**University of São Paulo**
**"Luiz de Queiroz" College of Agriculture**

# Functional characterization of *APC5* in *Arabidopsis thaliana*

**Luís Felipe Correa da Silva**

Dissertation presented to obtain the degree of Master in Science. Area: Plant Physiology and Biochemistry

**Piracicaba**
**2023**

**Luís Felipe Correa da Silva**
**Bachelor in Biological Science – Genetics**

# Functional characterization of *APC5* in *Arabidopsis thaliana*

Advisor:
Profª. Drª. **NUBIA BARBOSA ELOY**

Dissertation presented to obtain the degree of Master in Science. Area: Plant Physiology and Biochemistry

**Piracicaba**
**2023**

## DEDICATION

This work is dedicated to my mother, Verônica Quintanilha, who earlier left this world.

## ACKNOWLEDGMENTS

The years leading up to the writing of this work were, by far, the most challenging ones I've had so far. In addition to a historic pandemic, personal challenges were present in the most different spheres of my life. But looking back, I'm sure I did a good job, even with my limitations. If I got this far, fortunately, it was because I had the support and company of very special people.

I thank my family for their unconditional love and for always believing in my dreams and goals, and for helping me to put them into practice. I thank the woman who, in many aspects, is responsible for me being here, my mother, Verônica, who always motivated me and did not let me give up when I wanted to drop everything and go back to Rio de Janeiro. For always being strong, but without losing her sensibility, even in the most difficult moments of her life. I thank my brother, Guilherme, for always be by my side and reminding me that there are good people in the world. I thank my godmother, Renata, for the best advice and for always helping me put my dreams into practice. I thank my aunts Regina and Lourdes, for all the love, for always welcoming me, and for dedicating their time to caring for my mother with so much love. I thank my sister-in-law, Marilene, for taking care of my brother in my absence and for always being so sweet and kind.

I thank my advisor, Nubia, for believing in me, since the beginning, and for always being honest and sensitive with me. I thank my laboratory colleagues, Ingrid and Gabriela, for the shared learning and for sharing the day-to-day work at the bench, and also the good moments out of the lab. I thank my project and tattoo partner, Giovana, for all her sweetness, companionship, and assistance in the laboratory tasks. I thank my dear friend Perla, for being my partner from the beginning and for experiencing a frozen adventure with me. Her presence was crucial for many moments of this journey. I thank my advisor on MPIMP, Joachim Kopka, for welcoming me in his group and for all the knowledge sharing. I thank my colleagues from CEBTEC for the laughs and the daily companionship.

I thank my eternal Caríssimos, who taught me how good life can be when we have friends we love so much. I thank my eternal class of 2016.1, for making my undergraduate studies a

special time in my life. I thank my friends from LaBEP, for teaching me that science is always done with partnership, complicity, and respect. I thank Ana, João, and Pedro, for becoming my family in Piracicaba and for sharing the challenges of early adulthood with me. I thank my friend Nathália for being by my side on good and bad days, and her family, especially her mother, Aurea, for always welcoming me with so much affection and make me feel part of them. I thank my friends from AG Kopka, for welcoming me with so much warmth and for always being available to help and teach me, and for sharing good moments. I thank my beloved faces, João, Joel, Ligia, Luisa, Matheus, Nailah, and Thales, for all the love and for making me laugh at anything. I thank my friend, Larissa, for being my soulmate and for always making me see the light at the end of the tunnel.

I thank the professors Adriana Hermely and Mª Helena Goldman for agreeing to participate in this moment, evaluating and contributing to the improvement of this work.

I also thank the São Paulo Research Foundation (FAPESP) for the financial support through projects 2021/03212-5 and 2022/08534-3, which allowed this research to be carried out.

## EPÍGRAFE

*Como as folhas que o vento espalha*
*Quanto mais eu ando, mais entendo a grandeza de pertencer*

*- Rashid*

## RESUMO

### Caracterização funcional do *APC5* em *Arabidopsis thaliana*

Como organismos multicelulares, as plantas têm seu crescimento e desenvolvimento diretamente dependentes da divisão celular. Esse processo reúne dezenas de reações moleculares que garantem a proliferação celular nos mais variados tecidos vegetais. Nas quatro fases sequenciais que compõem o ciclo celular (S, G1, M e G2), a presença e/ou ausência de diferentes componentes moleculares do programa de divisão celular, como ciclinas, CDKs e securinas, garantem a progressão unidirecional do processo, de forma irreversível. O sistema ubiquitina-proteassoma (UPS) é uma cascata multi-enzimática que direciona substratos específicos para degradação por meio do proteassoma 26S. O Complexo Promotor da Anáfase/Ciclossomo (APC/C) é uma das enzimas responsáveis pelo reconhecimento do substrato a ser ubiquitinado pelo UPS. Em Arabidopsis thaliana, o APC/C possui 14 subunidades. Vários estudos relataram que perturbações nos níveis de expressão de algumas subunidades podem levar a alterações no fenótipo da planta, e comprometer processos como a gametogênese. O processo de divisão celular ocorre em sincronia com os outros processos metabólicos da célula, bem como de toda a planta. Alterações nos nutrientes ou mesmo nas demandas intracelulares podem levar a mudanças na concentração de metabólitos e em suas taxas de conversão, alterando os processos biológicos do organismo. Mudanças metabólicas ocorrem em uma taxa suficientemente rápida para escapar da regulação transcricional, obrigando a célula a usar outras maneiras de regular suas reações para lidar com essas mudanças, como a ação de metabólitos como reguladores. Dessa forma, este projeto tem como objetivo caracterizar funcionalmente o gene APC5 de A. thaliana, bem como investigar as alterações no metabolismo da planta devido a alterações nos níveis de expressão do APC5, possivelmente correlacionando as mudanças com processos já conhecidos que impulsionam o crescimento das plantas. Os resultados mostram que, embora diferenças metabólicas não tenham sido detectadas, a superexpressão do gene APC5 foi capaz de alterar a área total das rosetas de Arabidopsis, tornando-as menores em linhagens transgênicas quando comparadas ao tipo selvagem. Além disso, pode-se detectar que o promotor do gene está ativo nos meristemas apicais da planta, assim como no meristema das raízes laterais. Ensaios com a proteína APC5 fundida ao GFP mostram que ela está localizada no núcleo e no citoplasma.

Palavras-chave: APC/C, Ciclo celular, APC5, Desenvolvimento e crescimento vegetal

## ABSTRACT

### Functional characterization of *APC5* in *Arabidopsis thaliana*

As a multicellular organism, plants have their growth and development directly dependent on the cell division. This process brings together dozens of molecular reactions, which ensures cell proliferation in the most varied plant tissues. The four sequential phases that make up the cell cycle (S, G1, M and G2) have the presence and absence of different molecular components of the cell division program, such as cyclins, CDKs and securins, acting together in order to guarantee the unidirectional progression of the process, in an irreversible way . The ubiquitin-proteasome system (UPS) is a multi-enzymatic cascade that target specific substrates for degradation through 26S proteasome. The Anaphase Promoting Complex/Cyclosome (APC/C) is one of the enzymatic machines responsible for recognizing the substrate to be ubiquitinated by the UPS. In Arabidopsis thaliana, the APC/C has 14 subunits. Several studies have reported that perturbations in the expression levels of some subunits can lead to changes in the plant phenotype, and compromise process such as gametogenesis. The cell division process occurs in synchrony with the other metabolic processes of the cell, as well as of the entire plant. Changes in nutrients or even in intracellular demands can lead to changes in metabolites concentration and their conversion rates, altering the biological processes of the organism. Metabolic changes occur at a rate fast enough to escape transcriptional regulation, forcing the cell to use other ways to regulate its reactions to deal with these changes, such as the action of metabolites as regulators. In this way, this project aims to functionally characterize the APC5 gene from Arabidopsis thaliana, as well as to investigate the changes in the plant metabolism due to alterations in the expression levels of the APC5, possibly correlating the changes with already known process that drive plant growth. The results show that, although metabolic differences could not be detected, overexpression of the APC5 gene was able to change the total area of Arabidopsis rosettes, making them smaller in transgenic strains when compared to wild type. Furthermore, it can be detected that the promoter of the gene is active in the apical merisms of the plant, as well as in the meristem of the lateral roots. Assays with the APC5 protein fused to GFP show that it is located in the nucleus and cytoplasm.


