. **Science Advances**, v. 6, n. 5, 31 jan. 2020.

BRUSKOV, V. I. Heat-induced formation of reactive oxygen species and 8-oxoguanine, a biomarker of damage to DNA. **Nucleic Acids Research**, v. 30, n. 6, p. 1354–1363, 15 mar. 2002.

CADENAS, E.; DAVIES, K. J. A. Mitochondrial free radical generation, oxidative stress, and aging. **Free Radical Biology and Medicine**, v. 29, n. 3–4, p. 222–230, 1 ago. 2000.

CALMELS, N. et al. Functional and clinical relevance of novel mutations in a large cohort of patients with Cockayne syndrome. **Journal of medical genetics**, p. jmedgenet-2017-104877, 23 mar. 2018.

CAMERONI, E.; STETTLER, K.; SUTER, B. On the traces of XPD: cell cycle matters - untangling the genotype-phenotype relationship of XPD mutations. **Cell division**, v. 5, n. 1, p. 24, 15 jan. 2010.

CARRERO, D.; SORIA-VALLES, C.; LO, C. Hallmarks of progeroid syndromes: lessons from mice and reprogrammed cells. 2016.

CHARLET-BERGUERAND, N. et al. RNA polymerase II bypass of oxidative DNA damage is regulated by transcription elongation factors. **The EMBO journal**, v. 25, n. 23, p. 5481–5491, 29 nov. 2006.

CHATRE, L. et al. Reversal of mitochondrial defects with CSB-dependent serine protease inhibitors in patient cells of the progeroid Cockayne syndrome. **Proceedings of the National Academy of Sciences of the United States of America**, v. 112, n. 22, p. E2910–E2919, 2 jun. 2015.

CHODICK, G. et al. The Risk of Cataract among Survivors of Childhood and Adolescent Cancer: A Report from the Childhood Cancer Survivor Study. **Radiation Research**, v. 185, n. 4, p. 366–374, 1 abr. 2016.

CITTERIO, E. et al. ATP-Dependent Chromatin Remodeling by the Cockayne Syndrome B DNA Repair-Transcription-Coupling Factor. **Molecular and Cellular Biology**, v. 20, n. 20, p. 7643–7653, 15 out. 2000.

CLEAVER, J. E. et al. Conceptual developments in the causes of Cockayne syndrome. **Mechanisms of Ageing and Development**, v. 134, n. 5, p. 284–290, 2013.

CLEAVER, J. E. et al. Mitochondrial reactive oxygen species are scavenged by Cockayne syndrome B protein in human fibroblasts without nuclear DNA damage. **Proceedings of the National Academy of Sciences of the United States of America**, v. 111, n. 37, p. 13487–92, 16 set. 2014.

COCKAYNE, E. A. Dwarfism with retinal atrophy and deafness. **Archives of Disease in Childhood**, v. 11, n. 61, p. 1, 1936.

CORDISCO, S. et al. Cockayne Syndrome Type A Protein Protects Primary Human Keratinocytes from Senescence. **Journal of Investigative Dermatology**, v. 139, n. 1, p. 38–50, 1 jan. 2019.

CORRAL-DEBRINSKI, M. et al. Association of mitochondrial DNA damage with aging and coronary atherosclerotic heart disease. **Mutation Research/DNAging**, v. 275, n. 3–6, p. 169–180, 1 set. 1992.

COSTA, R. et al. The eukaryotic nucleotide excision repair pathway. **Biochimie**, v. 85, n. 11, p. 1083–1099, nov. 2003.

COVARRUBIAS, A. J. et al. NAD+ metabolism and its roles in cellular processes during ageing. **Nature Reviews Molecular Cell Biology 2020 22:2**, v. 22, n. 2, p. 119–141, 22 dez. 2020.

CROCHEMORE, C. et al. Epigenomic signature of the progeroid Cockayne syndrome exposes distinct and common features with physiological ageing. **bioRxiv**, p. 2021.05.23.445308, 24 maio 2021.

CROCI, L. et al. Local insulin-like growth factor I expression is essential for Purkinje neuron survival at birth. **Cell Death & Differentiation 2011 18:1**, v. 18, n. 1, p. 48–59, 2 jul. 2010.

CUGOLA, F. R. et al. The Brazilian Zika virus strain causes birth defects in experimental models. **Nature**, v. 534, n. 7606, p. 267–271, 9 jun. 2016.

DA COSTA, J. P. et al. A synopsis on aging-Theories, mechanisms and future prospects. **Ageing research reviews**, v. 29, p. 90–112, 1 ago. 2016.

DE BEER, J.; BARDOUTSOS, A.; JANSSEN, F. Maximum human lifespan may increase to 125 years. **Nature 2017 546:7660**, v. 546, n. 7660, p. E16–E17, 29 jun. 2017.

DE BLANK, P. M. K. et al. Impact of vision loss among survivors of childhood central nervous system astroglial tumors. **Cancer**, v. 122, n. 5, p. 730–739, 1 mar. 2016.

DE MAEYER, R. P. H.; CHAMBERS, E. S. The impact of ageing on monocytes and macrophages. **Immunology letters**, v. 230, p. 1–10, 1 fev. 2021.

DE WAARD, H. et al. Different effects of CSA and CSB deficiency on sensitivity to oxidative DNA damage. **Molecular and cellular biology**, v. 24, n. 18, p. 7941–8, set. 2004.

DIETRICH, J. Chemotherapy Associated Central Nervous System Damage. **Advances in Experimental Medicine and Biology**, v. 678, p. 77–85, 2010.

DIGIOVANNA, J. J.; KRAEMER, K. H. Shining a light on xeroderma pigmentosum. **The Journal of investigative dermatology**, v. 132, n. 3 Pt 2, p. 785–96, mar. 2012.

DOUGLAS, P. M.; DILLIN, A. Protein homeostasis and aging in neurodegeneration. **Journal of Cell Biology**, v. 190, n. 5, p. 719–729, 6 set. 2010.

DRÖGE, W. Oxidative Stress and Aging. **Advances in Experimental Medicine and Biology**, v. 543, p. 191–200, 2003.

DRÖGE, W.; SCHIPPER, H. M. Oxidative stress and aberrant signaling in aging and cognitive decline. **Aging Cell**, v. 6, n. 3, p. 361–370, 1 jun. 2007.

DUEZ, P. et al. Statistics of the Comet assay: A key to discriminate between genotoxic effects. **Mutagenesis**, v. 18, n. 2, p. 159–166, 1 mar. 2003.

EHNINGER, D.; NEFF, F.; XIE, K. Longevity, aging and rapamycin. **Cellular and Molecular Life Sciences**, v. 71, n. 22, p. 4325–4346, 1 nov. 2014.

FEHRER, C.; LEPPERDINGER, G. Mesenchymal stem cell aging. **Experimental Gerontology**, v. 40, n. 12, p. 926–930, 1 dez. 2005.

FIRSANOV, D. V.; KROPOTOV, A. V.; MIKHAILOV, V. M. [NADP increases the level of histone H2AX phosphorylation in mouse heart cells after ionizing radiation]. **Tsitologiia**, v. 53, n. 4, p. 355–358, 1 jan. 2011.

FORESTA, M. et al. Defective repair of 5-hydroxy-2'-deoxycytidine in Cockayne syndrome cells and its complementation by Escherichia coli formamidopyrimidine DNA glycosylase and endonuclease III. **Free Radical Biology and Medicine**, v. 48, n. 5, p. 681–690, mar. 2010.

FRAGA, M. F.; ESTELLER, M. Epigenetics and aging: the targets and the marks. **Trends in Genetics**, v. 23, n. 8, p. 413–418, 1 ago. 2007.

FRANCO, I. et al. Somatic mutagenesis in satellite cells associates with human skeletal muscle aging. **Nature Communications 2018 9:1**, v. 9, n. 1, p. 1–12, 23 fev. 2018.

FRIEDBERG, E. C. et al. **DNA Repair and Mutagenesis**. Second Edi ed. [s.l: s.n.].

FRONTINI, M.; PROIETTI-DE-SANTIS, L. Interaction between the Cockayne syndrome B and p53 proteins: implications for aging. **Aging**, v. 4, n. 2, p. 89–97, fev. 2012.

FROSINA, G. The bright and the dark sides of DNA repair in stem cells. **Journal of Biomedicine and Biotechnology**, v. 2010, 2010.

FULLERTON, H. J. et al. Recurrent stroke in childhood cancer survivors. **Neurology**, v. 85, n. 12, p. 1056–1064, 22 set. 2015.

FUSS, J. O.; TAINER, J. A. XPB and XPD helicases in TFIIH orchestrate DNA duplex opening and damage verification to coordinate repair with transcription and cell cycle via CAK kinase. **DNA repair**, v. 10, n. 7, p. 697–713, 15 jul. 2011.

GALLOWAY, P. G. et al. Hirano bodies contain tau protein. **Brain research**, v. 403, n. 2, p. 337–340, 17 fev. 1987.

GHIT, A. Immunofluorescence studies to dissect the impact of Cockayne syndrome A alterations on the protein interaction and cellular localization. **Journal of Genetic Engineering and Biotechnology**, v. 19, n. 1, p. 1–8, 1 dez. 2021.

GIAMBONA, A. et al. Very early prenatal diagnosis of Cockayne's syndrome by coelocentesis. **https://doi.org/10.1080/01443615.2021.2014429**, p. 1–8, 10 jan. 2022.

GOLDEN, T. R.; HINERFELD, D. A.; MELOV, S. Oxidative stress and aging: beyond correlation. **Aging Cell**, v. 1, n. 2, p. 117–123, 1 dez. 2002.

GONZAGA, E. R. Role of UV Light in Photodamage, Skin Aging, and Skin Cancer. **American Journal of Clinical Dermatology 2009 10:1**, v. 10, n. 1, p. 19–24, 21 ago. 2012.

GONZALEZ, C. et al. Modeling amyloid beta and tau pathology in human cerebral organoids. **Molecular Psychiatry 2018 23:12**, v. 23, n. 12, p. 2363–2374, 31 ago. 2018.

GOPALAKRISHNAN, K. et al. Hydrogen peroxide induced genomic instability in nucleotide excision repair-deficient lymphoblastoid cells. **Genome Integrity**, v. 1, n. 1, p. 1–14, 22 dez. 2010.

GOPINATH, S. D.; RANDO, T. A. Stem Cell Review Series: Aging of the skeletal muscle stem cell niche. **Aging Cell**, v. 7, n. 4, p. 590–598, 1 ago. 2008.

GORBUNOVA, V. et al. Changes in DNA repair during aging. **Nucleic acids research**, v. 35, n. 22, p. 7466–7474, dez. 2007.

GRÄFF, J.; TSAI, L. H. Histone acetylation: molecular mnemonics on the chromatin. **Nature Reviews Neuroscience 2013 14:2**, v. 14, n. 2, p. 97–111, 17 jan. 2013.

GRAHAM, V. et al. SOX2 Functions to Maintain Neural Progenitor Identity. **Neuron**, v. 39, n. 5, p. 749–765, 28 ago. 2003.

GRANDISON, R. C.; PIPER, M. D. W.; PARTRIDGE, L. Amino-acid imbalance explains extension of lifespan by dietary restriction in Drosophila. **Nature 2009 462:7276**, v. 462, n. 7276, p. 1061–1064, 2 dez. 2009.

GUARDIOLA, A. et al. Síndrome de cockayne: relato de caso. **Arquivos de Neuro-Psiquiatria**, v. 57, n. 1, p. 106–110, 1999.

HÄMÄLÄINEN, R. H. et al. mtDNA Mutagenesis Disrupts Pluripotent Stem Cell Function by Altering Redox Signaling. **Cell Reports**, v. 11, n. 10, p. 1614–1624, 16 jun. 2015.

HARMAN, D. Aging: A Theory Based On Free Radical And Radiation Chemistry. **Free Radical and Radiation Chemistry**, v. UCRL, n. 3078, 14 jul. 1955.

HARRISON, D. E. et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. **Nature 2009 460:7253**, v. 460, n. 7253, p. 392–395, 8 jul. 2009.

HAYFLICK, L.; MOORHEAD, P. S. The serial cultivation of human diploid cell strains. **Experimental Cell Research**, v. 25, n. 3, p. 585–621, 1 dez. 1961.

HENDERSON, T. O.; NESS, K. K.; COHEN, H. J. Accelerated aging among cancer survivors: from pediatrics to geriatrics. **American Society of Clinical Oncology educational book. American Society of Clinical Oncology. Annual Meeting**, n. 34, p. e423–e430, maio 2014.

HERBIG, U. et al. Cellular senescence in aging primates. **Science**, v. 311, n. 5765, p. 1257, 3 mar. 2006.

HERRANZ, D. et al. Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. **Nature Communications 2010 1:1**, v. 1, n. 1, p. 1–8, 12 abr. 2010.

HILDRESTRAND, G. A. et al. The capacity to remove 8-oxoG is enhanced in newborn neural stem/progenitor cells and decreases in juvenile mice and upon cell differentiation. **DNA repair**, v. 6, n. 6, p. 723–732, 1 jun. 2007.

HIPP, M. S.; KASTURI, P.; HARTL, F. U. The proteostasis network and its decline in ageing. **Nature Reviews Molecular Cell Biology 2019 20:7**, v. 20, n. 7, p. 421–435, 7 fev. 2019.

HOEIJMAKERS, J. H. Genome maintenance mechanisms for preventing cancer. **Nature**, v. 411, n. 6835, p. 366–74, 17 maio 2001.

HOU, Y. et al. Ageing as a risk factor for neurodegenerative disease. **Nature Reviews Neurology**, p. 1–17, 9 set. 2019.

HOUTKOOPER, R. H.; PIRINEN, E.; AUWERX, J. Sirtuins as regulators of metabolism and healthspan. **Nature Reviews Molecular Cell Biology 2012 13:4**, v. 13, n. 4, p. 225–238, 7 mar. 2012.

HUMPHREYS, V. et al. Age-related increases in DNA repair and antioxidant protection: a comparison of the Boyd Orr Cohort of elderly subjects with a younger population sample. **Age and ageing**, v. 36, n. 5, p. 521–526, set. 2007.

IMAM, S. Z. et al. Cockayne syndrome protein B interacts with and is phosphorylated by c-Abl tyrosine kinase. **Nucleic Acids Research**, v. 35, n. 15, p. 4941–4951, 1 ago. 2007.

ITOH, M. et al. Neurodegeneration in hereditary nucleotide repair disorders. **Brain and Development**, v. 21, n. 5, p. 326–333, 1 jul. 1999.

ITOH, T.; ONO, T.; YAMAIZUMI, M. A new UV-sensitive syndrome not belonging to any complementation groups of xeroderma pigmentosum or Cockayne syndrome: siblings showing biochemical characteristics of Cockayne syndrome without typical clinical manifestations. **Mutation Research/DNA Repair**, v. 314, n. 3, p. 233–248, 1 maio 1994.

JAARSMA, D. et al. Cockayne syndrome pathogenesis: Lessons from mouse models. **Mechanisms of Ageing and Development**, v. 134, n. 5–6, p. 180–195, 1 maio 2013.

JOVANOVIC, S. V; SIMIC, M. G. Mechanism of OH radical reactions with thymine and uracil derivatives. **Journal of the American Chemical Society**, v. 108, n. 19, p. 5968–72, 1 set. 1986.

KAJITANI, G. S. et al. Transcription blockage by DNA damage in nucleotide excision repair-related neurological dysfunctions. **Seminars in Cell & Developmental Biology**, v. 114, p. 20–35, 1 jun. 2021a.

KAJITANI, G. S. et al. Neurovascular dysfunction and neuroinflammation in a Cockayne syndrome mouse model. **Aging (Albany NY)**, v. 13, n. 19, p. 22710, 15 out. 2021b.

KAMENISCH, Y. et al. Proteins of nucleotide and base excision repair pathways interact in mitochondria to protect from loss of subcutaneous fat, a hallmark of aging. **Journal of Experimental Medicine**, v. 207, n. 2, p. 379–390, 15 fev. 2010.

KAPAHI, P.; KAEBERLEIN, M.; HANSEN, M. Dietary restriction and lifespan: Lessons from invertebrate models. **Ageing Research Reviews**, v. 39, p. 3–14, 1 out. 2017.

KARAM, S. M. et al. Cockayne syndrome: report of a Brazilian family with confirmation of impaired RNA synthesis after UV-irradiation. **Genetics and Molecular Biology**, v. 23, n. 2, p. 273–275, 2000.

KARIKKINETH, A. C. et al. Cockayne syndrome: Clinical features, model systems and pathways. **Ageing Research Reviews**, v. 33, p. 3–17, 1 jan. 2017.

KAUPPILA, T. E. S.; KAUPPILA, J. H. K.; LARSSON, N. G. Mammalian Mitochondria and Aging: An Update. **Cell metabolism**, v. 25, n. 1, p. 57–71, 10 jan. 2017.

KAUSHIK, S.; CUERVO, A. M. Proteostasis and aging. **Nature Medicine 2015 21:12**, v. 21, n. 12, p. 1406–1415, 8 dez. 2015.

KAWANISHI, S.; MURATA, M. Mechanism of DNA damage induced by bromate differs from general types of oxidative stress. **Toxicology**, v. 221, n. 2–3, p. 172–178, abr. 2006.

KENNEDY, B. K.; STEFFEN, K. K.; KAEBERLEIN, M. Ruminations on dietary restriction and aging. **Cellular and Molecular Life Sciences 2007 64:11**, v. 64, n. 11, p. 1323–1328, 31 mar. 2007.

KENNEDY, S. R. et al. Ultra-Sensitive Sequencing Reveals an Age-Related Increase in Somatic Mitochondrial Mutations That Are Inconsistent with Oxidative Damage. **PLOS Genetics**, v. 9, n. 9, p. e1003794, set. 2013.

KESLER, S. R. et al. Probability of Alzheimer's disease in breast cancer survivors based on gray-matter structural network efficiency. **Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring**, v. 9, p. 67–75, 1 jan. 2017.

KHOBTA, A.; EPE, B. Repair of oxidatively generated DNA damage in Cockayne syndrome. **Mechanisms of Ageing and Development**, v. 134, n. 5, p. 253–260, 2013.

KING, A. A. et al. Long-term neurologic health and psychosocial function of adult survivors of childhood medulloblastoma/PNET: a report from the Childhood Cancer Survivor Study. **Neuro-Oncology**, v. 19, n. 5, p. 689–698, 1 maio 2017.

KITSERA, N. et al. 8-Oxo-7,8-dihydroguanine in DNA does not constitute a barrier to transcription, but is converted into transcription-blocking damage by OGG1. **Nucleic Acids Research**, v. 39, n. 14, p. 5926–5934, 1 ago. 2011.

KOBLAN, L. W. et al. In vivo base editing rescues Hutchinson–Gilford progeria syndrome in mice. **Nature**, v. 589, n. 7843, p. 608–614, 28 jan. 2021.

KOMOR, A. C. et al. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. **Nature 2015 533:7603**, v. 533, n. 7603, p. 420–424, 20 abr. 2016.

KONDO, J.; INOUE, M. Application of Cancer Organoid Model for Drug Screening and Personalized Therapy. **Cells 2019, Vol. 8, Page 470**, v. 8, n. 5, p. 470, 17 maio 2019.

KOOB, M. et al. Neuroimaging In Cockayne Syndrome. 2010.

KOPPELMANS, V. et al. Late effects of adjuvant chemotherapy for adult onset non-CNS cancer; cognitive impairment, brain structure and risk of dementia. **Critical Reviews in Oncology/Hematology**, v. 88, n. 1, p. 87–101, 1 out. 2013.

KOROVILA, I. et al. Proteostasis, oxidative stress and aging. **Redox Biology**, v. 13, p. 550–567, 1 out. 2017.

KOWALD, A.; PASSOS, J. F.; KIRKWOOD, T. B. L. On the evolution of cellular senescence. **Aging Cell**, v. 19, n. 12, p. e13270, 1 dez. 2020.

KRAEMER, K. H. Xeroderma Pigmentosum. **Archives of Dermatology**, v. 123, n. 2, p. 241, 1 fev. 1987.

KRUK, P. A.; RAMPINO, N. J.; BOHR, V. A. DNA damage and repair in telomeres: relation to aging. **Proceedings of the National Academy of Sciences of the United States of America**, v. 92, n. 1, p. 258, 3 jan. 1995.

KUMARAVEL, T. S. et al. Comet Assay measurements: a perspective. **Cell Biology and Toxicology**, v. 25, n. 1, p. 53–64, 27 fev. 2009.

KUNO, A.; HOSODA, R.; HORIO, Y. SIRT1 protects the heart against doxorubicin-induced cardiotoxicity by mediating the DNA damage response via deacetylation of histone H2AX. **European Heart Journal**, v. 42, n. Supplement_1, 12 out. 2021.

KURTISHI, A. et al. Cellular Proteostasis in Neurodegeneration. **Molecular Neurobiology 2018 56:5**, v. 56, n. 5, p. 3676–3689, 4 set. 2018.

KYNG, K. J. et al. The transcriptional response after oxidative stress is defective in Cockayne syndrome group B cells. **Oncogene**, v. 22, n. 8, p. 1135–1149, 27 fev. 2003.

LARDENOIJE, R. et al. The epigenetics of aging and neurodegeneration. **Progress in Neurobiology**, v. 131, p. 21–64, 1 ago. 2015.

LATINI, P. et al. CSA and CSB proteins interact with p53 and regulate its Mdm2-dependent ubiquitination. **Cell Cycle**, v. 10, n. 21, p. 3719–3730, 1 nov. 2011.

LAUGEL, V. Cockayne syndrome: The expanding clinical and mutational spectrum. **Mechanisms of Ageing and Development**, v. 134, n. 5, p. 161–170, 2013.

LEE, J. H. et al. Cockayne syndrome group B deficiency reduces H3K9me3 chromatin remodeler SETDB1 and exacerbates cellular aging. **Nucleic Acids Research**, v. 47, n. 16, p. 8548–8562, 19 set. 2019.

LEHMANN, A. R.; MAYNE, L. The Response of Cockayne Syndrome Cells to UV-Irradiation. In: **Chromosome Damage and Repair**. Boston, MA: Springer US, 1981. p. 367–371.

LIMA, T. et al. Pleiotropic effects of mitochondria in aging. **Nature Aging 2022 2:3**, v. 2, n. 3, p. 199–213, 17 mar. 2022.

LIN, M. T.; BEAL, M. F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. **Nature**, v. 443, n. 7113, p. 787–795, 19 out. 2006.

LINDENBAUM, Y. et al. Xeroderma pigmentosum/cockayne syndrome complex: first neuropathological study and review of eight other cases. **European journal of paediatric neurology**, v. 5, n. 6, p. 225–42, jan. 2001.

LIOCHEV, S. I. Reactive oxygen species and the free radical theory of aging. **Free Radical Biology and Medicine**, v. 60, p. 1–4, 1 jul. 2013.

LODATO, M. A. et al. Aging and neurodegeneration are associated with increased mutations in single human neurons. **Science**, v. 359, n. 6375, p. 555–559, 2 fev. 2018.

LÓPEZ-OTÍN, C. et al. The Hallmarks of Aging. **Cell**, v. 153, n. 6, p. 1194, 6 jun. 2013.

MAESO-DÍAZ, R.; GRACIA-SANCHO, J. Aging and Chronic Liver Disease. **Seminars in liver disease**, v. 40, n. 4, p. 373–384, 1 nov. 2020.

MAIER, B. et al. Modulation of mammalian life span by the short isoform of p53. **Genes & Development**, v. 18, n. 3, p. 306–319, 1 fev. 2004.

MANI, C.; REDDY, P. H.; PALLE, K. DNA repair fidelity in stem cell maintenance, health, and disease. **Biochimica et biophysica acta. Molecular basis of disease**, v. 1866, n. 4, 1 abr. 2020.

MARIÑO, G. et al. Insulin-like growth factor 1 treatment extends longevity in a mouse model of human premature aging by restoring somatotroph axis function. **Proceedings of the National Academy of Sciences of the United States of America**, v. 107, n. 37, p. 16268–16273, 14 set. 2010.

MARTEIJN, J. A et al. Understanding nucleotide excision repair and its roles in cancer and ageing. **Nature reviews. Molecular cell biology**, v. 15, n. 7, 2014.

MARTINCORENA, I. et al. Somatic mutant clones colonize the human esophagus with age. **Science**, v. 362, n. 6417, p. 911–917, 23 nov. 2018.

MATTSON, M. P.; ARUMUGAM, T. V. Hallmarks of Brain Aging: Adaptive and Pathological Modification by Metabolic States. **Cell Metabolism**, v. 27, n. 6, p. 1176–1199, 5 jun. 2018.

MAYNARD, S. et al. Human Embryonic Stem Cells Have Enhanced Repair of Multiple Forms of DNA Damage. **Stem Cells**, v. 26, n. 9, p. 2266–2274, set. 2008.

MCHUGH, D.; GIL, J. Senescence and aging: Causes, consequences, and therapeutic avenues. **Journal of Cell Biology**, v. 217, n. 1, p. 65–77, 2 jan. 2018.

MCLEARY, F. et al. Mechanisms underlying select chemotherapeutic-agent-induced neuroinflammation and subsequent neurodegeneration. **European Journal of Pharmacology**, v. 842, p. 49–56, 5 jan. 2019.

MECOCCI, P. et al. Age-dependent increases in oxidative damage to DNA, lipids, and proteins in human skeletal muscle. **Free radical biology & medicine**, v. 26, n. 3–4, p. 303–308, fev. 1999.

MENCK, C. F.; MUNFORD, V. DNA repair diseases: what do they tell us about cancer and aging? **Genetics and Molecular Biology**, v. 37, n. 1, p. 220–233, 2014.

MERIGHI, A. et al. The Phosphorylated Form of the Histone H2AX (γH2AX) in the Brain from Embryonic Life to Old Age. **Molecules (Basel, Switzerland)**, v. 26, n. 23, 1 dez. 2021.

MILMAN, S.; HUFFMAN, D. M.; BARZILAI, N. The Somatotropic Axis in Human Aging: Framework for the Current State of Knowledge and Future Research. **Cell Metabolism**, v. 23, n. 6, p. 980–989, 14 jun. 2016.

MOSESSO, P. et al. Potassium bromate but not X-rays cause unexpectedly elevated levels of DNA breakage similar to those induced by ultraviolet light in Cockayne syndrome (CS-B) fibroblasts. **Cytogenetic and Genome Research**, v. 104, n. 1–4, p. 178–181, 2004.

MURAI, M. et al. Early postnatal ataxia and abnormal cerebellar development in mice lacking Xeroderma pigmentosum Group A and Cockayne Syndrome Group B DNA repair genes. **Proceedings of the National Academy of Sciences**, v. 98, n. 23, p. 13379–13384, 6 nov. 2001.

MUSI, N. et al. Tau protein aggregation is associated with cellular senescence in the brain. **Aging Cell**, v. 17, n. 6, p. e12840, 1 dez. 2018.

MUZAMMAL, M.; ZEESHAN ALI, M.; AHMED ABBASI, A. The molecular genetics of UV-Sensitive syndrome; a rare dermal anomaly مستودع البحوث والدراسات النفسية والتربوية The repository of psychological and educational studies View project Clinical, Molecular & Epidemiological Assesment of Genetic Diseases in Pakistan View project. **Article in Journal of the Pakistan Medical Association**, 2021.

NAGESHAPPA, S. et al. Altered neuronal network and rescue in a human MECP2 duplication model. **Molecular psychiatry**, v. 21, n. 2, p. 178–188, 1 fev. 2016.

NARDO, T. et al. A UV-sensitive syndrome patient with a specific CSA mutation reveals separable roles for CSA in response to UV and oxidative DNA damage. **Proceedings of the National Academy of Sciences of the United States of America**, v. 106, n. 15, p. 6209–14, 14 abr. 2009.

NATALE, V. A comprehensive description of the severity groups in Cockayne syndrome. **American Journal of Medical Genetics Part A**, v. 155, n. 5, p. 1081–1095, 1 maio 2011.

NESS, K. K. et al. Physiologic frailty as a sign of accelerated aging among adult survivors of childhood cancer: a report from the St Jude Lifetime cohort study. **Journal of clinical oncology : official journal of the American Society of Clinical Oncology**, v. 31, n. 36, p. 4496–4503, 30 dez. 2013.

NEWMAN, J. C.; BAILEY, A. D.; WEINER, A. M. Cockayne syndrome group B protein (CSB) plays a general role in chromatin maintenance and remodeling. **Proceedings of the National Academy of Sciences**, v. 103, n. 25, p. 9613–9618, 20 jun. 2006.

NICCOLI, T.; PARTRIDGE, L. Ageing as a Risk Factor for Disease. **Current Biology**, v. 22, n. 17, p. R741–R752, 11 set. 2012.

NIEDERNHOFER, L. J. et al. A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. **Nature 2006 444:7122**, v. 444, n. 7122, p. 1038–1043, 21 dez. 2006.

NIEDERNHOFER, L. J. et al. Nuclear Genomic Instability and Aging. **Annual review of biochemistry**, v. 87, p. 295–322, 20 jun. 2018.

NISHIWAKI, T. et al. Comparative study of nucleotide excision repair defects between XPD-mutated fibroblasts derived from trichothiodystrophy and xeroderma pigmentosum patients. **DNA repair**, v. 7, n. 12, p. 1990–8, 1 dez. 2008.

NOUSPIKEL, T. Nucleotide excision repair and neurological diseases. **DNA repair**, v. 7, n. 7, p. 1155–67, 1 jul. 2008.

NOUSPIKEL, T.; HANAWALT, P. C. Terminally Differentiated Human Neurons Repair Transcribed Genes but Display Attenuated Global DNA Repair and Modulation of Repair Gene Expression. **Molecular and Cellular Biology**, v. 20, n. 5, p. 1562–1570, 1 mar. 2000.

OLIVEIRA, D. et al. Different gene expression profiles in iPSC-derived motor neurons from ALS8 patients with variable clinical courses suggest mitigating pathways for neurodegeneration. **Human molecular genetics**, v. 29, n. 9, p. 1465–1475, 3 jun. 2020.

OSORIO, F. G. et al. Somatic Mutations Reveal Lineage Relationships and Age-Related Mutagenesis in Human Hematopoiesis. **Cell Reports**, v. 25, n. 9, p. 2308- 2316.e4, 27 nov. 2018.

PAL, S.; TYLER, J. K. Epigenetics and aging. **Science Advances**, v. 2, n. 7, 2016.

PARK, S. K. et al. Cockayne syndrome: a case with hyperinsulinemia and growth hormone deficiency. **Journal of Korean Medical Science**, v. 9, n. 1, p. 74–77, 19 jun. 2009.

PARLANTI, E. et al. The cross talk between pathways in the repair of 8-oxo-7,8-dihydroguanine in mouse and human cells. **Free Radical Biology and Medicine**, v. 53, n. 11, p. 2171–2177, 1 dez. 2012.

PASCUCCI, B. et al. An altered redox balance mediates the hypersensitivity of Cockayne syndrome primary fibroblasts to oxidative stress. **Aging Cell**, v. 11, n. 3, p. 520–529, jun. 2012.

PELLEGRINI, L.; LANCASTER, M. A. Modeling neurodegeneration with mutant-tau organoids. **Cell**, v. 184, n. 17, p. 4377–4379, 19 ago. 2021.

PORTO, E. M. et al. Base editing: advances and therapeutic opportunities. **Nature Reviews Drug Discovery 2020 19:12**, v. 19, n. 12, p. 839–859, 19 out. 2020.

RANES, M. et al. A ubiquitylation site in Cockayne syndrome B required for repair of oxidative DNA damage, but not for transcription-coupled nucleotide excision repair. **Nucleic Acids Research**, v. 44, n. 11, p. 5246–5255, 2016.

REES, H. A. et al. Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery. **Nature Communications 2017 8:1**, v. 8, n. 1, p. 1–10, 6 jun. 2017.

REID-BAYLISS, K. S. et al. Why Cockayne syndrome patients do not get cancer despite their DNA repair deficiency. **Proceedings of the National Academy of Sciences**, p. 201610020, 19 ago. 2016.

RILEY, J. C. Estimates of Regional and Global Life Expectancy, 1800–2001. **Population and Development Review**, v. 31, n. 3, p. 537–543, 1 set. 2005.

RISQUES, R. A.; KENNEDY, S. R. Aging and the rise of somatic cancer-associated mutations in normal tissues. **PLOS Genetics**, v. 14, n. 1, p. e1007108, 1 jan. 2018.

ROCHA, C. R. R. et al. The role of DNA repair in the pluripotency and differentiation of human stem cells. **Mutation research**, v. 752, n. 1, p. 25–35, jan. 2013.

ROPOLO, M. et al. Complementation of the oxidatively damaged DNA repair defect in Cockayne syndrome A and B cells by Escherichia coli formamidopyrimidine DNA glycosylase. **Free Radical Biology and Medicine**, v. 42, n. 12, p. 1807–1817, 15 jun. 2007.

ROSALES-CORRAL, S. A. et al. Antioxidant and Anti-Inflammatory Role of Melatonin in Alzheimer's Neurodegeneration. **Aging: Oxidative Stress and Dietary Antioxidants**, p. 177–193, 2014.

ROSSI, D. J. et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. **Proceedings of the National Academy of Sciences of the United States of America**, v. 102, n. 26, p. 9194–9199, 28 jun. 2005.

ROUHANI, F. J. et al. Mutational History of a Human Cell Lineage from Somatic to Induced Pluripotent Stem Cells. **PLOS Genetics**, v. 12, n. 4, p. e1005932, 1 abr. 2016.

SACCO, R. et al. Cockayne syndrome b maintains neural precursor function. **DNA Repair**, v. 12, n. 2, p. 110–120, 1 fev. 2013.

SALMON, A. B.; RICHARDSON, A.; PÉREZ, V. I. Update on the oxidative stress theory of aging: Does oxidative stress play a role in aging or healthy aging? **Free Radical Biology and Medicine**, v. 48, n. 5, p. 642–655, 1 mar. 2010.

SAPIEHA, P.; MALLETTE, F. A. Cellular Senescence in Postmitotic Cells: Beyond Growth Arrest. **Trends in Cell Biology**, v. 28, n. 8, p. 595–607, 1 ago. 2018.

SARKAR, D.; FISHER, P. B. Molecular mechanisms of aging-associated inflammation. **Cancer Letters**, v. 236, n. 1, p. 13–23, 8 maio 2006.

SCHEIBYE-KNUDSEN, M. et al. Cockayne syndrome group B protein prevents the accumulation of damaged mitochondria by promoting mitochondrial autophagy. **Journal of Experimental Medicine**, v. 209, n. 4, 2012.

SCHEIBYE-KNUDSEN, M. et al. A High-Fat Diet and NAD+ Activate Sirt1 to Rescue Premature Aging in Cockayne Syndrome. **Cell Metabolism**, v. 20, n. 5, p. 840–855, nov. 2014a.

SCHEIBYE-KNUDSEN, M. et al. A High-Fat Diet and NAD+ Activate Sirt1 to Rescue Premature Aging in Cockayne Syndrome. **Cell Metabolism**, v. 20, n. 5, p. 840–855, 4 nov. 2014b.

SCHEIBYE-KNUDSEN, M.; CROTEAU, D. L.; BOHR, V. A. Mitochondrial deficiency in Cockayne syndrome. **Mechanisms of ageing and development**, v. 134, n. 5–6, p. 275–83, 2013.

SCHMICKEL, R. D. et al. Cockayne Syndrome: A Cellular Sensitivity to Ultraviolet Light. **Pediatrics**, v. 60, n. 2, p. 135–139, 1 ago. 1977.

SELBY, C. P.; SANCAR, A. Cockayne syndrome group B protein enhances elongation by RNA polymerase II. **Proceedings of the National Academy of Sciences**, v. 94, n. 21, p. 11205–11209, 14 out. 1997.

SESTI, F. Oxidation of K+ Channels in Aging and Neurodegeneration. **Aging and Disease**, v. 7, n. 2, p. 130, 2016.

SETIA, H.; MUOTRI, A. R. Brain organoids as a model system for human neurodevelopment and disease. **Seminars in Cell & Developmental Biology**, v. 95, p. 93–97, 1 nov. 2019.

SHIELS, A.; HEJTMANCIK, J. F. Molecular Genetics of Cataract. **Progress in Molecular Biology and Translational Science**, v. 134, p. 203–218, 1 jan. 2015.

SIDDIQI, M. A.; BOTHE, E. Single- and double-strand break formation in DNA irradiated in aqueous solution: dependence on dose and OH radical scavenger concentration. **Radiation research**, v. 112, n. 3, p. 449–63, dez. 1987.

SIOMEK, A. et al. Higher Leukocyte 8-Oxo-7,8-Dihydro-2'-Deoxyguanosine and Lower Plasma Ascorbate in Aging Humans? **https://home.liebertpub.com/ars**, v. 9, n. 1, p. 143–150, 20 nov. 2006.

SOARES, J. P. et al. Aging and DNA damage in humans: a meta-analysis study. **Aging**, v. 6, n. 6, p. 432–439, 2014.

SOFFER, D. et al. Cockayne syndrome: unusual neuropathological findings and review of the literature. **Annals of neurology**, v. 6, n. 4, p. 340–348, 1979.

SPITZ, M. A. et al. Diagnostic and severity scores for Cockayne syndrome. **Orphanet Journal of Rare Diseases**, v. 16, n. 1, p. 1–10, 1 dez. 2021.

SPIVAK, G. Transcription-coupled repair: an update. **Archives of Toxicology**, p. 1–12, 22 ago. 2016.

SPIVAK, G.; HANAWALT, P. C. Host cell reactivation of plasmids containing oxidative DNA lesions is defective in Cockayne syndrome but normal in UV-sensitive syndrome fibroblasts. **DNA repair**, v. 5, n. 1, p. 13–22, 5 jan. 2006.