Keywords: APC/C, Cell cycle, APC5, Plant growth and development

# 1. INTRODUCTION

Plant growth is an important subject for agriculture and plant physiology, as it is directly associated with crop yield. In the history of plant science, several research groups around the world have focused their studies based on plant growth and development. Unlike most animals, plants have indeterminate growth, making it possible for them to keep growing even after their full mature phase. This characteristic is due to the maintenance of undifferentiated cells in the so-called meristems, which are in constant division and differentiation, giving rise to new tissues that will be incorporated into the plant body (Shi and Zhang, 2016). Growth in plants is defined as the irreversible increase in biomass, cell number, and plant volume resulting from the processes of cell division and expansion (Hilty et al., 2020).

The cell cycle is responsible for adding new cells to the growing organs. In plants, as well as in all eukaryotes, the cell cycle reunites the processes of cell division made up of the preparatory phases G1, S (DNA replication phase), and G2; and the proper cell division phases of mitosis (M phase) and cytokinesis. Cell division needs a myriad of reactions temporally and spatially coordinated to take place at exact phases of the cycle (Eloy et al., 2012; Tyson et al., 2002). This coordination is orchestrated at multiple levels and involves the activation of a special class of serine/threonine-specific protein kinases named cyclin-dependents kinases (CDKs), whose activity is dependent on a regulatory protein known as cyclin (Besson et al., 2008; Morgan, 1995). The different cyclin-CDK complexes formed in distinct phases of the cell cycle promote the phosphorylation of a variety of substrates, ultimately triggering processes such as DNA replication and mitosis. The phosphorylation complex recognizes the substrate through the exchangeable regulatory cyclin subunit, while the catalytic CDK recognizes the phosphorylation site in the substrate (Ser/Thr-Pro) (de Veylder et al., 2007; Lim and Kaldis, 2013). Even though higher eukaryotes have conserved CDKs, they also have several additional CDKs playing roles at different checkpoints during the cell cycle. Some of these CDKs are not conserved between animals and plants and show variations in amino acid sequences in the cyclin-binding domain (Joubès et al., 2000). Cyclin levels in cells are regulated both by transcriptional controls and proteolysis, consequently regulating the activity of the CDK-cyclin complex through the different phases of the cell cycle. The proteolysis of target proteins ensures that cell cycle progression occurs unidirectionally and irreversibly (Capron, 2003 ;Pan and Gao, 2023).

One of the main proteolysis pathways in the eukaryotic cell is the ubiquitin-proteosome system (UPS), which plays a major role in the protein fate. The UPS is a three-step process that results in the ubiquitination of a target protein, making it specifically degraded by the 26S proteasome. Each step of the UPS is performed by a specific enzyme: the E1-activating enzyme activates the ubiquitin molecule in an ATP-dependent way, the E2-ubiquitin conjugating enzyme conjugates the activated ubiquitin molecule to the substrate to be degraded, and the E3-ubiquitin ligase specifically recognizes this substrate. The relevance of this degradation pathway can be inferred from the number of genes in the Arabidopsis thaliana genome (approximately 6%) involved in the UPS. (Hua and Vierstra, 2011). Different species of eukaryotes displays a huge number of genes for E3 ligases, showing the importance of the ubiquitin system (Kulkarni, 2008). Two well-characterized E3-ligases are most inti-

mately dedicated to basic cell cycle control, namely the S-PHASE KINASE-ASSOCIATED PROTEIN 1 (SKP1)-CUL1-F-box (SCF)-related complex and the ANAPHASE-PROMOTING COMPLEX/CYCLOSOME (APC/C) (de Oliveira et al., 2022).

The primary function of APC/C, as the name suggests, is to promote anaphase, triggering the segregation of sister chromatids and by creating the metabolic environment for cycle progression. The proteolysis of Securin, an APC/C target, stops the inhibition of Separase, the enzyme responsible for breaking down the cohesion complex that holds the chromatids together, marking the beginning of the anaphase (Castro et al., 2005; de Lange et al., 2015; Jonak et al., 2017). However, before these events, the cell environment must be directed towards a specific composition of active and inactivated molecules, ensured by the targeting of cyclin A and B for degradation, which happens at prometaphase and at anaphase transition, respectively (Sullivan and Morgan, 2007). The APC/C activity also is present during the early-to-mid mitosis progression, through the same target-specific recognition, provided by the destruction (D) and KEN boxes present in the substrate sequences (Inzé and de Veylder, 2006; Eloy et al.,2012). Since its discovery 25 years ago, many aspects of APC/C regulation and roles in cell metabolism were uncovered, but our understanding of this cell machinery remains far from complete (Yamato, 2019). APC/C was reported to play roles in cellular differentiation, gametophytic development, shoot branching, and embryogenesis (de Oliveira et al., 2022). These findings have driven research interest in understanding what other roles the complex may play in plants (Eloy et al., 2011; Lima et al., 2010; Schwedersky et al., 2015).

This work aimed to characterize the gene encoding the subunit 5 of the APC/C (APC5) in Arabidopsis thaliana. For this, transgenic lines with higher APC5 expression levels were phenotypically and metabolically characterized. In addition, molecular tools were used to identify APC5 gene and protein localization.

# 2. THEORETICAL FRAMEWORK

## 2.1. Plant growth and development

Plants are sexually reproducing multicellular organisms, and, like animals, their life cycle starts as a single cell, the zygote. This cell undergoes different rounds of divisions and differentiation until reaching the mature form of the embryo. However, different from animals, the end of embryogenesis does not result in a downsized body architecture of the adult organism in most land plants. The organization of plant body (root, stem, and leaves) is established in embryogenesis, but its final form is determined during post-embryonic development, as the reproductive organs, for example, which in most animals are stablished already in the embryogenesis (Radoeva and Vaddepalli; 2019). Most animals, except species undergoing metamorphosis, complete their ontogenetic development during embryogenesis. In contrast, plants do not have an endpoint for ontogenetic development (Drost et al., 2017; Hariharan et al., 2016). Most of the true tissues and organs, including flowers, roots, stems, and vascular systems, develop after seed germination. They are sequentially added in the post-embryonic developmental program via functional units called phytomers by the action of meristems and throughout the plant life cycle (Javelle et al., 2011; McMaster, 2005; Perianez-Rodriguez et al., 2014) (Fig. **1**).

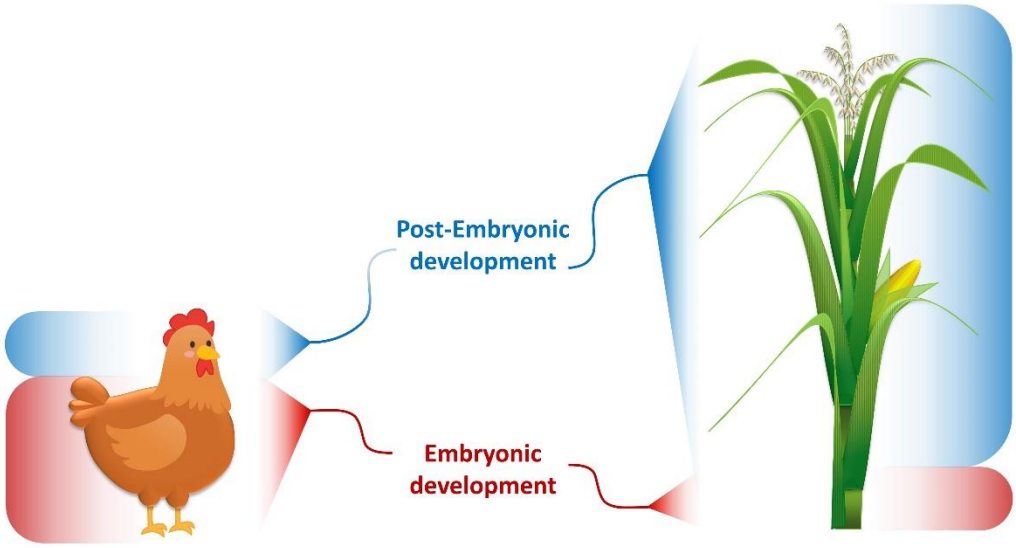


**Figure 1.** Comparation between animal and plant development.

**In most animals, the embryonic stage comprises the main step of the development, in a way that the structures reach their final forms but in smaller proportions when compared with an adult organism. In plant embryogenesis, however, the embryo develops initial structures that will be replaced by the final structures in the mature plant during the post-embryonic development.**

Meristems are formed by undifferentiated pluripotent cells able to continuously divide and give rise to new cells (Gaillochet, 2015; Müller-Xing and Xing, 2022). The main meristems, shoot apical and root apical meristems (SAM and RAM, respectively), are established during embryogenesis. In

eudicots, like Arabidopsis, the SAM is centrally established between the two cotyledons. During post-embryonic development, SAM will be responsible for adding aerial organs together with the post-formed axillary meristems (AMs), while RAM contributes to underground architecture. In the reproductive phase, SAM forms the inflorescence meristem (IM), which then generates the floral meristem (FM) that will produce the reproductive organs of the angiosperms (Brutnell and Langdale, 1998; Takeda and Aida, 2011; Xue et al., 2020). The maintenance of the meristematic regions is guaranteed by the asymmetric division of the meristematic cells. Of the two daughter cells formed, only one will enter the differentiation program, following successive divisions until it becomes a specific cell type. The other daughter cell will maintain its steam cell characteristics, ensuring that new cells can be constantly added to the plant (Fig. **2**).

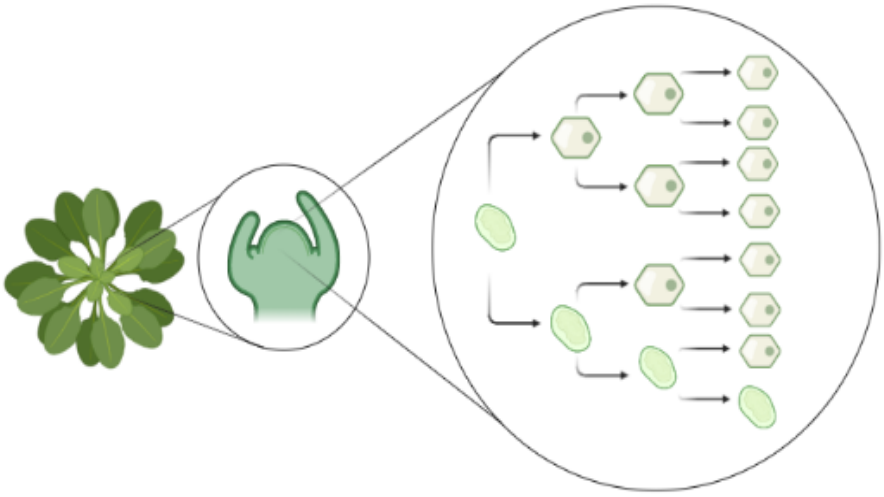

**Figure 2.** Schematic representation of the cell division by meristems.

**When a meristematic cell divides, one daughter cell maintains its undifferentiated meristematic characteristic and the stock of pluripotent cells. The other daughter cell will enter into the differentiation program, producing new differentiated cells. Figure made with BioRender®.**

The process of adding new cells has a big impact on the plant's body and results in its growth. Normally, plant growth is defined as the irreversible increase in biomass, volume and number of cells. Structural growth (number) is usually accompanied by expansive growth (volume), so that each daughter cell matches the mother cell volume. Thus, the term "cell proliferation" is used to describe the process of simultaneously increasing the number and size of cells, causing growth. Expansion rates are not always balanced with the rate of cell production, causing an overall increase in tissue, but a decrease in cell size (Hilty et al., 2021). DNA replication can also happen without cell division, in a process called “endoreduplication”, which generally results in larger cells with high ploidy level. As a result, the plant has an increase in tissue size, but not a proportional increase in cell number. These examples illustrate that the increase in size, biomass, and volume reflect events that go beyond the number of cells. Most of these events are ultimately under the control of the cell cycle. The

correct regulation and duration of the cycle are responsible for the constant size of cells in the meristem, as well as for cell proliferation and differentiation (Tsukaya, 2013; Katagiri et al., 2016; Robinson et al., 2018; Hilty et al., 2021).

## 2.2. Cell cycle

In multicellular organisms, growth and development are closely linked to the cell cycle. Cells undergoing cell division progress through a meticulously orchestrated sequence of events, including growth, DNA replication, and division, ultimately resulting in the formation of two genetically identical daughter cells (Ong, 2019). The cell cycle progression in eukaryotic cells is governed by a highly conserved regulatory system. This system is responsible for synchronizing the different molecular events necessary for the occurrence and progression of the cycle, but also for establishing connections with extracellular signals capable of regulating cell proliferation (Duronio and Xiong, 2013). Mitosis (nuclear division) is the most dramatic stage of the cell cycle, corresponding to the separation of daughter chromosomes and usually ending with cell division (cytokinesis). However, mitosis and cytokinesis last only about an hour, so approximately 95% of the cell cycle is spent in interphase - the period between mitoses. During interphase, the chromosomes are decondensed and distributed throughout the nucleus, which appears morphologically uniform. At the molecular level, however, interphase is the time during which both cell growth and DNA replication occur in preparation for cell division. APC/C functions essentially on the G2 to M transition. For example, to exit from M, mitotic cyclins must be destroyed by the APC/C in all organisms (Morgan and Roberts, 2002; Marrocco et al., 2010).

## 2.3. Ubiquitin-proteasome system

The timely, spatially, and specifically protein degradation is one of the main pathways to regulate the cell cycle. The eukaryotic cells have different ways to perform proteolysis at the different cell compartments, like cytosol, membrane, endoplasmic reticulum, and lysosome (Ciechanover, 2005a, Ciechanover, 2005b). Most of the cytosolic protein degradation occurs via the multienzyme pathway of the ubiquitin-proteasome system (UPS) (Park, 2020). By the UPS, proteins are first covalently tagged to multimers of ubiquitin, a 76 amino acids evolutionary conserved protein, and are afterwards degraded by a huge cytosolic protease: the 26S proteasome (Park, 2020; Li, 2022).

Three enzymes work sequentially to polyubiquitinate the target protein: the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2), and the ubiquitin ligase enzyme (E3). The activity of those enzymes allows the 26S proteasome to specifically recognize and degrade the target protein (Willems and De Veylder, 2022). However, the specificity of the system goes beyond the ability of the proteasome to degrade polyubiquitinated proteins. The E3 ligases recognize its substrates by degradation signals that contribute to the selectivity of the UPS, enabling the cell to regulate the dynamics of protein degradation (Nandi et al., 2006).