SUSIC, D.; FROHLICH, E. D. The aging hypertensive heart: a brief update. **Nature Clinical Practice Cardiovascular Medicine 2008 5:2**, v. 5, n. 2, p. 104–110, fev. 2008.

SWARTZ, J. M. et al. A Novel ERCC6 Splicing Variant Associated with a Mild Cockayne Syndrome Phenotype. **Hormone Research in Paediatrics**, v. 82, n. 5, p. 344–352, 27 nov. 2014.

TAKAHASHI, K.; YAMANAKA, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. **Cell**, v. 126, n. 4, p. 663–76, 25 ago. 2006.

TAKAHASHI, Y. et al. Decreased gene expression responsible for post-ultraviolet DNA repair synthesis in aging: a possible mechanism of age-related reduction in DNA repair capacity. **The Journal of investigative dermatology**, v. 124, n. 2, p. 435–442, 2005.

TAKESHIMA, H.; USHIJIMA, T. Accumulation of genetic and epigenetic alterations in normal cells and cancer risk. **npj Precision Oncology 2019 3:1**, v. 3, n. 1, p. 1–8, 6 mar. 2019.

THINNES, F. P. Why cancer survivors have a lower risk of Alzheimer disease. **Molecular Genetics and Metabolism**, v. 107, n. 3, p. 630–631, 1 nov. 2012.

TORNALETTI, S. et al. Effect of 8-oxoguanine on transcription elongation by T7 RNA polymerase and mammalian RNA polymerase II. **DNA Repair**, v. 3, n. 5, p. 483–494, 4 maio 2004.

TRAPP, C. et al. Deficiency of the Cockayne syndrome B (CSB) gene aggravates the genomic instability caused by endogenous oxidative DNA base damage in mice. **Oncogene 2007 26:27**, v. 26, n. 27, p. 4044–4048, 8 jan. 2007.

TRUJILLO, C. A. et al. Nested oscillatory dynamics in cortical organoids model early human brain network development. **bioRxiv**, p. 358622, 29 jun. 2018.

VAN AS, J. W.; VAN DEN BERG, H.; VAN DALEN, E. C. Platinum-induced hearing loss after treatment for childhood cancer. **Cochrane Database of Systematic Reviews**, v. 2016, n. 8, 3 ago. 2016.

VAN DER HORST, G. T. J. et al. Defective Transcription-Coupled Repair in Cockayne Syndrome B Mice Is Associated with Skin Cancer Predisposition. **Cell**, v. 89, n. 3, p. 425–435, 2 maio 1997.

VAN DER HORST, G. T. J. et al. UVB radiation-induced cancer predisposition in Cockayne syndrome group A (Csa) mutant mice. **DNA Repair**, v. 1, n. 2, p. 143–157, 28 fev. 2002.

VAN DER PLUIJM, I. et al. Impaired Genome Maintenance Suppresses the Growth Hormone–Insulin-Like Growth Factor 1 Axis in Mice with Cockayne Syndrome. **PLOS Biology**, v. 5, n. 1, p. e2, 2006.

VESSONI, A. T. et al. Cockayne syndrome-derived neurons display reduced synapse density and altered neural network synchrony. **Human molecular genetics**, v. 25, n. 7, p. 1271–80, abr. 2016.

VESSONI, A. T. et al. Cockayne Syndrome: The many challenges and approaches to understand a multifaceted disease. **Genetics and Molecular Biology**, v. 43, n. 1 suppl 1, 2020.

VITALE, I. et al. DNA Damage in Stem Cells. **Molecular Cell**, v. 66, n. 3, p. 306–319, 4 maio 2017.

VON ZGLINICKI, T.; WAN, T.; MIWA, S. Senescence in Post-Mitotic Cells: A Driver of Aging? **Antioxidants and Redox Signaling**, v. 34, n. 4, p. 308–323, 1 fev. 2021.

WANG, S. et al. Rescue of premature aging defects in Cockayne syndrome stem cells by CRISPR/Cas9-mediated gene correction. **Protein & Cell**, p. 1–22, 30 abr. 2019.

WEBSTER, A. D. B. et al. Growth retardation and immunodeficiency in a patient with mutations in the DNA ligase I gene. **The Lancet**, v. 339, n. 8808, p. 1508–1509, 20 jun. 1992.

WEEMS, J. C. et al. A role for the Cockayne Syndrome B (CSB)-Elongin ubiquitin ligase complex in signal-dependent RNA polymerase II transcription. **Journal of Biological Chemistry**, p. 100862, 9 jun. 2021.

WEICHHART, T. mTOR as Regulator of Lifespan, Aging, and Cellular Senescence: A Mini-Review. **Gerontology**, v. 64, n. 2, p. 127–134, 1 fev. 2018.

WEIDENHEIM, K. M.; DICKSON, D. W.; RAPIN, I. Neuropathology of Cockayne syndrome: Evidence for impaired development, premature aging, and neurodegeneration. **Mechanisms of ageing and development**, v. 130, n. 9, p. 619–36, set. 2009.

WHELAN, K. et al. Auditory complications in childhood cancer survivors: A report from the childhood cancer survivor study. **Pediatric Blood & Cancer**, v. 57, n. 1, p. 126–134, 15 jul. 2011.

WILSON, B. T. et al. The Cockayne Syndrome Natural History (CoSyNH) study: clinical findings in 102 individuals and recommendations for care. **Genetics in medicine : official journal of the American College of Medical Genetics**, v. 18, n. 5, p. 483–493, 1 maio 2016.

WONG, J. C. et al. Dendritic spine density is altered in a mouse model of Cockayne syndrome. **Neuropathology and Applied Neurobiology**, v. 39, n. 4, p. 437–440, 1 jun. 2013.

WOOD, R. D. et al. Human DNA repair genes. **Science**, v. 291, n. 5507, p. 1284–1289, 16 fev. 2001.

WOODS, C. G. Human microcephaly. **Current Opinion in Neurobiology**, v. 14, n. 1, p. 112–117, 1 fev. 2004.

WU, I. C. et al. High Oxidative Stress Is Correlated with Frailty in Elderly Chinese. **Journal of the American Geriatrics Society**, v. 57, n. 9, p. 1666–1671, 1 set. 2009.

XU, Y. et al. Rat Model of Cockayne Syndrome Neurological Disease. **Cell Reports**, v. 29, n. 4, p. 800-809.e5, 22 out. 2019.

XUE, X. J.; YUAN, X. B. Nestin is essential for mitogen-stimulated proliferation of neural progenitor cells. **Molecular and Cellular Neuroscience**, v. 45, n. 1, p. 26–36, 1 set. 2010.

YOSHIDA, Y.; YAMANAKA, S. Induced Pluripotent Stem Cells 10 Years Later. **Circulation Research**, v. 120, n. 12, 2017.

YUAN, X. et al. Activation of RNA Polymerase I Transcription by Cockayne Syndrome Group B Protein and Histone Methyltransferase G9a. **Molecular Cell**, v. 27, n. 4, p. 585–595, 17 ago. 2007.

ZHANG, X. et al. Mutations in UVSSA cause UV-sensitive syndrome and destabilize ERCC6 in transcription-coupled DNA repair. **Nature Genetics 2012 44:5**, v. 44, n. 5, p. 593–597, 1 abr. 2012.

ZSURKA, G. et al. Is There Still Any Role for Oxidative Stress in Mitochondrial DNA-Dependent Aging? **Genes 2018, Vol. 9, Page 175**, v. 9, n. 4, p. 175, 21 mar. 2018.

ZULEGER, N.; ROBSON, M. I.; SCHIRMER, E. C. The nuclear envelope as a chromatin organizer. **http://dx.doi.org/10.4161/nucl.2.5.17846**, v. 2, n. 5, p. 339–349, 2011.

APPENDIX A: Transcription blockage by DNA damage in nucleotide excision repair-related neurological dysfuntions

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Review

Transcription blockage by DNA damage in nucleotide excision repair-related neurological dysfunctions

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ABSTRACT

Human genetic syndromes deficient in nucleotide excision repair (NER), such as xeroderma pigmentosum and Cockayne syndrome, may present neurological abnormalities and premature aging symptoms. Unrepaired endogenously generated DNA damage that hampers transcription is a strong candidate that contributes to the development of these severe effects in neuronal tissue. Endogenous lesions include those generated due to byproducts of cellular metabolisms, such as reactive oxygen species. This review presents much of the evidence on the mechanisms related to neurodegenerative processes associated with DNA damage responses. The primary focus is on the effects of the transcription machinery, including the accumulation of DNA•RNA hybrids (R-loops) that, in turn, influence DNA damage and repair metabolism. Moreover, several neuronal tissues present higher expression of long genes, a genomic subset more affected by DNA lesions, which may explain part of the neurological abnormalities in these patients. Also, neuronal tissues have different DNA repair capabilities that might result in different neurological consequences, as observed in patients and NER deficient animal models. The better understanding of how the accumulation of transcription blocking lesions can lead to neurological abnormalities and premature aging-like phenotypes may assist us in finding potential biomarkers and therapeutic targets that might improve the lives of these patients, as well as other neurological disorders in the general population.

1. Introduction

Neurodegenerative disorders are characterized by tissue loss and decreased neuronal function. Aging is considered the main risk factor for most neurodegenerative diseases [1], and several markers for these syndromes overlap with hallmarks of aging. These include altered intercellular communication, cellular senescence, epigenetic alterations, and genomic instability [2]. The accumulation of DNA lesions and the molecular responses they elicit are regarded as central events for the development and progression of neurodegenerative disorders. These include Alzheimer's (AD), Parkinson's (PD), Amyotrophic Lateral Sclerosis (ALS), and many DNA repair-related diseases [1,3]. Nucleotide excision repair (NER) was the first DNA repair pathway to be reported as defective in syndromes presenting neurological abnormalities and premature aging-like symptoms [4]. Further studies on NER diseases indicated that their neurodegenerative phenotype is associated with transcription blockage by unrepaired DNA damage [5], suggesting that cellular strategies to cope with DNA lesions could be crucial to neural tissues. Therefore, this review will focus on NER diseases and how deficiencies in this pathway may affect the repair of transcription blocking lesions in neurological tissues, triggering neurodegeneration.

Xeroderma pigmentosum (XP), a rare autosomal recessive disease, was the first syndrome to be associated with a DNA repair defect [6]. It is mainly characterized by a severely increased skin cancer risk of

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Table 1
Clinical manifestations of DNA repair-deficient patients. Cancer, neurodegeneration, and photosensitivity are shown as present (colored) or absent (white) in the symptoms cluster associated with which protein.

PROTEINS	GENE ENSENBLE CODE	DNA REPAIR PATHWAY INVOLVED	ASSOCIATED CLINICAL DISORDERS	REFERENCE	Cancer	Neurodegeneration	Photosensitivity
XPA	***XPA*** ENSG00000136936	NER excision complex	Xeroderma Pigmentosum, most patients associated with severe neurological features, also called DeSanctis-Cacchione syndrome	[7, 12, 169, 222]	■	■	■
XPB	***ERCC3*** *ENSG00000163161*	NER excision complex	Xeroderma Pigmentosum may or may not present neurological features; Xeroderma Pigmentosum with Cockayne Syndrome; Trichothiodystrophy;	[7, 12, 35, 169, 222]	■	■	■
XPC	**XPC** ENSG00000154767	GG-NER	Xeroderma Pigmentosum	[7, 12, 169, 222]	■		■
XPD	***ERCC2*** *ENSG00000104884*	NER excision complex	Xeroderma Pigmentosum, may or may not present neurological features; Xeroderma Pigmentosum with Cockayne Syndrome; Trichothiodystrophy; COFS;	[7, 12, 35, 169, 222]	■	■	■
XPE	***DDB2*** *ENSG00000134574*	GG-NER	Xeroderma Pigmentosum;	[7, 12, 169, 222]	■		■
XPF	***ERCC4*** *ENSG00000175595*	NER excision complex	Xeroderma Pigmentosum, may or may not present neurological features; XFE Syndrome; Cockayne Syndrome;	[7, 12, 169, 222]	■	■	■
XPG	***ERCC5*** *ENSG00000134899*	NER excision complex	Xeroderma Pigmentosum, may or may not present neurological features; Xeroderma Pigmentosum with Cockayne Syndrome;	[7, 12, 169, 222]	■	■	■
XPV / DNA Pol η	***POLH*** *ENSG00000170734*	Translesion synthesis	Xeroderma Pigmentosum	[7, 12, 169, 222]	■		■
ERCC1	***ERCC1*** ENSG00000012061	NER excision complex	Xeroderma Pigmentosum with neurological features; COFS;	[7, 12, 169, 222]	■	■	■
CSA	**ERCC8** ENSG00000049167	TC-NER	Cockayne Syndrome; UV-Sensitive Syndrome	[7, 12, 13, 29, 32, 169, 222]		■	■
CSB	***ERCC6*** *ENSG00000225830*	TC-NER	Cockayne Syndrome; UV-Sensitive Syndrome; COFS;	[7, 12, 13, 29, 32, 169, 222]		■	■
UVSSA	**UVSSA** ENSG00000163945	TC-NER	UV-Sensitive Syndrome	[29, 32, 33]			■

sun-exposed areas, sometimes accompanied by neurological symptoms [7]. XP is mainly caused by a deficiency in the genes *XPA* to *XPG* (as described in Table 1) involved with the NER pathway, a versatile DNA repair mechanism responsible for removing a wide variety of double helix-distorting lesions, such as ultraviolet light (UV) induced pyrimidine dimers and other lesions, such as lipid peroxidation generated alkylations [8,9]. Mutations in XP genes may thus lead to NER impairment, accumulation of DNA lesions, and a drastic increase in mutagenesis, especially in sun-exposed areas, which explains the increased skin cancer risk.

NER is composed of more than 30 proteins that coordinately recognize and remove the damaged strand of DNA. There are two NER subpathways differentiated by the damage recognition process. The global genome repair (GG-NER) scans for lesions throughout the genome, while the transcription-coupled repair (TC-NER) is initiated in response to a blockage of the RNA polymerase, which functions as a DNA damage signal. After lesion recognition by either sub-pathway, the damaged DNA region is opened by helicases, and nucleases cleave the DNA strand

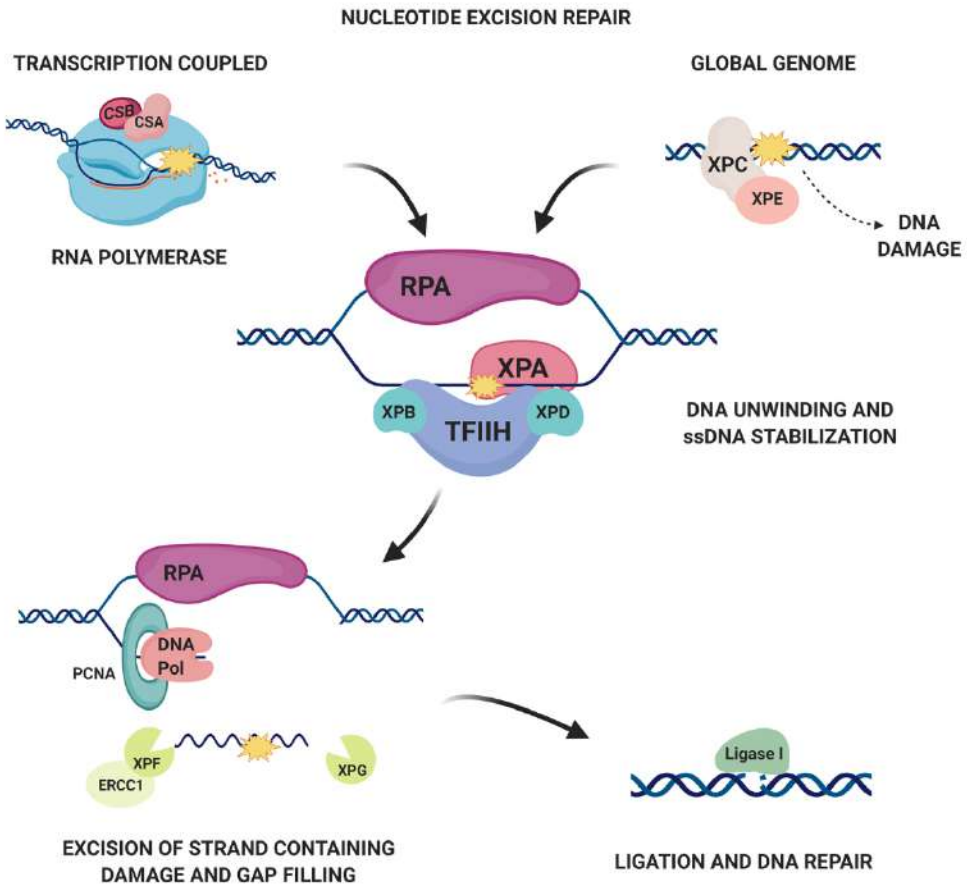

Fig. 1. Scheme representing the nucleotide excision repair pathway. This DNA repair pathway consists of damage recognition, unwinding of the double helix, incision on both sides of the lesion for the removal of the damaged strand, and gap-filling DNA synthesis followed by ligation. Nucleotide excision repair (NER) can be divided into two recognition subpathways: Transcription coupled (TC-NER) and Global genome repair (GG-NER). GG-NER removes lesions throughout the genome in a chromatin region-independent approach, depending on the XPC-RAD23B complex and XPE to recognize the lesion and mediate the recruitment of the excision complex. TC-NER occurs during transcription once a lesion stalls the RNA polymerase. CSA and CSB proteins recognize the blocked RNA polymerase and recruit the excision complex. After the recruitment of the excision complex, both GG-NER and TC-NER follow the same path. In this complex, TFIIH contains the helicases XPB and XPD that are responsible for unwinding the DNA at the damage site (approximately 30 bp), while RPA coats the single-strand DNA. The XPF/ERCC1 and XPG endonucleases are then recruited and are responsible for making the incision on 5' and 3' of the damaged strand, respectively. DNA polymerases δ and ε associated with PCNA perform the gap-filling synthesis, and DNA ligase I conclude the repair process. Evidence indicates that the defects in the sub-pathway represented on the left (TC-NER, when DNA lesions are blocking transcription), together with the following steps downward, are responsible for potential neurological abnormalities syndromes.

at both sides of the lesion. The damaged strand is excised, resulting in a gapped DNA, subsequently filled by DNA polymerases. DNA ligases complete the restoration of the original DNA molecule [7]. A detailed description of the NER pathway has previously been reviewed [10], and it is illustrated in Fig. 1.

XP also has a variant, milder, form of the disease (XP variant, XP-V), in which patients have a normal NER, but are defective in the *POLH* gene that encodes polη, a Y-family translesion DNA polymerase. The lack of the capacity to correctly bypass UV-induced pyrimidine dimers induces mutations in cells of XP-V patients, explaining the phenotype with increased skin cancer.

In addition to the increased skin cancer incidence, approximately 20–30% of XP patients also exhibit neurological symptoms, including loss of neurons in the brain cortex, hearing loss, swallowing difficulties, and intellectual disabilities. The severity of the symptoms is quite diverse and related to the mutated site of the NER gene, and the subpathway involved [7,11,12]. XP patients with mutations in the GG-NER genes *XPC*, *XPE/DDB2,* and the translesion synthesis DNA polymerase, *POLH/XPV,* do not present neurological symptoms, while mutations in *XPA*, *XPB/ERCC3*, *XPD/ERCC2*, *XPF/ERCC4,* and *XPG/ERCC5* genes, encoding proteins that act in the excision step, may result in severe neurological symptoms (Table 1).

The causal factors that give rise to this wide range of symptom severity observed in NER deficient patients are unclear. For example, patients with missense mutations in the *XPG* gene have increased frequency of skin cancer, but a mild XP-G neurological phenotype. However, patients carrying homozygous mutations that result in a truncated XPG protein or abrogate XPG expression have more severe neurological symptoms [13,14]. Interestingly, cells derived from these more affected patients are more sensitive to oxidative stress, which correlates to the neurological phenotype observed in these patients. On the other hand, the lack of XPG may also affect other repair processes besides NER [15–17]. Other XP proteins, such as XPB, XPD, and XPF, also perform functions other than in NER. XPB and XPD act as the helicase components of the transcription factor II H (TFIIH), involved in the transcription of numerous genes [18,19]. XPF, along with ERCC1, form an endonuclease complex that participates in several other DNA repair processes other than NER [20–22]. On the other hand, XPA, a scaffolding protein with no enzymatic functions, participates exclusively in the NER excision complex [23,24]. This implies that while the other functions of XP proteins may also impact the severity of the patient phenotype, deficiency in NER alone may be sufficient to cause neurodegeneration [23]. Curiously, mutations in TC-NER genes cause another NER-related neurodegenerative disease, Cockayne Syndrome (CS) [25].

Cockayne syndrome is another NER related autosomal recessive disease caused by mutations in *CSA/ERCC8* or *CSB/ERCC6* genes, involved in damage recognition by TC-NER (Fig. 1). Vessoni et al. present a comprehensive list of CS-causing CSA and CSB mutations [26]. CS patients are commonly born with no overt phenotype, but with a compromised development during early childhood. CS children gradually develop typical features of the disease, including small, deep-sunken eyes, loss of subcutaneous fat, dwarfism and sensitivity to UV irradiation (without skin cancer predisposition). Developmental and neurological problems are also observed, such as microcephaly, cerebral calcification (especially basal ganglia and cerebellum), progressive neurodegeneration, sensorineural deafness, segmental progeroid (premature aging-like) phenotype and a drastically reduced life expectancy [7,27–30]. Curiously, mutations in CS genes may also result in the UV-Sensitive Syndrome, in which patients exhibit a far milder phenotype, presenting solely higher skin sensitivity to UV radiation [31–33]. Moreover, mutations in the *UVSSA* gene, which encodes a protein that stabilizes processes in TC-NER, can also lead to UV-sensitive syndrome.

On the other hand, mutations in CS, as well as other NER genes (*ERCC1, XPF, XPG*), may also lead to the development of cerebro-oculo-facial-skeletal syndrome (COFS), considered a more severe form of CS, presenting microcephaly and developmental delay during the fetal phase, arthrogryposis, kyphosis, and congenital cataracts soon after birth [34]. Other neurodegenerative pathologies associated with NER mutations are trichothiodystrophy (TTD) and XPF-ERCC1 (XFE) syndromes. Symptoms of TTD patients are similar to CS, with additional fragile and brittle hair due to sulfur deficiency and reduced levels of cysteine-rich matrix proteins [35]. XFE is a severe progeroid syndrome caused by mutations in the helicase domain of XPF protein, affecting the XPF-ERCC1 endonuclease protein complex [36]. The total inefficiency of NER characterizes the syndrome, and the patients exhibit neurologic, hepatobiliary, musculoskeletal, and hematopoietic symptoms, distinguishing this syndrome from XP and CS [36].

The initial questions on what could lead to neurological abnormalities and premature aging symptoms in CS and some of the XP patients were raised more than forty years ago when cells of these patients showed high sensitivity to UV-irradiation [4,37,38]. Clues for the answers to these questions started to emerge a few years later, with the observation that XP and CS cells have defects in RNA synthesis after DNA damage [5,39], additionally to discoveries showing that actively transcribed genes are preferentially repaired. This preferential repair was later found to be due to a system that came to be known as TC-NER [40,41].

As previously discussed, NER defective patients with severe neurological symptoms have mutations in genes that act mainly on the TC-NER sub-pathway or in the excision complex. In contrast, mutations in GG-NER specific genes are associated with skin cancer phenotypes, with no neurological abnormalities. This leads to the hypothesis that unrepaired DNA damage that blocks transcription may result in the symptoms related to neurological and developmental abnormalities and premature aging. These DNA lesions cannot be those induced by sunlight in neurological tissues; instead, they are endogenously generated. The accumulation of these lesions during a lifetime hampers transcription and triggers cellular responses such as cell death or senescence, resulting in the severe symptoms observed in the patients.

The source of these endogenously generated lesions is still unknown, but reactive oxygen species (ROS) generated as byproducts of cell metabolism, including cellular respiration, are often speculated to be the main culprits [42]. This aerobic metabolism and its byproducts are especially relevant to the brain, as it consumes over 20% of the body oxygen, despite representing only 2% of the total body mass [43]. Furthermore, many genes with neural-specific function and expression are known to be long genes [44]. As DNA lesions caused by endogenous factors are expected to be mostly stochastically generated, the rate of lesion formation in genes is likely correlated to its length. This correlation suggests that the neural-specific long genes are more susceptible to be damaged, and further indicates the relevance of DNA damage and repair to neurological tissues.

This review will discuss several known aspects of transcription blockage, cellular effects caused by unrepaired endogenously generated DNA damage, and how they may affect neurological tissues and neuron-specific genes, leading to neurological abnormalities. We will mainly consider studies involving NER deficiency, as previously commented. Several other human syndromes related to defective DNA damage responses (DDR) and other DNA repair pathways such as ataxia-telangiectasia (AT) [45], Fanconi anemia [46], Werner syndrome and Huntington disease (HD) [47] also present symptoms related to neurological abnormalities and/or premature aging [48], which supports the relevance of DNA damage processing and may share similar pathways for the clinical phenotypes with NER syndromes. However, these syndromes will only be mentioned in specific discussions of this review, along with other genetic or aging-related neurodegenerative disorders.

2. NER participates in the repair of DNA lesions caused by endogenous factors

DNA lesions can be caused by endogenous factors such as spontaneous decay of the DNA molecule [49] or as a result of several cellular metabolic processes, including ROS, natural byproducts of metabolism capable of reacting with the DNA molecule. The increase in ROS is strongly associated with neurodegenerative diseases and aging [50–52]. However, it is not yet clear whether imbalanced ROS is the cause or consequence of many of these diseases, including those associated with deficiencies in DNA repair. ROS react and alter the DNA structure, compromising the function of the genetic material, and interfering with essential cellular processes, such as transcription and replication [53,54].

ROS promote oxidation of DNA by several different mechanisms and induce different types of lesions [55]. Oxidized DNA bases are typically repaired via base excision repair (BER), but some can also be substrates for NER, including 8-oxodGuo (8-oxo-7,8-dihydro-2'-deoxyguanosine), thymine glycol and G[8−5 m]T intra-strand crosslinks. Cyclopurines, as well as malondialdehyde and ethylene adducts, are not only substrates for NER but have also been found to block transcription [56–60]. NER deficient cells have also been shown to be more sensitive to oxidizing agents such as hydrogen peroxide, potassium bromate, and photo-activated methylene blue [14,61–65]. CS proteins, in particular, are involved with the repair of several DNA lesions induced by oxidation [66], and augmented levels of 8-oxodGuo have been detected in cultured fibroblasts derived from CS-B patients, as well as in primary keratinocytes obtained from CS-A patients [67–69].

Cyclopurines are also lesions inefficiently repaired in CS mutated cells, as 8,5′-cyclo-2′-deoxyadenosines accumulate in the kidney, liver, and brain of CSB knockout (KO) animals [70]. Recently, higher levels of cyclopurine deoxynucleosides were also found to be accumulated in the brain of XPA KO mice in an age-dependent manner [71]. Accumulation of 5',8-cyclo-2'-deoxynucleosides was observed in cells submitted to the hypoxia environment, and this was particularly high in XPA-knockdown, NER impaired cells [72]. Interestingly, low O_2 tensions may be exacerbated in the brain, as partial pressure and oxygen concentration in the tissue are both low and non-uniform [73]. Thus, cyclopurine lesions are candidates for explaining the progressive neurodegeneration observed in NER deficient patients. This hypothesis is supported by the fact that this type of lesion is not a substrate for DNA repair pathways other than NER, unlike 8-oxodGuo and some adducts formed by lipid peroxidation aldehydes that may also be repaired by BER and Homologous Recombination (HR). Additionally, these lesions are very stable and can block transcription, increasing their cytotoxicity [57,74–76]. However, as endogenous, *in vivo* cyclopurine levels are generally low, the role of these lesions for neurodegenerative disorders is still highly uncertain [76].

During oxidative stress, the cellular membrane can also be oxidated, generating lipid peroxidation. The products of lipoperoxidation, such as isoprostanes, hydroperoxides, and aldehydes, can react with DNA generating adducts. These lesions are generally known as exocyclic adducts, such as malonaldehyde, 4-hydroxy-2-nonenal (HNE), 2,4-decadienal, acrolein, and crotonaldehyde. These adducts are eventually repaired and found in urine, representing relevant biomarkers for human diseases, including cancer and neurological disorders [77,78]. HNE-DNA adducts are inefficiently repaired in fibroblasts derived from CS-B patients [65,68,79,80], and, more recently, 3, N4-ethenocytosine was shown to block transcription, and this blockage is exacerbated in B-lymphoblastoid cells derived from CS-A, CS-B, XP-A, XP-B, and XP-D patients, as well as CS-A and CS-B patient SV-40 transformed fibroblasts [56]. ERCC1$^{-/-}$ mice, as well as fibroblasts acquired from this knockout model, have also been found to be highly sensitive to lipid peroxidation products [81], suggesting that these lesions are also possible candidates for contributing to the neurodegeneration phenotype in NER disorders.

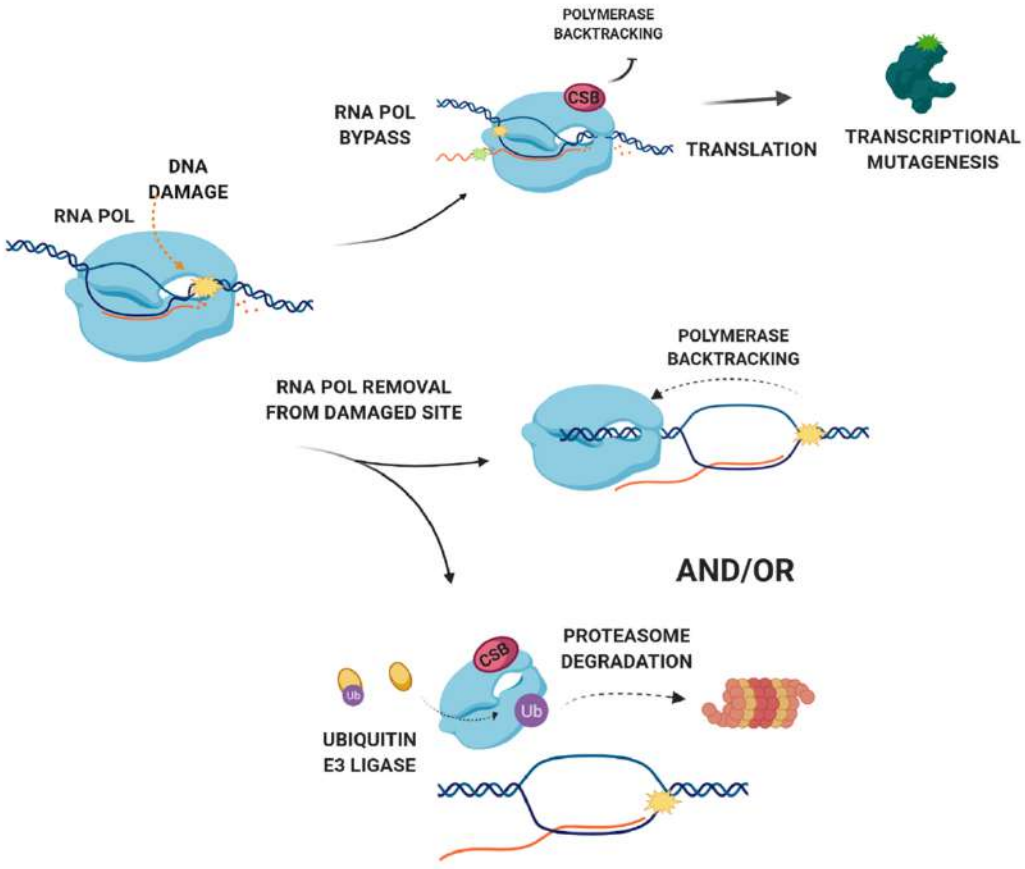

Fig. 2. Possible outcomes of RNA polymerases stalled at DNA damage. The blocked RNA polymerase can be recognized by a CSB protein, which may inhibit polymerase backtracking and promote an error-prone lesion bypass that can lead to transcriptional mutagenesis. However, when confronted with a bulky lesion, RNA Pol is unable to bypass the lesion, and the transcription machinery has to be removed from the lesion, allowing for DNA repair. This may be achieved by backtracking the transcription machinery, eliminating it by polyubiquitylation followed by proteasomal degradation, or both.

3. DNA damage as obstacles for transcription

Lesions can affect transcription in diverse manners, from reducing RNA Pol II transcriptional rate and compromising the fidelity of the process, to more severe ones, such as stalling the RNA polymerase or aborting transcription. Therefore, both transcription blocking lesions (TBLs) and non-bulky lesions unable to fully arrest RNA Pol II have an impact on gene expression [82,83]. The impact of DNA lesions on the transcription process depends on several factors, including the nature of the lesion, cell type, cell cycle phase, chromatin organization and frequency of transcription initiation [83], all of which also influence the rate of repair of TBLs, especially by the NER pathway.

RNA Pol II stalling leads to an extensively orchestrated response that involves not only the recruitment of the DNA repair machinery to the lesion, but also interferes in genome-wide transcription, both in the region of the lesion (cis) as well as in other genome regions (trans) [83–87]. One example of transcription modulation in trans is observed after UV irradiation, which leads to genome-wide transcriptional alterations. These alterations are related to a decrease in hypophosphorylated RNA Pol II, involved in transcription initiation, as well as an increase in the hyperphosphorylated form of this polymerase, involved in transcription elongation. These effects are more prominent in NER deficient cells [88].

The decrease of RNA Pol II in the promoter regions after UV irradiation may be related to an initial increase in transcription elongation, mediated by the elongation factor p-TEFb [85]. Following UV irradiation, p-TEFb is released from its inhibitory complex, and RNA Poll II switches to elongation mode on promoter-proximal pause sites. This process culminates in wave-like transcription elongation in almost all active genes and the induction of DDR genes. This widespread *de novo* RNA synthesis enhances DNA damage-sensing and, consequently, TC-NER recruitment [89,90].

Another evidence of genome-wide reorganization of RNA Pol II following DNA lesion induction was shown using ChIP-seq analysis after a sub-lethal dose of UVB irradiation. As soon as 2–4 h of irradiation, a reduction of more than 90% of RNA Pol II occupancy of the promoters of all expressed genes was observed, proceeded by a complete recovery 5–6 h after irradiation. Interestingly, a small set of genes had increased RNA Pol II occupancy in their promoters after UVB irradiation. These genes were associated with DDR, signal transduction, and apoptosis, many of which are regulated by the transcription factor p53 [91].

The shift in transcription induced by TBLs also plays a role in the pre-RNA maturation process and may culminate in DNA-damage-induced alternative splicing. ATR mediates the phosphorylation of the RNA Pol II C-terminal domain, slowing-down the elongation process and consequently inducing alternative splicing [92,93]. The spliceosome released from the nascent RNA activates ATM and subsequently induces the formation of R-loops (DNA•RNA hybrids), which are also implicated in alternative splicing [94]. Alternative splicing isoforms were observed as part of a complex time-dependent transcriptional response to UV irradiation, involving different DDR genes. Immediately after irradiation, a switch to the expression of short mRNA isoforms and alternative last exon splicing (ALE) in various genes involved with DDR was observed. For instance, the gene *ASCC3* has long and short ALE isoforms with opposite effects on transcription recovery after DNA damage. The long isoform encodes a protein responsible for maintaining the transcriptional suppression in late stages of DDR, while the short isoform functions as a non-coding RNA required for transcription recovery [95]. Some non-coding RNAs are also transcribed in response to DNA-damage, including lincRNA-p21 and PANDA, which participate in p53 mediated gene expression [96,97].

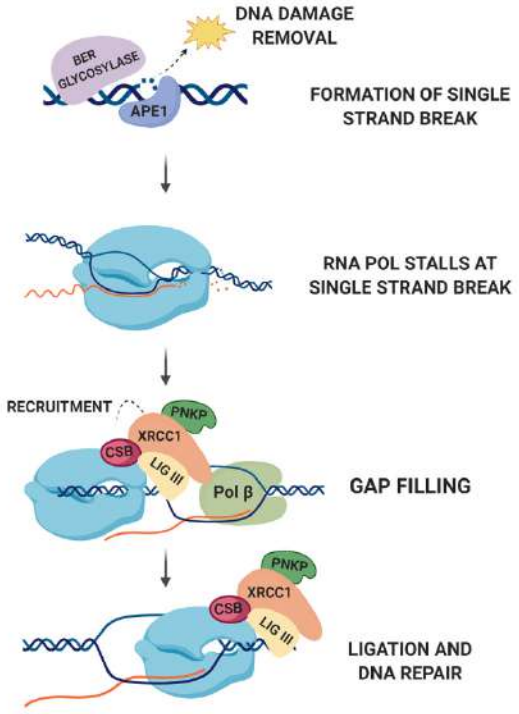

Fig. 3. Transcription and base excision repair. There are numerous DNA glycosylases responsible for detecting several different base modifications and initiating base excision repair (BER). The glycosylase recognizes and removes the damaged base, and the AP site is hydrolyzed by an AP endonuclease, such as APE1. The gap with a 3'OH and a 5'deoxyribose-phosphate will be the substrate for the next steps of BER. However, when this process occurs in a transcribed region, the RNA polymerase may stall in this single-stranded site. If an RNA Pol II stalls in these sites, recruitment of XRCC1/Ligase III complex occurs in a CSB-dependent manner and facilitates the assembly of the remaining BER factors, including PNKP and DNA Polymerase β, which fills the gap and is followed by DNA ligation through Ligase III, completing DNA repair and re-enabling transcription.