The importance of ubiquitination is exemplified by the fact that 6% of the Arabidopsis genome encodes UPS genes (Hua and Viestra, 2011). Genomic analysis of the different genes acting in the ubiquitination cascade in different species of eukaryotes shows that few genes encode E1, tens are encoding for E2, and hundreds of different E3 genes can be found (Kulkarni, 2008). In rice (Oryza sativa), six and 36 genes express ubiquitin-activating enzyme and ubiquitin-conjugating enzyme, respectively, while more than 1100 genes exist for ubiquitin ligase enzymes (Al-Saharin et al., 2022).

The first step of the UPS pathway, as mentioned before, is the ubiquitin activation by the E1 enzyme that uses one ATP molecule for adenylating the ubiquitin C-terminus glycine, forming the intermediate ubiquitin-AMP and releasing PPi. Next, a thioesterification reaction links the ubiquitin to the E1 cysteine residue, forming a ubiquitin-E1 thioester and releasing the AMP molecule. The ubiquitin-E1 thioester can be recognized by a myriad of E2, allowing the transference of the ubiquitin from E1 to E2 by another thioester linkage at an E2 cysteine residue (Yuan, 2021). In the following step, the interaction between E2 and E3 enzymes is required and ensured by a conserved core in all E2 and by the terminal groups (Soss, 2011). The E2-E3 interaction allows the target protein, specifically recognized by an E3 enzyme, to be covalently linked to the ubiquitin carried by the E2 via an isopeptide bond between the target protein lysin ε-amino and the ubiquitin C-terminal carboxyl group (Scaglione, 2013; Zhen, 2014; Liu, 2020). The transfer of the ubiquitin from the E2 to the E3´s substrate occurs in two ways, according to the ubiquitin ligase enzyme involved. The E3 ligase can directly stimulate the nucleophilic attack of the substrate lysine on the E2 ubiquitin thioester linkage, leading to a direct covalent attachment of the ubiquitin C-terminus to the substrate lysine. Furthermore, the E3 can stimulate the transfer of ubiquitin from the E2 to its own cysteine active site, and then promote the ubiquitin attachment on a substrate lysin (Buetow and Huang, 2016). As mentioned before, most of the UPS genes in eukaryotes encodes E3 ligases, resulting in different enzymes that can recognize a variety of substrates. In plants, the E3 ligases can be grouped, according to their catalytic domain: U-Box, homology to E6-associated carboxyl terminus (HECT), and really interesting new gene (RING). U-Box and HECT are mostly monomeric. The RING domain can be found in monomeric enzymes, like RING ubiquitin ligase and RING between RING (RBR) ligase, and in multi-subunits E3 enzymes such as cullin RING ligases (CRLs) (de Oliveira et al., 2022).

The ubiquitin molecule can be attached as a single moiety (monoubiquitylation) or as several ubiquitin molecules linked to each other (polyubiquitylation). Polyubiquitylation can happen between the ubiquitin N-terminus or one of the seven lysine residues of the protein (K6, K11, K27, K29, K33, K48, and K63) and the C-terminus of the following ubiquitin molecule. According to the residue in which the polymeric ubiquitin chain is formed, the chain achieves different topologies with different cellular functions (Morimoto et al., 2016). Chains linked via K63 play non-degradative roles in cell metabolism, while polyubiquitin chains linked via K48 are the canonical signal for protein degradation (Manohar et al., 2019).

The final step of the UPS is the protein degradation by the 26S proteasome, a compartmental protease of the AAA+ (ATPases associated with various cellular activities) family (Zhang, 2020; Khan, 2021). By hydrolyzing one ATP molecule, the proteasome 26S can disrupt the complex structures of its substrates, allowing the unfolded polypeptide to enter the degradation chamber for proteo-

lytic cleavage (de la Peña, 2018; Bard, 2018). This sophisticated protease complex hydrolyses proteins in the cytoplasm and nucleus and is approximately 2.5 MDa. The 26S proteasome structure can be divided into a 20S core catalytic particle (CP) and one or two 19S regulatory particles (RP) that can bind to one or both ends of the 20S, forming the enzymatically active complex (Thompson, 2008; Ehlinger, 2013; de la Peña, 2018). The CP is formed by two outer α-rings and two inner β-rings, which are made up of seven structurally similar α and β subunits, respectively. β1, β2, and β5 subunits contain the catalytic threonine residues located in the N terminus and show an N-terminal nucleophile hydrolase activity. Two pairs of each of those β-type subunits form a degradation chamber, with the catalytic threonine residues on the surface and accessible only through narrow axial pores, excluding folded and even large unfolded polypeptides. Those pores are gated by the 19S particle, which unfolds the substrate by ATPases activity and mechanically translocate it into the degradation chamber (Tanaka, 2009; Watanabe, 2022; Bard, 2018).

The recognition of the substrate by the 26S proteosome relies on two characteristics of the substrate: a targeting signal at the polyubiquitin chain and an unstructured initiation region. The recruitment of the substrate is mediated by ubiquitin receptors, which can be intrinsic or extrinsic. The intrinsic receptors are placed at the proteasome subunits that compose the RP particle, and at least three types of receptors can be found. The subunit Rpn10 recognizes the ubiquitin by a ubiquitin-interacting motifs (UIM) domain and a single α-helix and are flexibly linked to an N-terminal von Willebrand factor A (VWA) domain docked tightly into the proteasome structure. The ubiquitin recognition by the Rpn13 is provided by a pleckstrin-like receptor for ubiquitin (PRU) domain, which is docked directly into the proteasome RP and interacts with ubiquitin through three loops. Finally, the Rpn1 uses two grooves flanked by α-helices in its toroid repeat region to make the recognition. The extrinsic receptors, in turn, can provide substrates through dynamic interactions with both the proteasome and ubiquitin chains, combining ubiquitin-chain recognition through ubiquitin-associated (UBA) domains with proteasome binding via a flexibly N-terminal ubiquitin-like (UBL) domain (Jiang et al., 2018; Bard et al., 2020). The flexible domain of the extrinsic receptors probably enables the accommodation of a wide variety of substrate conformations and ubiquitin modifications than the intrinsic receptors. Also, the extrinsic receptors can select substrates for degradation upstream to the ubiquitination, recognizing and binding disorientated regions on the substrate, and directly interacting with E3 ligases. Thus, proteins lacking the recognition signal (polyubiquitination and the disordered region) that would not be recognized by the intrinsic ubiquitin receptors still can be directed to proteolysis by the 26S proteasome (Bard et al., 2020).

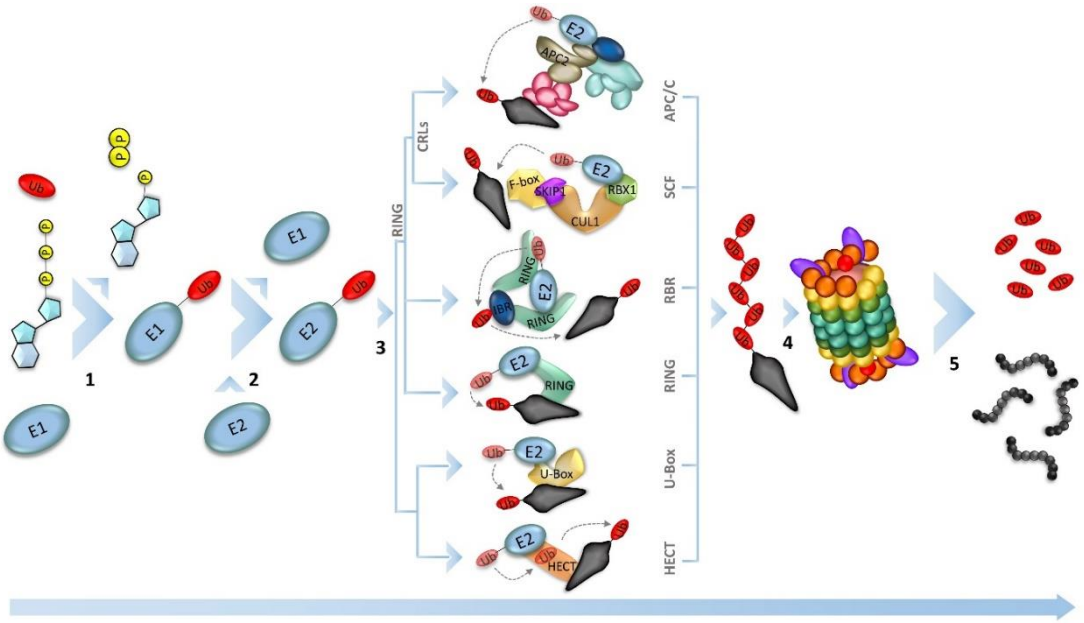


**Figure 2.** The UPS

**Ubiquitin-Proteasome System (UPS). The proteolysis in the UPS happens by sequential reactions catalyzed by three different enzymes. The process starts with the activation of the ubiquitin by the ubiquitin-activating enzyme (E1), using one ATP molecule (1). Next, the activated ubiquitin is transferred to the ubiquitin-conjugating enzyme (E2), which interacts with the ubiquitin ligase (E3) and conjugates the ubiquitin to the substrate that is recognized by the E3 (2). Plant genomes encode hundreds of E3 ligases (the main E3 ligases found in plants are represented in 3), that will target different substrates, making them recognizable by the proteasome 26S, and leading to substrate degradation (5).**

## 2.4. The *A. thaliana* anaphase promoting complex/cyclosome

The E3 ligases are grouped according to the presence of a RING, U-box, or HECT domain, which leads to different ways of binding to a partner E2 conjugating enzyme (Yang, 2021). Among the E3 ligases found in eukaryotes, CRLs are the largest class, playing roles in protein ubiquitination associated with the cell cycle, transcription, signal transduction, and development (Bosu and Kipreos, 2008). Those multisubunit enzymes are characterized by holding a CUL protein, which is responsible for bringing together the RING domain-containing protein and a variable adaptor that recruits the target protein. At the C-terminus region, the CUL protein recruits the RING BOX PROTEIN 1 (RBX1) and the E2 conjugating enzyme, while at the N-terminus region, the substrate binds to an adaptor (Zheng et al, 2002).

In plants, the CRLs are the best characterized E3s as most of these enzymes are related to plant growth and development. According to the CUL present in the CRLs structure, four subtypes of CRLs are found in plants: CUL1, also called SCF (S-Phase Kinase-Associated Protein 1 (SKP1)-CUL1-F-box, CUL3, CUL4, and the cullin-like protein APC2 (Ban and Estelle, 2018). The SCF and the

APC/C are the two best characterized E3s in the plant cell cycle, each of them interacting with a certain type of cyclins and acting on the cell cycle progression (Inzé and De Veylder, 2006).

The APC/C is essential for the mitosis exit, since its main function, as the name suggests, is to promote anaphase onset. After being activated, the complex can specifically target securin for degradation. Securin acts as an inhibitor of separase, which is responsible for breaking the bonds that hold the chromatids together in the kinetochore. Once the inhibition of this enzyme no longer occurs, its activity promotes the separation of chromatids and the beginning of anaphase (Castro et al., 2005; de Lange et al., 2015; Jonak et al., 2017; Kernan et al., 2018). Securins are highly spread among animals and fungi genomes, however, these proteins have not been reported in plants. Recently, two proteins, PATRONUS 1 and PATRONUS 2 (PANS1 and PANS2), were shown to be essential for plant viability and also interact with separase, suggesting that they act like securins in plants. Moreover, for chromatid separation to occur, PANS1 needs to be selectively targeted by the APC/C for degradation (Cromer et al., 2019).

From the ubiquitin ligases, the APC/C is one of the most complex, being composed of dozens of subunits according to the species. In A. thaliana, the APC/C consists of 14 core subunits, APC1, APC2, APC3a, APC3b, APC4, APC5, APC6, APC7, APC8, APC10, APC11, APC13, APC15, and CDC26, resulting in a ≈1.2 MDa ubiquitination machine. Each subunit is encoded by a single gene, apart from APC3, which is encoded by two genes: APC3a/CDC27a and APC3b/CDC27b/HOBBIT (HBT) (Xu, 2019). The subunits can be grouped into four modules, according to their functions in the complex. APC2 and APC11, with a CUL and a Zn+2-binding RING domain, respectively, are the catalytic subunits responsible for transferring the ubiquitin molecule to the substrate. The tetrico-peptide repeats (TPR) domain subunits APC3, APC6, APC7, and APC8 modulate the interactions with the activators proteins. The APC13 and APC15 are known as TRP-accessory subunits by their interaction with the TRP subunits. APC1, which contains a proteasome-cyclosome (PC) repeat important for APC/C stability, APC4, and APC5 form the scaffolding group. These proteins bridge the catalytic and TRP modules, probably acting in space optimization between the two regions to guarantee efficient catalysis. The TRP and scaffold modules, together, forms the backbone of the complex. The activators module includes the cell division cycle 20 (CDC20) and cell cycle switch 52 (CCS52/CDH1), which contain a tryptophan-aspartate (WD) repeat domain, necessary for effective substrate ubiquitination, and the APC10/Doc1, an important coactivator of the APC/C due to its action in recognizing and recruiting D-box containing proteins (Zheng et al., 2011; Xu et al., 2019; Saleme et al., 2021; de Oliveira et al., 2022). Moreover, the APC/C coactivators can increase the affinity for the cognate E2 and its catalytic efficiency (Voorhis and Morgan, 2014).

APC/C function relies on a spatiotemporal regulation performed by different polypeptides that can interact with the APC/C either to activate or inactivate the complex. As mentioned before, the coactivators CDC20 confer substrate specificity and catalytic activity. Moreover, those effects can also be provided by CCS52/CDH1, which is also intrinsically related to the complex structure. In A. thaliana genome, five genes putatively encode for CDC20 (CDC20.1 – CDC20.5). While CDC20.1 and CDC20.2 are functionally redundant during mitoses, but not in meiosis, the CDC20.3 – CDC20.5 genes seem to be pseudogenes that have lost their function as canonical CDC20 genes. Regarding

the CCS52, two A types (CCS52A1 and CCS52A2) and one plant-specific B type (CCS52B) are found in Arabidopsis. In this species, as in other plants, the ULTRAVIOLET-B-INSENSITIVE 4 (UVI4) binds to the CCS52A1 co-activator, inhibiting the complex function, as well as the UVI4 homolog, named OMISSION OF SECOND DIVISION 1 (OSD1)/GIGAS CELL1 (GIG1), which interacts with activators like CDC20.1, CDC20.5, CCS52A1, CCS52A2, and CCS52B (McLean et al., 2011; Heyman and de Veylder, 2012; Saleme et al., 2021).

In addition to the already known regulators, the APC/C of plants has another specific regulator, whose function is still quite unclear. The small protein named SAMBA, first identified by tandem affinity purification (TAP) in Arabidopsis, can interact with the APC/C and regulate its function (Eloy et at., 2012). However, this regulation seems to be species-specific. While the knockout Arabidopsis plants displayed an enlarged meristem size, with larger seeds, leaves, and roots, the Samba CRISPR/Cas9 mutants in maize, produce plants with growth defects and reduced leaf size due to alterations in the rate of cell division and expansion (Eloy et al., 2012; Gong et al., 2022).