4. DNA damage repair is coupled with transcription

The stalled transcription machinery needs to be translocated to allow the DNA repair factors to access the lesion site. In order to do so, RNA Pol II can be either pushed back in a reverse translocation (backtracking) or be degraded (Fig. 2). RNA Pol II degradation is initiated with the polyubiquitylation of RPB1 – the largest subunit of the RNA Pol II – by E3 ubiquitin ligases, followed by its recognition and proteasomal degradation, which decreases the total amount of RNA Pol II in the cell, provoking a cellular decrease in transcription [98]. Even though both processes have been extensively studied, it is not clear whether they occur alternatively or in addition to each other [83,99,100].

RNA Pol II backtracking can be prevented by CSB, as this protein recognizes the obstruction and promotes forward elongation in a non-damaged template or non-bulky lesions, such as thymine glycol or 8-oxodGuo, contributing to the bypass of the lesion [59,101]. However, this bypass may occur in an error-prone mode by the RNA Pol II, causing transcriptional mutagenesis (Fig. 2), a process that can compromise translational fidelity by altering RNA nucleotides in codons and thus change the amino acid sequence [102,103]. Bulky lesions, on the other hand, preclude CSB from pushing RNA Pol II forward, and the persistent interaction between arrested RNA Pol II and CSB is likely the signal to initiate TC-NER [101,104,105].

Interestingly, TC-NER occurs preferentially in 5' regions of the genes [106] and might be explained by a *de novo* synthesis of RNA after TC-NER mediated RNA Pol II release from the DNA template [90], which may point to a more critical role of these regions and of small genes in DDR [85]. Indeed, small genes were found to be more differentially expressed after induction of TBLs [107,108]. For instance, the expression of activating transcription factor 3 (ATF3), a member of the ATF/cAMP response element (CRE), is increased immediately after genotoxic stress. ATF3 is then recruited to CRE/ATF-binding sites, repressing promoters of specific genes [109,110]. During transcriptional restart, ATF3 is ubiquitylated by CSB and degraded, a process that does not occur in CSB deficient cells, which results in the downregulation of genes that, when dysfunctional, are related to microcephaly, Purkinje cell degeneration and cognitive problems [109]. TC-NER being more active at smaller genes and proximal promoter regions may have its drawbacks, however, as it implies a slower repair and delayed transcriptional recovery of the terminal region of longer genes, as discussed more thoroughly further in this review [84].

An essential process in response to TBLs is the transcription reactivation that is essential to maintain cellular homeostasis. Whether transcription restarts from the blocked lesion, or *de novo* synthesis is necessary, or if both mechanisms co-exist is still unclear [85]. Sigurdsson et al. showed that transcription from a backtracked RNA Pol II, *in vitro*, can occur after the cleavage of nascent RNA by a specific exonuclease activity of the RNA Pol II assisted by elongation factors [111]. However, it was also demonstrated that RNA Pol II is released from the template after TC-NER [100], suggesting that transcription reactivation starts back from the promoter, providing time for DNA repair progression [84].

Besides facilitating bypassing natural pause sites and non-bulky lesions, recent works suggest that CSB is related to the initiation of transcription-related repair processes other than NER [83,112], such as BER and double-strand break (DSB) repair. CSB and transcription were recently found to stimulate the recruitment of the BER scaffolding protein XRCC1 to single-strand break (SSB) sites generated by BER glycosylases, in a process proposed as TC-BER (Fig. 3) [113]. This mechanism is corroborated by the fact that oxidized bases are preferentially repaired in transcribed genes of mammalian cells [114]. Furthermore, abasic sites have been shown to block transcription elongation [115], and 8-oxodGuo lesions are more likely to be repaired in transcribed regions in human cells [116].

CSB has also been shown to be related to an alternative mechanism of HR, now called transcription-coupled DSB repair [117,118]. CSB recruits HR proteins (BRCA1, RPA, and Rad51) to damaged chromatin, but represses non-homologous end-joining (NHEJ) pathway [117,118]. Interestingly, NHEJ also has a transcription-dependent feature. The NHEJ proteins Ku70, XRCC4, and Polynucleotide kinase/phosphatase (PNKP) form a multiprotein complex with RNA Pol II and utilize nascent RNA as a template for error-free NHEJ repair [119]. Further emerging pieces of evidence demonstrate that actively transcribed regions are more prone to have DSBs repaired [120,121]. Similarly to TC-NER, deficiency in DSB repair leads to a wide range of diseases associated with premature aging and neurodegeneration, such as AT and Werner syndrome, suggesting that DSB repair may also be related to RNA Pol II arrest on active genes [120].

Additionally, another PNKP-dependent, transcription-coupled repair system has been recently depicted. This novel pathway has been associated with the neurodegenerative syndrome Huntington's disease (HD), caused by CAG triplet repeat expansion in exon 1 of the *HTT* gene, which codes for the Huntingtin (HTT) protein. The HTT protein forms a protein complex with RNA polymerase II subunit A, HTT, Ataxin-3 (ATXN3), Ligase 3 (LIG3), and PNKP. Deficiency in HTT impaired this complex function, resulting in the accumulation of DNA breaks in actively transcribed genes of differentiated neurons. This resulted in chronic activation of ATM and p53, possibly leading to neuronal dysfunction or neurodegeneration [47]. Curiously, PNKP also participates in SSB repair and interacts with XRCC1 [122]. However, it is yet unknown whether the HTT-dependent transcription-coupled repair mechanism is related to SSB repair.

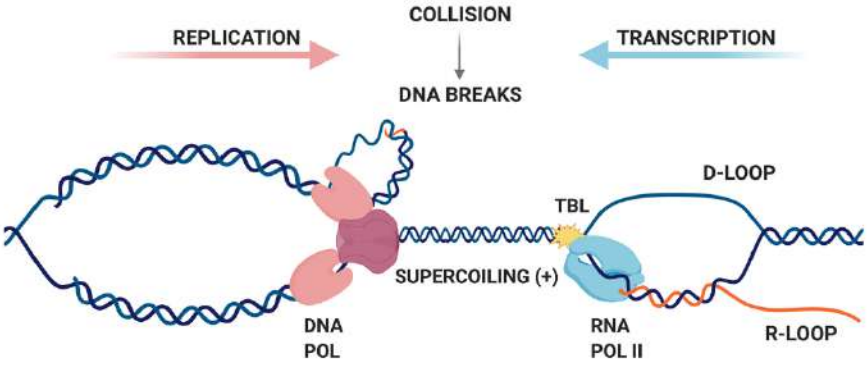

Fig. 4. Transcription-replication head-on collision. Transcription exposes the DNA to some modifications that can increase genomic instability. For instance, during the elongation process, RNA Pol promotes positive supercoiling ahead and negative supercoiling behind it, promoting the increase in DNA non-B structures such as R-loops and G-quadruplex. Transcription machinery stalled in a DNA lesion may also lead to R-loop formation. In the R-loop structure, the single-stranded DNA forms a D-loop structure that is more susceptible to the action of nucleases, ROS, and other DNA damaging agents. Furthermore, in replicating cells, the stalled RNA Pol may collide with the replication complex, leading to harmful consequences such as double-strand breaks, mainly when it occurs in a head-on orientation, demonstrating the importance of proteins involved in maintaining transcription homeostasis and avoiding transcription-replication conflicts (e.g., topoisomerases, helicases, DNA repair enzymes, RBPs).

5. Transcription as a source of DNA damage and R-loops

Despite the role of transcription in sensing some types of DNA lesions and initiating DDR, transcription can also act as a source of genomic instability [123,124]. Indeed, a high amount of mutagenesis and recombination occur in regions with intense transcription activation [124–126]. Transcription unwinds the DNA structure and promotes chromatin remodeling, and the negative supercoiling behind the RNA Pol II generates unstable regions of single-strand DNA more susceptible to chemical interactions culminating in DNA damage.

Transcription also leads to the formation of non-B DNA structures such as R-loops, which are increased by transcriptional arrest and can, in turn, affect RNA metabolism and promote genomic instability [123]. R-loops architecture consists of a DNA strand paired with the nascent RNA, while the other single DNA strand is left unpaired and vulnerable to enzymatic or chemical attacks (Fig. 4).

R-loops can also be a critical barrier during the replication process, leading to the collision of the replication fork with the transcription machinery [123,124]. These collisions are considered the most dangerous source of DNA damage associated with replication [127–130]. Collisions among replication forks and transcription complexes can culminate in DNA replication fork stalling, leading to the induction of DNA strand breaks, mutations, and DNA recombination (Fig. 4) [131]. These collisions can occur in a co-directional orientation, with both following the same direction in the DNA molecule or head-on, wherein the machinery collide in opposite directions. A head-on collision is more detrimental to the cells, generating topological stress and promoting RNA Pol II stalling and increasing the number of R-loops. In contrast, the co-directional collision appears to be less harmful and may assist cells in tolerating R-loops, demonstrating that the orientation of the collision is essential to its consequences [131,132].

The accumulation of unscheduled R-loops has been suggested to be more threatening for cells than the replication-transcription collisions alone [132]. Thus, despite the role of those structures in regulating some physiological functions, cells display several mechanisms to avoid the accumulation of those hybrids [133]. During transcription, various factors involved in RNA processing and exportation (such as THO complex and the ASF/SF2 processing factor) protect the nascent RNA, preventing it from hybridizing with the DNA template [134], while proteins such as Senataxin and RNAseH1 aid in removing RNA•DNA hybrids [135,136]. Other factors, including chromatin structure and control over DNA supercoiling, are also crucial in this homeostasis.

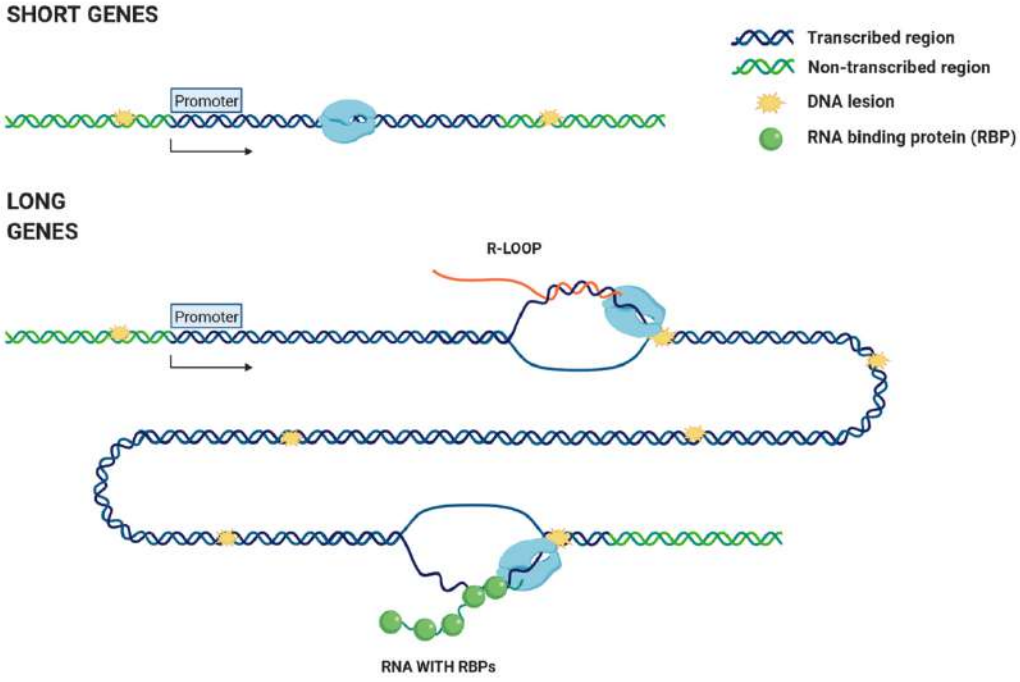

Fig. 5. Long genes are more prone to contain DNA lesions than short genes. DNA lesions are predicted to be generated in a stochastic manner, implying that the longer the gene, the higher the probability to contain DNA lesions. These lesions may induce a transcriptional arrest, which can cause R-loop formation, especially in the absence of RNA binding proteins such as SFPQ, which prevents the annealing of the nascent RNA with the DNA template, protecting the genome from the harmful consequences of the R-loops.

Dysfunction in any of these regulatory mechanisms might lead to transcriptional induction of DNA damage, compromising genomic stability and may promote neurodegeneration, as observed in patients containing mutations in genes involved in R-loop resolution that lead to genetic neurodegenerative disorders, such as ALS and Aicardi–Goutières syndrome [133–137].

Curiously, TC-NER factors, XPF/ERCC1, and XPG nucleases participate in R-loops processing, with DSBs as byproducts in a process that requires the CSB protein. Thus, TC-NER may play a direct role in R-loop resolution, although this process in itself may also cause genomic instability due to the DNA breaks generated by the nucleases [138]. Moreover, CSB proteins are recruited to R-loops accumulated after ROS production, inducing transcription-coupled homologous recombination. When CSB is recruited to R-loops in telomeric regions, ROS induced DNA breaks are suppressed [118,139]. NER proteins' role in the resolution of R-loops could be one of the disrupted cellular functions that contribute to the neurodegenerative problems observed in NER-related diseases.

Transcriptional activation has also been found to mediate DSB formation in human primary neurons as well as in multiple brain regions of mice in response to increased neuronal activity after sensory stimulation, indicating that DSBs occur during gene expression in neurons independently of replication [140,141]. DSB induction was also more severe and prolonged in a transgenic mice model of AD than in wild type mice [140], further suggesting that homeostasis of the transcription process and its consequences, especially DNA lesions and their repair, are critical to neuronal health.

6. Transcription elongation poses a challenge in longer genes

Long genes are more likely to present roadblocks for transcription, considering the higher probability of a TBL occurring in a more extended sequence. Indeed, long genes were shown to be more downregulated in DNA repair-deficient Ercc1$^{\Delta/-}$ mice [142].

Even though long genes are more prone to hindrances in transcription, the transcription machinery during elongation of long genes does not present many differences compared to the transcription elongation machinery present in shorter genes [143]. However, the transcription machinery may not be the only responsible for overcoming obstacles for transcription, as RNA-binding proteins (RBPs) may also assist in the bypass of these obstacles. RBPs bind co-transcriptionally to nascent long introns or other parts of the nascent RNA and are crucial for the transcription of longer genes (Fig. 5) [144,145].

Interestingly, defects in several RBPs also result in neuropathies [146], as exemplified by the induction of neuronal apoptosis in developing mouse brain caused by the loss of the RBP SFPQ. It has been proposed that the involvement of RBPs in neuropathies could be due to the disruption of long gene transcription. SFPQ mediates the recruitment of cyclin-dependent kinase 9 to the RNA Pol II elongation complex, where it binds co-transcriptionally to long introns, downregulating the expression of genes longer than 100 kb [144]. TDP-43, a protein associated with ALS, is another RBP recently shown to regulate the expression of genes with total intron length of 50 kb or more, as well as alternative splicing and R-loop formation [147].

Topoisomerase function also has a significant impact on long gene transcription. Inhibition of topoisomerase 1 causes downregulation of genes longer than 200 kb in neurons [148]. Moreover, *de novo* mutations in topoisomerase genes were associated with neurodevelopmental disorders, such as autism [149].

Transcription of long genes could also be affected by DNA methylation regulation, as it was found that the density of methylated cytosines is higher in long genes than in the rest of the genome. Knockout of the *MeCP2* gene, which encodes for a methyl-DNA binding protein associated with the autism-like Rett syndrome, causes an upregulation of long genes in mouse brain tissue. The only factor associated with this differential expression was gene length, but not histone modifications or sequence composition. Treatment with the topoisomerase inhibitor Topotecan managed to reverse the overexpression of long genes and partially rescue the phenotype of MeCP2-knockdown cultured neurons [150].

7. Long genes are more transcriptionally active in neural tissues

Neural tissues harbor a significant amount of tissue-specific gene expression [44,151], with the cerebral cortex expressing the second-largest amount of tissue-specific genes [152]. Interestingly, the genes expressed in the nervous system, particularly in the brain, are commonly longer than genes expressed in other tissues [44,148,150,153]. Moreover, long genes are enriched for brain-specific function [150].

In several neural tissues, the correlation between gene length and expression levels is strongly positive, indicating that longer genes, in general, are more likely to be expressed in the nervous system. Exceptions to this pattern can be found on more peripheral neural tissues, such as the retina, vomeronasal organ, and other olfactory associated neurons. Amygdala, frontal cortex, and hippocampus are the three tissues with the most positive correlation between gene length and expression [153]. Gene expression of genes with lengths of up to 10 kb is depleted in the cerebellum and cortex. However, in genes with more than 100 kb, gene expression levels are up to 10 times higher in these two neural tissues than in other tissues [150].

Intron length seems to act as a regulator of expression due to the transcription time of long genes. Transcription elongation rate can vary from 0.5 to 5 kb/sec, depending on chromatin characteristics [107]. For long genes, the time allowed for transcription could be very significant for expression control. Empirical evidence for intron length as a temporal regulator of gene expression was shown when the introns of the *Hes7* gene were removed in mice, resulting in severe development alterations in embryos [154].

Moreover, longer sequences present more robust gene expression levels, especially when regulatory regions are longer. It was shown *in silico* that if the regulatory sequence of a gene is longer than its basal mRNA levels are less subjected to alterations, even after changes in the DNA sequence [155]. This could contribute to the maintenance of the highly expressed sequences in the brain, as longer genes are more expressed in this tissue than in others. However, these long genes expressed in the neuronal tissues may be more susceptible to the effects of endogenously generated DNA damage in blocking transcription and eventually to the formation of unscheduled R-loops. This could explain part of the contribution TC-NER proteins may have in the resolution of transcription blockage in neuronal tissue, and thus aid in explaining neurological abnormalities in patients where these proteins are absent.

8. DNA repair pathways in the nervous system

Neurons are post-mitotic cells, and as they are generally non-dividing, they are less prone to genome mutagenesis and are unable to perform HR. Interestingly, while a deficiency in either HR or non-homologous end joining (NHEJ) induces embryonic lethality, each repair pathway influences different developmental stages of the nervous system. The absence of HR results in apoptosis of proliferating neural precursor cells, while the NHEJ requirement is only observed in later developmental stages, in differentiating neural cells [156].

Long-patch BER is also associated with the S-phase of the cell cycle [157], with its activity and components, namely FEN-1, PCNA, and Ligase I being reduced in differentiated neurons. Repair of hydrogen peroxide-induced DNA damage was also found to be inefficient in these cells [143]. Supplementation of the BER components DNA ligase I and DNA pol β into post-mitotic rat neurons restored gaps in DNA damaged templates [158], further suggesting a long patch BER attenuation and possibly indicating that neurons rely mainly on short-patch BER for this type of repair [159].

Notably, although neurons present lower levels of DNA pol β than

other cell types, this protein represents more than 90% of polymerases in post-mitotic neurons [160], suggesting that despite its decreased activity, BER is crucial to the homeostasis of these cells [161]. This is corroborated by the exacerbation of a neurodegenerative phenotype in an AD mouse model caused by the reduction of DNA pol β [162].

Despite their relevance to tissue homeostasis, BER [163] and NHEJ display decreased activity in aging brain cells [163,164] and other tissues [165]. Interestingly, the expression of OGG1 and UDG glycosylases was found to be increased in the brains of individuals over 70 years old [166]. The upregulation of these BER players might be an adaptive stress response to the increased levels of oxidative stress and DNA damage formation, as other increased stress responses, including an increase in cellular antioxidants, were also observed in the brains of these older individuals [166].

NER has been proposed to be attenuated in neurons and other post-mitotic cells, such as adipocytes and myotubes, since these cells present a diminished unscheduled DNA synthesis in response to genotoxic agents, including UV and methyl-methanesulfonate, a decrease found to be even more significant in aged rats [167,168]. However, it was later demonstrated that NER is functional in regions containing the transcribed genes of neurons, with global genome NER acting in the non-transcribed strand and TC-NER in the actively transcribed strand [169]. Furthermore, as neurons accumulate more DNA damage during aging due to the diminished repair pathways and higher metabolic rate, transcriptional mutagenesis is expected to be more common than in other cell types. Since these are life-long cells, the synthesis of misfolded proteins could have further implications. In fact, transcriptional mutagenesis may promote the formation of prion in neurons [170] and the aggregation of misfolded proteins in AD and PD [171].

Similarly to mature neurons, mature oligodendrocytes are considered post-mitotic cells and thus have no functional HR repair [172,173]. However, oligodendrocyte-progenitor cells (OPCs) remain mitotically active, acting as a source of oligodendrocyte and myelin replenishment throughout the lifespan [174,175], and thus have access to HR repair.

The other glial cells, astrocytes, and microglia, both proliferate in their mature stage, with cell density remaining generally stable during aging in adulthood [176,177] and microglial cells having a higher population turnover than other CNS cell types, with self-renewal maintained by coupled temporal and spatial proliferation and apoptosis [177]. Astrocyte and microglial turnover is also affected by endogenous and exogenous stress signals. These include DNA damage, environmental toxins, and damaged neurons, which can induce astrocyte and microglia activation and/or proliferation [178,179]. It is therefore expected that microglia can also use HR repair, although, to our knowledge, no studies have focused on understanding the mechanisms of DSB repair in this cell type. On the other hand, HR and NHEJ have been implicated in astrocyte resistance to ionizing radiation and chemotherapy, respectively [180,181].

Although there are few studies on the repair pathways of glial cells, cytotoxicity in neuronal and glial cells to DNA damaging agents capable of generating TBLs has revealed various responses. In general, astrocytes are more resistant to DNA damaging agents than other glial cells and neurons. Surprisingly, the repair of pyrimidine dimers, lesions repaired by the NER pathway, was attenuated in neurons and astrocytes when compared to fibroblasts, suggesting that a reduced global genome NER may be a common feature of CNS cells [182]. However, despite a similar repair of photoproducts, neurons display an increased sensitivity compared to astrocytes to chloroacetaldehyde, a compound that generates ethenobase and DNA lesions induced by oxidative stress, generally repaired by NER and BER, respectively. The ablation of either repair pathway increased neuronal, but not astrocytic, sensitivity to chloroacetaldehyde [183]. The enhanced resistance of astroglial cells to cytotoxic agents may be the result of other mechanisms, including mitochondrial DNA specific repair [184] and antioxidant defenses such as glutathione (GSH) and SOD proteins [185]. Interestingly, the co-culturing of astrocytes and neurons can increase neuronal resistance to oxidative stress through paracrine signaling and the transport of GSH precursors from astrocytes to neurons [185,186].

9. Brain tissue-specific effects of transcription blocking lesions and the lack of DNA repair

As previously mentioned, TBLs can activate several signaling pathways, especially DDR, including the phosphorylation and nuclear localization of p53 and activation of ATM and ATR kinases. These molecular mechanisms can induce several cellular effects, including cellular dysfunction, senescence, inflammation, and cell death [187–189]. A higher degree of neuronal cell death is observed in NER neurodegenerative diseases - the cerebellum of CS patients displays the loss of Purkinje cells, while XPA patients may exhibit neuronal cell death throughout the CNS [190]. NER deficient animal models, such as $Ercc1^{\Delta/-}$ and $CSB^{m/m}/XPA^{-/-}$ mice also display neuronal loss throughout their lives [142,191]. Moreover, a neuron-specific $CSB^{m/m}/XPA^{-/-}$ mouse model has demonstrated that TC-NER and NER deficiencies in neuronal cells result in a cognitive decline and severe loss of brain tissue [192]. Therefore, as neurons cannot regenerate and have decreased DNA repair, it would be expected that DNA damage-induced neuronal death is the primary driver of neurodegenerative diseases [193].

However, although NER whole-body KO mice feature a more modest loss of brain tissue than neuron-specific KO, whole-body KO mice display a substantially reduced lifespan and cognitive decline [191, 192]. Furthermore, aging-related neurodegenerative diseases, neurological deterioration, and cognitive impairment better correlate with synapse loss and myelin deterioration than neuronal death [194–196]. This indicates that other factors play a role in the neurodegenerative phenotype.

One of the proposed factors is cellular senescence, a characteristic that has been observed in several aging-related neurological diseases and tissues of progeroid NER deficient models [23,197–199]. Surprisingly, despite being post-mitotic cells, DNA damage in neurons has been shown to induce a senescence-like phenotype through a p21 dependent pathway [200]. Similar to regular senescent cells, they also produce and secrete an increased amount of ROS as well as display activation of the pro-inflammatory factors IL-6 and p38 [200].

Other brain cells, including astrocytes, microglia, OPCs, and vascular cells, can also enter a senescent-like state, thereby affecting brain physiology [201–203]. Microglial senescence has been shown to impair myelin debris clearance [204], and elimination of aged microglia followed by microglia repopulation, despite not altering neuroinflammatory gene expression, reversed age-induced changes in the expression of genes involved in neuronal/synaptic function, restored neuronal morphology and rescued deficits in long-term potentiation [205]. Interestingly, the use senolytics, molecules that specifically induce cell death of senescent cells, has also been shown to reverse some effects of neurodegenerative diseases. Targeting senescent cells ameliorated several aging phenotypes, including senescence-associated secretory phenotype-related IL-6 levels, fur condition, and general frailty in NER deficient, progeroid $Xpd^{TTD/TTD}$ and $Ercc1^{\Delta/-}$ mice, as well as naturally aged ones [206,207]. Targeting senescent, dysfunctional OPCs in an AD mice model also led to a decrease in Aβ load and neuroinflammation, restoring some of the cognitive deficits in these mice [208].

The inflammatory status of the brain cells, whether induced by a senescence-like response or other stimuli, can significantly alter brain physiology through several effects, such as the disruption of the blood-brain barrier [209], demyelination [210], and by generating ROS and reactive nitrogen species, hence increasing the DNA damage load in CNS cells [211]. This affects gene transcription and promotes other DDR, including motor neuron and oligodendrocyte cell death [83,212,213]. Interestingly, PARP-1 and DNA damage has been shown to activate microglia [214,215], and the more severe NER deficient models display

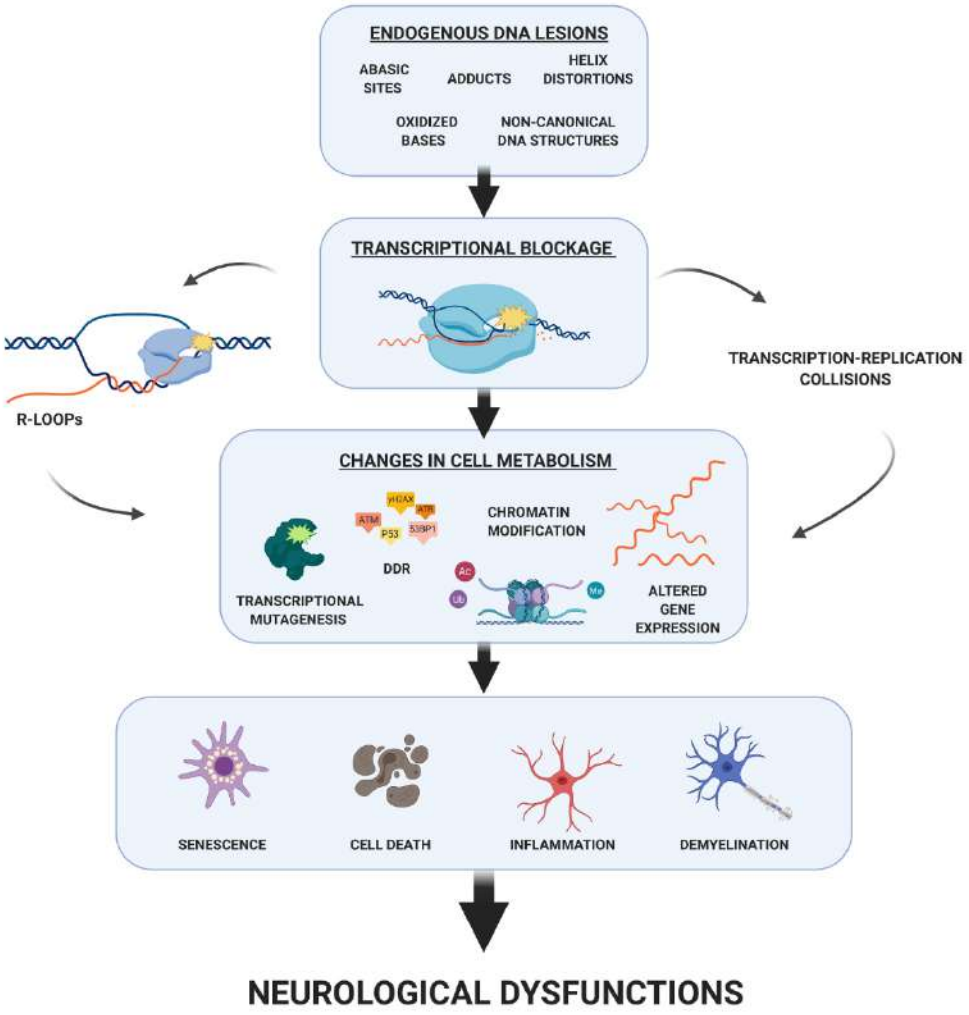

Fig. 6. Cascade effects of transcription blocking DNA lesions in neurological dysfunctions. Transcription blocking lesions are more relevant to transcriptionally active genes, and are especially problematic to long genes, as gene size influences the chance of the RNA polymerase elongation encountering a transcription blocking lesion. If unrepaired, these lesions can cause RNA polymerase arrest, which may further promote genomic instability, as well as changes in cellular metabolism. These, in turn, can lead to senescence, cell death, inflammation and demyelination, important features in the neurological dysfunctions associated with NER diseases and other aging-associated neurodegenerative diseases.

high astro and microglial cell activation [192]. Ercc1$^{\Delta/-}$ mice microglia has been shown to be in an activated and primed-like state, capable of inducing pro-inflammatory cytokines with lower amounts of stimuli. This microglia activation is partially induced by neuronal genotoxic stress, as forebrain neuron-specific Ercc$^{\Delta/-}$ mice exhibited a delayed, but prominent activation of microglia in that brain region [216], indicating that accumulation of NER related lesions can promote a neuroinflammatory phenotype.

10. Final considerations

As previously discussed, DNA damage not only affects transcription by inducing DDR through regulation of gene expression and alternative splicing [82,92,110,217], but also by blocking the RNA polymerase, which has a more significant impact on longer genes [107]. These transcriptional alterations caused by TBLs may themselves give rise to many cellular dysfunctions or cell death [83]. Oxidation induced DNA damage has also been found to accumulate in the non-transcribed portions of gene promoters (i.e., sections with no active TC-NER) in the brains of aged individuals. DNA damage accumulation can be correlated to the downregulation of several genes associated with synaptic transmission, vesicular transport, and ERK/MAPK signaling, pathways that, when altered, can have functional consequences to neuronal activity, including reduced synaptic plasticity and memory formation [166].

Transcriptional alterations have also been observed in SV40-transformed fibroblasts from CS-B patients, and are related not only to transcription blockage by DNA damage, but also gene regulation by the lack of CSB protein. Interestingly, many of the genes with reduced expression in the absence of CSB are neuronal genes, indicating a direct activity of this protein on neuronal gene regulation. These cells were also not able to reprogram to neuron-like cells, independent of DNA damage [218]. Similar impaired neuronal differentiation was observed in a CSB-knockdown immortalized human neural progenitor cell line [219]. While these observations may be linked to neurological abnormalities in CS patients, they do not explain their phenotype, as CS patients have cells that differentiate into neurons during development. Immortalized cells were used in both of these works, which may have affected the neuron differentiation process.

The development of induced pluripotent stem (iPS) cells from primary fibroblasts originated from CS-B patients (CSB-iPS cells) allowed for a more detailed investigation of neurological differentiation in the absence of this protein. Increased oxidative stress and elevated cell death rate were observed in CSB-iPS cells, in agreement with ROS induced DNA damage participating in premature aging and neurological abnormalities observed in CS patients [220]. In contrast to the immortalized cell lines, CSB-iPS cells can reprogram to neurons. These cells, however, display dysregulation of gene expression related to synapse formation, which culminates in lower synapse density and decreased synchrony of electrophysiological activity [221].

While we have focused on reviewing the possible neurodegenerative effects of TBLs mainly through NER deficient models, NER proteins also contribute to cellular homeostasis through functions other than DNA repair. These include transcription regulation and chromatin remodeling, as proteins involved with CS have been shown to modify DNA

conformation by altering DNA histone interaction and repositioning nucleosome. Moreover, CS cells display loss of proteostasis indicated by endoplasmatic reticulum stress and a high load of misfolded and carbonylated proteins [30,222–224]. Nevertheless, these other functions of NER proteins also affect gene transcription, whether directly or indirectly.

Moreover, despite having focused on nuclear DNA damage in this work, mitochondrial DNA oxidation is also crucial to the developmental mechanisms of neurodegenerative diseases, since mitochondrial dysfunction and increased ROS generation in this type of disorder have been reported [225]. Although NER is generally responsible for repairing the nuclear genetic material, some evidence indicates that NER proteins may also be found in the mitochondria and assist in protecting the mitochondrial genome from lesions generated by oxidation. This is especially demonstrated for CSA and CSB, which, in response to redox processes, localize in the mitochondria and associate with OGG1, promoting mitochondrial DNA damage repair [226–228].

Lastly, although responses to TBLs – cell death, senescence, inflammation, and transcriptional alterations – have their distinct mechanisms, they also share molecular crosstalks, including the p53, p21, ERK/MAPK pathways, which are pleiotropic factors that can influence multiple cellular and CNS tissue outcomes [188,200,229]. A summary of the neurological effects caused by TBLs is presented in Fig. 6. Moreover, cardiovascular disease symptoms have also been reported on DNA repair-deficient mouse models [198,230], and one should consider that these abnormalities may also be related to neurodegenerative processes.

Elucidation of the processes responsible for generating TBLs, as well as their tolerance and repair pathways may, therefore, assist in understanding not only NER syndromes, or age-related neurodegenerative diseases, but also natural human aging. Moreover, investigations using the models described have to potential to reveal novel biomarkers for neurological diseases, as well as new approaches for therapy and improvement of life quality in affected individuals.

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Conflict of interest

None.

References

[1] Y. Hou, X. Dan, M. Babbar, Y. Wei, S.G. Hasselbalch, D.L. Croteau, V.A. Bohr, Ageing as a risk factor for neurodegenerative disease, Nat. Rev. Neurol. 15 (2019) 565–581, https://doi.org/10.1038/s41582-019-0244-7.

[2] C. López-Otín, M.A. Blasco, L. Partridge, M. Serrano, G. Kroemer, The hallmarks of aging, Cell 153 (2013) 1194–1207, https://doi.org/10.1016/j.cell.2013.05.039.

[3] A.A. Abugable, J.L.M. Morris, N.M. Palminha, R. Zaksauskaite, S. Ray, S.F. El-Khamisy, DNA repair and neurological disease: From molecular understanding to the development of diagnostics and model organisms, DNA Repair (Amst.) 81 (2019), 102669, https://doi.org/10.1016/j.dnarep.2019.102669.

[4] A. Andrews, S. Barret, J. Robbins, Relation of DNA repair processes to pathological ageing of the nervous system in Xeroderma Pigmentosum, Lancet 307 (1976) 1318–1320, https://doi.org/10.1016/s0140-6736(76)92652-0.

[5] L. Mayne, A.R. Lehmann, Failure of RNA synthesis to recover after UV irradiation: an early defect in cells from individuals with Cockayne syndrome and xeroderma pigmentosum, Mutat. Res. 96 (1982) 140, https://doi.org/10.1016/0027-5107(82)90047-1.

[6] J.E. Cleaver, Defective repair replication of DNA in xeroderma pigmentosum, Nature 218 (1968) 652–656, https://doi.org/10.1038/218652a0.

[7] C.F.M. Menck, V. Munford, DNA repair diseases: what do they tell us about cancer and aging? Genet. Mol. Biol. 37 (2014) 220–233, https://doi.org/10.1590/s1415-47572014000200008.

[8] B. Tudek, D. Zdżalik-Bielecka, A. Tudek, K. Kosicki, A. Fabisiewicz, E. Speina, Lipid peroxidation in face of DNA damage, DNA repair and other cellular processes, Free Radic. Biol. Med. 107 (2017) 77–89, https://doi.org/10.1016/j.freeradbiomed.2016.11.043.

[9] A.P. Schuch, N.C. Moreno, N.J. Schuch, C.F.M. Menck, C.C.M. Garcia, Sunlight damage to cellular DNA: Focus on oxidatively generated lesions, Free Radic. Biol. Med. 107 (2017) 110–124, https://doi.org/10.1016/j.freeradbiomed.2017.01.029.

[10] R. Costa, V. Chiganças, R.S. Galhardo, H. Carvalho, C.F.M. Menck, The eukaryotic nucleotide excision repair pathway, Biochimie 85 (2003) 1083–1099, https://doi.org/10.1016/j.biochi.2003.10.017.

[11] G. Spivak, Nucleotide excision repair in humans, DNA Repair (Amst.) 36 (2015) 13–18, https://doi.org/10.1016/j.dnarep.2015.09.003.

[12] J.J. Digiovanna, K.H. Kraemer, Shining a light on xeroderma pigmentosum, J. Invest. Dermatol. 132 (2012) 785–796, https://doi.org/10.1038/jid.2011.426.

[13] S. Moriwaki, M. Takigawa, N. Igarashi, Y. Nagai, H. Amano, O. Ishikawa, S. Khan, K.H. Kraemer, Xeroderma pigmentosum complementation group G patient with a novel homozygous missense mutation and no neurological abnormalities, Exp. Dermatol. 21 (2012) 304–307, https://doi.org/10.1111/j.1600-0625.2012.01446.x.

[14] D.T. Soltys, C.R.R. Rocha, L.K. Lerner, T.A. de Souza, V. Munford, F. Cabral, T. Nardo, M. Stefanini, A. Sarasin, J.B. Cabral-Neto, C.F.M. Menck, Novel XPG (ERCC5) mutations affect DNA repair and cell survival after ultraviolet but not oxidative stress, Hum. Mutat. 34 (2013) 481–489, https://doi.org/10.1002/humu.22259.