How the substrate is recognized by APC/C is still not completely clear. The characterized recognition process so far is the one that occurs through the interaction of WD40 β-propeller domain of complex activators with regions known as degrons, present in the substrate sequence (He et al., 2013). The most well-understood APC/C degrons in plants are the D- (RXXL) and KEN-box. Although some details are still not known, recognition of degrons seems to be an effective way to guarantee the specificity of the proteolytic process in the cell, since a great part of APC/C substrates have these sequences in their primary structure (Qin, 2016).

### 2.5. APC/C in plant growth and development

Plant development and growth are highly complex processes tightly regulated by various signaling pathways, gene expression networks, and environmental cues. APC/C most well-understood role is in the cell cycle control by mediating the degradation of specific cell cycle regulators proteins like cyclins (Petersen et al., 2000; Harper et al., 2002; Capron et al, 2003; Buschhorn and Peters, 2006). However, APC/C has been implicated in several key aspects of plant development, such as cell differentiation, organ formation, hormone signaling, and embryogenesis (de Oliveira et al., 2022). It has also been shown that some APC/C substrates are related to stress tolerance. PATRONUS 1/COPPER MODIFIED RESISTANCE1 (PANS1/CMR1), for example, is involved in pollen viability and female gametophyte development, but it is also required for Cu tolerance and survival under abiotic stresses, mainly salinity stress (Juraniec et al., 2014, 2016).

Different works reported that the modulation of the APC/C subunits, or even of its activators and inhibitors in plants can change the phenotype. Some are the increased cell proliferation upon overexpression of APC3a/CDC27a, the dwarf phenotype caused by a mutation on APC3b/HOBBIT (Blilou, 2002), the increase of cell proliferation by APC10 overexpression (Eloy, 2011), and the smaller root meristem size in uvi4 mutants (Heyman et al., 2011).

The specific features of the APC/C in plants suggest that its developmental function depends on gene structure and expression. This is evidenced by the distinctive way in which the plant APC/C

forms flexible subcomplexes potentially necessary for particular plant growth responses, and essential for adapting to shifting environmental conditions (Lima et al., 2010).

## 2.6. Plant metabolism

Cellular metabolism refers to all biochemical reactions that occur within the cell involving synthesis and degradation processes. Metabolites are highly diverse in terms of structure and physical/chemical properties when compared to proteins and transcripts, having a wide variation in in pathways of action and functions. In plant cells, metabolism is commonly subdivided into primary and secondary. Primary metabolism gathers the fundamental reactions for maintaining essential functions of the plant cell, and consequently the whole organism, such as respiration, photosynthesis, and protein synthesis. Primary metabolic processes use building blocks such as amino acids, sugars, and fatty acids. In contrast, secondary metabolism is responsible for “non-essential” reactions that benefit plant survival and reproduction in the environment, which may be present in some species and absent in others (Hatcher et al., 2020; Pichersky & Gang, 2000; Wink, 2008).

The metabolome refers to the entire set of metabolites produced and modified by an organism. Metabolomics is the branch of science that focuses on quantifying (in relative or absolute amounts) and identifythe metabolites of an organism, in normal conditions or when biological systems are altered. This "-omics" technology provides us with a high technical/experimental power, as it allows us to detect small changes in the complex network of metabolites of an organism as a whole, or even at the level of systems, organs, and cells. Quantitative plant metabolomics based on mass spectometry (MS) has established itself as a powerful tool for addressing interesting biological questions related to the plant environment and agriculture. As plants are sessile organisms, they cannot escape fluctuations in environmental conditions and/or plant-pathogen or plant-predator interactions, which negatively affect their growth and development. Therefore, plant survival depends mainly on the initiation of complex adaptive responses that involve stress detection, signal transduction, the activation of a series of genes, and synthesis of metabolites related to stress. Central metabolism is involved in the regulation of the various developmental processes that allow plants to survive such environmental threats and the measurement of known primary metabolites (e.g., carbohydrates, amino acids, and organic acids) has contributed widely to elucidating how and to what extent plant metabolism readjusts to a changing environment. On the other hand, the measurement of specific secondary metabolites, such as phytohormones, which are key metabolites in signaling and communication between an organism and the abiotic/biotic environment, has contributed to improving our current understanding of the plant defense and growth system (Hirayama & Shinozaki, 2010; Jorge et al., 2016b; Lisec et al., 2006; Obata & Fernie, 2012).

Metabolomics plays a crucial role in gene characterization in plants by providing comprehensive insights into the metabolic changes associated with gene expression and regulation. By analyzing the metabolite profiles of plant tissues or mutants with altered gene expression, metabolomics enables the identification of metabolic pathways influenced by specific genes. Metabolomics also facilitates the functional annotation of unknown genes or gene products by correlating metabolite changes with gene

expression patterns. Additionally, metabolomics helps unravel the regulatory networks that govern gene expression in plants, by identifying metabolites acting as signaling molecules or intermediates. Overall, metabolomics provides a valuable framework for understanding the metabolic consequences of gene expression changes, advancing our knowledge of gene function and their impact on plant phenotype and responses (Tohge, 2005; Saito, 2010; Pan et al., 2012; van der Hooft and Mohimani, 2020).

## 3. CONCLUSIONS

By the end of this work, it was shown that the *APC5* could be overexpressed in *Arabidopsis thaliana*, and the increase in gene levels were able to negatively modulate the size of the two independent overexpression lines, APC5OE$^{3.1}$ an APC5OE$^{4.1}$, resulting in smaller rosette area. Metabolomic profiling could not detect significant differences in the metabolic composition of the overexpression lines when compared to the wild type. The subcellular localization of the gene product was identified by GFP imaging, and the APC5 protein was located at the cell nucleus and cytoplasm. By the GUS histochemical assay, it was showed that the gene promoter has a timely activity at the root meristem, being more active at the beginning of the root development, specifically at the lateral roots. The western blot suggests that the putative degron present in the protein sequence may not be truly active, not leading the APC5 for 26S proteasome degradation. And finally, the essentiality of the gene was demonstrated through the SALK_024997 lineage since no homozygote for the T-DNA insertion in the *APC5* sequence could be found.

## REFERENCES


Al-Saharin, R., Hellmann, H., & Mooney, S. (2022). Plant E3 Ligases and Their Role in Abiotic Stress Response. Cells, 11(5), 890. doi: 10.3390/cells11050890. PMID: 35269512; PMCID: PMC8909703.

Ban, Z., & Estelle, M. (2021). CUL3 E3 ligases in plant development and environmental response. Nature Plants, 7(1), 6-16. doi: 10.1038/s41477-020-00833-6. Epub 2021 Jan 15. PMID: 33452490; PMCID: PMC8932378.

Bansal, S., & Tiwari, S. (2019). Mechanisms for the temporal regulation of substrate ubiquitination by the anaphase-promoting complex/cyclosome. Cell Division, 14, 14. doi: 10.1186/s13008-019-0057-5. PMID: 31889987; PMCID: PMC6927175.

Bard, J. A. M., Goodall, E. A., Greene, E. R., Jonsson, E., Dong, K. C., & Martin, A. (2018). Structure and function of the 26S proteasome. Annual Review of Biochemistry, 87, 697-724. doi: 10.1146/annurev-biochem-062917-011931. PMID: 29652515; PMCID: PMC6422034.