[15] K.S. Trego, S.B. Chernikova, A.R. Davalos, J.J.P. Perry, L.D. Finger, C. Ng, M. Tsai, S.M. Yannone, J.A. Tainer, J. Campisi, P.K. Cooper, The DNA repair endonuclease XPG interacts directly and functionally with the WRN helicase defective in Werner syndrome, Cell Cycle 10 (2011) 1998–2007, https://doi.org/10.4161/cc.10.12.15878.

[16] K.S. Trego, T. Groesser, A.R. Davalos, C. Wiese, J. Campisi, P.K. Cooper, K. S. Trego, T. Groesser, A.R. Davalos, A.C. Parplys, W. Zhao, M.R. Nelson, Non-catalytic roles for XPG with BRCA1 and BRCA2 in homologous recombination and genome stability article non-catalytic roles for XPG with BRCA1 and BRCA2 in homologous recombination and genome stability, Mol. Biol. Evol. 61 (2016) 535–546, https://doi.org/10.1016/j.molcel.2015.12.026.

[17] S.E. Tsutakawa, A.H. Sarker, C. Ng, A.S. Arvai, D.S. Shin, B. Shih, Human XPG nuclease structure, assembly, and activities with insights for neurodegeneration and cancer from pathogenic mutations, Proc. Natl. Acad. Sci. 117 (2020) 14127–14138, https://doi.org/10.1073/pnas.1921311117.

[18] F. Tirode, D. Busso, I. Cedex, C.U. De, Strasbourg, reconstitution of the transcription factor TFIIH : assignment of functions for the three enzymatic subunits, XPB, XPD, and cdk7, Mol. Cell. 3 (1999) 87–95, https://doi.org/10.1016/s1097-2765(00)80177-x.

[19] J.O. Fuss, J.A. Tainer, XPB and XPD helicases in TFIIH orchestrate DNA duplex opening and damage verification to coordinate repair with transcription and cell cycle via CAK kinase, DNA Repair (Amst.) 10 (2011) 697–713, https://doi.org/10.1016/j.dnarep.2011.04.028.

[20] A. Ahmad, A.R. Robinson, A. Duensing, E. Van Drunen, H.B. Beverloo, D. B. Weisberg, P. Hasty, J.H.J. Hoeijmakers, L.J. Niedernhofer, E.R.C.C.1-X.P. F. Endonuclease, Facilitates DNA double-strand break repair, Mol. Cell. Biol. 28 (2008) 5082–5092, https://doi.org/10.1128/MCB.00293-08.

[21] D.K. Douwel, R.A.C.M. Boonen, D.T. Long, A.A. Szypowska, M. Ra, J.C. Walter, P. Knipscheer, XPF-ERCC1 acts in unhooking DNA interstrand crosslinks in cooperation, Mol. Cell. 54 (2014) 460–471, https://doi.org/10.1016/j.molcel.2014.03.015.

[22] C. Takahata, Y. Masuda, A. Takedachi, K. Tanaka, S. Iwai, I. Kuraoka, Repair synthesis step involving ERCC1-XPF participates in DNA repair of the Top1-DNA damage complex, Carcinogenesis 36 (2015) 841–851, https://doi.org/10.1093/carcin/bgv078.

[23] L.J. Niedernhofer, Tissue-specific accelerated aging in nucleotide excision repair deficiency, Mech. Ageing Dev. 129 (2008) 408–415, https://doi.org/10.1016/j.mad.2008.04.010.

[24] N. Sugitani, R.M. Sivley, K.E. Perry, J.A. Capra, W.J. Chazin, XPA : a key scaffold for human nucleotide excision repair, DNA Repair (Amst.) 44 (2016) 123–135, https://doi.org/10.1016/j.dnarep.2016.05.018.

[25] K.S. Reid-Bayliss, S.T. Arron, L. a Loeb, V. Bezrookove, J.E. Cleaver, Why Cockayne syndrome patients do not get cancer despite their DNA repair deficiency, Proc. Natl. Acad. Sci. 113 (2016) 10151–10156, https://doi.org/10.1073/pnas.1610020113.

[26] A.T. Vessoni, C. Chaves, C. Guerra, G.S. Kajitani, L.L. Souza, C. Carrião, M. Garcia, Cockayne syndrome : the many challenges and approaches to understand a multifaceted disease, Genet. Mol. Biol. 43 (2020), e20190085.

[27] K.M. Weidenheim, D.W. Dickson, I. Rapin, Neuropathology of Cockayne syndrome: evidence for impaired development, premature aging, and neurodegeneration, Mech. Ageing Dev. 130 (2009) 619–636, https://doi.org/10.1016/j.mad.2009.07.006.

[28] M.A. Nance, S.A. Berry, Cockayne syndrome: review of 140 cases, Cockayne syndrome: review of 140 cases, Am. J. Med. Genet. 42 (1992) 68–84, https://doi.org/10.1002/ajmg.1320420115.

[29] A.C. Karikkineth, M. Scheibye-Knudsen, E. Fivenson, D.L. Croteau, V.A. Bohr, Cockayne syndrome: Clinical features, model systems and pathways, Ageing Res. Rev. 33 (2017) 3–17, https://doi.org/10.1016/j.arr.2016.08.002.

[30] M.C. Alupei, P. Maity, P.R. Esser, I. Krikki, F. Tuorto, R. Parlato, M. Penzo, A. Schelling, V. Laugel, L. Montanaro, K. Scharffetter-Kochanek, S. Iben, Loss of proteostasis is a pathomechanism in Cockayne syndrome, Cell Rep. 23 (2018) 1612–1619, https://doi.org/10.1016/j.celrep.2018.04.041.
[31] K. Horibata, Y. Iwamoto, I. Kuraoka, N.G.J. Jaspers, A. Kurimasa, M. Oshimura, M. Ichihashi, K. Tanaka, Complete absence of Cockayne syndrome group B gene product gives rise to UV-sensitive syndrome but not Cockayne syndrome, Proc. Natl. Acad. Sci. 101 (2004) 15410–15415, https://doi.org/10.1073/pnas.0404587101.
[32] G. Spivak, The many faces of Cockayne syndrome, Proc. Natl. Acad. Sci. 101 101 (2004) 15273–15274, https://doi.org/10.1073/pnas.0406894101.
[33] G. Spivak, UV-sensitive syndrome, Mutat. Res. 577 (2005) 162–169, https://doi.org/10.1016/j.mrfmmm.2005.03.017.
[34] V. Laugel, C. Dalloz, E.S. Tobias, J.L. Tolmie, D. Martin-Coignard, V. Drouin-Garraud, V. Valayannopoulos, A. Sarasin, H. Dollfus, Cerebro-oculo-facio-skeletal syndrome: Three additional cases with CSB mutations, new diagnostic criteria and an approach to investigation, J. Med. Genet. 45 (2008) 564–571, https://doi.org/10.1136/jmg.2007.057141.
[35] M. Stefanini, E. Botta, M. Lanzafame, D. Orioli, Trichothiodystrophy: from basic mechanisms to clinical implications, DNA Repair (Amst.) 9 (2010) 2–10, https://doi.org/10.1016/j.dnarep.2009.10.005.
[36] L.J. Niedernhofer, G.A. Garinis, A. Raams, A.S. Lalai, A.R. Robinson, E. Appeldoorn, H. Odijk, R. Oostendorp, A. Ahmad, W. Van Leeuwen, A.F. Theil, W. Vermeulen, G.T.J. Van Der Horst, P. Meinecke, W.J. Kleijer, J. Vijg, N.G. J. Jaspers, J.H.J. Hoeijmakers, A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis, Nature 444 (2006) 1038–1043, https://doi.org/10.1038/nature05456.
[37] A.D. Andrews, S.F. Barrett, F.W. Yoder, J.H. Robbins, Cockayne's syndrome fibroblasts have increased sensitivity to ultraviolet light but normal rates of unscheduled dna synthesis, J. Invest. Dermatol. 70 (1978) 237–239, https://doi.org/10.1111/1523-1747.ep12541383.
[38] A.D. Andrews, S.F. Barrett, J.H. Robbins, Xeroderma pigmentosum neurological abnormalities correlate with colony forming ability after ultraviolet radiation, Proc. Natl. Acad. Sci. U. S. A 75 (1978) 1984–1988, https://doi.org/10.1073/pnas.75.4.1984.
[39] A.R. Lehmann, Three complementation groups in Cockayne syndrome, Mutat. Res. - Fundam. Mol. Mech. Mutagen. 106 (1982) 347–356, https://doi.org/10.1016/0027-5107(82)90115-4.
[40] V.A. Bohr, C. Smith, D.S. Okumoto, P.C. Hanawalt, DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall (https://doi:), Cell 40 40 (1985) 359–369, https://doi.org/10.1016/0092-8674(85)90150-3.
[41] I. Mellon, G. Spivak, P.C. Hanawalt, Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene, Cell 51 (1987) 241–249, https://doi.org/10.1016/0092-8674(87)90151-6.
[42] D.L. Croteau, V.A. Bohr, Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells, J. Biol. Chem. 272 (1997) 25409–25412, https://doi.org/10.1074/jbc.272.41.25409.
[43] D.D. Clark, L. Sokoloff, Basic Neurochemistry: Molecular, Cellular and Medical Aspects, sixth ed., Lippincott, 1999.
[44] J. Lonsdale, J. Thomas, M. Salvatore, R. Phillips, E. Lo, S. Shad, R. Hasz, G. Walters, F. Garcia, N. Young, B. Foster, M. Moser, E. Karasik, B. Gillard, K. Ramsey, S. Sullivan, J. Bridge, H. Magazine, J. Syron, J. Fleming, L. Siminoff, H. Traino, M. Mosavel, L. Barker, S. Jewell, D. Rohrer, D. Maxim, D. Filkins, P. Harbach, E. Cortadillo, B. Berghuis, L. Turner, E. Hudson, K. Feenstra, L. Sobin, J. Robb, P. Branton, G. Korzeniewski, C. Shive, D. Tabor, L. Qi, K. Groch, S. Nampally, S. Buia, A. Zimmerman, A. Smith, R. Burges, K. Robinson, K. Valentino, D. Bradbury, M. Cosentino, N. Diaz-Mayoral, M. Kennedy, T. Engel, P. Williams, K. Erickson, K. Ardlie, W. Winckler, G. Getz, D. DeLuca, D. MacArthur, M. Kellis, A. Thomson, T. Young, E. Gelfand, M. Donovan, Y. Meng, G. Grant, D. Mash, Y. Marcus, M. Basile, J. Liu, J. Zhu, Z. Tu, N.J. Cox, D.L. Nicolae, E.R. Gamazon, H.K. Im, A. Konkashbaev, J. Pritchard, M. Stevens, T. Flutre, X. Wen, E.T. Dermitzakis, T. Lappalainen, R. Guigo, J. Monlong, M. Sammeth, D. Koller, A. Battle, S. Mostafavi, M. McCarthy, M. Rivas, J. Maller, I. Rusyn, A. Nobel, F. Wright, A. Shabalin, M. Feolo, N. Sharopova, A. Sturcke, J. Paschal, J.M. Anderson, E.L. Wilder, L.K. Derr, E.D. Green, J.P. Struewing, G. Temple, S. Volpi, J.T. Boyer, E.J. Thomson, M.S. Guyer, C. Ng, A. Abdallah, D. Colantuoni, T.R. Insel, S.E. Koester, A.R. Little, P.K. Bender, T. Lehner, Y. Yao, C.C. Compton, J.B. Vaught, S. Sawyer, N.C. Lockhart, J. Demchok, H.F. Moore, The genotype-tissue expression (GTEx) project, Nat. Genet. 45 (2013) 580–585, https://doi.org/10.1038/ng.2653.
[45] K.R. Choy, D.J. Watters, Neurodegeneration in Ataxia-Telangiectasia : multiple roles of ATM kinase in cellular homeostasis, Dev. Dyn. 247 (2018) 33–46, https://doi.org/10.1002/dvdy.24522.
[46] A. Gueiderikh, F. Rosselli, J.B.C. Neto, A never-ending story: the steadily growing family of the FA and FA-like genes, Genet. Mol. Biol. 40 (2017) 398–407, https://doi.org/10.1590/1678-4685-GMB-2016-0213.
[47] R. Gao, A. Chakraborty, C. Geater, S. Pradhan, K.L. Gordon, J. Snowden, S. Yuan, A.S. Dickey, S. Choudhary, T. Ashizawa, L.M. Ellerby, A.R. La Spada, L. M. Thompson, T.K. Hazra, P.S. Sarkar, Mutant huntingtin impairs PNKP and ATXN3, disrupting DNA repair and transcription, Elife 8 (2019) 1–31, https://doi.org/10.7554/eLife.42988.
[48] L.J. Niedernhofer, A.U. Gurkar, Y. Wang, J. Vijg, J.H.J. Hoeijmakers, P. D. Robbins, Nuclear genomic instability and aging, Annu. Rev. Biochem. 87 (2018) 295–322, https://doi.org/10.1146/annurev-biochem-062917-012239.
[49] T. Lindahl, Instability and decay of the primary structure of DNA, Nature 362 (1993) 709–715, https://doi.org/10.1038/362709a0.
[50] F. Bozzo, A. Mirra, M.T. Carrì, Oxidative stress and mitochondrial damage in the pathogenesis of ALS: new perspectives, Neurosci. Lett. 636 (2017) 3–8, https://doi.org/10.1016/j.neulet.2016.04.065.
[51] F. Collin, Chemical basis of reactive oxygen species reactivity and involvement in neurodegenerative diseases, Int. J. Mol. Sci. 20 (2019) 2407, https://doi.org/10.3390/ijms20102407.
[52] A. Singh, R. Kukreti, L. Saso, S. Kukreti, Oxidative stress: a key modulator in neurodegenerative diseases, Molecules 24 (2019) 1583, https://doi.org/10.3390/molecules24081583.
[53] M. Ljungman, The transcription stress response, Cell Cycle 6 (2007) 2252–2257, https://doi.org/10.4161/cc.6.18.4751.
[54] B.R. Berquist, D.M. Wilson, Pathways for repairing and tolerating the spectrum of oxidative DNA lesions, Cancer Lett. 327 (2012) 61–72, https://doi.org/10.1016/j.canlet.2012.02.001.
[55] J. Cadet, K.J.A. Davies, M.H. Medeiros, P. Di Mascio, J.R. Wagner, Formation and repair of oxidatively generated damage in cellular DNA, Free Radic. Biol. Med. 107 (2017) 13–34, https://doi.org/10.1016/j.freeradbiomed.2016.12.049.
[56] I.A. Chaim, A. Gardner, J. Wu, T. Iyama, D.M. Wilson, L.D. Samson, A novel role for transcription-coupled nucleotide excision repair for the in vivo repair of 3,N4-ethenocytosine, Nucleic Acids Res. 45 (2017) 3242–3252, https://doi.org/10.1093/nar/gkx015.
[57] P.J. Brooks, D.S. Wise, D.A. Berry, J.V. Kosmoski, M.J. Smerdon, R.L. Somers, H. Mackie, A.Y. Spoonde, E.J. Ackerman, K. Coleman, R.E. Tarone, J.H. Robbins, The oxidative DNA lesion 8,5'-(S)-cyclo-2'-deoxyadenosine is repaired by the nucleotide excision repair pathway and blocks gene expression in mammalian cells, J. Biol. Chem. 275 (2000) 22355–22362, https://doi.org/10.1074/jbc.M002259200.
[58] S.D. Cline, J.N. Riggins, S. Tornaletti, L.J. Marnett, P.C. Hanawalt, Malondialdehyde adducts in DNA arrest transcription by T7 RNA polymerase and mammalian RNA polymerase II, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 7275–7280, https://doi.org/10.1073/pnas.0402252101.
[59] S. Tornaletti, Transcription arrest at DNA damage sites, Mutat. Res. Fundam. Mol. Mech. Mutagen. 577 (2005) 131–145, https://doi.org/10.1016/j.mrfmmm.2005.03.014.
[60] J. Wang, H. Cao, C. You, B. Yuan, R. Bahde, S. Gupta, C. Nishigori, L. J. Niedernhofer, P.J. Brooks, Y. Wang, Endogenous formation and repair of oxidatively induced G[8–5m]T intrastrand cross-link lesion, Nucleic Acids Res. 40 (2012) 7368–7374, https://doi.org/10.1093/nar/gks357.
[61] B. Pascucci, T. Lemma, E. Iorio, S. Giovannini, B. Vaz, I. Iavarone, A. Calcagnile, L. Narciso, P. Degan, F. Podo, V. Roginskya, B.M. Janjic, B. Van Houten, M. Stefanini, E. Dogliotti, M. D'Errico, An altered redox balance mediates the hypersensitivity of Cockayne syndrome primary fibroblasts to oxidative stress, Aging Cell 11 (2012) 520–529, https://doi.org/10.1111/j.1474-9726.2012.00815.x.
[62] M. D'Errico, E. Parlanti, M. Teson, P. Degan, T. Lemma, A. Calcagnile, I. Iavarone, P. Jaruga, M. Ropolo, A.M. Pedrini, D. Orioli, G. Frosina, G. Zambruno, M. Dizdaroglu, M. Stefanini, E. Dogliotti, The role of CSA in the response to oxidative DNA damage in human cells, Oncogene 26 (2007) 4336–4343, https://doi.org/10.1038/sj.onc.1210232.
[63] C. Maria Berra, C.S. De Oliveira, C.C. Machado Garcia, C.R. Reily Rocha, L. Koch Lerner, L.C. De Andrade Lima, M. Da Silva Baptista, C.F. Martins Menck, Nucleotide excision repair activity on DNA damage induced by photoactivated methylene blue, Free Radic. Biol. Med. 61 (2013) 343–356, https://doi.org/10.1016/j.freeradbiomed.2013.03.026.
[64] L.K. Lerner, N.C. Moreno, C.R.R. Rocha, V. Munford, V. Santos, D.T. Soltys, C.C. M. Garcia, A. Sarasin, C.F.M. Menck, XPD/ERCC2 mutations interfere in cellular responses to oxidative stress, Mutagenesis 34 (2019) 1–14, https://doi.org/10.1093/mutage/gez020.
[65] M. D'Errico, B. Pascucci, E. Iorio, B. Van Houten, E. Dogliotti, The role of CSA and CSB protein in the oxidative stress response, Mech. Ageing Dev. 134 (2013) 261–269, https://doi.org/10.1016/j.mad.2013.03.006.
[66] M.D. D'Errico, B. Pascucci, E. Iorio, B. Van Houten, E. Dogliotti, The role of CSA and CSB protein in the oxidative stress response, Mech. Ageing Dev. 134 (2013) 261–269, https://doi.org/10.1016/j.mad.2013.03.006.
[67] G.L. Dianov, C. Bischoff, M. Sunessen, V.A. Bohr, Repair of 8-oxoguanine in DNA is deficient in Cockayne syndrome group B cells, Nucleic Acids Res 27 (1999) 1365–1368, https://doi.org/10.1038/sj.onc.1205994.
[68] J. Tuo, M. Müftüoglu, C. Chen, P. Jaruga, R.R. Selzer, R.M. Brosh, H. Rodriguez, M. Dizdaroglu, V.A. Bohr, The Cockayne syndrome Group B gene product is involved in general genome base excision repair of 8-hydroxyguanine in DNA, J. Biol. Chem. 276 (2001) 45772–45779, https://doi.org/10.1074/jbc.M107888200.
[69] S. Cordisco, L. Tinaburri, M. Teson, D. Orioli, R. Cardin, P. Degan, M. Stefanini, G. Zambruno, L. Guerra, E. Dellambra, Cockayne syndrome type A protein protects primary human keratinocytes from senescence, J. Invest. Dermatol. 139 (2019) 38–50, https://doi.org/10.1016/j.jid.2018.06.181.
[70] G. Kirkali, N.C. de Souza-Pinto, P. Jaruga, V.A. Bohr, M. Dizdaroglu, Accumulation of (5'S)-8,5'-cyclo-2'-deoxyadenosine in organs of Cockayne syndrome complementation group B gene knockout mice, DNA Repair (Amst.) 8 (2009) 274–278, https://doi.org/10.1016/j.dnarep.2008.09.009.
[71] T. Mori, H. Nakane, T. Iwamoto, M.G. Krokidis, C. Chatgilialoglu, K. Tanaka, T. Kaidoh, M. Hasegawa, S. Sugiura, High levels of oxidatively generated DNA damage 8,5′-cyclo-2′-deoxyadenosine accumulate in the brain tissues of

xeroderma pigmentosum group A gene-knockout mice, DNA Repair (Amst.) 80 (2019) 52–58, https://doi.org/10.1016/j.dnarep.2019.04.004.
[72] M.G. Krokidis, E. Parlanti, M. D'Errico, B. Pascucci, A. Pino, A. Alimonti, D. Pietraforte, A. Masi, C. Ferreri, C. Chatgilialoglu, Purine DNA lesions at different oxygen concentration in DNA repair-impaired human cells (EUE-siXPA), Cells 8 (2019) 1377, https://doi.org/10.3390/cells8111377.
[73] M. Erecińska, I.A. Silver, Tissue oxygen tension and brain sensitivity to hypoxia, Respir. Physiol. 128 (2001) 263–276, https://doi.org/10.1016/S0034-5687(01)00306-1.
[74] I. Kuraoka, C. Bender, A. Romieu, J. Cadet, R.D. Wood, T. Lindahl, Removal of oxygen free-radical-induced 5′,8-purine cyclodeoxynucleosides from DNA by the nucleotide excision-repair pathway in human cells (https://doi:), Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 3832–3837, https://doi.org/10.1073/pnas.070471597.
[75] P.J. Brooks, T.F. Cheng, L. Cooper, Do all of the neurologic diseases in patients with DNA repair gene mutations result from the accumulation of DNA damage? DNA Repair (Amst.) 7 (2008) 834–848, https://doi.org/10.1016/j.dnarep.2008.01.017.
[76] P.J. Brooks, Blinded by the UV light: how the focus on transcription-coupled NER has distracted from understanding the mechanisms of Cockayne syndrome neurologic disease, DNA Repair (Amst.) 12 (2013) 656–671, https://doi.org/10.1016/j.dnarep.2013.04.018.Blinded.
[77] M.H.G. Medeiros, Exocyclic DNA adducts as biomarkers of lipid oxidation and predictors of disease. Challenges in developing sensitive and specific methods for clinical studies, Chem. Res. Toxicol. 22 (2009) 419–425, https://doi.org/10.1021/tx800367d.
[78] I.A. Blair, DNA adducts with lipid peroxidation products, J. Biol. Chem. 283 (2008) 15545–15549, https://doi.org/10.1074/jbc.R700051200.
[79] L. Maddukuri, E. Speina, M. Christiansen, D. Dudzińska, J. Zaim, T. Obtułowicz, S. Kabaczyk, M. Komisarski, Z. Bukowy, J. Szczegielniak, A. Wójcik, J. T. Kuśmierek, T. Stevnsner, V.A. Bohr, B. Tudek, Cockayne syndrome group B protein is engaged in processing of DNA adducts of lipid peroxidation product trans-4-hydroxy-2-nonenal, Mutat. Res. Fundam. Mol. Mech. Mutagen. 43 (2009) 7514–7521, https://doi.org/10.1016/j.mrfmmm.2009.03.007.
[80] J. Tuo, P. Jaruga, H. Rodriguez, M. Dizdaroglu, V.A. Bohr, The cockayne syndrome group B gene product is involved in cellular repair of 8-hydroxyadenine in DNA, J. Biol. Chem. 277 (2002) 30832–30837, https://doi.org/10.1074/jbc.M204814200.
[81] J. Czerwińska, M. Nowak, P. Wojtczak, D. Dziuban-Lech, J.M. Cieśla, D. Kołata, B. Gajewska, A. Barańczyk-Kuźma, A.R. Robinson, H.L. Shane, S.Q. Gregg, L. H. Rigatti, M.J. Yousefzadeh, A.U. Gurkar, S.J. McGowan, K. Kosicki, M. Bednarek, E. Zarakowska, D. Gackowski, R. Oliński, E. Speina, L. J. Niedernhofer, B. Tudek, ERCC1-deficient cells and mice are hypersensitive to lipid peroxidation, Free Radic. Biol. Med. 124 (2018) 79–96, https://doi.org/10.1016/j.freeradbiomed.2018.05.088.
[82] L.H. Gregersen, J.Q. Svejstrup, The cellular response to transcription-blocking DNA damage, Trends Biochem. Sci. 43 (2018) 327–341, https://doi.org/10.1016/j.tibs.2018.02.010.
[83] H. Lans, J.H.J. Hoeijmakers, W. Vermeulen, J.A. Marteijn, The DNA damage response to transcription stress, Nat. Rev. Mol. Cell Biol. 20 (2019) 765–784, https://doi.org/10.1038/s41580-019-0169-4.
[84] L.C. Andrade-Lima, A. Veloso, M. Ljungman, Transcription blockage leads to new beginnings, Biomolecules 5 (2015) 1600–1617, https://doi.org/10.3390/biom5031600.
[85] M.E. Geijer, J.A. Marteijn, What happens at the lesion does not stay at the lesion: transcription-coupled nucleotide excision repair and the effects of DNA damage on transcription in cis and trans, DNA Repair (Amst.). 71 (2018) 56–78, https://doi.org/10.1016/j.dnarep.2018.08.007.
[86] L. Proietti-De-Santis, P. Drané, J.M. Egly, Cockayne syndrome B protein regulates the transcriptional program after UV irradiation, EMBO J. 25 (2006) 1915–1923, https://doi.org/10.1038/sj.emboj.7601071.
[87] R. Madabhushi, L. Pan, L.H. Tsai, DNA damage and its links to neurodegeneration, Neuron 83 (2014) 266–282, https://doi.org/10.1586/14737175.2015.1028369.Focused.
[88] D.A.P. Rockx, R. Mason, A. Van Hoffen, M.C. Barton, E. Citterio, D.B. Bregman, A. A. Van Zeeland, H. Vrieling, L.H.F. Mullenders, UV-induced inhibition of transcription involves repression of transcription initiation and phosphorylation of RNA polymerase II, Proc. Natl. Acad. Sci. U. S. A 97 (2000) 10503–10508, https://doi.org/10.1073/pnas.180169797.
[89] A. Bugai, A.J.C. Quaresma, C.C. Friedel, T. Lenasi, R. Düster, C.R. Sibley, K. Fujinaga, P. Kukanja, T. Hennig, M. Blasius, M. Geyer, J. Ule, L. Dölken, M. Barboric, P-TEFb activation by RBM7 shapes a pro-survival transcriptional response to genotoxic stress, Mol. Cell. 74 (2019) 254–267.e10, https://doi.org/10.1016/j.molcel.2019.01.033.
[90] M.D. Lavigne, D. Konstantopoulos, K.Z. Ntakou-Zamplara, A. Liakos, M. Fousteri, Global unleashing of transcription elongation waves in response to genotoxic stress restricts somatic mutation rate, Nat. Commun. 8 (2017) 2076, https://doi.org/10.1038/s41467-017-02145-4.
[91] Á. Gyenis, D. Umlauf, Z. Újfaludi, I. Boros, T. Ye, L. Tora, UVB induces a genome-wide acting negative regulatory mechanism that operates at the level of transcription initiation in human cells, PLoS Genet. 10 (2014), e1004483, https://doi.org/10.1371/journal.pgen.1004483.
[92] M.J. Muñoz, M.S.P. Santangelo, M.P. Paronetto, M. de la Mata, F. Pelisch, S. Boireau, K. Glover-Cutter, C. Ben-Dov, M. Blaustein, J.J. Lozano, G. Bird, D. Bentley, E. Bertrand, A.R. Kornblihtt, DNA damage regulates alternative splicing through inhibition of RNA polymerase II elongation, Cell 137 (2009) 708–720, https://doi.org/10.1016/j.cell.2009.03.010.
[93] M.J. Muñoz, N. Nieto Moreno, L.E. Giono, A.E. Cambindo Botto, G. Dujardin, G. Bastianello, S. Lavore, A. Torres-Méndez, C.F.M. Menck, B.J. Blencowe, M. Irimia, M. Foiani, A.R. Kornblihtt, Major roles for pyrimidine dimers, nucleotide excision repair, and ATR in the alternative splicing response to UV irradiation, Cell Rep. 18 (2017) 2868–2879, https://doi.org/10.1016/j.celrep.2017.02.066.
[94] M. Tresini, D.O. Warmerdam, P. Kolovos, L. Snijder, M.G. Vrouwe, J.A. A. Demmers, W.F.J. Van Ijcken, F.G. Grosveld, R.H. Medema, J.H.J. Hoeijmakers, L.H.F. Mullenders, W. Vermeulen, J.A. Marteijn, The core spliceosome as target and effector of non-canonical ATM signalling, Nature 523 (2015) 53–58, https://doi.org/10.1038/nature14512.
[95] L. Williamson, M. Saponaro, S. Boeing, P. East, R. Mitter, T. Kantidakis, G. P. Kelly, A. Lobley, J. Walker, B. Spencer-Dene, M. Howell, A. Stewart, J. Q. Svejstrup, UV irradiation induces a non-coding RNA that functionally opposes the protein encoded by the same gene, e13, Cell 168 (2017) 843–855, https://doi.org/10.1016/j.cell.2017.01.019.
[96] M. Huarte, M. Guttman, D. Feldser, M. Garber, M.J. Koziol, D. Kenzelmann-Broz, A.M. Khalil, O. Zuk, I. Amit, M. Rabani, L.D. Attardi, A. Regev, E.S. Lander, T. Jacks, J.L. Rinn, A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response, Cell 142 (2010) 409–419, https://doi.org/10.1016/j.cell.2010.06.040.
[97] T. Hung, Y. Wang, M.F. Lin, A.K. Koegel, Y. Kotake, G.D. Grant, H.M. Horlings, N. Shah, C. Umbricht, P. Wang, Y. Wang, B. Kong, A. Langerød, A.L. Børresen-Dale, S.K. Kim, M. Van De Vijver, S. Sukumar, M.L. Whitfield, M. Kellis, Y. Xiong, D.J. Wong, H.Y. Chang, Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters, Nat. Genet. 43 (2011) 621–629, https://doi.org/10.1038/ng.848.
[98] R. Anindya, O. Aygün, J.Q. Svejstrup, Damage-induced ubiquitylation of human RNA polymerase II by the ubiquitin ligase Nedd4, but not Cockayne syndrome proteins or BRCA1, Mol. Cell. 28 (2007) 386–397, https://doi.org/10.1016/j.molcel.2007.10.008.
[99] L. Mullenders, DNA damage mediated transcription arrest: step back to go forward, DNA Repair (Amst.) 36 (2015) 28–35, https://doi.org/10.1016/j.dnarep.2015.09.005.
[100] Y.Y. Chiou, J. Hu, A. Sancar, C.P. Selby, RNA polymerase II is released from the DNA template during transcription-coupled repair in mammalian cells, J. Biol. Chem. 293 (2018) 2476–2486, https://doi.org/10.1074/jbc.RA117.000971.
[101] J. Xu, I. Lahiri, W. Wang, A. Wier, M.A. Cianfrocco, J. Chong, A.A. Hare, P. B. Dervan, F. DiMaio, A.E. Leschziner, D. Wang, Structural basis of DNA lesion recognition for eukaryotic transcription-coupled nucleotide excision repair, DNA Repair (Amst.) 71 (2018) 43–55, https://doi.org/10.1016/j.dnarep.2018.08.006.
[102] D. Brégeon, P.W. Doetsch, Transcriptional mutagenesis: causes and involvement in tumor development, Nat. Rev. Cancer 11 (2011) 218–227, https://doi.org/10.1038/nrc3006.Transcriptional.
[103] M. Ezerskyte, J.A. Paredes, S. Malvezzi, J.A. Burns, G.P. Margison, M. Olsson, D. A. Scicchitano, K. Dreij, O6-methylguanine-induced transcriptional mutagenesis reduces p53 tumor-suppressor function, Proc. Natl. Acad. Sci. U. S. A 115 (2018) 4731–4736, https://doi.org/10.1073/pnas.1721764115.
[104] C.P. Selby, A. Sancar, Cockayne syndrome group B protein enhances elongation by RNA polymerase II, Proc. Natl. Acad. Sci. U. S. A 94 (1997) 11205–11209, https://doi.org/10.1073/pnas.94.21.11205.
[105] F. Brueckner, U. Hennecke, T. Carrel, P. Cramer, CPD damage recognition by transcribing RNA polymerase II, Science 315 (2007) 859–862, https://doi.org/10.1126/science.1135400.
[106] J. Hu, S. Adar, C.P. Selby, J.D. Lieb, A. Sancar, Genome-wide analysis of human global and transcription-coupled excision repair of UV damage at single-nucleotide resolution, Genes Dev. 29 (2015) 948–960, https://doi.org/10.1101/gad.261271.115.
[107] L.C. Andrade-Lima, A. Veloso, M.T. Paulsen, C.F.M. Menck, M. Ljungman, DNA repair and recovery of RNA synthesis following exposure to ultraviolet light are delayed in long genes, Nucleic Acids Res 43 (2015) 2744–2756, https://doi.org/10.1093/nar/gkv148.
[108] B.C. McKay, L.J. Stubbert, C.C. Fowler, J.M. Smith, R.A. Cardamore, J.C. Spronck, Regulation of ultraviolet light-induced gene expression by gene size, Proc. Natl. Acad. Sci. U. S. A 101 (2004) 6582–6586, https://doi.org/10.1073/pnas.0308181101.
[109] A. Epanchintsev, F. Costanzo, M.A. Rauschendorf, M. Caputo, T. Ye, L.M. Donnio, L. Proietti-de-Santis, F. Coin, V. Laugel, J.M. Egly, Cockayne's syndrome A and B proteins regulate transcription arrest after genotoxic stress by promoting ATF3 degradation, Mol. Cell. 68 (2017) 1054–1066.e6, https://doi.org/10.1016/j.molcel.2017.11.009.
[110] U. Kristensen, A. Epanchintsev, M.A. Rauschendorf, V. Laugel, T. Stevnsner, V. A. Bohr, F. Coin, J.M. Egly, Regulatory interplay of Cockayne syndrome B ATPase and stress-response gene ATF3 following genotoxic stress, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 2261–2270, https://doi.org/10.1073/pnas.1220071110.
[111] S. Sigurdsson, A.B. Dirac-Svejstrup, J.Q. Svejstrup, Evidence that transcript cleavage Is essential for RNA polymerase II transcription and cell viability, Mol. Cell. 38 (2010) 202–210, https://doi.org/10.1016/j.molcel.2010.02.026.
[112] T. Stevnsner, M. Muftuoglu, M.D. Aamann, V.A. Bohr, The role of Cockayne syndrome group B (CSB) protein in base excision repair and aging, Mech. Ageing Dev. 129 (2008) 441–448, https://doi.org/10.1016/j.mad.2008.04.009.
[113] H. Menoni, F. Wienholz, A.F. Theil, R.C. Janssens, H. Lans, A. Campalans, J. P. Radicella, J.A. Marteijn, W. Vermeulen, The transcription-coupled DNA repair-

initiating protein CSB promotes XRCC1 recruitment to oxidative DNA damage, Nucleic Acids Res. 46 (2018) 7747–7756, https://doi.org/10.1093/nar/gky579.
[114] D. Banerjee, S.M. Mandal, A. Das, M.L. Hegde, S. Das, K.K. Bhakat, I. Boldogh, P. S. Sarkar, S. Mitra, T.K. Hazra, Preferential repair of oxidized base damage in the transcribed genes of mammalian cells, J. Biol. Chem. 286 (2011) 6006–6016, https://doi.org/10.1074/jbc.M110.198796.
[115] S. Tornaletti, L.S. Maeda, P.C. Hanawalt, Transcription arrest at an abasic site in the transcribed strand of template DNA, Chem. Res. Toxicol. 19 (2006) 1215–1220, https://doi.org/10.1021/tx060103g.
[116] J. Guo, P.C. Hanawalt, G. Spivak, Comet-FISH with strand-specific probes reveals transcription-coupled repair of 8-oxoGuanine in human cells, Nucleic Acids Res. 41 (2013) 7700–7712, https://doi.org/10.1093/nar/gkt524.
[117] N.L. Batenburg, E.L. Thompson, E.A. Hendrickson, X. Zhu, Cockayne syndrome group B protein regulates DNA double-strand break repair and checkpoint activation, EMBO J. 34 (2015) 1399–1416, https://doi.org/10.15252/embj.201490041.
[118] Y. Teng, T. Yadav, M. Duan, J. Tan, Y. Xiang, B. Gao, J. Xu, Z. Liang, Y. Liu, S. Nakajima, Y. Shi, A.S. Levine, L. Zou, L. Lan, ROS-induced R loops trigger a transcription-coupled but BRCA1/2-independent homologous recombination pathway through CSB, Nat. Commun. 9 (2018) 4115, https://doi.org/10.1038/s41467-018-06586-3.
[119] A. Chakraborty, N. Tapryal, T. Venkova, N. Horikoshi, R.K. Pandita, A.H. Sarker, P.S. Sarkar, T.K. Pandita, T.K. Hazra, Classical non-homologous end-joining pathway utilizes nascent RNA for error-free double-strand break repair of transcribed genes, Nat. Commun. 7 (2016) 1–12, https://doi.org/10.1038/ncomms13049.
[120] A. Marnef, S. Cohen, G. Legube, Transcription-coupled DNA double-strand break repair: active genes need special care, J. Mol. Biol. 429 (2017) 1277–1288, https://doi.org/10.1016/j.jmb.2017.03.024.
[121] N. Puget, K.M. Miller, G. Legube, Non-canonical DNA/RNA structures during Transcription-Coupled Double-Strand Break Repair: Roadblocks or Bona fide repair intermediates? DNA Repair (Amst.) 81 (2019), 102661 https://doi.org/10.1016/j.dnarep.2019.102661.
[122] M. Weinfeld, R.S. Mani, I. Abdou, R.D. Aceytuno, J.N.M. Glover, Tidying up loose ends : the role of polynucleotide kinase/phosphatase in DNA strand break repair, Trends Biochem. Sci. 36 (2011) 262–271, https://doi.org/10.1016/j.tibs.2011.01.006.
[123] H. Gaillard, A. Aguilera, Transcription as a threat to genome integrity, Annu. Rev. Biochem. 85 (2016) 291–317, https://doi.org/10.1146/annurev-biochem-060815-014908.
[124] B. Gómez-González, A. Aguilera, Transcription-mediated replication hindrance: a major driver of genome instability, Genes Dev. 33 (2019) 1008–1026, https://doi.org/10.1101/gad.324517.119.
[125] H. Gaillard, E. Herrera-Moyano, A. Aguilera, Transcription-associated genome instability, Chem. Rev. 113 (2013) 8638–8661, https://doi.org/10.1021/cr400017y.
[126] S. Jinks-robertson, A.S. Bhagwat, Transcription-associated mutagenesis, Annu. Rev. Genet. 48 (2014) 341–359, https://doi.org/10.1146/annurev-genet-120213-0920.
[127] F. Prado, A. Aguilera, Impairment of replication fork progression mediates RNA polII transcription-associated recombination, EMBO J. 24 (2005) 1267–1276, https://doi.org/10.1038/sj.emboj.7600602.
[128] P. Gottipati, T.N. Cassel, L. Savolainen, T. Helleday, Transcription-associated recombination is dependent on replication in mammalian cells, Mol. Cell. Biol. 28 (2008) 154–164, https://doi.org/10.1128/mcb.00816-07.
[129] S. Paul, S. Million-Weaver, S. Chattopadhyay, E. Sokurenko, H. Merrikh, Accelerated gene evolution through replication-transcription conflicts, Nature 495 (2013) 512–515, https://doi.org/10.1038/nature11989.
[130] S. Hamperl, K.A. Cimprich, Conflict resolution in the genome: how transcription and replication make it work, Cell 167 (2016) 1455–1467, https://doi.org/10.1016/j.cell.2016.09.053.
[131] T. García-Muse, A. Aguilera, Transcription-replication conflicts: How they occur and how they are resolved, Nat. Rev. Mol. Cell Biol. 17 (2016) 553–563, https://doi.org/10.1038/nrm.2016.88.
[132] S. Hamperl, M.J. Bocek, J.C. Saldivar, T. Swigut, K.A. Cimprich, Transcription-replication conflict orientation modulates R-loop levels and activates distinct DNA damage responses, Cell 170 (2017) 774–786.e19, https://doi.org/10.1016/j.cell.2017.07.043.
[133] J.M. Santos-Pereira, A. Aguilera, R loops: new modulators of genome dynamics and function, Nat. Rev. Genet. 16 (2015) 583–597, https://doi.org/10.1038/nrg3961.
[134] P. Huertas, A. Aguilera, Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination, Mol. Cell. 12 (2003) 711–721, https://doi.org/10.1016/j.molcel.2003.08.010.
[135] B.L. Fogel, E. Cho, A. Wahnich, F. Gao, O.J. Becherel, X. Wang, F. Fike, L. Chen, C. Criscuolo, G. De Michele, A. Filla, A. Collins, A.F. Hahn, R.A. Gatti, G. Konopka, S. Perlman, M.F. Lavin, D.H. Geschwind, G. Coppola, Mutation of senataxin alters disease-specific transcriptional networks in patients with ataxia with oculomotor apraxia type 2, Hum. Mol. Genet. 23 (2014) 4758–4769, https://doi.org/10.1093/hmg/ddu190.
[136] Y.W. Lim, L.A. Sanz, X. Xu, S.R. Hartono, F. Chédin, Genome-wide DNA hypomethylation and RNA:DNA hybrid accumulation in Aicardi–Goutières syndrome, Elife (2015), https://doi.org/10.7554/eLife.08007.
[137] A. Aguilera, B. Gómez-González, DNA-RNA hybrids: the risks of DNA breakage during transcription, Nat. Struct. Mol. Biol. 24 (2017) 439–443, https://doi.org/10.1038/nsmb.3395.
[138] J. Sollier, C.T. Stork, M.L. García-Rubio, R.D. Paulsen, A. Aguilera, K.A. Cimprich, Transcription-Coupled nucleotide excision repair factors promote R-loop-induced genome instability, Mol. Cell. 56 (2014) 777–785, https://doi.org/10.1016/j.molcel.2014.10.020.
[139] J. Tan, M. Duan, T. Yadav, L. Phoon, X. Wang, J. Zhang, L. Zou, L. Lan, An R-loop-initiated CSB–RAD52–POLD3 pathway suppresses ROS-induced telomeric DNA breaks, Nucleic Acids Res. 48 (2019) 1–16, https://doi.org/10.1093/nar/gkz1114.
[140] E. Suberbielle, P.E. Sanchez, A.V. Kravitz, X. Wang, K. Ho, K. Eilertson, N. Devidze, A.C. Kreitzer, L. Mucke, Physiologic brain activity causes DNA double-strand breaks in neurons, with exacerbation by amyloid-β, Nat. Neurosci. 16 (2013) 613–621, https://doi.org/10.1038/nn.3356.
[141] R. Madabhushi, F. Gao, A.R. Pfenning, L. Pan, S. Yamakawa, J. Seo, R. Rueda, T. X. Phan, H. Yamakawa, P.C. Pao, R.T. Stott, E. Gjoneska, A. Nott, S. Cho, M. Kellis, L.H. Tsai, Activity-induced DNA breaks govern the expression of neuronal early-response genes, Cell 161 (2015) 1592–1605, https://doi.org/10.1016/j.cell.2015.05.032.
[142] W.P. Vermeij, M.E.T. Dollé, E. Reiling, D. Jaarsma, C. Payan-Gomez, C. R. Bombardieri, H. Wu, A.J.M. Roks, S.M. Botter, B.C. Van Der Eerden, S. A. Youssef, R.V. Kuiper, B. Nagarajah, C.T. Van Oostrom, R.M.C. Brandt, S. Barnhoorn, S. Imholz, J.L.A. Pennings, A. De Bruin, Gyenis, J. Pothof, J. Vijg, H. Van Steeg, J.H.J. Hoeijmakers, Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice, Nature 537 (2016) 427–431, https://doi.org/10.1038/nature19329.
[143] J.-M. Oh, C. Di, C.C. Venters, J. Guo, C. Arai, B.R. So, A.M. Pinto, Z. Zhang, L. Wan, I. Younis, G. Dreyfuss, U1 snRNP telescripting regulates a size–function-stratified human genome, Nat. Struct. Mol. Biol. 24 (2017) 993–999, https://doi.org/10.1038/nsmb.3473.
[144] A. Takeuchi, K. Iida, T. Tsubota, M. Hosokawa, M. Denawa, J.B. Brown, K. Ninomiya, M. Ito, H. Kimura, T. Abe, H. Kiyonari, K. Ohno, M. Hagiwara, Loss of Sfpq causes long-gene transcriptopathy in the brain, Cell Rep. 23 (2018) 1326–1341, https://doi.org/10.1016/j.celrep.2018.03.141.
[145] J.K. Nussbacher, R. Tabet, G.W. Yeo, C. Lagier-Tourenne, Disruption of RNA metabolism in neurological diseases and emerging therapeutic interventions, Neuron 102 (2019) 294–320, https://doi.org/10.1016/j.neuron.2019.03.014.
[146] H.L. Zhou, M. Mangelsdorf, J.H. Liu, L. Zhu, J.Y. Wu, RNA-binding proteins in neurological diseases, Sci. China Life Sci. 57 (2014) 432–444, https://doi.org/10.1007/s11427-014-4647-9.
[147] M. Polymenidou, C. Lagier-Tourenne, K.R. Hutt, S.C. Huelga, J. Moran, T. Y. Liang, S. Ling, E. Sun, E. Wancewicz, C. Mazur, H. Kordasiewicz, Y. Sedaghat, J.P. Donohue, L. Shiue, C.F. Bennett, G.W. Yeo, D.W. Cleveland, Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43, Nat. Neurosci. 14 (2011) 459–468, https://doi.org/10.1038/nn.2779.
[148] I.F. King, C.N. Yandava, A.M. Mabb, J.S. Hsiao, H.S. Huang, B.L. Pearson, J. M. Calabrese, J. Starmer, J.S. Parker, T. Magnuson, S.J. Chamberlain, B. D. Philpot, M.J. Zylka, Topoisomerases facilitate transcription of long genes linked to autism, Nature 501 (2013) 58–62, https://doi.org/10.1038/nature12504.
[149] B. Devlin, B.E. Boone, S.E. Levy, J. Lihm, J.D. Buxbaum, Y. Wu, L. Lewis, Y. Han, E. Boerwinkle, R.A. Gibbs, M. Fromer, K. Shakir, T. Fennell, K. Garimella, E. Banks, R. Poplin, S. Gabriel, M. De Pristo, S. Sunyaev, M.J. Daly, Patterns and rates of exonic de novo mutations in autism spectrum disorders, Nature 485 (2012) 242–246, https://doi.org/10.1038/nature11011.
[150] H.W. Gabel, B. Kinde, H. Stroud, C.S. Gilbert, D.A. Harmin, N.R. Kastan, M. Hemberg, D.H. Ebert, M.E. Greenberg, Disruption of DNA-methylation-dependent long gene repression in Rett syndrome, Nature 522 (2015) 89–93, https://doi.org/10.1038/nature14319.
[151] A.R. Sonawane, J. Platig, M. Fagny, C.Y. Chen, J.N. Paulson, C.M. Lopes-Ramos, D.L. DeMeo, J. Quackenbush, K. Glass, M.L. Kuijjer, Understanding tissue-specific gene regulation, Cell Rep. 21 (2017) 1077–1088, https://doi.org/10.1016/j.celrep.2017.10.001.
[152] L. Fagerberg, B.M. Hallstrom, P. Oksvold, C. Kampf, D. Djureinovic, J. Odeberg, M. Habuka, S. Tahmasebpoor, A. Danielsson, K. Edlund, A. Asplund, E. Sjostedt, E. Lundberg, C.A.K. Szigyarto, M. Skogs, J. Ottosson Takanen, H. Berling, H. Tegel, J. Mulder, P. Nilsson, J.M. Schwenk, C. Lindskog, F. Danielsson, A. Mardinoglu, A. Sivertsson, K. Von Feilitzen, M. Forsberg, M. Zwahlen, I. Olsson, S. Navani, M. Huss, J. Nielsen, F. Ponten, M. Uhlen, Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics, Mol. Cell. Proteom. 13 (2014) 397–406, https://doi.org/10.1074/mcp.M113.035600.
[153] M.J. Zylka, J.M. Simon, B.D. Philpot, Gene length matters in neurons, Neuron 86 (2015) 353–355, https://doi.org/10.1016/j.neuron.2015.03.059.
[154] Y. Takashima, T. Ohtsuka, A. González, H. Miyachi, R. Kageyama, Intronic delay is essential for oscillatory expression in the segmentation clock, Proc. Natl. Acad. Sci. U. S. A 108 (2011) 3300–3305, https://doi.org/10.1073/pnas.1014418108.
[155] K.A. Barr, J. Reinitz, O. Radulescu, An in silico analysis of robust but fragile gene regulation links enhancer length to robustness, BioRxiv (2019) 1–36, https://doi.org/10.1101/677641.
[156] K.E. Orii, Y. Lee, N. Kondo, P.J. McKinnon, Selective utilization of nonhomologous end-joining and homologous recombination DNA repair pathways during nervous system development, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 10017–10022, https://doi.org/10.1073/pnas.0602436103.
[157] H.E. Krokan, M. Bjoras, Base excision repair, Cold Spring Harb. Perspect. Biol. 5 (2013) 1–17, https://doi.org/10.1101/cshperspect.a012583.
[158] K.S. Rao, DNA repair in aging rat neurons, Neuroscience 145 (2007) 1330–1340, https://doi.org/10.1016/j.neuroscience.2006.09.032.

[159] P. Sykora, J.-L. Yang, L.K. Ferrarelli, J. Tian, T. Tadokoro, A. Kulkarni, L. Weissman, G. Keijzers, D.M. Wilson, M.P. Mattson, V.A. Bohr, Modulation of DNA base excision repair during neuronal differentiation, Neurobiol. Aging 34 (2013) 1717–1727, https://doi.org/10.1016/j.physbeh.2017.03.040.
[160] J. Waser, H. Ulrich, C.C. Kuenzle, S. Spadari, D.N.A. Polymerase, β from brain neurons is a repair enzyme, Eur. J. Biochem 97 (1979) 361–368, https://doi.org/10.1111/j.1432-1033.1979.tb13122.x.
[161] P. Sykora, D.M. Wilson, V.A. Bohr, Base excision repair in the mammalian brain: implication for age related neurodegeneration, Mech. Ageing Dev. 134 (2013) 440–448, https://doi.org/10.1016/j.mad.2013.04.005.
[162] P. Sykora, M. Misiak, Y. Wang, S. Ghosh, G.S. Leandro, D. Liu, J. Tian, B. A. Baptiste, W.N. Cong, B.M. Brenerman, E. Fang, K.G. Becker, R.J. Hamilton, S. Chigurupati, Y. Zhang, J.M. Egan, D.L. Croteau, D.M. Wilson, M.P. Mattson, V. A. Bohr, DNA polymerase β deficiency leads to neurodegeneration and exacerbates Alzheimer disease phenotypes, Nucleic Acids Res 43 (2015) 943–959, https://doi.org/10.1093/nar/gku1356.
[163] U. Swain, K.S. Rao, Age-dependent decline of DNA base excision repair activity in rat cortical neurons, Mech. Ageing Dev. 133 (2012) 186–194, https://doi.org/10.1016/j.mad.2012.01.001.
[164] V.N. Vyjayanti, K.S. Rao, DNA double strand break repair in brain: reduced NHEJ activity in aging rat neurons, Neurosci. Lett. 393 (2006) 18–22, https://doi.org/10.1016/j.neulet.2005.09.053.
[165] V. Gorbunova, A. Seluanov, Z. Mao, C. Hine, Changes in DNA repair during aging, Nucleic Acids Res 35 (2007) 7466–7474, https://doi.org/10.1093/nar/gkm756.
[166] T. Lu, Y. Pan, S. Kao, C. Li, I. Kohane, J. Chan, B.A. Yanker, Gene regulation and DNA damage in the ageing human brain, Lett. Nat. 429 (2004) 883–891, https://doi.org/10.1053/j.gastro.2008.09.055.
[167] T. Nouspikel, P.C. Hanawalt, DNA repair in terminally differentiated cells, DNA Repair (Amst.) 1 (2002) 59–75, https://doi.org/10.1016/S1568-7864(01)00005-2.
[168] T. Nouspikel, DNA repair in differentiated cells: Some new answers to old questions, Neuroscience 145 (2007) 1213–1221, https://doi.org/10.1016/j.neuroscience.2006.07.006.
[169] T. Nouspikel, Nucleotide excision repair and neurological diseases, DNA Repair (Amst.) 7 (2008) 1155–1167, https://doi.org/10.1016/j.dnarep.2008.03.015.
[170] P.W. Doetsch, Translesion synthesis by RNA polymerases: occurrence and biological implications for transcriptional mutagenesis, Mutat. Res. Fundam. Mol. Mech. Mutagen. 510 (2002) 131–140, https://doi.org/10.1016/S0027-5107(02)00258-0.
[171] T.T. Saxowsky, P.W. Doetsch, RNA polymerase encounters with DNA damage: transcription-coupled repair or transcriptional mutagenesis? Chem. Rev. 106 (2006) 474–488, https://doi.org/10.1021/cr040466q.
[172] M. Bradl, H. Lassmann, Oligodendrocytes: biology and pathology, Acta Neuropathol. 119 (2010) 37–53, https://doi.org/10.1007/s00401-009-0601-5.
[173] K. Tse, K. Herrup, DNA damage in the oligodendrocyte lineage and its role in brain aging, Mech. Ageing Dev. 191 (2017) 37–50, https://doi.org/10.1016/j.physbeh.2017.03.040.
[174] J. Lasiene, A. Matsui, Y. Sawa, F. Wong, P.J. Horner, Age-related myelin dynamics revealed by increased oligodendrogenesis and short internodes, Aging Cell 8 (2009) 201–213, https://doi.org/10.1111/j.1474-9726.2009.00462.x.
[175] K. Psachoulia, F. Jamen, K.M. Young, W.D. Richardson, Cell cycle dynamics of NG2 cells in the postnatal and ageing brain, Neuron Glia Biol. 5 (2009) 57–67, https://doi.org/10.1017/S1740925X09990354.
[176] A. Grosche, J. Grosche, M. Tackenberg, D. Scheller, G. Gerstner, A. Gumprecht, T. Pannicke, P.G. Hirrlinger, U. Wilhelmsson, K. Hüttmann, W. Härtig, C. Steinhäuser, M. Pekny, A. Reichenbach, Versatile and simple approach to determine astrocyte territories in mouse neocortex and hippocampus, PLoS One 8 (2013), e69143, https://doi.org/10.1371/journal.pone.0069143.
[177] K. Askew, K. Li, A. Olmos-Alonso, F. Garcia-Moreno, Y. Liang, P. Richardson, T. Tipton, M.A. Chapman, K. Riecken, S. Beccari, A. Sierra, Z. Molnár, M.S. Cragg, O. Garaschuk, V.H. Perry, D. Gomez-Nicola, Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain, Cell Rep. 18 (2017) 391–405, https://doi.org/10.1016/j.celrep.2016.12.041.
[178] I. Rozovsky, C.E. Finch, T.E. Morgan, Age-related activation of microglia and astrocytes: in vitro studies show persistent phenotypes of aging, increased proliferation, and resistance to down-regulation, Neurobiol. Aging 19 (1998) 97–103, https://doi.org/10.1016/S0197-4580(97)00169-3.
[179] A.D. Rogove, W. Lu, S.E. Tsirka, Microglial activation and recruitment, but not proliferation, suffice to mediate neurodegeneration, Cell Death Differ. 9 (2002) 801–806, https://doi.org/10.1038/sj.cdd.4401041.
[180] B. McEllin, C.V. Camacho, B. Mukherjee, B. Hahm, N. Tomimatsu, R.M. Bachoo, S. Burma, PTEN loss compromises homologous recombination repair in astrocytes: implications for GBM therapy with temoolomide or PARP inhibitors, Cancer Res. 70 (2010) 5457–5464, https://doi.org/10.1146/annurev-cellbio-092910-154240.Sensory.
[181] M.A. Bylicky, G.P. Mueller, R.M. Day, Radiation resistance of normal human astrocytes: the role of non-homologous end joining DNA repair activity, J. Radiat. Res. 60 (2019) 37–50, https://doi.org/10.1093/jrr/rry084.
[182] A. Yamamoto, Y. Nakamura, N. Kobayashi, T. Iwamoto, A. Yoshioka, H. Kuniyasu, T. Kishimoto, T. Mori, Neurons and astrocytes exhibit lower activities of global genome nucleotide excision repair than do fibroblasts, DNA Repair (Amst.) 6 (2007) 649–657, https://doi.org/10.1016/j.dnarep.2006.12.006.
[183] G.E. Kisby, H. Lesselroth, A. Olivas, L. Samson, B. Gold, K. Tanaka, M.S. Turker, Role of nucleotide- and base-excision repair in genotoxin-induced neuronal cell death, DNA Repair (Amst.) 3 (2004) 617–627, https://doi.org/10.1016/j.dnarep.2004.02.005.
[184] S.B. Hollensworth, C.C. Shen, J.E. Sim, D.R. Spitz, G.L. Wilson, S.P. Ledoux, Glial cell type-specific responses to menadione-induced oxidative stress, Free Radic. Biol. Med. 28 (2000) 1161–1174, https://doi.org/10.1016/S0891-5849(00)00214-8.
[185] G.J. McBean, Cysteine, glutathione, and thiol redox balance in astrocytes, Antioxidants 6 (2017) 1–13, https://doi.org/10.3390/antiox6030062.
[186] X.F. Wang, M.S. Cynader, Astrocytes provide cysteine to neurons by releasing glutathione, J. Neurochem. 74 (2000) 1434–1442, https://doi.org/10.1046/j.1471-4159.2000.0741434.x.
[187] R.S. Morrison, Y. Kinoshita, The role of p53 in neuronal cell death, Cell Death Differ. 7 (2000) 868–879, https://doi.org/10.1038/sj.cdd.4400741.
[188] J. Jebelli, C. Hooper, J.M. Pocock, Microglial p53 activation is detrimental to neuronal synapses during activation-induced inflammation: implications for neurodegeneration, Neurosci. Lett. 583 (2014) 92–97, https://doi.org/10.1016/j.neulet.2014.08.049.
[189] C. Kang, Q. Xu, T.D. Martin, M.Z. Li, M. Demaria, L. Aron, T. Lu, B.A. Yankner, J. Campisi, S.J. Elledge, The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4, Science 349 (80–.) (2015) 1–26, https://doi.org/10.1111/mec.13536.Application.
[190] M. Itoh, M. Hayashi, K. Shioda, M. Minagawa, F. Isa, K. Tamagawa, Y. Morimatsu, M. Oda, Neurodegeneration in hereditary nucleotide repair disorders, Brain Dev. 21 (1999) 326–333, https://doi.org/10.1016/S0387-7604(99)00033-9.
[191] M.C. De Waard, I. Van Der Pluijm, N. Zuiderveen Borgesius, L.H. Comley, E. D. Haasdijk, Y. Rijksen, Y. Ridwan, G. Zondag, J.H.J. Hoeijmakers, Y. Elgersma, T.H. Gillingwater, D. Jaarsma, Age-related motor neuron degeneration in DNA repair-deficient Ercc1 mice, Acta Neuropathol. 120 (2010) 461–475, https://doi.org/10.1007/s00401-010-0715-9.
[192] D. Jaarsma, I. van der Pluijm, M.C. de Waard, E.D. Haasdijk, R. Brandt, M. Vermeij, Y. Rijksen, A. Maas, H. van Steeg, J.H.J. Hoeijmakers, G.T.J. van der Horst, Age-related neuronal degeneration: Complementary roles of nucleotide excision repair and transcription-coupled repair in preventing neuropathology, PLoS Genet. 7 (2011), e1002405, https://doi.org/10.1371/journal.pgen.1002405.
[193] M.L. Fishel, M.R. Vasko, M.R. Kelley, DNA repair in neurons: so if they don't divide what's to repair? Mutat. Res. Fundam. Mol. Mech. Mutagen. 614 (2007) 24–36, https://doi.org/10.1016/j.mrfmmm.2006.06.007.
[194] T.W. Gould, R.R. Buss, S. Vinsant, D. Prevette, W. Sun, C.M. Knudson, C. E. Milligan, R.W. Oppenheim, Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS, J. Neurosci. 26 (2006) 8774–8786, https://doi.org/10.1523/JNEUROSCI.2315-06.2006.
[195] I. Brasnjevic, P.R. Hof, H.W.M. Steinbusch, C. Schmitz, Accumulation of nuclear DNA damage or neuron loss: Molecular basis for a new approach to understanding selective neuronal vulnerability in neurodegenerative diseases, DNA Repair (Amst.) 7 (2008) 1087–1097, https://doi.org/10.1016/j.dnarep.2008.03.010.
[196] M. Hetman, A. Vashishta, G. Rempala, Neurotoxic mechanisms of DNA damage: focus on transcriptional inhibition, J. Neurochem. 114 (2010) 1537–1549, https://doi.org/10.1016/j.cortex.2009.08.003.Predictive.
[197] G. Weeda, I. Donker, J. De Wit, H. Morreau, R. Janssens, C.J. Vissers, A. Nigg, H. Van Steeg, D. Bootsma, J.H.J. Hoeijmakers, Disruption of mouse ERCC1 results in a novel repair syndrome with growth failure, nuclear abnormalities and senescence, Curr. Biol. 7 7 (1997) 427–439, https://doi.org/10.1016/S0960-9822(06)00190-4.
[198] M. Durik, M. Kavousi, I. Van Der Pluijm, A. Isaacs, C. Cheng, K. Verdonk, A. E. Loot, H. Oeseburg, U.M. Bhaggoe, F. Leijten, R. Van Veghel, R. De Vries, G. Rudez, R. Brandt, Y.R. Ridwan, E.D. Van Deel, M. De Boer, D. Tempel, I. Fleming, G.F. Mitchell, G.C. Verwoert, K.V. Tarasov, A.G. Uitterlinden, A. Hofman, H.J. Duckers, C.M. Van Duijn, B.A. Oostra, J.C.M. Witteman, D. J. Duncker, A.H.J. Danser, J.H. Hoeijmakers, A.J.M. Roks, Nucleotide excision DNA repair is associated with age-related vascular dysfunction, Circulation 126 (2012) 468–478, https://doi.org/10.1161/CIRCULATIONAHA.112.104380.
[199] D.J. Baker, R.C. Petersen, Cellular senescence in brain aging and neurodegenerative diseases: evidence and perspectives, J. Clin. Invest. 128 (2018) 1208–1216, https://doi.org/10.1172/JCI95145.
[200] D. Jurk, C. Wang, S. Miwa, M. Maddick, V. Korolchuk, A. Tsolou, E.S. Gonos, C. Thrasivoulou, M. Jill Saffrey, K. Cameron, T. von Zglinicki, Postmitotic neurons develop a p21-dependent senescence-like phenotype driven by a DNA damage response, Aging Cell. 11 (2012) 996–1004, https://doi.org/10.1111/j.1474-9726.2012.00870.x.
[201] Y. Yamazaki, D.J. Baker, M. Tachibana, C.-C. Liu, J.M. van Deursen, T.G. Brott, G. Bu, T. Kanekiyo, Vascular cell senescence contributes To blood-brain barrier breakdown, Stroke 47 (2016) 1068–1077, https://doi.org/10.1016/j.physbeh.2017.03.040.
[202] J. Cohen, C. Torres, Astrocyte senescence: evidence and significance, Aging Cell. 18 (2019) 1–14, https://doi.org/10.1111/acel.12937.
[203] M.R. Stojiljkovic, Q. Ain, T. Bondeva, R. Heller, C. Schmeer, O.W. Witte, Phenotypic and functional differences between senescent and aged murine microglia, Neurobiol. Aging 74 (2019) 56–69, https://doi.org/10.1016/j.neurobiolaging.2018.10.007.
[204] P. Thériault, S. Rivest, Microglia: senescence impairs clearance of myelin debris, Curr. Biol. 26 (2016) R772–R775, https://doi.org/10.1016/j.cub.2016.06.066.
[205] M.R.P. Elmore, L.A. Hohsfield, E.A. Kramár, L. Soreq, R.J. Lee, S.T. Pham, A. R. Najafi, E.E. Spangenberg, M.A. Wood, B.L. West, K.N. Green, Replacement of microglia in the aged brain reverses cognitive, synaptic, and neuronal deficits in mice, Aging Cell 17 (2018), e12832, https://doi.org/10.1111/acel.12832.
[206] M.P. Baar, R.M.C. Brandt, D.A. Putavet, J.D.D. Klein, K.W.J. Derks, B.R. M. Bourgeois, S. Stryeck, Y. Rijksen, H. van Willigenburg, D.A. Feijtel, I. van der

Pluijm, J. Essers, W.A. van Cappellen, W.F. van IJcken, A.B. Houtsmuller, J. Pothof, R.W.F. de Bruin, T. Madl, J.H.J. Hoeijmakers, J. Campisi, P.L.J. de Keizer, Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging, Cell 169 (2017) 132–147.e16, https://doi.org/10.1016/j.cell.2017.02.031.

[207] H. Fuhrmann-Stroissnigg, Y.Y. Ling, J. Zhao, S.J. McGowan, Y. Zhu, R.W. Brooks, D. Grassi, S.Q. Gregg, J.L. Stripay, A. Dorronsoro, L. Corbo, P. Tang, C. Bukata, N. Ring, M. Giacca, X. Li, T. Tchkonia, J.L. Kirkland, L.J. Niedernhofer, P. D. Robbins, Identification of HSP90 inhibitors as a novel class of senolytics, Nat. Commun. 8 (2017) 422, https://doi.org/10.1038/s41467-017-00314-z.

[208] P. Zhang, Y. Kishimoto, I. Grammatikakis, K. Gottimukkala, R.G. Cutler, S. Zhang, K. Abdelmohsen, V.A. Bohr, J. Misra Sen, M. Gorospe, M.P. Mattson, Senolytic therapy alleviates Aβ-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model, Nat. Neurosci. 22 (2019) 719–728, https://doi.org/10.1038/s41593-019-0372-9.

[209] M.A. Yenari, L. Xu, N.T. Xian, Y. Qiao, R.G. Giffard, Microglia potentiate damage to blood-brain barrier constituents: Improvement by minocycline in vivo and in vitro, Stroke 37 (2006) 1087–1093, https://doi.org/10.1161/01.STR.0000206281.77178.ac.

[210] O.A. Bizzozero, G. DeJesus, K. Callahan, A. Pastuszyn, Elevated protein carbonylation in the brain white matter and gray matter of patients with multiple sclerosis, J. Neurosci. Res. 81 (2005) 687–695, https://doi.org/10.1002/jnr.20587.

[211] S. Di Meo, T.T. Reed, P. Venditti, V.M. Victor, Role of ROS and RNS sources in physiological and pathological conditions, Oxid. Med. Cell. Longev. 2016 (2016), https://doi.org/10.1155/2016/1245049.

[212] S. Yan, M. Sorrell, Z. Berman, Functional interplay between ATM/ATR-mediated DNA damage response and DNA repair pathways in oxidative stress, Cell. Mol. Life Sci. 71 (2014) 3951–3967, https://doi.org/10.1007/s00018-014-1666-4.

[213] S.A. Liddelow, K.A. Guttenplan, L.E. Clarke, F.C. Bennett, C.J. Bohlen, L. Schirmer, M.L. Bennett, A.E. Münch, W.S. Chung, T.C. Peterson, D.K. Wilton, A. Frouin, B.A. Napier, N. Panicker, M. Kumar, M.S. Buckwalter, D.H. Rowitch, V. L. Dawson, T.M. Dawson, B. Stevens, B.A. Barres, Neurotoxic reactive astrocytes are induced by activated microglia, Nature 541 (2017) 481–487, https://doi.org/10.1038/nature21029.

[214] M.F. Farez, F.J. Quintana, R. Gandhi, G. Izquierdo, M. Lucas, H.L. Weiner, Toll-like receptor 2 and poly(ADP-ribose) polymerase 1 promote central nervous system neuroinflammation in progressive EAE, Nat. Immunol. 10 (2009) 958–964, https://doi.org/10.1038/ni.1775.

[215] Y. Shen, M.Z. McMackin, Y. Shan, A. Raetz, S. David, G. Cortopassi, Frataxin deficiency promotes excess microglial DNA damage and inflammation that is rescued by PJ34, PLoS One 11 (2016) 1–18, https://doi.org/10.1371/journal.pone.0151026.

[216] D.D.A. Raj, D. Jaarsma, I.R. Holtman, M. Olah, F.M. Ferreira, W. Schaafsma, N. Brouwer, M.M. Meijer, M.C. De Waard, I. Van der Pluijm, R. Brandt, K.L. Kreft, J.D. Laman, G. De Haan, K.P.H. Biber, J.H.J. Hoeijmakers, B.J.L. Eggen, H.W.G. M. Boddeke, Priming of microglia in a DNA-repair deficient model of accelerated aging (https://doi:), Neurobiol. Aging 35 (2014) 2147–2160, https://doi.org/10.1016/j.neurobiolaging.2014.03.025.

[217] M.J. Muñoz, N. Nieto Moreno, L.E. Giono, A.E. Cambindo Botto, G. Dujardin, G. Bastianello, S. Lavore, A. Torres-Méndez, C.F.M. Menck, B.J. Blencowe, M. Irimia, M. Foiani, A.R. Kornblihtt, Major roles for pyrimidine dimers, nucleotide excision repair, and ATR in the alternative splicing response to UV irradiation, Cell Rep. 18 (2017) 2868–2879, https://doi.org/10.1016/j.celrep.2017.02.066.

[218] Y. Wang, P. Chakravarty, M. Ranes, G. Kelly, P.J. Brooks, E. Neilan, A. Stewart, G. Schiavo, J.Q. Svejstrup, Dysregulation of gene expression as a cause of Cockayne syndrome neurological disease, Proc. Natl. Acad. Sci. U. S. A 111 (2014) 14454–14459, https://doi.org/10.1073/pnas.1412569111.

[219] F. Ciaffardini, S. Nicolai, M. Caputo, G. Canu, E. Paccosi, M. Costantino, M. Frontini, a S. Balajee, L. Proietti-De-Santis, The cockayne syndrome B protein is essential for neuronal differentiation and neuritogenesis. Cell Death Dis. 5 (2014), e1268 https://doi.org/10.1038/cddis.2014.228.

[220] L.N. de S. Andrade, J.L. Nathanson, G.W. Yeo, C.F.M. Menck, A.R. Muotri, Evidence for premature aging due to oxidative stress in iPSCs from Cockayne syndrome, Hum. Mol. Genet. 21 (2012) 3825–3834, https://doi.org/10.1093/hmg/dds211.

[221] A.T. Vessoni, R.H. Herai, J.V. Karpiak, A.M.S. Leal, C.A. Trujillo, A. Quinet, L. F. Agnez Lima, C.F.M. Menck, A.R. Muotri, Cockayne syndrome-derived neurons display reduced synapse density and altered neural network synchrony, Hum. Mol. Genet. 25 (2016) 1271–1280, https://doi.org/10.1093/hmg/ddw008.

[222] I. Kamileri, I. Karakasilioti, G.A. Garinis, Nucleotide excision repair: new tricks with old bricks, Trends Genet. 28 (2012) 566–573, https://doi.org/10.1016/j.tig.2012.06.004.

[223] S. Koch, O.G. Gonzalez, R. Assfalg, A. Schelling, P. Schäfer, K. Scharffetter-Kochanek, S. Iben, Cockayne syndrome protein A is a transcription factor of RNA polymerase I and stimulates ribosomal biogenesis and growth, Cell Cycle 13 (2014) 2029–2037, https://doi.org/10.4161/cc.29018.

[224] J. Bradsher, J. Auriol, L.P. De Santis, S. Iben, J.L. Vonesch, I. Grummt, J.M. Egly, CSB is a component of RNA pol I transcription, Mol. Cell. 10 (2002) 819–829, https://doi.org/10.1016/S1097-2765(02)00678-0.

[225] A.H. Bhat, K.B. Dar, S. Anees, M.A. Zargar, A. Masood, M.A. Sofi, S.A. Ganie, Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight, Biomed. Pharmacother. 74 (2015) 101–110, https://doi.org/10.1016/j.biopha.2015.07.025.

[226] M.D. Aamann, M.M. Sorensen, C. Hvitby, B.R. Berquist, M. Muftuoglu, J. Tian, N. C. De Souza-Pinto, M. Scheibye-Knudsen, D.M. Wilson, T. Stevnsner, V.A. Bohr, Cockayne syndrome group B protein promotes mitochondrial DNA stability by supporting the DNA repair association with the mitochondrial membrane, FASEB J. 24 (2010) 2334–2346, https://doi.org/10.1096/fj.09-147991.

[227] Y. Kamenisch, M. Fousteri, J. Knoch, A.K. Von Thaler, B. Fehrenbacher, H. Kato, T. Becker, M.E.T. Dollé, R. Kuiper, M. Majora, M. Schaller, G.T.J. Van Der Horst, H. Van Steeg, M. Röcken, D. Rapaport, J. Krutmann, L.H. Mullenders, M. Berneburg, Proteins of nucleotide and base excision repair pathways interact in mitochondria to protect from loss of subcutaneous fat, a hallmark of aging, J. Exp. Med. 207 (2010) 379–390, https://doi.org/10.1084/jem.20091834.

[228] M. Prates Mori, N.C. de Souza-Pinto, Role of mitochondrial dysfunction in the pathophysiology of DNA repair disorders, Cell Biol. Int. 42 (2018) 643–650, https://doi.org/10.1002/cbin.10917.

[229] J.R. Chang, M. Ghafouri, R. Mukerjee, A. Bagashev, T. Chabrashvili, B.E. Sawaya, Role of p53 in neurodegenerative diseases, Neurodegener. Dis. 9 (2012) 68–80, https://doi.org/10.1159/000329999.

[230] H. Wu, B.S. van Thiel, P.K. Bautista-Niño, E. Reiling, M. Durik, F.P.J. Leijten, Y. Ridwan, R.M.C. Brandt, H. van Steeg, M.E.T. Dollé, W.P. Vermeij, J.H. J. Hoeijmakers, J. Essers, I. van der Pluijm, A.H. Jan Danser, A.J.M. Roks, Dietary restriction but not angiotensin II type 1 receptor blockade improves DNA damage-related vasodilator dysfunction in rapidly aging Ercc1Δ/- mice, Clin. Sci. 131 (2017) 1941–1953, https://doi.org/10.1042/CS20170026.