Barford, D. (2011). Structure, function and mechanism of the anaphase promoting complex (APC/C). Quarterly Reviews of Biophysics, 44(2), 153-190. doi: 10.1017/S0033583510000259. PMID: 21092369.

Besson, A., Dowdy, S. F., & Roberts, J. M. (2008). CDK Inhibitors: Cell Cycle Regulators and Beyond. Developmental Cell, 14(2), 159-169. doi: 10.1016/j.devcel.2008.01.013.

Blilou, I., Frugier, F., Folmer, S., Serralbo, O., Willemsen, V., Wolkenfelt, H., Eloy, N. B., Ferreira, P. C., Weisbeek, P., & Scheres, B. (2002). The Arabidopsis HOBBIT gene encodes a CDC27 homolog that links the plant cell cycle to progression of cell differentiation. Genes & Development, 16(19), 2566-2575. doi: 10.1101/gad.237302. PMID: 12368267; PMCID: PMC187454.

Bosu, D. R., & Kipreos, E. T. (2008). Cullin-RING ubiquitin ligases: Global regulation and activation cycles. Cell Division, 3, 7. doi: 10.1186/1747-1028-3-7. PMID: 18282298; PMCID: PMC2266742.

Boyes DC, Zayed AM, Ascenzi R, McCaskill AJ, Hoffman NE, Davis KR, Görlach J. Growth stage-based phenotypic analysis of Arabidopsis: a model for high throughput functional genomics in plants. Plant Cell. 2001 Jul;13(7):1499-510. doi: 10.1105/tpc.010011. PMID: 11449047; PMCID: PMC139543.

Boyes, D. C., Zayed, A. M., Ascenzi, R., McCaskill, A. J., Hoffman, N. E., Davis, K. R., & Görlach, J. (2001). Growth stage-based phenotypic analysis of Arabidopsis: A model for high throughput functional genomics in plants. The Plant Cell, 13(7), 1499-1510. doi: 10.1105/tpc.010011. PMID: 11449047; PMCID: PMC139543.

Brutnell, T. P., & Langdale, J. A. (1998). Signals in leaf development. In J. A. Callow (Ed.), Advances in Botanical Research (Vol. 28, pp. 161-195). Academic Press. ISBN 9780120059287. ISSN 0065-2296. doi: 10.1016/S0065-2296(08)60296-7.

Buetow, L., & Huang, D. (2016). Structural insights into the catalysis and regulation of E3 ubiquitin ligases. Nature Reviews Molecular Cell Biology, 17, 626-642. doi: 10.1038/nrm.2016.91.

Buschhorn, B. A., & Peters, J. M. (2006). How APC/C orders destruction. Nature Cell Biology, 8, 209-211. doi: 10.1038/ncb0306-209.

Capron, A., Serralbo, O., Fülöp, K., Frugier, F., Parmentier, Y., Dong, A., Lecureuil, A., Guerche, P., Kondorosi, E., Scheres, B., & Genschik, P. (2003). The Arabidopsis anaphase-promoting complex or cyclosome: Molecular and genetic characterization of the APC2 subunit. The Plant Cell, 15(10), 2370-2382. doi: 10.1105/tpc.013847. Epub 2003 Sep 24. Erratum in: The Plant Cell, 31(6), 1386-1387. PMID: 14508008; PMCID: PMC197302.

Capron, A., Serralbo, O., Fülöp, K., Frugier, F., Parmentier, Y., Dong, A., et al. (2003). The arabidopsis anaphase-promoting complex or cyclosome: Molecular and genetic characterization of the APC2 subunit. The Plant Cell, 15, 2370-2382. doi: 10.1105/tpc.013847.

Castro, A., Bernis, C., Vigneron, S., et al. (2005). The anaphase-promoting complex: A key factor in the regulation of cell cycle. Oncogene, 24, 314–325. doi: 10.1038/sj.onc.1207973

Castro, A., Bernis, C., Vigneron, S., Labbé, J. C., & Lorca, T. (2005). The anaphase-promoting complex: A key factor in the regulation of cell cycle. Oncogene, 24(3), 314-325. doi: 10.1038/sj.onc.1207973. PMID: 15678131.

Ciechanover, A. (2005). Intracellular protein degradation: From a vague idea thru the lysosome and the ubiquitin-proteasome system and onto human diseases and drug targeting. Cell Death and Differentiation, 12(9), 1178-1190. doi: 10.1038/sj.cdd.4401692. PMID: 16094394.

Ciechanover, A. (2005). Proteolysis: From the lysosome to ubiquitin and the proteasome. Nature Reviews Molecular Cell Biology, 6(1), 79-87. doi: 10.1038/nrm1552. PMID: 15688069.

Clough, S. J., & Bent, A. F. (1998). Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. The Plant Journal, 16(6), 735-743. doi: 10.1046/j.1365-313x.1998.00343.x. PMID: 10069079.

Cromer, L., Jolivet, S., Singh, D. K., Berthier, F., De Winne, N., De Jaeger, G., et al. (2019). Patronus is the elusive plant securin, preventing chromosome separation by antagonizing separase. Proceedings of the National Academy of Sciences, 116(32), 16018-16027. doi: 10.1073/pnas.1906237116.

Cronin, N. B., Yang, J., Zhang, Z., Kulkarni, K., Chang, L., Yamano, H., & Barford, D. (2015). Atomic-Resolution Structures of the APC/C Subunits Apc4 and the Apc5 N-Terminal Domain. Journal of Molecular Biology, 427(20), 3300-3315. doi: 10.1016/j.jmb.2015.08.023. Epub 2015 Sep 4. PMID: 26343760; PMCID: PMC4590430.

Cronin, N. B., Yang, J., Zhang, Z., Kulkarni, K., Chang, L., Yamano, H., & Barford, D. (2015). Atomic-Resolution Structures of the APC/C Subunits Apc4 and the Apc5 N-Terminal Domain. Journal of Molecular Biology, 427(20), 3300-3315. doi: 10.1016/j.jmb.2015.08.023. PMID: 26343760; PMCID: PMC4590430.

Davey NE, Morgan DO. Building a Regulatory Network with Short Linear Sequence Motifs: Lessons from the Degrons of the Anaphase-Promoting Complex. Mol Cell. 2016 Oct 6;64(1):12-23. doi: 10.1016/j.molcel.2016.09.006. PMID: 27716480; PMCID: PMC5098475.

Davey, N. E., & Morgan, D. O. (2016). Building a Regulatory Network with Short Linear Sequence Motifs: Lessons from the Degrons of the Anaphase-Promoting Complex. Molecular Cell, 64(1), 12-23. doi: 10.1016/j.molcel.2016.09.006.

de la Peña, A. H., Goodall, E. A., Gates, S. N., Lander, G. C., & Martin, A. (2018). Substrate-engaged 26S proteasome structures reveal mechanisms for ATP-hydrolysis-driven translocation. Science, 362(6418), eaav0725. doi: 10.1126/science.aav0725. PMID: 30309908; PMCID: PMC6519459.

de Lange, J., Faramarz, A., Oostra, A. B., de Menezes, R. X., van der Meulen, I. H., Rooimans, M. A., et al. (2015). Defective sister chromatid cohesion is synthetically lethal with impaired APC/C function. Nature Communications, 6(1), 8399. doi: 10.1038/ncomms9399

de Oliveira, P. N., da Silva, L. F., & Eloy, N. B. (2022). The role of APC/C in Cell Cycle Dynamics, growth and development in cereal crops. Frontiers in Plant Science, 13. Retrieved from https://doi.org/10.3389/fpls.2022.987919.

De Veylder, L., Beeckman, T., & Inzé, D. (2007). The ins and outs of the plant cell cycle. Nature Reviews Molecular Cell Biology, 8(8), 655-665. doi: 10.1038/nrm2227. PMID: 17643126.