APPENDIX B: Cockayne Syndrome: The Many Challenges and Approaches to Understand a Multifaceted Disease

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Review Article

Cockayne Syndrome: The many challenges and approaches to understand a multifaceted disease

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Abstract

The striking and complex phenotype of Cockayne syndrome (CS) patients combines progeria-like features with developmental deficits. Since the establishment of the *in vitro* culture of skin fibroblasts derived from patients with CS in the 1970s, significant progress has been made in the understanding of the genetic alterations associated with the disease and their impact on molecular, cellular, and organismal functions. In this review, we provide a historic perspective on the research into CS by revisiting seminal papers in this field. We highlighted the great contributions of several researchers in the last decades, ranging from the cloning and characterization of CS genes to the molecular dissection of their roles in DNA repair, transcription, redox processes and metabolism control. We also provide a detailed description of all pathological mutations in genes *ERCC6* and *ERCC8* reported to date and their impact on CS-related proteins. Finally, we review the contributions (and limitations) of many genetic animal models to the study of CS and how cutting-edge technologies, such as cell reprogramming and state-of-the-art genome editing, are helping us to address unanswered questions.

Keywords: Cockayne syndrome, transcription-coupled nucleotide excision repair, neurodegeneration, progeroid syndrome, DNA repair.

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The epidemiology of Cockayne Syndrome (CS) and the discovery of the CS genes

Edward Alfred Cockayne first described CS in 1936. He diagnosed it in two young siblings (born to healthy parents) that displayed a set of very similar characteristics that included skin photosensitivity, short stature, prominent superior maxillae, disproportionally large hands and feet, sunken eyes with retinal atrophy, hearing impairment, below-average intelligence, a severely limited speaking ability, and muscle contraction, conditions that would later become characteristic of CS patients (Cockayne, 1936, 1946; Laugel, 2013). Subsequent reports also confirmed that patients with CS feature complex and heterogeneous neuropathology that includes calcification of the basal ganglia, cerebellar atrophy, loss of Purkinje and granular cells, hyperchromatic macroglial cells, microcephaly, and patchy demyelination (Guardiola *et al.*, 1999; Karam *et al.*, 2000; Weidenheim *et al.*, 2009; Wilson *et al.*, 2015; Karikkineth *et al.*, 2017).

CS is an autosomal recessive disorder with a prevalence of ~2.7 per million births in Western Europe and in Japan (Kleijer *et al.*, 2008; Kubota *et al.*, 2015). The phenotype of the patients can range from mild to very severe and is subdivided into three types. The classical type (type I) corresponds to the moderate phenotype in which life expectancy is 16 years. In type II (the most severe and with the earliest onset), life expectancy is 5 years, whereas in the third type (mild and atypical), the phenotype manifests itself later in life, with life expectancy above 30 years. In all cases, pneumonia/respiratory ailments are the most common causes of death (Natale, 2010).

In vitro culture of skin fibroblasts derived from patients with CS in the 1970s was the first step toward the development of experimental models of the disease. CS fibroblasts are characterized by extreme sensitivity to ultraviolet light (UV) despite a normal ability to excise pyrimidine-dimers from the genome (Schmickel *et al.*, 1977; Andrews *et al.*, 1978). In fact, CS cells display a marked defect in the recovery of RNA synthesis after UV irradiation (Mayne and Lehmann, 1982) owing to a failure in the repair of transcrip-

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tionally active genes (Venema *et al.*, 1990; van Hoffen *et al.*, 1993). By evaluation of post-UV RNA synthesis recovery in multinucleated cells obtained by the fusion of cells from different CS donors, three complementation groups (A, B, and C) were initially identified (Tanaka *et al.*, 1981; Lehmann, 1982). Group C identified by Lehmann corresponded to a patient that had combined features of CS and *xeroderma pigmentosum* (XP). Patients that fall in this category (termed XP/CS) manifest, in addition to CS features, the classical XP characteristics (skin pigmentation and extremely high skin cancer predisposition) and harbor mutations in the genes *XPB*, *XPD*, *XPG*, or (more recently identified) *XPF* or *ERCC1* (Weeda *et al.*, 1990; Kashiyama *et al.*, 2013; Lehmann *et al.*, 2014; Moriel-Carretero *et al.*, 2015).

In the 1990s, the genes corresponding to groups A and B were cloned, characterized, and termed *CSA* and *CSB*, respectively. *CSB* was originally termed *ERCC6* (excision repair cross-complementation group 6) because it was found to complement the nucleotide excision repair (NER) defect of the Chinese Hamster Ovary mutant cell line UV61, a representative of complementation group 6 of rodent cell lines defective in excision repair (Troelstra *et al.*, 1990). Two years later, Hoeijmaker's group demonstrated that the expression of this gene could reverse UV sensitivity and rescue post-UV RNA synthesis in a cell line (CS1AN) assigned to CS group B but not in group A cells (Troelstra *et al.*, 1992). Another two years later, using episomal vectors to drive the expression of a cDNA library, Friedberg's group was able to identify the gene capable of reversing the UV sensitivity of CS cells from group A (but not B) and to reactivate the expression of a UV-inactivated reporter gene (Henning *et al.*, 1995). They discovered that the *CSA* protein, encoded by *ERCC8* (Itoh *et al.*, 1996), can interact with the CSB protein. Both genes play a critical role in the transcription-coupled nucleotide excision repair (TC-NER) of damaged DNA, which is described below in more detail.

CSA and CSB in TC-NER

Cells evolved complex and refined mechanisms to prevent genome instability in response to the presence of exogenously and endogenously generated DNA lesions. One of these mechanisms, conserved from bacteria to humans, is the NER pathway (Schärer, 2013; Marteijn *et al.*, 2014; Gregersen and Svejstrup, 2018). This system drives the repair of bulky distorting DNA lesions (such as those induced by UV and by some redox processes) in four sequential steps: i) detection of a lesion, ii) excision of a DNA single-strand fragment containing the lesion, iii) DNA synthesis by a polymerase to fill the gap, and iv) nick sealing by a ligase (Costa *et al.*, 2003; Reardon and Sancar, 2005; Menck and Munford, 2014). In bacteria, three proteins (UvrA, UvrB, and UvrC) are critical for the detection and excision of the lesion (Seeberg and Strike, 1976; Sancar and Rupp, 1983), whereas in humans, more than 30 proteins acting in an orchestrated manner are required for these steps, as reviewed elsewhere in detail (Menck and Munford, 2014). NER is subdivided into two sub-pathways: global genome repair (GG-NER) and transcription-coupled repair (TC-NER). They differ in how DNA lesions are detected, although the excision and DNA re-synthesis steps are shared by the two pathways (Menck and Munford, 2014). In humans, GG-NER is initiated by the XPC protein (Sugasawa *et al.*, 1998), which is constantly scanning the whole genome for the presence of helix-distorting lesions (Hoogstraten *et al.*, 2008), and the detection of UV products is facilitated by XPE/DDB2 (Cleaver *et al.*, 2009). In TC-NER, as shown in Figure 1, the triggering event is the arrest of RNA polymerase II (RNA pol II) owing to the presence of a lesion in the actively transcribed strand of a gene (Bohr *et al.*, 1985; Hanawalt and Spivak, 2008). It is in this pathway that the CSA and CSB proteins play a critical part. Upon RNA pol II blockade, the binding of CSB to RNA pol II is stabilized (Tantin *et al.*, 1997; van den Boom *et al.*, 2004; Fousteri *et al.*, 2006) and CSB then wraps DNA around itself, altering its conformation and recruiting histone acetyltransferase p300 and core NER factors (such as RPA, XPG, and TFIIH) to RNA pol II arrest sites (Fousteri *et al.*, 2006), as presented in Figure 1 (upper panel). CSB also recruits an E3-ubiquitin ligase complex, highlighted in green, composed of CSA (which contains WD motifs involved in protein–protein interactions), DDB1, Cullin 4A, and ROC1/Rbx1 proteins (Groisman *et al.*, 2003; Fousteri *et al.*, 2006). Although CSA is not necessary for the recruitment of NER factors, it is required for recruiting HMGN1, XAB2, and TFIIS to RNA pol II arrest sites (Fousteri *et al.*, 2006). XAB2 is an XPA-interacting protein (Nakatsu *et al.*, 2000) and might act as a scaffolding factor for protein assembly during TC-NER (Fousteri and Mullenders, 2008), while the nucleosome-binding protein, HMGN1, was suggested to promote chromatin changes that allow for the incision step (Fousteri and Mullenders, 2008).

The fates of the nascent transcript and that of the stalled RNA pol II are still debated. One hypothesis is that transcript cleavage may occur in an elongation factor for RNA pol II (ELL)-dependent manner (Gregersen and Svejstrup, 2018). As for the stalled RNA pol II — which occupies a space that ranges from 25 nucleotides downstream of the lesion to 10 nucleotides upstream (Spivak and Ganesan, 2014), thus impairing the assembly of NER factors — it may undergo reverse translocation/backtracking (Donahue *et al.*, 1994; Fousteri *et al.*, 2006) or be targeted for proteasomal degradation by ubiquitination (Harreman *et al.*, 2009), as shown in Figure 1 (lower panel).

Upon lesion resolution, the CSA-E3 ubiquitin ligase complex performs a critical function in the recovery of transcription by targeting CSB for proteasomal degradation (Groisman *et al.*, 2006). To avoid premature degradation of CSB, UVSSA, which binds firmly to stalled RNA pol II, recruits USP7, an enzyme that promotes deubiquitination of CSB (Schwertman *et al.*, 2012), highlighted in blue in Figure 1. Therefore, once the DNA lesion is removed, USP7-mediated deubiquitination of CSB ceases, and CSB is finally targeted for degradation.

In the absence of the CSA or CSB protein, the arrest of RNA pol II persists, an event that leads to p53 activation and

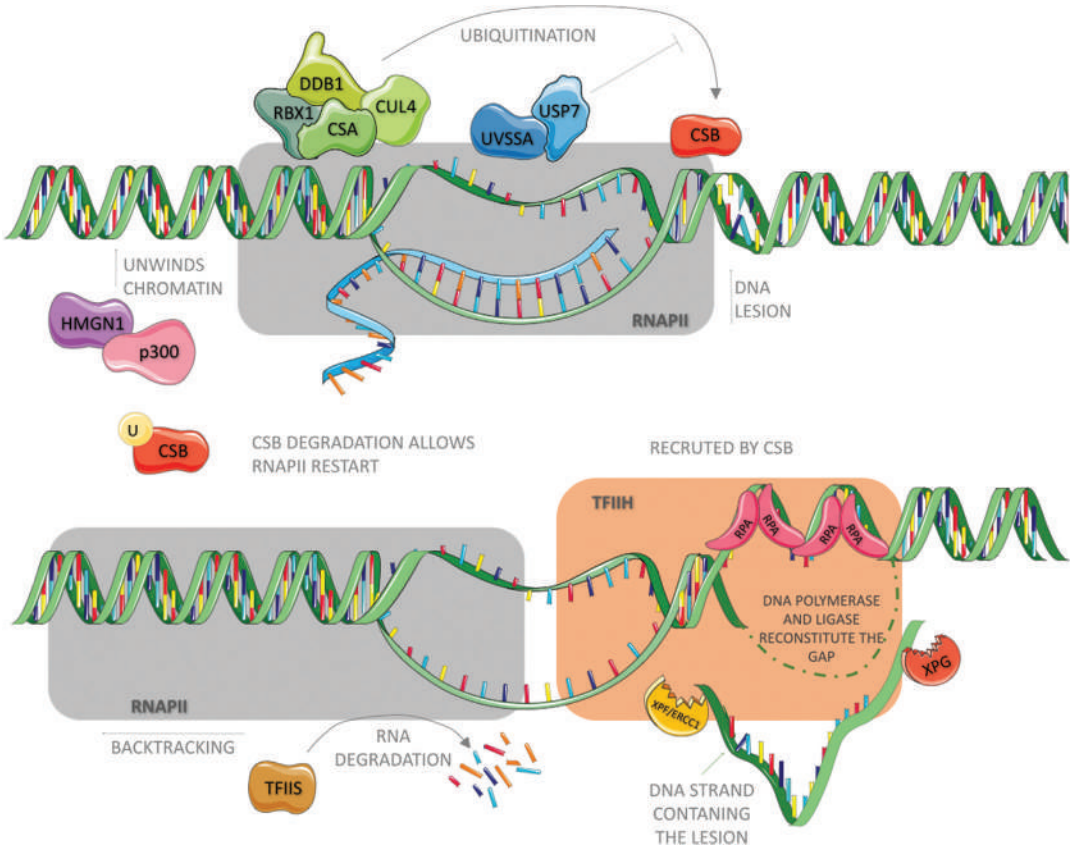

Figure 1 - Physical blockage of RNA polymerase II facilitates CSB binding to it. The following is responsible for recruiting p300, CSA and the other NER factors. CSA, together with CUL4, RBX1 and DDB1 are constantly ubiquitinating CSB, however UVSSA-USP7 complex are constantly removing ubiquitin tags from it. CSA also recruits HMGN1, which together with p300 unwinds chromatin upstream RNApol II, allowing it to backtrack and expose the lesion site to NER factors. TFIIS stimulates RNA cleavage by RNApol II during this process. NER factors unwinds DNA around the lesion. While RPA protects ssDNA from degradation, XPG and XPF-ERCC1 endonucleases cleave the strand containing the lesion. DNA polymerase and ligase then fill up the gap. CSB degradation is necessary to RNA synthesis recovery.

cell death, thus explaining the extreme sensitivity of CS cells to UV damage (Ljungman and Zhang, 1996). Besides its participation in TC-NER, proteins CSA and CSB play several other important roles. In the following sections, we provide detailed descriptions of the structures of these proteins, their functions outside of TC-NER, and a comprehensive review of pathological mutations, their consequences for protein function, and their association with patients' clinical characteristics.

The structure and functions of proteins CSA and CSB, or how pathological mutations are (not) associated with clinical phenotypes

CSB is a 168 kDa protein composed of 1493 amino acid residues and is encoded by the *ERCC6* gene located in chromosomal region 10q11 (Troelstra *et al.*, 1992, 1993). It belongs to the SWI2/SNF2 family of helicases, and just as all the proteins in this family, it does not have the capacity to open the DNA double helix (Selby and Sancar, 1997a). In contrast, the SWI2/SNF2 proteins temporarily modify DNA conformation via ATP hydrolysis, thereby altering the DNA contact with histones and nucleosome positioning. Therefore, SWI2/SNF2 proteins are considered chromatin remodelers (Lusser and Kadonaga, 2003; Beerens *et al.*, 2005). In addition to its known classic function in TC-NER, CSB takes part in the regulation of transcription and assists with nuclear and mitochondrial base excision repair (BER). It has been reported that CSB interacts with proteins XPB, XPD, XPG, TFIIH, RNA pol I and II, and glycosylases (Tantin, 1998; Bradsher *et al.*, 2002; Tuo *et al.*, 2002; Sarker *et al.*, 2005; Stevnsner *et al.*, 2008; Kamenisch *et al.*, 2010), although some of these interactions were described only once and need additional confirmation of their biological relevance.

The CSB structure mainly includes the following domains: an acidic domain, SNF2/ATPase region, ubiquitin-binding domain, and a nuclear localization signal (Liu *et al.*, 2015). The acidic domain comprising amino acid residues 356 to 394 is located in the N-terminal portion, which is mostly negatively charged (Troelstra *et al.*, 1992). In other proteins, this domain facilitates protein–protein interactions, especially those of nuclear and DNA-binding proteins, such as transcriptional activators and chromatin remodelers (Melcher, 2000; Carpenter *et al.*, 2005; Wu *et al.*, 2017). Given that CSB is classified as a chromatin remodeler, it has been hypothesized that its acidic domain facilitate this activity, but

the possible underlying mechanisms have not yet been fully elucidated (Brosh *et al.*, 1999).

Mutations in the acidic domain of CSB in UV61 cells do not compromise the ability to repair lesions caused by UV, 4-QNO (4-Nitroquinoline 1-oxide), and NA-AAF (*N*-acetoxy-2-acetylaminofluorene), or cell viability after exposure to these agents (Brosh *et al.*, 1999; Sunesen *et al.*, 2000). These data indicate that the integrity of this domain is not essential for this protein's function in TC-NER. Similar results were obtained by Lake *et al.* (2010) with UV irradiation of CS1AN-SV cells expressing CSB protein lacking the first 454 amino acid residues in the N-terminal portion, demonstrating that the absence of this region does not compromise the ability of the protein to associate with chromatin but instead makes such associations much more frequent even without UV exposure. It was also observed that this deletion increases the ATPase activity of CSB, indicating that the N-terminal portion acts as a negative regulator of its association with chromatin via ATP hydrolysis (Lake *et al.*, 2010). CSB protein structure and homozygous and heterozygous pathological alterations are illustrated in Figure 2 A and B, whereas Table S1 lists all ERCC6 mutations reported in the literature.

The nuclear localization signal is found within regions 466–481 and 1038–1055 (amino acid positions) (Lange *et al.*, 2007). In a recent work, Iyama *et al.* (2018) identified through computational analysis the existence of a third region of nuclear localization signal, in addition to the three nucleolar localization signals that cooperate for the distribution of the protein between the nucleus and nucleolus.

Among these regions there is also the SNF2/ATPase domain, which is highly conserved in the SWI2/SNF2 family (Pazin and Kadonaga, 1997). This domain extends from amino acid residue 510 to residue 960 and contains seven ATPase motifs: I, Ia, II, II, IV, V, and VI (Troelstra *et al.*, 1992), essential for the functioning of the protein (Brosh *et al.*, 1999; Selzer *et al.*, 2002). The function of the ATPase region is the most relevant for the activity of CSB, because this function provides energy for its association with (and remodeling of) chromatin by altering the positioning of nucleosomes (Citterio *et al.*, 2000). Through this activity, CSB enables the repair of DNA lesions by promoting the access of other proteins, such as CSA and NER factors, to the site of

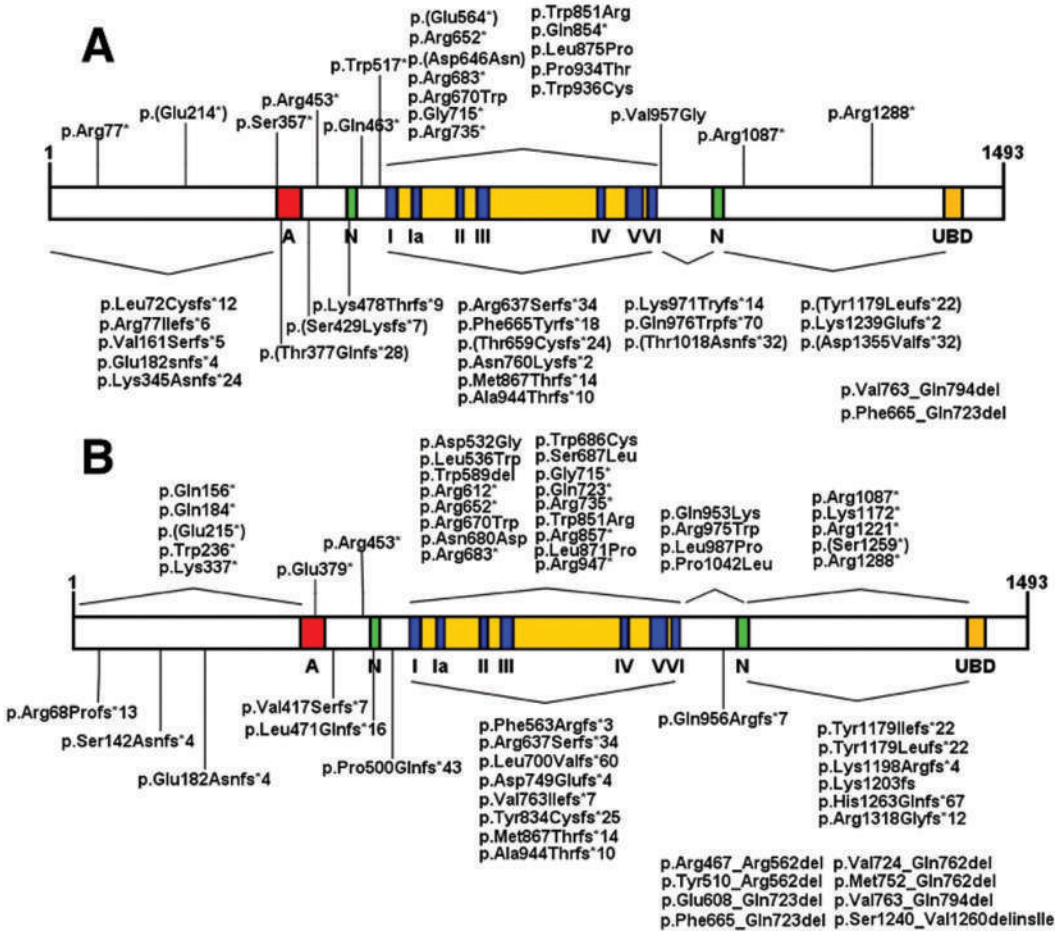

Figure 2 - Representation of CSB protein and domains. Acidic region (A), nuclear localization signal (N), helicase motifs (I, Ia –VI) and ubiquitin binding domain (UBD). (A) Homozygous mutations are indicated: frameshifts and nonsense mutations are indicated above the protein, while deletions and missense mutations are indicated below the protein. (B) Represents heterozygous mutations.

stalled RNA pol II (Stadler and Richly, 2017). During the transcription process, CSB alters *in situ* chromatin conformation, favoring the binding of transcription factors (Lake *et al.*, 2014).

To understand and characterize the functional importance of each ATPase motif, several cell lines carrying mutations in different and highly conserved regions in these motifs have been created. In general, amino acid substitutions in these regions decrease cell survival, RNA synthesis recovery, and DNA repair after exposure to UV, as well as increase the sensitivity to 4-NQO (Brosh *et al.*, 1999; Muftuoglu *et al.*, 2002; Selzer *et al.*, 2002). Notably, mutations in domains I and II, named "Walker A" and "Walker B," respectively, can completely inactivate the ATPase activity (Citterio *et al.*, 1998; Christiansen *et al.*, 2003). Mutations in motifs V and VI also compromise the ATPase activity, although to a lesser extent (Christiansen *et al.*, 2003). Different motifs can also contribute in different ways to other activities carried out by CSB. Tuo *et al.* (2001) demonstrated that cells mutated in motifs V and VI are more sensitive to γ-radiation than wild-type cells, and DNA lesions such as 7,8-dyhydro-2'-deoxyguanosine (8-oxoGua) accumulate in CSB-null and VI mutant-CSB cells after exposure to γ-radiation, indicating a possible relation between CSB and the BER pathway (Tuo *et al.*, 2002a).

The ubiquitin-binding domain (UBD) is located in the C-terminal region of the CSB protein (amino acid residues 1400–1428). UBD-CSB–deficient cells have a phenotype similar to that of cells that do not express the CSB protein at all. Although the TC-NER complex is fully assembled around the lesion and RNA pol II in these cells, the repair does not proceed because of the inability of CSB to leave the lesion site (Anindya *et al.*, 2010). The replacement of the CSB UBD by another UBD, such as UBA2 of Rad23, an otherwise unrelated *Saccharomyces cerevisiae* DNA repair gene, also enables CSB dissociation from the lesion region and progression of the repair process, thereby demonstrating the need for CSB ubiquitination for the correct functioning of the protein in this TC-NER (Anindya *et al.*, 2010). Cells lacking UBD in the CSB protein are sensitive to oxidatively induced DNA damage (Ranes *et al.*, 2016), suggesting that this domain is important for the repair of this kind of lesion. The conserved amino acid lysine at position 911 was recently found to be a ubiquitination site that is also required for this function, but is dispensable for TC-NER (Ranes *et al.*, 2016).

By constructing several CSB mutants with different deletions in the C-terminal region, Sin *et al.* (2016) found that the integrity of the amino acid sequence in this region is important for this sumoylation of this protein and association with chromatin. Aside from this region, a functional UBD domain is necessary for RNA Pol II interaction and CSA recruitment to the nucleus (Sin *et al.*, 2016). In addition, Groisman (2006) demonstrated that the degradation of CSB depends on the action of an E3-ubiquitin ligase complex that contains CSA.

CSA is a 44 kDa protein 396 amino acid residues long and is encoded by the *ERCC8* gene located in chromosomal region 5q12.1 (Henning *et al.*, 1995). It belongs to the WD-repeat family because it contains 7 WD40 domains that are repeated in its structure. These domains consist of approximately 40 amino acid residues that start with a conserved glycine and histidine sequence and terminate in tryptophan and aspartic acid (WD), a seven-bladed propeller structure with its N terminus attached to DDB1 via a helix-loop-helix motif (Fischer *et al.*, 2011). Proteins with the WD40 domain characteristically interact with other proteins and are typically known for their ability to form protein complexes (Xu and Min, 2011). Although they do not have a catalytic activity, they are involved in a variety of cellular functions, such as the regulation of transcription and chromatin conformation, apoptosis, signal transduction, and cell cycle control, among others (Xu and Min, 2011). CSA protein structure and homozygous/heterozygous pathological alterations are illustrated in Figure 3A and B. Table S2 shows all the ERCC8 mutations reported in the literature.

It is known that CSA is part of the E3 ubiquitin ligase complex, along with DDB1, RBX1, and CUL4A (Groisman *et al.*, 2003; Fischer *et al.*, 2011), responsible for the ubiquitination and degradation of TC-NER proteins when the repair is finalized (Groisman *et al.*, 2006). In addition, CSA interacts and complexes with several proteins involved in transcription, ribosomal biogenesis, and TC-NER (reviewed by Aamann *et al.*, 2014; Koch *et al.*, 2014). Nonetheless, the mechanisms via which these interactions occur, and which CSA regions are involved, are not yet completely understood.

The three-dimensional structure and conserved amino acid residues in specific regions of the protein are of great importance for the regulatory activity and the interactions. Mutations in these regions that lead to the alteration of one of these factors may inevitably impair these functions (Muftuoglu *et al.*, 2002; Christiansen *et al.*, 2003).

To identify a possible relation between the mutations and the phenotype of patients with CS, we mapped all the mutations in the *ERCC8* and *ERCC6* genes and their impact on proteins CSA and CSB, respectively, that is already reported in the literature. In total, we found 102 mutations in *ERCC6* (50 homozygous and 52 heterozygous) and 37 mutations in *ERCC8* (23 homozygous and 14 heterozygous). These numbers indicate that 70% of all the cases of the disease are caused by CSB mutations and 30% by CSA mutations. Analyzing only homozygous mutations, we observed the lack of an obvious correlation between the type of mutation or the affected region with the severity of CS (Tables S1 and S2).

Nevertheless, as discussed by Laugel (2013), the large prevalence of type I CS clinical classification (moderate phenotype) is noted in CSA patients, with most of the mutations located in the WD domains. Regarding CSB, mutations are predominant among the domains (such as domains III and IV, for example) and are mostly nonsense mutations and frameshifts, with lower prevalence of missense mutations and deletions. Unlike CSA cases, type II CS clinical manifestation is

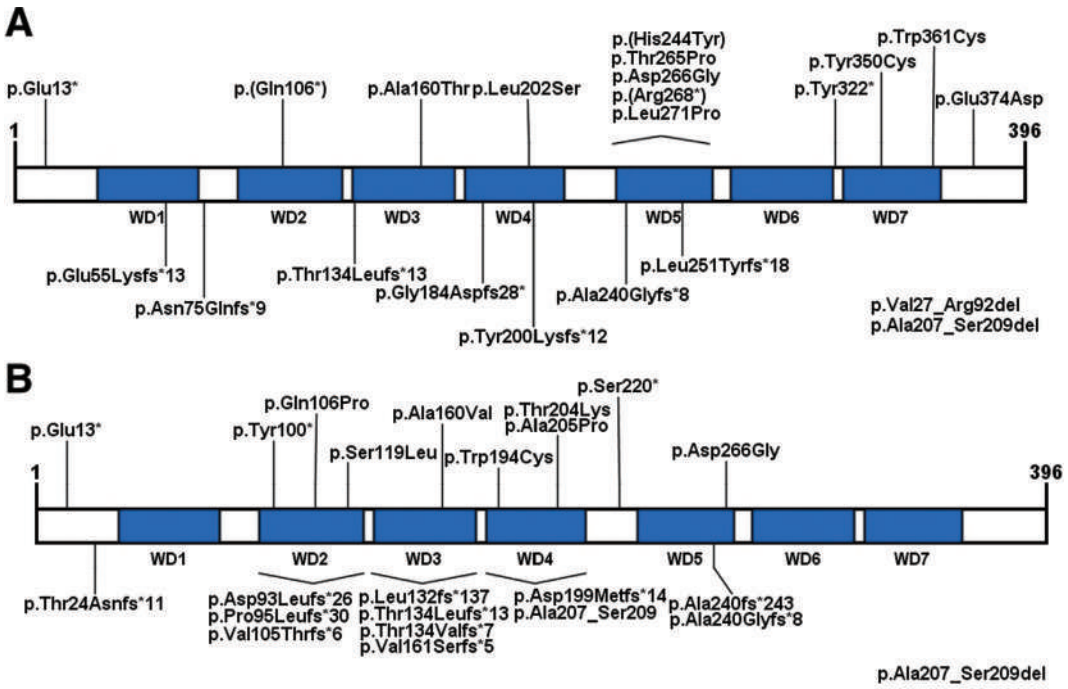

Figure 3 - Representation of CSA protein and its seven WD-repeat domains. (A) Homozygous mutations are indicated: frameshifts and nonsense mutations are indicated above the protein, while deletions and missense mutations are indicated below the protein. (B) Represents heterozygous mutations.

predominant among CSB patients (Laugel, 2013; Calmels *et al.*, 2018).

The mutations that give rise to truncated CS proteins and the phenotype of the patients are also non-correlating variables. Proteins with mutations in the amino acid end sequences, such as R1087X, all have the integral ATPase domains but still lead to the manifestation of type II CS (more severe phenotype). On the other hand, the most striking example is the total absence of functional CSB resulting from the R77X mutation, as reported by Horibata *et al.* (2004), not leading to CS manifestation but instead causing UV-sensitive syndrome (UVSS) (Spivak, 2005). Another interesting observation is that the same mutation may cause different forms of CS in different patients. Other examples of one specific mutation resulting in different phenotypes exist in the literature. For example, Colella *et al.* (2000) described two siblings with the R735X mutation in CSB that do not cause CS but instead induce De Sanctis–Cacchione syndrome (a variant form of XP), whereas Mallery *et al.* (1998) described an association of the same mutation with type I of CS. In addition, Jaakkola *et al.* (2010) discovered the R1288X mutation (in CSB), which causes a severe neurological disorder known as cerebro-ocular-facial-skeletal syndrome (COFS) in several members of the same family, while the same mutation was also associated with type II CS manifestation (Laugel *et al.*, 2009). This unexpected difference in phenotypes suggests that the genetic background may be a key factor that also needs to be taken into account in studies on the development, diversification of forms, and manifestations of the disease.

CS as a transcription syndrome

A deficiency in TC-NER and the consequent inability to recover RNA synthesis upon DNA damage in actively transcribed strands of genes could contribute to the neurological CS phenotype. Nonetheless, patients with UVSS (which can be caused by mutations in genes *CSA*, *CSB*, or *UVSSA*) lack TC-NER and are sun sensitive but do not manifest any of the neurological symptoms of CS (Itoh *et al.*, 1996; Horibata *et al.*, 2004; Spivac, 2005; Nardo *et al.*, 2009; Zhang *et al.*, 2012; Brooks, 2013).

In 1997, both the Hoeijmakers and Sancar groups discovered that CSB associates with RNA pol II, suggesting that CSB is implicated in transcription (Selby and Sancar, 1997a,b; van Gool *et al.*, 1997). In fact, CSB was shown to increase the rate of transcription elongation up to threefold in an *in vitro* assay that reconstituted the transcription system (Selby and Sancar, 1997b). Similar observations were made by Balajee *et al.* (1997). These results prompted the authors to suggest that CS may be mainly a transcription, rather than a DNA repair related syndrome.

Five years later, Bradsher *et al.* (2002) stated that CSB was found in the nucleolus in a complex containing RNA pol I, which regulates ribosomal RNA (rRNA) transcription. The authors noticed that a CSB mutant cell line showed 8- to 10-fold lower rates of rRNA synthesis than did wild-type cells, confirming a critical role of CSB not only in mRNA synthesis (by RNA pol II) but also in rRNA synthesis (by RNA pol I). Of note, the CSA protein turned out to be associated with RNA pol I in the nucleolus, and a knockdown of CSA reduced rRNA synthesis (Koch *et al.*, 2014). This malfunction in RNA pol I transcription has also been linked to

endoplasmic-reticulum stress, leading to an unfolded protein response and the loss of proteostasis, which may be linked to the CS phenotype (Alupei *et al.*, 2018).

In agreement with the idea that CS is a transcription syndrome, Newman *et al.* (2006) found that CSB-null fibroblasts feature a gene dysregulation pattern similar to that induced by HDAC inhibitors. Wang *et al.* (2014) reported dysregulation of several genes (linked to neurons) in CSB-mutant and CSA-mutant fibroblasts and in *post-mortem* brain tissue of patients. The authors also noticed that *in vitro* transdifferentiation of fibroblasts into neurons and neuroblast differentiation are impaired in CSB-deficient cells. These results strongly indicate that CSB is critical for neuronal differentiation and maintenance, and that gene expression defects might underlie the neurodegenerative and the neurodevelopmental defects observed in patients.

By taking advantage of cell reprogramming, Andrade *et al.* (2012) for the first time reprogrammed CSB-mutated primary fibroblasts into induced-pluripotent stem cells. Aside from making it possible to obtain a cell type that can be used to model CS development *in vitro*, the authors noticed dysregulation of hundreds of targets (including p53 and TXNIP) in these cells (Andrade *et al.*, 2012). More recently, by combining cell reprogramming with neuron differentiation protocols, Vessoni *et al.* (2016) were, for the first time, able to obtain live neurons from patients' skin fibroblasts. By RNA sequencing, the authors were able to find that pathological mutations in the *ERCC6* gene changed the expression of almost 5000 transcripts in neurons of CSB-deficient patients compared to unaffected controls. Pathways related to axonogenesis, the action potential of neurons, neurotransmission, as well as transcripts related to the growth hormone–IGF-1 pathway were found to be dysregulated in the CSB-deficient neurons. Collectively, these results confirm that CSB deficiency heavily impacts the transcriptional process of the cell types relevant for the disease, even in the absence of exogenous DNA-damaging agents. Such extensive transcriptional dysregulation may underlie the complex and heterogeneous CS phenotype.

Nonetheless, as mentioned before, some mutations in genes *XPB*, *XPD*, *XPF*, *XPG*, or *ERCC1*, which participate in NER (Menck and Munford, 2014), may result in a combined phenotype of CS and XP (Lehmann 1982, 2014; Weeda *et al.*, 1990; Moriel-Carretero *et al.*, 2015). How can we explain the CS phenotype in all these cases? A likely answer to this question may depend on a multiprotein complex, TFIIH, which is indispensable for NER and for transcription. TFIIH consists of two functional subcomplexes (Core and CAK) that participate in initiation, promoter escape, and early elongation (Compe and Egly, 2012). The core subcomplex consists of seven proteins, including 3' to 5' ATP-dependent helicase XPB, and XPD, a 5' to 3' ATP-dependent helicase that binds to the core and CAK complexes together and facilitates optimal transcription (Tirode *et al.*, 1999; Egly and Coin, 2011). In this sense, two mutations in the *XPB* gene (associated with the XP/CS phenotype) reduce transcriptional activity in a reconstituted transcription assay *in vitro* (Coin *et al.*, 1999). Moreover, *XPD* or *XPB* mutations associated with CS were found to disrupt the interactions among CSB, TFIIH, and RNA pol I (Bradsher *et al.*, 2002). The XPG nuclease was also found to associate with and stabilize TFIIH, and mutations in the *XPG* gene related to an XP-G/CS phenotype abrogate the XPG–TFIIH interaction (Ito *et al.*, 2007; Lehmann *et al.*, 2014; Narita *et al.*, 2015). The recently described XP/CS patients with mutations in *XPF* or *ERCC1* (Kashiyama *et al.*, 2013) pose a challenge to the "CS as a transcription syndrome" point of view, because XPF/ERCC1 is implicated in NER and not in basal transcription. Although XPF is recruited to the promoter of inducible genes (to facilitate chromatin modification for transcription) in the absence of exogenous DNA damage, other NER factors are recruited as well, including XPC, in which mutations do not result in neurological abnormalities (Le May *et al.*, 2010). XPF mutations that cause the XP/CS phenotype were recently found to cause persistent recruitment of NER proteins to DNA damage sites, which may induce the stalling of RNA and DNA polymerases, thereby interfering with the transcription and replication processes (Sabatella *et al.*, 2018). Nevertheless, endogenous levels of DNA lesions that are substrates for NER have been reported to accumulate in mammalian cells and tissues to the levels that would not be consistent with the notion of defective TC-NER as a cause of the neurological symptoms of CS (Brooks, 2013). Therefore, more studies are needed to clarify the mechanism behind XPF/ERCC1 mutations and the development of the CS phenotype in these patients (Kashiyama *et al.*, 2013).

CS and redox processes

The manifestation of CS occurs only when some NER proteins are mutated, while the complete deactivation of this pathway, via mutations in *XPA*, leads to the development of XP. Consequently, the roles of proteins CSA and CSB in addition to those known classic functions come into question. In addition, the main symptoms of patients with CS, e.g., neurological aberrations, cannot be explained only by the inefficient repair of UV damage because neurons are not exposed to this type of radiation. Due to the systemic presence of reactive species and their ability to chemically and structurally modify biomolecules, especially DNA, these compounds have been investigated as possible contributing factors of CS.

Experiments with keratinocytes derived from patients with CS suggest that these cells contain high concentrations of reactive oxygen species (ROS) with redox balance alterations under baseline conditions, characteristics that are related to the senescence phenotype of these cells (Cordisco *et al.*, 2018). The induction of redox processes by the exposure of mice and $CSB^{-/-}$ mouse embryonic fibroblasts to ionizing radiation or paraquat revealed high sensitivity to the toxic effects of these agents, while such high sensitivity is not observed in CSA-mutant mice (de Waard *et al.*, 2003, 2004). The sensitivity to the redox processes was also observed in $CSA^{-/-}$ keratinocytes and fibroblasts treated with potassium bromide (D'Errico *et al.*, 2007) and in $CSB^{-/-}$ cells exposed to

MMS (methyl methansulfonate) and 5-hydroxymethyl-2'deoxyuridine (Wong *et al.*, 2007).

Moreover, lipid peroxidation products such as HNE (4-hydroxynonenal) at high cellular concentrations perform direct modifications on the CSB protein, compromising its ATPase activity required for the DNA repair by TC-NER (Maddukuri *et al.*, 2009). On the other hand, Boetefuer *et al.* (2018) demonstrated that this activity is not needed for CSB–chromatin association when CS1AN-sv cells are exposed to menadione. In that condition, these associations are loci-specific and are stimulated by the PARP1 protein, thereby indicating possible participation of CSB in the transcriptional regulation in response to oxidative stress (Boetefuer *et al.*, 2018a). These findings are in agreement with previous results that point to the involvement of a CSB function in this process, thereby showing that CSB localization and interaction with transcriptional repressor CTCF in promoter regions are greater in cells under oxidative stress (Lake *et al.*, 2016; Boetefuer *et al.*, 2018b).

Although DNA oxidation products are typically repaired by the BER pathway, it has been demonstrated that BER and NER proteins not only show crosstalk, but also that some oxidatively generated lesions are substrates for the NER pathway (D'Errico *et al.*, 2007; Berra *et al.*, 2013). For example, 8-oxoGua, thymine glycol, malondialdehyde, and etheno adducts induce distorting modifications in the double helix and have the potential to block transcription (reviewed by Tornaletti, 2005; Chaim *et al.*, 2017), with cyclopurines being DNA oxidation products that are repaired only by NER (Brooks *et al.*, 2000). Numerous experiments have confirmed the importance of the CSA and CSB proteins for the repair of oxidized bases, by demonstrating that in the absence of CSA or CSB there is accumulation of 8-oxoGua in DNA (Dianov *et al.*, 1999; Tuo *et al.*, 2001; D'Errico *et al.*, 2007; Aamann *et al.*, 2014a; Cordisco *et al.*, 2018). 8-OH-Ade, 5-hydroxycytosine, and cyclopurines are also lesions that are inefficiently repaired in cells harboring mutations in CS genes (Tuo *et al.*, 2002b, 2003; D'Errico *et al.*, 2013). Because of the inefficient repair in the cells of important tissues such as the brain, the accumulation of these lesions in DNA may be one of the factors that cause and aggravate the neurological symptoms of the disease, as seen in patients with neurodegenerative diseases, such as Alzheimer's, Parkinson's, Huntington's disease, and amyotrophic lateral sclerosis (Ayala-Peña, 2013; Coppedè *et al.*, 2016; Abolhassani *et al.*, 2017).

The mutational impact of oxidatively induced DNA damage in CS was investigated by Lodato *et al.* (2018). By means of single-cell whole-genome sequencing followed by genome-wide somatic single-nucleotide variant identification, they detected an increased number of mutations in the neurons of patients with CS as compared to the control. In addition, C > A variants, a signature for mutations induced by oxidatively induced DNA damage, are found in a higher frequency among patients with CS (Lodato *et al.*, 2018).

It is noteworthy that in response to irradiation or hydrogen peroxide, CSA is translocated to the nuclear matrix by a CSB-dependent mechanism (Kamiuchi *et al.*, 2002), but as discussed above, these two proteins perform different functions in chromatin remodeling and in recruitment of the factors associated with the repair and blocking of RNA pol II *in vivo* (Fousteri *et al.*, 2006). This cooperation between CSA and CSB was not observed in response to treatment with alkylating agents and is independent of XPA and XPC, thus suggesting that TC-NER plays a key part in this cellular response mechanism (Kamiuchi *et al.*, 2002).

Brain biopsies of CS and XPA patients yielded distinct results in response to the accumulation of oxidized bases in DNA and SOD expression, with only XPA-mutant patients showing upregulation of 8-oxoGua in the nucleus and alteration in SOD expression (Hayashi *et al.*, 2005).

In addition to the participation of these proteins in the repair of oxidation-induced lesions through the NER pathway, there is also a contribution to the removal of DNA damage via direct and indirect activity in the BER pathway (Khobta and Epe, 2013). This contribution is mediated by the direct modulation through interactions of CSB with BER protein glycosylases, APE1, NEIL1 and NEIL2, and association with the OGG1 complex, which stimulates the incision activity of these proteins and drives the repair (Wong *et al.*, 2007; Muftuoglu *et al.*, 2009; Aamann *et al.*, 2014b). $Csb^{m/m}/Ogg1^{-/-}$ mice show high concentrations of 8-oxoGua in comparison with $Ogg1^{-/-}$ animals, thereby confirming the importance of the cooperation between these two proteins in the removal of these lesions (Osterod *et al.*, 2002; Trapp *et al.*, 2007). Pastoriza-Gallego *et al.* (2007) also demonstrated that the 8-oxoGua lesions exert different effects on gene expression depending upon the promoter and sequence context, and that both proteins, Csb and Ogg1, are required for full repair.

Recent evidence also indicates that CSB recruits the XRCC1 protein to single-strand break regions after oxidatively induced DNA damage for subsequent processing by BER (Menoni *et al.*, 2018). In the indirect modulation of the BER pathway, CSB acts as an important factor in the expression of *hOGG1*, inducing its transcription. It has also been observed that in CSB-deficient cells, the amounts of *OGG1* mRNA and protein are low (Dianov *et al.*, 1999; Tuo *et al.*, 2002a; Aamann *et al.*, 2014b).

Some studies point to a possible implication of CSB in the repair of oxidized bases in genomic and mitochondrial DNA, to CSB migration to mitochondria, and modulation of p53 activity in response to a redox process (Stevnsner *et al.*, 2008; Frontini and Proietti-De-Santis, 2009; Aamann *et al.*, 2010).

The repair of oxidized bases also has a fundamental role for the mitochondria. These organelles produce ATP through the electron transport chain, a process that not only provides cells with energy, but also produces ROS as a by-product of respiration (reviewed by Kowaltowski *et al.*, 2009). For this reason, mitochondria are also considered the primary source of ROS in cells, having a much more oxidizing environment within the mitochondrial matrix as compared with the cellular cytosol (Hu *et al.*, 2008).

Because of proximity to the initial site of formation and constant exposure to ROS, mitochondrial DNA (mtDNA) is the main target of these molecules, and just as nuclear DNA, it is susceptible to oxidation in the DNA bases and undergoes other modifications in its structure (reviewed by Muftuoglu *et al.*, 2014). Besides, the accumulation of lesions in mtDNA causes instability and compromises its functioning, which can lead to mutations and affect genes that are important for mitochondrial metabolism. In addition, various aging-related diseases, such as cardiovascular diseases, Parkinson's disease, Alzheimer's disease, and cancer are associated with significant amounts of lesions in mitochondria, thus being considered one of the causes of the pathologies related to mitochondrial dysfunction (Mecocci *et al.*, 1994; Ide *et al.*, 2001; Stichel *et al.*, 2007; Wallace, 2012).

Mitochondria have some specific repair pathways, such as BER and mismatch repair (MMR). As in the repair of nuclear DNA, the CSA and CSB proteins are present and participate in this process, even though there is no mitochondrial NER (Kamenisch *et al.*, 2010). It has been demonstrated that CSA and CSB, after induction of redox processes by the exposure to H_2O_2, UV, or menadione, are directed toward mitochondria and interact with OGG1 (Aamann *et al.*, 2010; Kamenisch *et al.*, 2010; reviewed by Prates Mori and de Souza-Pinto, 2018). In mitochondria, CSB may act as a modulator of the BER pathway, in association with the inner mitochondrial membrane for mtDNA lesion removal (Stuart *et al.*, 2005; Aamann *et al.*, 2010). In fact, $CSB^{m/m}$ mice accumulate 2.5-fold more 8-oxoGua in mtDNA than wild-type animals (Osenbroch *et al.*, 2009). The mutation in $CSB^{m/m}$ mice is the same as that in the *ERCC6* gene of the CS1AN patient, K337 → stop, which generates a truncated nonfunctional protein (Troelstra *et al.*, 1992; van der Horst *et al.*, 1997).

In addition to the influence on DNA repair, CSB is involved in gene expression by engaging in the elongation by RNA polymerase II (Selby and Sancar, 1997a). In *in vitro* transcription, through interactions with proteins of the mitochondrial nucleoid complex, CSB promotes POLRMT transcriptional elongation and allows it to access mtDNA by TFAM removal from the region to be transcribed (Berquist *et al.*, 2012).

Regarding CS mitochondrial metabolism, Scheibye-Knudsen *et al.* (2012) reported a significant increase of this metabolism in both $CSB^{m/m}$ mice and CSB-deficient cells, and this phenomenon is also manifested in CSA- or CSB- deficient fibroblasts and $CSA^{-/-}/XPA^{-/-}$ mice (Pascucci *et al.*, 2012; Brace *et al.*, 2016). This increase in energy metabolism is accompanied by a large production of ROS through electron leakage (Pascucci *et al.*, 2012; Cleaver *et al.*, 2014), and is the main source of DNA damage induction (Scheibye-Knudsen *et al.*, 2012) and of changes in the membrane potential, excessive fragmentation, and mitochondrial fission (Pascucci *et al.*, 2012, 2016).

Under normal cell conditions, organelles and defective proteins are eliminated by autophagy. On the other hand, dysfunctional or damaged mitochondria are subjected to a special form of autophagy, called mitophagy (Fivenson *et al.*, 2017). This mechanism contributes to cell homeostasis, and its malfunction is present in several aging-related diseases (reviewed by Redmann *et al.*, 2014). Human and murine CSB-mutant fibroblasts feature a dysfunction in this pathway owing to defects in the recruitment of P62, an essential player in mitophagy (Scheibye-Knudsen *et al.*, 2012).

Thus, dysfunctional mitochondria, with large amounts of mtDNA lesions, can produce more ROS via their metabolism. Consequently, ROS accumulate in the cells, promoting apoptosis (Pinto and Moraes, 2015; van Houten *et al.*, 2016), which might be associated with reduced amounts of subcutaneous fat in $CSA^{-/-}$ and $CSB^{m/m}$ mice (Kamenisch *et al.*, 2010). Treatment with pharmacological activators of autophagy, such as rapamycin (Scheibye-Knudsen *et al.*, 2012) and an HDAC inhibitor (Majora *et al.*, 2018), attenuates the accumulation of damaged mitochondria in CSB-deficient cells and the loss of subcutaneous fat in $CSB^{m/m}$ mice.

Models for CS study

To better understand the mechanisms of CS progression, several animal models have been developed for research on the disease, e.g., mice, *Caenorhabditis elegans*, and zebrafish. In general, such models carry knockout mutations or mutations identical to those found in humans, enabling a more accurate analysis in different cells and tissues of an organism.

The first models generated to study CS were created on the basis of mice with the C57BL/6 background - $CSB^{m/m}$ mice (van der Horst *et al.*, 1997), and $CSA^{-/-}$ mice (van der Horst *et al.*, 2002). In $CSA^{-/-}$ cells, the interruption of the gene sequence in exon 2 of the *Ercc8* gene results in protein absence (van der Horst *et al.*, 2002). When exposed to UV, the fibroblasts of these animals manifest higher UV sensitivity, and they have an inability to resume RNA synthesis and defective TC-NER, these being the classic characteristic of CS (Lehmann, 1982; van der Horst *et al.*, 1997, 2002).

The $CSB^{m/m}$ and $CSA^{-/-}$ models have similar phenotypic characteristics, although neither has the severe neurodegenerative phenotype and lifespan reduction seen in patients with CS. Despite differences between them, these mouse models have a few characteristics that resemble their human counterpart, such as the tendency toward reduced body weight via a decrease in the amounts of subcutaneous and visceral fat (Gorgels *et al.*, 2007; Scheibye-Knudsen *et al.*, 2012), mild neurological dysfunction with changes in myelin (Jaarsma *et al.*, 2011), activation of glial cells in white-matter regions (Jaarsma *et al.*, 2011), progressive deafness (Nagtegaal *et al.*, 2015), photophobia and sensitivity to UV exposure (van der Horst *et al.*, 1997*,* 2002). In contrast to what is manifested in humans, the mouse models are prone to skin and eye cancers when exposed to UV, a feature that can be explained by the inefficiency of murine GG-NER in dealing with UV-induced cyclobutane pyrimidine dimer-like lesions, which are preferentially repaired by TC-NER (van der Horst *et al.*, 1997, 2002; de Boer and Hoeijmakers, 1999).

$CSB^{m/m}$ cells (from several tissues, mainly the brain and kidneys) also contain high levels of formamidopyrimidines and 8-oxoGua in DNA when compared to wild-type mice (Muftuoglu *et al.*, 2009). In addition to nuclear DNA damage, mtDNA damage and mutational load in the mtDNA of $CSA^{-/-}$ and $CSB^{m/m}$ mice increased during aging and is related to subcutaneous fat loss, one of the main characteristics of CS, probably mediated by the apoptosis caused by mitochondrial dysfunction (Kamenisch *et al.*, 2010) and by cell senescence, a feature commonly present in progeroid models (Carrero *et al.*, 2016).

The combined deletion of other NER proteins such as XPA in these CS mice leads to a more severe phenotype. These animals manifest severe neurological symptoms, ataxia, compromised growth, low weight, a lack of motor coordination, kyphosis, and abnormal behavior and development with premature death within 20 days (Murai *et al.*, 2001; van der Pluijm *et al.*, 2007). Inactivation of GG-NER by an XPC knockout in $CSB^{m/m}$ mice leads to a similar phenotype, with dysmyelination, slow development, and low body weight: characteristics that resemble those of patients with CS (Laposa *et al.*, 2007; van der Pluijm *et al.*, 2007; Revet *et al.*, 2012). $CSA^{-/-}/XPA^{-/-}$ mice also show evidence of neurological problems, life expectancy reduced to ~1 month, an increase in cellular oxidative phosphorylation, and lipodystrophy (Brace *et al.*, 2013*,* 2016).

The mechanism that underlies this aggravation of the CS phenotype by the double inactivation of CS and XP genes in mice has yet to be uncovered. Since CS and XP proteins act in the same DNA repair pathway, it is assumed that this severe CS phenotype in mice is due to the disruption of both NER-related and other functions of the CS proteins, such as transcription regulation (Brooks, 2013) and/or interactions with other DNA repair pathways (Murai *et al.*, 2001). Inactivation of NER by an *XPC* or *XPA* gene knockout can result in the accumulation of transcription-blocking DNA lesions, and the loss of either CS protein may further interfere with transcription and further increase the DNA damage load, which can in turn have detrimental effects, such as cell death or early cell senescence, both of which have been observed in progeroid mice (Weeda *et al.*, 1997; van der Pluijm *et al.*, 2007; Brooks, 2013).

Combined inactivation of TC-NER and NER in neurons has also been reported to generate an age-related progressive neurodegenerative phenotype. $CSB^{m/m}$ mice featuring a neuron-specific conditional XPA knockout have a shorter lifespan, behavioral abnormalities, and brain atrophy. These characteristics are possibly related to a synergistic effect of the TC-NER and NER pathways or to other functions of the inactivated proteins, such as chromatin remodeling or other functions in transcription (Newman *et al.*, 2006; Jaarsma *et al.*, 2011; Wang *et al.*, 2018). Cell type–specific conditional knockouts may also help to better understand the participation of other processes in the progression of the disease, along with their molecular mechanisms, e.g., the role of oligodendrocytes (Howng *et al.*, 2010) or other glial cells (Raj *et al.*, 2014) in CS dysmyelination and in the neurodegenerative phenotype.

Mutations in XPG or ERCC1 can yield mice with the characteristics similar to those of CS and other diseases on the CS spectrum, such as XP/CS and COFS (Jaarsma *et al.*, 2013). These NER nuclease–deficient models have several neurodegenerative features, altered metabolism, and a reduced lifespan (Weeda *et al.*, 1997; Barnhoorn *et al.*, 2014). Notably, despite the lipodystrophy observed in all these animal models, dietary restriction and methionine restriction (which are metabolic interventions known to increase the lifespan of several species) reversed some of the neurodegeneration indicators of the $CSA^{-/-}/XPA^{-/-}$ and $ERCC1^{\Delta/-}$ models, with dietary restriction nearly doubling the lifespan of $ERCC1^{\Delta/-}$ mice. This finding indicates that metabolic interventions are a possible therapy for NER-related progeroid diseases (Brace *et al.*, 2016; Vermeij *et al.*, 2016).

The current murine models for CS research do not fully reproduce the phenotype seen in humans because these animals manifest milder symptoms of the disease when only a single TC-NER protein is mutated. One of the possible explanations is the adaptation of NER to deal with DNA damage that would be repaired initially by CSA and CSB. This explains the severe symptoms when both TC-NER and NER are inactivated (van der Pluijm *et al.*, 2007). In this case, it is important to consider the evolutionary distance that separates mice from humans. This distance entails differences in gene expression and metabolic and physiological profiles, among others (Seok *et al.*, 2013; Lin *et al.*, 2014). Such differences contribute to the following phenomenon: the phenotype resulting from the same mutation and the activity of the repair pathway are not identical between the two species. Table 1 summarizes the mouse models carrying mutations in CS genes and other single-NER-mutation models that recapitulate CS-like phenotypes.

Different animal models of lower complexity have also been developed to study the role of CSA and CSB proteins, and are an interesting alternative for the research into protein functions. Among these models are *Caenorhabditis elegans* and zebrafish.

C. elegans has advantages, such as the ease of laboratory maintenance, of genetic manipulation, of tissue differentiation, rapid reproduction with several offspring, a generally fixed and genetically determined number of cells, and a short life cycle, allowing for the study of its development within short periods. It has been demonstrated that the NER pathway is well conserved in *C. elegans*, resembling the repair mechanisms of mammals (Meyer *et al.*, 2007; reviewed by Lans and Vermeulen, 2011), and because they do not repair DNA by photoreactivation (Hartman *et al.*, 1989). Rather, the lesions caused by UV are repaired exclusively by NER. CSA and CSB analogs, csa-1 (Babu *et al.*, 2014) and csb-1 (CeCSB) (Lee *et al.*, 2002), respectively, were found to be a part of this pathway. Animals mutated in csa-1 are hypersensitive to UV-B light exposure (Babu *et al.*, 2014), and csb-1–deficient animal germ cells show apoptosis induction and morphological abnormalities after exposure to this agent (Lee *et al.*,

Table 1 - Reported neurological and metabolic characteristics of Cockayne Syndrome and related mouse models.

Mouse model	Genetic background	Neurological abnormalities	Metabolic characteristics	Lifespan	Other reported features	References
$Csb^{m/m}$	C57BL/6J	Mild, progressive neurodegeneration with no behavioral phenotype, mild astro and microgliosis, hearing loss associated with loss of neurosensorial cells, late retinal degeneration	Increased metabolism and mitochondrial numbers, Progressive lipodistrophy	> 2 years	Increased skin cancer predisposition after UV exposure	Van der Horst *et al*., 1997; Jaarsma *et al*., 2011
$Csa^{-/-}$	C57BL/6J	Mild, progressive neurodegeneration with no behavioral phenotype, mild astro and microgliosis, hearing loss associated with loss of neurosensorial cells	Increased metabolism	> 2 years	Increased skin cancer predisposition after UV exposure	van der Horst *et al*., 2002; Gorgels *et al*, 2007; Jaarsma *et al*., 2011; Brace *et al*., 2016
$Csb^{m/m}/Xpa^{-/-}$	C57BL/6J; CBA/C57BL6/CD-1/129Ola hybrid	Profound early postnatal ataxia, abnormal cerebellar development and degeneration, loss of purkinje cells, progressive neurological dysfunction, motor coordination problems, early retinal degeneration	Severe post-natal growth deficiency, Severe lipodistrophy, decreased oxidative metabolism	3 weeks	Suppression of the GH/IGF1 somatotroph axis increased antioxidant responses, hypoglycemia	Murai *et al*., 2001, van der Pluijm *et al*., 2007; Jaarsma *et al*., 2011
$Csb^{-/-}$ / $Xpa^{c/-}$ CamKIIα-Cre	C57BL/6J	Progressive neurological dysfunction, Purkinje cell death, neurodegeneration, motor coordination problems starting at 6 months	Weight reduction in adult animals	90 weeks	-	Jaarsma *et al*., 2011
$Csb^{m/m}/Xpc^{-/-}$	C57BL/6J, 129Ola /C57BL/6J hybrid	Depletion of purkinje cells, neurodegeneration in cerebellum, motor coordination problems, dysmielination	Severe post-natal growth deficiency, Severe lipodistrophy	3 weeks	-	van der Pluijm *et al*., 2007; Laposa *et al*., 2007; Revet *et al*., 2012
$Csb^{m/m}/Ogg1^{-/-}$	C57BL/6J	No neurological phenotype has been observed	Not described	> 2 years	Accumulation of oxidative stress related DNA damage and mutations in various tissues	Osterod *et al*., 2002; Pastoriza-Gallego *et al*., ?2007; Fusser *et al*., 2011
$Csa^{-/-}/Xpa^{-/-}$	C57BL/6J	Early, progressive neurological dysfunction, dysmyelination, abnormal cerebellar development and degeneration, motor coordination problems, severe astro and microgliosis	Post-natal growth deficiency, increased metabolism, severe lipodistrophy, senescence of fat tissue, no fat tissue inflammation	20 weeks	-	Brace *et al*., 2013; Brace *et al*., 2016
$Xpg^{-/-}$	C57BL/6J	Purkinje cell degeneration, cerebellum neurodegeneration	Severe post-natal growth deficiency, Severe lipodistrophy	3 weeks	Abnormalities of the gastro-intestinal tract, early fibroblast senescence	Harada *et al*., 1999; Sun *et al*., 2001; Barnhoorn *et al*., 2014
$Xpg^{-/-}$	C57BL6/FVB F1 hybrid	Purkinje cell death, neurodegeneration, motor coordination problems axonal spheroids, severe astrogliosis	Post-natal growth deficiency, loss of subcutaneous fat	18 weeks	Skeletal abnormalities, mild anisokaryosis, increased mean nuclear size of liver cells, supression of GH/IGF1 axis	Barnhoorn *et al*, 2014; Vermeij, 2016
$Ercc1^{-/-}$	Hybrid C57Bl/6/129	Early, progressive neurological dysfunction, neurodegeneration and motor coordination problems	Severe post-natal growth deficiency, decreased oxidative metabolism, severe lipodistrophy, increase in fat tissue inflammation	3 weeks	Liver nuclear abnormalities, p53 upregulation in liver and kidney, early fibroblast senescence	Mcwhir *et al*., 1993, Weeda *et al*., 1997
$Ercc1^{\Delta/-}$	C57Bl6J/FVB hybrid	Early, progressive neurological dysfunction, myelin abnormalities, abnormal cerebellar development and degeneration, Motor coordination problems, severe astro and microgliosis	Post-natal growth deficiency, decreased oxidative metabolism, severe lipodistrophy, increase in fat tissue inflammation	22-25 weeks	Early fibroblast and vascular tissue senescence, reduced bone density	Weeda *et al*., 1997; Nevedomskaya *et al*., 2010; de Waard *et al*., 2010; Durik *et al*., 2012; Vermeij *et al*., 2016;

2002). Moreover, a knockout of either csa-1 or csb-1 in *C. elegans* resulted in increased oxygen consumption and in changes in the transcription of genes related to mitochondrial ATP production, ubiquitin pathways, and transcriptional regulation (Scheibye-Knudsen *et al.*, 2016).

In general, *C. elegans* is a good model for the investigation of the DNA damage response to UV via the NER pathway, because this species makes it possible to reproduce and examine the effects of irradiation or other DNA-damaging agents on an entire organism and throughout its developmental stages within a short period of time.

The zebrafish is a vertebrate model that is widely used in research on the effects of exposure to genotoxic agents, carcinogenesis processes, and mainly embryonic development (Spitsbergen and Kent, 2003; Titus *et al.*, 2009). This animal has orthologous DNA repair genes in the pathways present in higher eukaryotes, e.g., BER, NER, MMR, Non-homologous end joining and Homologous recombination (Pei and Strauss, 2013). In NER, 44 genes are responsible for the functioning mechanisms of damage removal. Despite this observation, there are still few zebrafish studies where these characteristics are exploited from the perspective of DNA repair alone.

Zebrafish with the CSB depleted by morpholino oligonucleotides at the larval stage show an increased frequency of morphological abnormalities, which may recapitulate some of the congenital and developmental manifestations seen in patients with CS. Ionizing radiation can further increase morphological aberrations in CSB-depleted zebrafish, thereby pointing to an important role of CSB in the defense against oxidative DNA damage (Wei *et al.*, 2015).

Due to the complexity of CS and the unique characteristics of patients with CS, which cannot be fully recapitulated in any animal model, a combination of human cell models with various animal models offers complimentary approaches to elucidate the various characteristics of this syndrome. In this sense, the use of somatic cell reprogramming coupled with genome editing allows investigators to obtain relevant and functional cell types (such as neurons) carrying patient-specific mutations. Moreover, this method offers an opportunity to investigate how the genetic background of different patients interacts with the one given pathological mutation. Such an approach may clarify why certain patients that share the same mutation in a CS gene have different phenotypes (Colella *et al.*, 2000).

Conclusion

Almost 60 years of accumulated research in the NER field, especially regarding progeroid CS, provides extensive knowledge about the structure of proteins CSA and CSB and their participation in TC-NER and other mechanisms, such as transcription, repair, and mitochondrial functioning. For the advances made so far, human cellular models and animal models have been invaluable. They have revealed that the CS phenotype is likely a combination of altered gene transcription, metabolic adjustment, redox imbalance, and DNA repair defects, although the relative importance of each of these mechanisms for the disease is still largely unknown. Moreover, the full spectrum of the syndrome is not completely mirrored in animals, and this situation undoubtedly hinders further research. This difficulty is also associated with the impossibility of correlation between the mutations in *ERCC6*/*ERCC8* genes and the phenotype of the patients, suggesting that the genetic background can heavily influence the manifestation of the symptoms.

Recently, the ability to generate pluripotent stem cells from patients with CS enabled for the first time the recapitulation of the full genetic signature of a patient in a cell type relevant for the disease, e.g., neurons. This observation expands and complements the previous CS models because it enables the investigation of metabolism, of the DNA damage response, and of gene expression in a patient's cells. These data were previously impossible or very difficult to obtain. Still, one needs to keep in mind that CS is a systemic disease, and for this reason, investigating how dysfunction in one tissue/organ impacts others is critical. All these difficulties reveal how complex and diverse CS is and imply that a multimodel approach will therefore help to better recapitulate certain characteristics of CS. Moreover, although not entirely equivalent to normal aging, CS has clinical and cellular similarities to some aging-related diseases. Thus, improvements of (and new approaches to) CS models may have a broad impact on the study of these diseases as well.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

ATV, CCCG, GSK, LLSN, CCMG wrote the manuscript, and all authors approved the final version.

References

Aamann MD, Sorensen MM, Hvitby C, Berquist BR, Muftuoglu M, Tian J, de Souza-Pinto NC, Scheibye-Knudsen M, Wilson DM, Stevnsner T *et al.* (2010) Cockayne syndrome group B protein promotes mitochondrial DNA stability by supporting the DNA repair association with the mitochondrial membrane. FASEB J 24:2334–2346.

Aamann MD, Hvitby C, Popuri V, Muftuoglu M, Lemminger L, Skeby CK, Keijzers G, Ahn B, Bjoras M, Bohr VA *et al.* (2014a)

Cockayne Syndrome group B protein stimulates NEIL2 DNA glycosylase activity. Mech Ageing Dev 135:1–14.

Aamann MD, Muftuoglu M, Bohr VA and Stevnsner T (2014b) Multiple interaction partners for Cockayne syndrome proteins. Mech Ageing Dev 134:212–224.

Abolhassani N, Leon J, Sheng Z, Oka S, Hamasaki H, Iwaki T and Nakabeppu Y (2017) Molecular pathophysiology of impaired glucose metabolism, mitochondrial dysfunction, and oxidative DNA damage in Alzheimer's disease brain. Mech Ageing Dev 161:95–104.

Alupei MC, Maity P, Esser P, Krikki I, Tuorto F, Parlato R, Penzo M, Schelling A, Laugel V, Montanaro L *et al.* (2018) Loss of proteostasis is a pathomechanism in Cockayne syndrome. Cell Rep 23:1612–1619.

Andrade LN, Nathanson JL, Yeo GW, Menck CFM and Muotri AR (2012) Evidence for premature aging due to oxidative stress in iPSCs from Cockayne syndrome. Hum Mol Genet 21:3825–3834.

Andrews AD, Barrett SF, Yoder FW and Robbins JH (1978) Cockayne's Syndrome fibroblasts have increased sensitivity to ultraviolet light but normal rates of unscheduled DNA synthesis. J Invest Dermatol 70:237–239.

Anindya R, Mari PO, Kristensen U, Kool H, Giglia-Mari G, Mullenders LH, Fousteri M, Vermeulen W, Egly JM and Svejstrup JQ (2010) A ubiquitin-binding domain in cockayne syndrome b required for transcription-coupled nucleotide excision repair. Mol Cell 38:637–648.

Ayala-Peña S (2013) Role of oxidative DNA damage in mitochondrial dysfunction and Huntington's disease pathogenesis. Free Radic Biol Med 62:102–110.

Babu V, Hofmann K and Schumacher B (2014) A *C. elegans* homolog of the Cockayne syndrome complementation group A gene. DNA Repair (Amst) 24:57–62.

Balajee AS, May A, Dianov GL, Friedberg EC and Bohr VA (1997) Reduced RNA polymerase II transcription in intact and permeabilized Cockayne syndrome group B cells. Proc Natl Acad Sci U S A 94:4306–4311.

Barnhoorn S, Uittenboogaard LM, Jaarsma D, Vermeij WP, Tresini M, Weymaere M, Menoni H, Brandt RM, de Waard MC, Botter SM *et al.* (2014) Cell-autonomous progeroid changes in conditional mouse models for repair endonuclease XPG Deficiency. PLoS Genet 10:e1004686.

Beerens N, Hoeijmakers JHJ, Kanaar R, Vermeulen W and Wyman C (2005) The CSB protein actively wraps DNA. J Biol Chem 280:4722–4729.

Berquist BR, Canugovi C, Sykora P, Wilson DM and Bohr VA (2012) Human Cockayne syndrome B protein reciprocally communicates with mitochondrial proteins and promotes transcriptional elongation. Nucleic Acids Res 40:8392–8405.

Berra CM, de Oliveira CS, Garcia CCM, Rocha CRR, Lerner LK, Lima LC, Baptista M da S and Menck CFM (2013) Nucleotide excision repair activity on DNA damage induced by photoactivated methylene blue. Free Radic Biol Med 61:343–356.

Boetefuer EL, Lake RJ, Dreval K and Fan HY (2018a) Poly(ADP-ribose) polymerase 1 (PARP1) promotes oxidative stress–induced association of Cockayne syndrome group B protein with chromatin. J Biol Chem 293:17863-17874.

Boetefuer EL, Lake RJ and Fan HY (2018b) Mechanistic insights into the regulation of transcription and transcription-coupled DNA repair by Cockayne syndrome protein B. Nucleic Acids Res 46:7471–7479.

Bohr VA, Smith CA, Okumoto DS and Hanawalt PC (1985) DNA repair in an active gene: Removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. Cell 40:359–369.

Brace LE, Vose SC, Vargas DF, Zhao S, Wang XP and Mitchell JR (2013) Lifespan extension by dietary intervention in a mouse model of Cockayne syndrome uncouples early postnatal development from segmental progeria. Aging Cell 12:1144–1147.

Bradsher J, Auriol J, Proietti de Santis L, Iben S, Vonesch JL, Grummt I and Egly JM (2002) CSB Is a Component of RNA Pol I Transcription. Mol Cell 10:819–829.

Brace LE, Vose SC, Stanya K, Gathungu RM, Marur VR, Longchamp A, Treviño-Villarreal H, Mejia P, Vargas D, Inouye K *et al.* (2016) Increased oxidative phosphorylation in response to acute and chronic DNA damage. Aging Mech Dis 2:16022.

Brooks PJ (2013) Blinded by the UV light: how the focus on transcription-coupled NER has distracted from understanding the mechanisms of Cockayne syndrome neurologic disease. DNA Repair (Amst) 12:656–671.

Brooks PJ, Wise DS, Berry DA, Kosmoski JV, Smerdon MJ, Somers RL, Mackie H, Spoonde AY, Ackerman EJ, Coleman K *et al.* (2000) The oxidative DNA lesion 8,5'-(S)-Cyclo-2'-deoxyadenosine is repaired by the nucleotide excision repair pathway and blocks gene expression in mammalian cells. J Biol Chem 275:22355–22362.

Brosh RM Jr, Balajee AS, Selzer RR, Sunesen M, Proietti De Santis L and Bohr VA (1999) The ATPase domain but not the acidic region of Cockayne syndrome group B gene product is essential for DNA repair. Mol Biol Cell 10:3583–3594.

Calmels N, Botta E, Jia N, Fawcett H, Nardo T, Nakazawa Y, Lanzafame M, Moriwaki S, Sugita K, Kubota M *et al.* (2018) Functional and clinical relevance of novel mutations in a large cohort of patients with Cockayne syndrome. J Med Genet 55:329-343.

Carpenter AE, Memedula S, Plutz MJ and Belmont AS (2005) Common effects of acidic activators on large-scale chromatin structure and transcription. Mol Cell Biol 25:958–968.

Carrero D, Soria-Valles C and López-Otín C (2016) Hallmarks of progeroid syndromes: Lessons from mice and reprogrammed cells. Dis Model Mech 9:719–735.

Chaim IA, Gardner A, Wu J, Iyama T, Wilson DM and Samson LD (2017) A novel role for transcription-coupled nucleotide excision repair for the in vivo repair of 3,N^4-ethenocytosine. Nucleic Acids Res 45:3242–3252.

Christiansen M, Stevnsner T, Modin C, Martensen PM, Brosh RM and Bohr VA (2003) Functional consequences of mutations in the conserved SF2 motifs and post-translational phosphorylation of the CSB protein. Nucleic Acids Res 31:963–973.

Citterio E, Rademakers S, van der Horst GT, van Gool AJ, Hoeijmakers JH and Vermeulen W (1998) Biochemical and biological characterization of wild-type and ATPase-deficient Cockayne syndrome B repair protein. J Biol Chem 273:11844–11851.

Citterio E, van den Boom V, Schnitzler G, Kanaar R, Bonte E, Kingston RE, Hoeijmakers JHJ and Vermeulen W (2000) ATP-dependent chromatin remodeling by the Cockayne syndrome B DNA repair-transcription-coupling factor. Mol Cell Biol 20:7643–7653.

Cleaver JE, Lam ET and Revet I (2009) Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. Nat Rev Genet 10:756–768.

Cleaver JE, Brennan-Minnella AM, Swanson RA, Fong K, Chen J, Chou KM, Chen YW, Revet I and Bezrookove V (2014) Mitochondrial reactive oxygen species are scavenged by Cockayne syndrome B protein in human fibroblasts without nu-

clear DNA damage. Proc Natl Acad Sci U S A 111:13487-13492.
Cockayne EA (1936) Dwarfism with retinal atrophy and deafness. Arch Dis Child 11:1–8.
Cockayne EA (1946) Dwarfism with retinal atrophy and deafness. Arch Dis Child 11:52–54.
Coin F, Bergmann E, Tremeau-Bravard A and Egly J (1999) Mutations in XPB and XPD helicases found in xeroderma pigmentosum patients impair the transcription function of TFIIH. EMBO J 18:1357–1366.
Colella S, Nardo T, Botta E, Lehmann AR and Stefanini M (2000) Identical mutations in the CSB gene associated with either Cockayne syndrome or the DeSanctis-Cacchione variant of Xeroderma pigmentosum. Hum Mol Genet 9:1171–1175.
Compe E and Egly JM (2012) TFIIH: When transcription meets DNA repair. Nat Rev Mol Cell Biol 13:343–354.
Coppedè F, Tannorella P, Stoccoro A, Chico L, Siciliano G, Bonuccelli U and Migliore L (2016) Methylation analysis of DNA repair genes in Alzheimer's disease. Mech Ageing Dev 161:105–111.
Cordisco S, Tinaburri L, Teson M, Orioli D, Cardin R, Degan P, Stefanini M, Zambruno G, Guerra L and Dellambra E (2018) Cockayne syndrome type A protein protects primary human keratinocytes from senescence. J Invest Dermatol 139:1–13.
Costa RM, Chigançcas V, Galhardo RS, Carvalho H and Menck CFM (2003) The eukaryotic nucleotide excision repair pathway. Biochimie 85:1083–1099.
D'Errico M, Parlanti E, Teson M, Degan P, Lemma T, Calcagnile A, Iavarone I, Jaruga P, Ropolo M, Pedrini AM *et al.* (2007) The role of CSA in the response to oxidative DNA damage in human cells. Oncogene 26:4336–4343.
D'Errico M, Pascucci B, Iorio E, van Houten B and Dogliotti E (2013) The role of CSA and CSB protein in the oxidative stress response. Mech Ageing Dev 134:261–269.
de Boer J and Hoeijmakers JH (1999) Cancer from the outside, aging from the inside: Mouse models to study the consequences of defective nucleotide excision repair. Biochimie 81:127–137.
de Waard H, de Wit J, Gorgels TGMF, van den Aardweg G, Andressoo JO, Vermeij M, Van Steeg H, Hoeijmakers JHJ and van der Horst GTJ (2003) Cell type-specific hypersensitivity to oxidative damage in CSB and XPA mice. DNA Repair (Amst) 2:13–25.
de Waard H, de Wit J, Andressoo JO, van Oostrom CTM, Riis B, Weimann A, Poulsen HE, van Steeg H, Hoeijmakers JHJ and van der Horst GTJ (2004) Different effects of CSA and CSB deficiency on sensitivity to oxidative DNA damage. Mol Cell Biol 24:7941–7948.
Dianov G, Bishoff C, Sunesen M and Bohr VA (1999) Repair of 8-oxoguanine is deficient in Cockayne syndrome group B. Nucleic Acids Res 27:1365–1368.
Donahue BA, Yint S, Taylort JS, Reines D and Hanawalt PC (1994) Transcript cleavage by RNA polymerase II arrested by a cyclobutane pyrimidine dimer in the DNA template. Proc Natl Acad Sci U S A 91:8502–8506.
Durik M, Kavousi M, van der Pluijm I, Isaacs A, Cheng C, Verdonk K, Loot AE, Oeseburg H, Bhaggoe UM, Leijten F *et al.* (2013) Nucleotide Excision DNA Repair is Associated with Age-Related Vascular Dysfunction. Circulation 126:468–478.
Egly JM and Coin F (2011) A history of TFIIH: Two decades of molecular biology on a pivotal transcription/repair factor. DNA Repair (Amst) 10:714–721.
Fischer ES, Scrima A, Böhm K, Matsumoto S, Lingaraju GM, Faty M, Yasuda T, Cavadini S, Wakasugi M, Hanaoka F *et al.* (2011) The molecular basis of CRL4DDB2/CSA ubiquitin ligase architecture, targeting, and activation. Cell 147:1024–1039.
Fivenson EM, Lautrup S, Sun N, Sheibye-Knudsen M, Stevnsner T, Nilsen H, Bohr VA and Fang EF (2017) Mitophagy in neurodegeneration and aging. Neurochem Int 109:202–209.
Fousteri M and Mullenders LHF (2008) Transcription-coupled nucleotide excision repair in mammalian cells: Molecular mechanisms and biological effects. Cell Res 18:73–84.
Fousteri M, Vermeulen W, van Zeeland AA and Mullenders LHF (2006) Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. Mol Cell 23:471–482.
Frontini M and Proietti-De-Santis L (2009) Cockayne syndrome B protein (CSB): Linking p53, HIF-1 and p300 to robustness, lifespan, cancer and cell fate decisions. Cell Cycle 8:693–696.
Fusser M, Nesse GJ, Khobta A, Xia N, Li H, Klungland A and Epe B (2011) Spontaneous mutagenesis in csbm/mogg1-/- mice is attenuated by dietary resveratrol. Carcinogenesis 32:80–85.
Gorgels TG, van der Pluijm I, Brandt RM, Garinis GA, van Steeg H, van den Aardweg G, Jansen GH, Ruijter JM, Bergen AA, van Norren D *et al.* (2007) Retinal degeneration and ionizing radiation hypersensitivity in a mouse model for Cockayne syndrome. Mol Cell Biol 27:1433–1441.
Gregersen LH and Svejstrup JQ (2018) The cellular response to transcription-blocking DNA damage. Trends Biochem Sci 43:327–341.
Groisman R, Polanowska J, Kuraoka I, Sawada J, Saijo M, Drapkin R, Kisselev AF, Tanaka K and Nakatani Y (2003) The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. Cell 113:357–367.
Groisman R, Kuraoka I, Chevallier O, Gaye N, Magnaldo T, Tanaka K, Kisselev AF, Harel-Bellan A and Nakatani Y (2006) CSA-dependent degradation of CSB by the ubiquitin-proteasome pathway establishes a link between complementation factors of the Cockayne syndrome. Genes Dev 20:1429–1434.
Guardiola A, Álvares-da-Silva CR, Grisolia J and Silbermann R (1999) Síndrome de Cockayne. Arq Neuro-Psiquiatr 57:106–110.
Hanawalt PC and Spivak G (2008) Transcription-coupled DNA repair: Two decades of progress and surprises. Nature 9:958–970.
Harreman M, Taschner M, Sigurdsson S, Anindya R, Reid J, Somesh B, Kong SE, Banks CAS, Conaway RC, Conaway JW *et al.* (2009) Distinct ubiquitin ligases act sequentially for RNA polymerase II polyubiquitylation. Proc Natl Acad Sci U S A 106:20705–20710.
Hartman PS, Hevelone J, Dwarakanath V and Mitchell DL (1989) Excision repair of UV radiation-induced DNA damage in *Caenorhabditis elegans*. Genetics 122:379–385.
Hayashi M, Araki S, Kohyama J, Shioda K and Fukatsu R (2005) Oxidative nucleotide damage and superoxide dismutase expression in the brains of Xeroderma pigmentosum group A and Cockayne syndrome. Brain Dev 27:34–38.
Henning KA, Li L, Iyer N, McDaniel LD, Reagan MS, Legerski R, Schultz RA, Stefanini M, Lehmann AR, Mayne LV *et al.* (1995) The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH. Cell 82:555–564.

Hoogstraten D, Bergink S, Ng JM, Verbiest VH, Luijsterburg MS, Geverts B, Raams A, Dinant C, Hoeijmakers JHJ, Vermeulen W *et al.* (2008) Versatile DNA damage detection by the global genome nucleotide excision repair protein XPC. J Cell Sci 121:2850–2859.

Horibata K, Iwamoto Y, Kuraoka I, Jaspers NG, Kurimasa A, Oshimura M, Ichihashi M and Tanaka K (2004) Complete absence of Cockayne syndrome group B gene product gives rise to UV-sensitive syndrome but not Cockayne syndrome. Proc Natl Acad Sci U S A 101:15410–15415.

Howng SY, Avila RL, Emery B, Traka M, Lin W, Watkins T, Cook S, Bronson R, Davisson M, Barres BA *et al.* (2010) ZFP191 is required by oligodendrocytes for CNS myelination. Genes Dev 24:301–311.

Hu J, Dong L and Outten CE (2008) The redox environment in the mitochondrial intermembrane space is maintained separately from the cytosol and matrix. J Biol Chem 283:29126–29134.

Ide T, Tsutsui H, Hayashidani S, Kang D, Suematsu N, Nakamura KI, Utsumi H, Hamasaki N and Takeshita A (2001) Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. Circ Res 88:529–535.

Ito S, Kuraoka I, Chymkowitch P, Compe E, Takedachi A, Ishigami C, Coin F, Egly JM and Tanaka K (2007) XPG stabilizes TFIIH, allowing transactivation of nuclear receptors: implications for Cockayne syndrome in XP-G/Patients with CS. Mol Cell 26:231–243.

Itoh T, Shiomi T, Shiomi N, Harada Y, Wakasugi M, Matsunaga T, Nikaido O, Friedberg EC and Yamaizumi M (1996) Rodent complementation group 8 (ERCC8) corresponds to Cockayne syndrome complementation group A. Mutat Res 362:167–174.

Iyama T, Okur MN, Golato T, McNeill DR, Lu H, Hamilton R, Raja A, Bohr VA and Wilson III DM (2018) Regulation of the intranuclear distribution of the Cockayne syndrome proteins. Sci Rep 8:1–12.

Jaakkola E, Mustonen A, Olsen P, Miettinen S, Savuoja T, Raams A, Jaspers NG, Shao H, Wu BL and Ignatius J (2010) ERCC6 founder mutation identified in Finnish patients with COFS syndrome. Clin Genet 78:541–547.

Jaarsma D, van der Pluijm I, de Waard MC, Haasdijk ED, Brandt R, Vermeij M, Rijksen Y, Maas A, van Steeg H, Hoeijmakers JHJ *et al.* (2011) Age-related neuronal degeneration: Complementary roles of nucleotide excision repair and transcription-coupled repair in preventing neuropathology. PLoS Genet 7:e1002405.

Jaarsma D, van der Pluijm I, van der Horst GTJ and Hoeijmakers JH (2013) Cockayne syndrome pathogenesis: Lessons from mouse models. Mech Ageing Dev 134:180–195.

Kamenisch Y, Fousteri M, Knoch J, von Thaler AK, Fehrenbacher B, Kato H, Becker T, Dollé ME, Kuiper R, Majora M *et al.* (2010) Proteins of nucleotide and base excision repair pathways interact in mitochondria to protect from loss of subcutaneous fat, a hallmark of aging. J Exp Med 207:379–90.

Kamiuchi S, Saijo M, Citterio E, de Jager M, Hoeijmakers JH and Tanaka K (2002) Translocation of Cockayne syndrome group A protein to the nuclear matrix: Possible relevance to transcription-coupled DNA repair. Proc Natl Acad Sci U S A 99:201–206.

Karam SM, Costa JC, Jardim L, Pires RF, Lehmann AR and Giugliani R (2000) Cockayne syndrome: Report of a Brazilian family with confirmation of impaired RNA synthesis after UV-irradiation. Genet Mol Biol 275:273–275.

Karikkineth AC, Scheibye-Knudsen M, Fivenson E, Croteau DL and Bohr VA (2017) Cockayne syndrome: Clinical features, model systems and pathways. Ageing Res Rev 33:3–17.

Kashiyama K, Nakazawa Y, Pilz DT, Guo C, Shimada M, Sasaki K, Fawcett H, Wing JF, Lewin SO, Carr L *et al.* (2013) Malfunction of nuclease ERCC1-XPF results in diverse clinical manifestations and causes Cockayne syndrome, Xeroderma pigmentosum, and Fanconi anemia. Am J Hum Genet 92:807–819.

Khobta A and Epe B (2013) Repair of oxidatively generated DNA damage in Cockayne syndrome. Mech Ageing Dev 134:253–260.

Kleijer WJ, Laugel V, Berneburg M, Nardo T, Fawcett H, Gratchev A, Jaspers NGJ, Sarasin A, Stefanini M and Lehmann AR (2008) Incidence of DNA repair deficiency disorders in western Europe: Xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. DNA Repair (Amst) 7:744–750.

Koch S, Gonzalez OG, Assfalg R, Schelling A, Schäfer P, Scharffetter-Kochanek K and Iben S (2014) Cockayne syndrome protein A is a transcription factor of RNA polymerase I and stimulates ribosomal biogenesis and growth. Cell Cycle 13:2029–2037.

Kowaltowski AJ, de Souza-Pinto NC, Castilho RF and Vercesi AE (2009) Mitochondria and reactive oxygen species. Free Radic Biol Med 47:333–343.

Kubota M, Ohta S, Ando A, Koyama A, Terashima H, Kashii H, Hoshino H, Sugita K and Hayashi M (2015) Nationwide survey of Cockayne syndrome in Japan: Incidence, clinical course and prognosis. Pedriatrics Int 57:339–347.

Lake RJ, Geyko A, Hemashettar G, Zhao Y and Fan HY (2010) UV-induced association of the CSB remodeling protein with chromatin requires ATP-dependent relief of N-terminal autorepression. Mol Cell 37:1–20.

Lake RJ, Boetefuer EL, Tsai PF, Jeong J, Choi I, Won KJ and Fan HY (2014) The sequence-specific transcription factor c-jun targets Cockayne syndrome protein B to regulate transcription and chromatin structure. PLoS Genet 10:e1004284.

Lake RJ, Boetefuer EL, Won KJ and Fan HY (2016) The CSB chromatin remodeler and CTCF architectural protein cooperate in response to oxidative stress. Nucleic Acids Res 44:2125–2135.

Lange A, Mills RE, Lange CJ, Stewart M, Devine SE and Corbett AH (2007) Classical nuclear localization signals: Definition, function, and interaction with importin α. J Biol Chem 282:5101–5105.

Lans H and Vermeulen W (2011) Nucleotide excision repair in *Caenorhabditis elegans*. Mol Biol Int 2011:1–12.

Laposa RR, Huang EJ and Cleaver JE (2007) Increased apoptosis, p53 up-regulation, and cerebellar neuronal degeneration in repair-deficient Cockayne syndrome mice. Proc Natl Acad Sci U S A 104:1389–1394.

Laugel V (2013) Cockayne syndrome: The expanding clinical and mutational spectrum. Mech Ageing Dev 134:161–170.

Laugel V, Dalloz AC, Durand M, Sauvanaud F, Kristensen U, Vincent MC, Pasquier L, Odent S, Gener B, Tobias ES *et al.* (2009) Mutation update for the CSB/ERCC6 and CSA/ERCC8 genes involved in Cockayne syndrome. Hum Mutat 31:113–126.

Le May N, Mota-Fernandes D, Vélez-Cruz R, Iltis I, Biard D and Egly JM (2010) NER factors are recruited to active promoters and facilitate chromatin modification for transcription in the absence of exogenous genotoxic attack. Mol Cell 38:54–66.

Lee MH, Ahn B, Choi IS and Koo HS (2002) The gene expression and deficiency phenotypes of Cockayne syndrome B protein in *Caenorhabditis elegans*. FEBS Lett 522:47–51.

Lehmann AR (1982) Three complementation groups in Cockayne syndrome. Mutat Res 106:347–356.

Lehmann J, Schubert S, Schäfer A, Apel A, Laspe P, Schiller S, Ohlenbusch A, Gratchev A and Emmert S (2014) An unusual mutation in the XPG gene leads to an internal in-frame deletion and a XP/CS complex phenotype. Br J Dermatol 171:903–905.

Lin S, Lin Y, Nery JR, Urich MA, Breschi A, Davis CA, Dobin A, Zaleski C, Beer MA, Chapman WC *et al.* (2014) Comparison of the transcriptional landscapes between human and mouse tissues. Proc Natl Acad Sci U S A 111:17224–17229.

Liu W, Xie Y, Ma J, Luo X, Nie P, Zuo Z, Lahrmann U, Zhao Q, Zheng Y and Zhao Y (2015) Sequence analysis IBS: An illustrator for the presentation and visualization of biological sequences. Bioinformatics 31:3359–3361.

Ljungman M and Zhang F (1996) Blockage of RNA polymerase as a possible trigger for UV light-induced apoptosis. Oncogene 13:823–831.

Lodato MA, Rodin RE, Bohrson CL, Coulter ME, Barton AR, Kwon M, Sherman MA, Vitzthum CM, Lovelace LJ, Yandava CN *et al.* (2018) Aging and neurodegeneration are associated with increased mutations in single human neurons. Science 359:555–559.

Lusser A and Kadonaga JT (2003) Chromatin remodeling by ATP-dependent molecular machines. BioEssays 25:1192–1200.

Maddukuri L, Speina E, Christiansen M, Dudzinska D, Zaim J, Obtulowicz T, Kabaczyk S, Komisarki M, Bukowy Z, Szczegielniak J *et al.* (2009) Cockayne syndrome group B protein is engaged in processing of DNA adducts of lipid peroxidation product trans-4-hydroxy-2- nonenal. Mutat Res 666:23–31.

Majora M, Sondenheimer K, Knechten M, Uthe I, Esser C, Schiavi A, Ventura N and Krutmann J (2018) HDAC inhibition improves autophagic and lysosomal function to prevent loss of subcutaneous fat in a mouse model of Cockayne syndrome. Sci Transl Med 10:1–13.

Mallery DL, Tanganelli B, Colella S, Steingrimsdottir H, van Gool AJ, Troelstra C, Stefanini M and Lehmann AR (1998) Molecular analysis of mutations in the CSB (ERCC6) gene in patients with Cockayne syndrome. Am J Hum Genet 62:77–85.

Marteijn JA, Lans H, Vermeulen W and Hoeijmakers JHJ (2014) Understanding nucleotide excision repair and its roles in cancer and ageing. Nat Rev Mol Cell Biol 15:465–481.

Mayne LV and Lehmann AR (1982) Failure of RNA synthesis to recover after UV irradiation: An early defect in cells from individuals with Cockayne's syndrome and Xeroderma pigmentosum. Cancer Res 42:1473–1478.

McWhir J, Selfridge J, Harrison DJ, Squires S and Melton DW (1993) Mice with DNA repair gene (ERCC-1) deficiency have elevated levels of p53, liver nuclear abnormalities and dia before weaning. Nat Genet 5:217–224.

Mecocci P, MacGarvey U and Beal MF (1994) Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann Neurol 36:747–751.

Melcher K (2000) The strength of acidic activation domains correlates with their affinity for both transcriptional and non-transcriptional protiens. J Mol Biol 301:1097–1112.

Menck CFM and Munford V (2014) DNA repair diseases: What do they tell us about cancer and aging? Genet Mol Biol 37:220–233.

Menoni H, Wienholz F, Theil AF, Janssens RC, Lans H, Campalans A, Radicella JP, Marteijn JA and Vermeulen W (2018) The transcription-coupled DNA repair-initiating protein CSB promotes XRCC1 recruitment to oxidative DNA damage. Nucleic Acids Res 46:7747–7756.

Meyer JN, Boyd WA, Azzam GA, Haugen AC, Freedman JH and van Houten B (2007) Decline of nucleotide excision repair capacity in aging *Caenorhabditis elegans*. Genome Biol 8:R70.

Moriel-Carretero M, Herrera-Moyano E and Aguilera A (2015) A unified model for the molecular basis of Xeroderma pigmentosum-Cockayne syndrome. Rare Dis 3:e1079362.

Muftuoglu M, Selzer R, Tuo J, Brosh RM and Bohr VA (2002) Phenotypic consequences of mutations in the conserved motifs of the putative helicase domain of the human Cockayne syndrome group B gene. Gene 283:27–40.

Muftuoglu M, de Souza-Pinto NC, Dogan A, Aamann M, Stevnsner T, Rybanska I, Kirkali G, Dizdaroglu M and Bohr VA (2009) Cockayne syndrome group B protein stimulates repair of formamidopyrimidines by NEIL1 DNA glycosylase. J Biol Chem 284:9270–9279.

Muftuoglu M, Mori MP and de Souza-Pinto NC (2014) Formation and repair of oxidative damage in the mitochondrial DNA. Mitochondrion 17:164–181.

Murai M, Enokido Y, Inamura N, Yoshino M, Nakatsu Y, van der Horst GT, Hoeijmakers JH, Tanaka K and Hatanaka H (2001) Early postnatal ataxia and abnormal cerebellar development in mice lacking Xeroderma pigmentosum Group A and Cockayne syndrome Group B DNA repair genes. Proc Natl Acad Sci U S A 98:13379–13384.

Nagtegaal AP, Rainey RN, van der Pluijm I, Brandt RM, van der Horst GT, Borst JG and Segil N (2015) Cockayne syndrome group B (Csb) and group A (Csa) deficiencies predispose to hearing loss and cochlear hair cell degeneration in mice. J Neurosci 35:4280–4286.

Nakatsu Y, Asahina H, Citterio E, Rademakers S, Vermeulen W, Kamiuchi S, Yeo J, Khaw M, Saijo M, Kodo N *et al.* (2000) XAB2, a novel tetratricopeptide repeat protein involved in transcription-coupled DNA repair and rranscription. J Biol Chem 275:34931–34937.

Nardo T, Oneda R, Spivak G, Vaz B, Mortier L, Thomas P, Orioli D, Laugel V, Stary A, Hanawalt PC *et al.* (2009) A UV-sensitive syndrome patient with a specific CSA mutation reveals separable roles for CSA in response to UV and oxidative DNA damage. Proc Natl Acad Sci U S A 106:6209–6214.

Narita T, Narita K, Takedachi A, Saijo M and Tanaka K (2015) Regulation of transcription elongation by the XPG-TFIIH complex is implicated in Cockayne syndrome. Mol Cell 35:3178–3188.

Natale V (2010) A comprehensive description of the severity groups in Cockayne Syndrome. Am J Med Genet Part A 155:1081–1095.

Nevedomskaya E, Meissner A, Goraler S, De Waard M, Ridwan Y, Zondag G, Van Der Pluijm I, Deelder AM and Mayboroda OA (2010) Metabolic profiling of accelerated aging ERCC1d/- mice. J Proteome Res 9:3680–3687.

Newman JC, Bailey AD and Weiner AM (2006) Cockayne syndrome group B protein (CSB) plays a general role in chromatin maintenance and remodeling. Proc Natl Acad Sci U S A 103:9613–9618.

Osenbroch PO, Auk-Emblem P, Halsne R, Strand J, Forstrøm RJ, van der Pluijm I and Eide L (2009) Accumulation of mitochondrial DNA damage and bioenergetic dysfunction in CSB defective cells. FEBS J 276:2811–2821.

Osterod M, Larsen E, Le Page F, Hengstler JG, van der Horst GTJ, Boiteux S, Klungland A and Epe B (2002) A global DNA repair mechanism involving the Cockayne syndrome B (CSB) gene product can prevent the *in vivo* accumulation of endogenous oxidative DNA base damage. Oncogene 21:8232–8239.

Pascucci B, Lemma T, Iorio E, Giovannini S, Vaz B, Iavarone I, Calcagnile A, Narciso L, Degan P, Podo F *et al.* (2012) An altered redox balance mediates the hypersensitivity of Cockayne syndrome primary fibroblasts to oxidative stress. Aging Cell 11:520–529.

Pascucci B, D'Errico M, Romagnoli A, De Nuccio C, Savino M, Pietraforte D, Lanzafame M, Calcagnile AS, Fortini P, Baccarini S *et al.* (2016) Overexpression of Parkin rescues the defective mitochondrial phenotype and the increased apoptosis of Cockayne Syndrome A cells. Oncotarget 8:102852–102867.

Pastoriza-Gallego M, Armier J and Sarasin A (2007) Transcription through 8-oxoguanine in DNA repair-proficient and Csb^{-}/$Ogg1^{-}$ DNA repair-deficient mouse embryonic fibroblasts is dependent upon promoter strength and sequence context. Mutagenesis 22:343–351.

Pazin MJ and Kadonaga JT (1997) SWI2/SNF2 and related proteins: ATP-driven motors that disrupt protein- DNA interactions? Cell 88:737–740.

Pei DS and Strauss PR (2013) Zebrafish as a model system to study DNA damage and repair. Mutat Res 743–744:151–159.

Pinto M and Moraes CT (2015) Mechanisms linking mtDNA damage and aging. Free Radic Biol Med 85:250–258.

Prates Mori M and de Souza-Pinto NC (2018) Role of mitochondrial dysfunction in the pathophysiology of DNA repair disorders. Cell Biol Int 42:643–650.

Raj DD, Jaarsma D, Holtman IR, Olah M, Ferreira FM, Schaafsma W, Brouwer N, Meijer MM, de Waard MC, van der Pluijm I *et al.* (2014) Priming of microglia in a DNA-repair deficient model of accelerated aging. Neurobiol Aging 35:2147–2160.

Ranes M, Boeing S, Wang Y, Wienholz F, Menoni H, Walker J, Encheva V, Chakravarty P, Mari PO, Stewart A *et al.* (2016) A ubiquitylation site in Cockayne syndrome B required for repair of oxidative DNA damage, but not for transcription-coupled nucleotide excision repair. Nucleic Acids Res 44:5246–5255.

Reardon JT and Sancar A (2005) Nucleotide excision repair. Prog Nucleic Acid Res 79:183–235.

Revet I, Feeney L, Tang A, Huang EJ and Cleaver JE (2012) Dysmyelination not demyelination causes neurological symptoms in preweaned mice in a murine model of Cockayne syndrome. Proc Natl Acad Sci U S A 109:4627–32.

Sabatella M, Theil AF, Ribeiro-Silva C, Slyskova J, Thijssen K, Voskamp C, Lans H and Vermeulen W (2018) Repair protein persistence at DNA lesions characterizes XPF defect with Cockayne syndrome features. Nucleic Acids Res 46:9563–9577.

Sancar A and Rupp WD (1983) A novel repair enzyme: UVRABC excision nuclease of *Escherichia coli* cuts a DNA strand on both sides of the damaged region. Cell 33:249–260.

Sarker AH, Tsutakawa SE, Kostek S, Ng C, Shin DS, Peris M, Campeau E, Tainer JA, Nogales E and Cooper PK (2005) Recognition of RNA polymerase II and transcription bubbles by XPG, CSB, and TFIIH: Insights for transcription-coupled repair and Cockayne syndrome. Mol Cell 20:187–198.

Schärer OD (2013) Nucleotide excision repair in eukaryotes. Cold Spring Harb Perspect Biol 5:a012609.

Scheibye-Knudsen M, Ramamoorthy M, Sykora P, Maynard S, Lin PC, Minor RK, Wilson DM, Cooper M, Spencer R, de Cabo R *et al.* (2012) Cockayne syndrome group B protein prevents the accumulation of damaged mitochondria by promoting mitochondrial autophagy. J Exp Med 209:855–869.

Scheibye-Knudsen M, Tseng A, Jensen MB, Scheibye-Alsing K, Fang EF, Iyama T, Bharti SK, Marosi K, Froetscher L, Kassahun H *et al.* (2016) Cockayne syndrome group A and B proteins converge on transcription-linked resolution of non-B DNA. Proc Natl Acad Sci U S A 113:12502–12507.

Schmickel RD, Chu EH, Trosko JE and Chang CC (1977) Cockayne syndrome: A cellular sensitivity to ultraviolet light. Pediatrics 60:135–139.

Schwertman P, Lagarou A, Dekkers DHW, Raams A, van der Hoek A, Laffeber C, Hoeijmakers JH, Demmers JAA, Fousteri M, Vermeulen W *et al.* (2012) UV-sensitive syndrome protein UVSSA recruits USP7 to regulate transcription-coupled repair. Nat Genet 44:598-602.

Seeberg E and Strike P (1976) Excision repair of ultraviolet-irradiated deoxyribonucleic acid in plasmolyzed cells of *Escherichia coli*. J Bacteriol 125:787–795.

Selby CP and Sancar A (1997a) Cockayne syndrome group B protein enhances elongation by RNA polymerase II. Proc Natl Acad Sci U S A 94:11205–11209.

Selby CP and Sancar A (1997b) Human transcription-repair coupling factor CSB / ERCC6 is a DNA- stimulated ATPase but is not a helicase and does not disrupt the ternary transcription complex of stalled RNA polymerase II. J Biol Chem 272:1885–1890.

Selzer RR, Nyaga S, Tuo J, May A, Muftuoglu M, Christiansen M, Citterio E, Brosh RM Jr and Bohr VA (2002) Differential requirement for the ATPase domain of the Cockayne syndrome group B gene in the processing of UV-induced DNA damage and 8-oxoguanine lesions in human cells. Nucleic Acids Res 30:782–793.

Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L *et al.* (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 110:3507–3512.

Sin Y, Tanaka K and Saijo M (2016) The C-terminal region and SUMOylation of Cockayne syndrome group B protein play critical roles in transcription-coupled nucleotide excision repair. J Biol Chem 291:1387–1397.

Spitsbergen JM and Kent ML (2003) The state of the art of the zebrafish model for toxicology and toxicologic pathology research - advantages and current limitations. Toxicol Pathol 31:62–87.

Spivak G (2005) UV-sensitive syndrome. Mutat Res 577:162–169.

Spivak G and Ganesan AK (2014) The complex choreography of transcription-coupled repair. DNA Repair (Amst) 19:64–70.

Stadler J and Richly H (2017) Regulation of DNA repair mechanisms: How the chromatin environment regulates the DNA damage response. Int J Mol Sci 18:1–15.

Stevnsner T, Muftuoglu M, Aamann MD and Bohr VA (2008) The role of Cockayne syndrome group B (CSB) protein in base excision repair and aging. Mech Ageing Dev 129:441–448.

Stichel CC, Zhu XR, Bader V, Linnartz B, Schmidt S and Lübbert H (2007) Mono- and double-mutant mouse models of Parkinson's disease display severe mitochondrial damage. Hum Mol Genet 16:2377–2393.

Stuart JA, Mayard S, Hashiguchi K, Souza-Pinto NC and Bohr VA (2005) Localization of mitochondrial DNA base excision repair to an inner membrane-associated particulate fraction. Nucleic Acids Res 33:3722–3732.

Sugasawa K, Ng JM, Masutani C, Iwai S, van der Spek PJ, Eker APM, Hanaoka F, Bootsma D and Hoeijmakers JHJ (1998) Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. Mol Cell 2:223–232.

Sunesen M, Selzer RR, Brosh Jr. RM, Balajee AS, Stevnsner T and Bohr VA (2000) Molecular characterization of an acidic region deletion mutant of Cockayne syndrome group B protein. Nucleic Acids Res 28:3151–3159.

Taghdiri M, Dastsooz H, Fardaei M, Mohammadi S, Fard MAF and Faghihi MA (2017) A novel mutation in ERCC8 gene causing Cockayne syndrome. Front Pediatr 5:169.

Tanaka K, Kawai K, Kumahara Y, Ikenaga M and Okada Y (1981) Genetic complementation groups in Cockayne syndrome. Somatic Cell Genet 7:445–455.

Tantin D (1998) RNA polymerase II elongation complexes containing the Cockayne syndrome group B protein interact with a molecular complex containing the transcription factor IIH components xeroderma pigmentosum B and p62. J Biol Chem 273:27794–27799.

Tantin D, Kansal A and Carey M (1997) Recruitment of the putative transcription-repair coupling factor CSB/ERCC6 to RNA polymerase II elongation complexes. Mol Cell Biol 17:6803–6814.

Tirode F, Busso D, Coin F and Egly JM (1999) Reconstitution of the transcription factor TFIIH: assignment of functions for the three enzymatic subunits, XPB, XPD, and cdk7. Mol Cell 3:87–95.

Titus TA, Yan YL, Wilson C, Starks AM, Frohnmayer JD, Canestro C, Rodriguez-Mari A, He X and Postlethwait JH (2009) The Fanconi anemia/BRCA gene network in zebrafish: Embryonic expression and comparative genomics. Mutat Res 668:117–132.

Tornaletti S (2005) Transcription arrest at DNA damage sites. Mutat Res 577:131–145.

Trapp C, Reite K, Klungland A and Epe B (2007) Deficiency of the Cockayne syndrome B (CSB) gene aGG-NERavates the genomic instability caused by endogenous oxidative DNA base damage in mice. Oncogene 26:4044–4048.

Troelstra C, Odijk LH, de Wit J, Westerveld A, Thompson LH, Bootsma D and Hoeijmakers JHJ (1990) Molecular cloning of the human DNA excision repair. Mol Cell Biol 10:5806–5813.

Troelstra C, van Gool A, de Wit J, Vermeulen W, Bootsma D and Hoeijmakers JHJ (1992) ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. Cell 71:939–953.

Troelstra C, Hesen W, Bootsma D and Hoeijmakers JHJ (1993) Structure and expression of the excision repair gene ERCC6, involved in the human disorder Cockayne's syndrome group B. Nucleic Acids Res 21:419–426.

Tuo J, Müftüoglu M, Chen C, Jaruga P, Selzer RR, Brosh RM, Rodriguez H, Dizdaroglu M and Bohr VA (2001) The Cockayne syndrome group b gene product is involved in general genome base excision repair of 8-Hydroxyguanine in DNA. J Biol Chem 276:45772–45779.

Tuo J, Chen C, Zeng X, Christiansen M and Bohr VA (2002a) Functional crosstalk between hOgg1 and the helicase domain of Cockayne syndrome group B protein. DNA Repair (Amst) 1:913–927.

Tuo J, Jaruga P, Rodriguez H, Dizdaroglu M and Bohr VA (2002b) The cockayne syndrome group B gene product is involved in cellular repair of 8-hydroxyadenine in DNA. J Biol Chem 277:30832–30837.

Tuo J, Jaruga P, Rodriguez H, Bohr VA and Dizdaroglu M (2003) Primary fibroblasts of Cockayne syndrome patients are defective in cellular repair of 8-hydroxyguanine and 8-hydroxyadenine resulting from oxidative stress. FASEB J 17:668–674.

van den Boom V, Citterio E, Hoogstraten D, Zotter A, Egly JM, van Cappellen WA, Hoeijmakers JHJ, Houtsmuller AB and Vermeulen W (2004) DNA damage stabilizes interaction of CSB with the transcription elongation machinery. J Cell Biol 166:27–36.

van der Horst GTJ, van Steeg H, Berg RJW, van Gool AJ, de Wit J, Weeda G, Morreau H, Beems RB, van Kreijl CF, de Gruijl FR *et al.* (1997) Defective transcription-coupled repair in Cockayne syndrome B mice is associated with skin cancer predisposition. Cell 89:425–435.

van der Horst GTJ, Meira L, Gorgels TG, de Wit J, Velasco-Miguel S, Richardson JA, Kamp Y, Vreeswijk MPG, Smit B, Bootsma D *et al.* (2002) UVB radiation-induced cancer predisposition in Cockayne syndrome group A (Csa) mutant mice. DNA Repair (Amst) 1:143–157.

van der Pluijm I, Garinis GA, Brandt RMC, Gorgels TG, Wijnhoven SW, Diderich KEM, De Wit J, Mitchell JR, van Oostrom C, Beems R *et al.* (2007) Impaired genome maintenance suppresses the growth hormone-insulin-like growth factor 1 axis in mice with Cockayne syndrome. PLoS Biol 5:0023–0038.

van Gool AJ, Citterio E, Rademakers S, van Os R, Vermeulen W, Constantinou A, Egly JM, Bootsma D and Hoeijmakers JH (1997) The Cockayne syndrome B protein, involved in transcription- coupled DNA repair, resides in an RNA polymerase II-containing complex. EMBO J 16:5955–5965.

van Hoffen A, Natarajan AT, Mayne LV, Zeeland AA Van, Mullendersl LHF and Venemal J (1993) Deficient repair of the transcribed strand of active in Cockayne's syndrome cells. Nucleic Acids Res 21:5890–5895.

van Houten B, Hunter SE and Meyer JN (2016) Mitochondrial DNA damage induced autophagy, cell death, and disease. Front Biosci 21:42–54.

Venema J, Mullenders LH, Natarajan AT, van Zeeland AA and Mayne LV (1990) The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. Proc Natl Acad Sci U S A 87:4707–4711.

Vermeij WP, Dollé MET, Reiling E, Jaarsma D, Payan-Gomez C, Bombardieri CR, Wu H, Roks AJM, Botter SM, van der Eerden BC *et al.* (2016) Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice. Nature 537:427–431.

Vessoni AT, Herai RH, Karpiak JV, Leal AMS, Trujillo CA, Quinet A, Lima LFA, Menck CFM and Muotri AR (2016) Cockayne Syndrome-derived neurons display reduced synapse density and altered neural network synchrony. Hum Mol Genet 25:1271–1280.

Wallace DC (2012) Mitochondria and cancer. Nat Rev Cancer 12:685–698.

Wang W, Xu J, Chong J and Wang D (2018) Structural basis of DNA lesion recognition for eukaryotic transcription- coupled nucleotide excision repair. DNA Repair (Amst) 71:43–55.

Wang Y, Chakravarty P, Ranes M, Kelly G, Brooks PJ, Neilan E, Stewart A, Schiavo G and Svejstrup JQ (2014) Dysregulation of gene expression as a cause of Cockayne syndrome neurological disease. Proc Natl Acad Sci U S A 111:14454–14459.

Weeda G, van Ham RCA, Vermeulen W, Bootsma D, van der Eb AJ and Hoeijmakers JHJ (1990) A presumed DNA helicase encoded by ERCC-3 is involved in the human repair disorders Xeroderma pigmentosum and Cockayne's syndrome. Cell 62:777–791.

Weeda G, Donker I, de Wit J, Morreau H, Janssens R, Vissers CJ, Nigg A, van Steeg H, Bootsma D and Hoeijmakers JHJ (1997) Disruption of mouse ERCC1 results in a novel repair syndrome with growth failure, nuclear abnormalities and senescence. Curr Biol 7:427–439.

Wei L, Nakajima S, Böhm S, Bernstein KA, Shen Z and Tsang M (2015) DNA damage during the G0 / G1 phase triggers RNA-templated, Cockayne syndrome B-dependent homologous recombination. Proc Natl Acad Sci U S A 112:E3495–E3504.

Weidenheim KM, Dickson DW and Rapin I (2009) Neuropathology of Cockayne syndrome: Evidence for impaired development, premature aging, and neurodegeneration. Mech Ageing Dev 130:619–636.

Wilson BT, Stark Z, Sutton RE, Danda S, Ekbote AV, Elsayed SM, Gibson L, Goodship JA, Jackson AP, Keng WT *et al.* (2015) The Cockayne Syndrome Natural History (CoSyNH) study: Clinical findings in 102 individuals and recommendations for care. Genet Med 18:483–493.

Wong HK, Muftuoglu M, Beck G, Imam SZ, Bohr VA and Wilson DM (2007) Cockayne syndrome B protein stimulates apurinic endonuclease 1 activity and protects against agents that introduce base excision repair intermediates. Nucleic Acids Res 35:4103–4113.

Wu M, Wei W, Chen J, Cong R, Shi T, Li J, Wong J and Du JX (2017) Acidic domains differentially read histone H3 lysine 4 methylation status and are widely present in chromatin-associated proteins. Sci China Life Sci 60:138–151.

Xu C and Min J (2011) Structure and function of WD40 domain proteins. Protein Cell 2:202–214.

Yu S, Chen L, Ye L, Fei L, Tang W and Tian Y (2014) Identification of two missense mutations of ERCC6 in three chinese sisters with Cockayne syndrome by Whole Exome Sequencing. PloS One 9:1–11.

Zhang X, Horibata K, Saijo M, Ishigami C, Ukai A, Kanno S, Tahara H, Neilan EG, Honma M, Nohmi T *et al.* (2012) Mutations in UVSSA cause UV-sensitive syndrome and destabilize ERCC6 in transcription-coupled DNA repair. Nat Genet 44:593–597.

Supplementary material

The following online material is available for this article:
Table S1 - Homozygous and heterozygous *ERCC6 (CSB)* mutations and their effects on patients phenotype.
Table S2 - Homozygous and heterozygous *ERCC8 (CSA)* mutations and their effects on patients phenotype.

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