Duronio, R. J., & Xiong, Y. (2013). Signaling pathways that control cell proliferation. Cold Spring Harbor Perspectives in Biology, 5(3), a008904. doi: 10.1101/cshperspect.a008904. PMID: 23457258; PMCID: PMC3578363.

Ehlinger, A., & Walters, K. J. (2013). Structural insights into proteasome activation by the 19S regulatory particle. Biochemistry, 52(21), 3618-3628. doi: 10.1021/bi400417a. PMID: 23672618; PMCID: PMC3864829.

Eloy, N. B., de Freitas Lima, M., Van Damme, D., Vanhaeren, H., Gonzalez, N., De Milde, L., Hemerly, A. S., Beemster, G. T., Inzé, D., & Ferreira, P. C. (2011). The APC/C subunit 10 plays an essential role in cell proliferation during leaf development. The Plant Journal, 68(2), 351-363. doi: 10.1111/j.1365-313X.2011.04691.x. Epub 2011 Aug 8. PMID: 21711400.

Eloy, N. B., Gonzalez, N., Van Leene, J., Maleux, K., Vanhaeren, H., De Milde, L., Dhondt, S., Vercruysse, L., Witters, E., Mercier, R., Cromer, L., Beemster, G. T., Remaut, H., Van Montagu, M. C., De Jaeger, G., Ferreira, P. C., & Inzé, D. (2012). SAMBA, a plant-specific anaphase-promoting complex/cyclosome regulator is involved in early development and A-type cyclin stabilization. Proceedings of the National Academy of Sciences of the United States of America, 109(34), 13853-13858. doi: 10.1073/pnas.1211418109. Epub 2012 Aug 6. PMID: 22869741; PMCID: PMC3427114.

Frank, U., Kublik, S., Mayer, D., et al. (2019). A T-DNA mutant screen that combines high-throughput phenotyping with the efficient identification of mutated genes by targeted genome sequencing. BMC Plant Biology, 19, 539. doi: 10.1186/s12870-019-2162-7.

Gaillochet, C., & Lohmann, J. U. (2015). The never-ending story: From pluripotency to plant developmental plasticity. Development, 142(13), 2237-2249. doi: 10.1242/dev.117614. PMID: 26130755; PMCID: PMC4510588.

Gong, P., Bontinck, M., Demuynck, K., De Block, J., Gevaert, K., Eeckhout, D., Persiau, G., Aesaert, S., Coussens, G., Van Lijsebettens, M., Pauwels, L., De Jaeger, G., Inzé, D., & Nelissen, H. (2022). SAMBA controls cell division rate during maize development. Plant Physiology, 188(1), 411-424. doi: 10.1093/plphys/kiab514. PMID: 34791456; PMCID: PMC8774815.

Hajk-Georg Drost, Philipp Janitza, Ivo Grosse, Marcel Quint (2017). Cross-kingdom comparison of the developmental hourglass. Current Opinion in Genetics & Development, 45, 69-75. doi: 10.1016/j.gde.2017.03.003.

Hariharan, I. K., Wake, D. B., & Wake, M. H. (2015). Indeterminate Growth: Could It Represent the Ancestral Condition? Cold Spring Harbor Perspectives in Biology, 8(2), a019174. doi: 10.1101/cshperspect.a019174. PMID: 26216720; PMCID: PMC4743077.

Harper, J. W., Burton, J. L., & Solomon, M. J. (2002). The anaphase promoting complex: It's not just for mitosis anymore. Genes & Development, 16, 2179-2206. doi: 10.1101/gad.1013102.

He, J., Chao, W. C., Zhang, Z., Yang, J., Cronin, N., & Barford, D. (2013). Insights into degron recognition by APC/C coactivators from the structure of an Acm1-Cdh1 complex. Molecular Cell, 50(5), 649-660. doi: 10.1016/j.molcel.2013.04.024. PMID: 23707760; PMCID: PMC3690534.

Heyman, J., Van den Daele, H., De Wit, K., Boudolf, V., Berckmans, B., Verkest, A., Alvim Kamei, C. L., De Jaeger, G., Koncz, C., & De Veylder, L. (2011). Arabidopsis ULTRAVIOLET-B-INSENSITIVE4 maintains cell division activity by temporal inhibition of the anaphase-promoting complex/cyclosome. The Plant Cell, 23(12), 4394-4410. doi: 10.1105/tpc.111.091793. PMID: 22167059; PMCID: PMC3269873.

Hilty, J., Muller, B., Pantin, F., & Leuzinger, S. (2021). Plant growth: The What, the How, and the Why. New Phytologist, 232, 25-41. https://doi.org/10.1111/nph.17610.

Hua, Z., & Vierstra, R. D. (2011). The cullin-RING ubiquitin-protein ligases. Annual Review of Plant Biology, 62, 299-334. doi: 10.1146/annurev-arplant-042809-112256. PMID: 21370976.

Inzé, D., & De Veylder, L. (2006). Cell cycle regulation in plant development. Annual Review of Genetics, 40, 77-105. doi: 10.1146/annurev.genet.40.110405.090431. PMID: 17094738.

Javelle, M., Vernoud, V., Rogowsky, P. M., & Ingram, G. C. (2011). Epidermis: The formation and functions of a fundamental plant tissue. New Phytologist, 189(1), 17-39. doi: 10.1111/j.1469-8137.2010.03514.x. PMID: 21054411.

Jiang, T.-X., Zhao, M., & Qiu, X.-B. (2018). Substrate receptors of proteasomes. Biological Reviews, 93(3), 1765-1777. doi: 10.1111/brv.12419.

Jonak, K., Zagoriy, I., Oz, T., Graf, P., Rojas, J., Mengoli, V., & Zachariae, W. (2017). APC/C-Cdc20 mediates deprotection of centromeric cohesin at meiosis II in yeast. Cell Cycle, 16(12), 1145-1152. doi: 10.1080/15384101.2017.1320628. PMID: 28514186; PMCID: PMC5499901.

Joubès, J., Chevalier, C., Dudits, D., Heberle-Bors, E., Inzé, D., Umeda, M., & Renaudin, J. P. (2000). CDK-related protein kinases in plants. Plant Molecular Biology, 43(5-6), 607-620. doi: 10.1023/a:1006470301554. PMID: 11089864.

Juraniec, M., Heyman, J., Schubert, V., Salis, P., De Veylder, L., & Verbruggen, N. (2016). Arabidopsis COPPER MODIFIED RESISTANCE1/PATRONUS1 is essential for growth adaptation to stress and required for mitotic onset control. New Phytologist, 209, 177-191. doi: 10.1111/nph.13589.

Juraniec, M., Lequeux, H., Hermans, C., Willems, G., Nordborg, M., Schneeberger, K., Salis, P., Vromant, M., Lutts, S., & Verbruggen, N. (2014). Towards the discovery of novel genetic component involved in stress resistance in Arabidopsis thaliana. New Phytologist, 201, 810-824.

Katagiri, Y., Hasegawa, J., Fujikura, U., Hoshino, R., Matsunaga, S., & Tsukaya, H. (2016). The coordination of ploidy and cell size differs between cell layers in leaves. Development, 143(7), 1120-1125. doi: 10.1242/dev.130021.

Kernan, J., Bonacci, T., & Emanuele, M. J. (2018). Who guards the guardian? Mechanisms that restrain APC/C during the cell cycle. Biochimica et Biophysica Acta - Molecular Cell Research, 1865, 1924-1933. doi: 10.1016/j.bbamcr.2018.09.011.

Kevei, Z., Baloban, M., Da Ines, O., Tiricz, H., Kroll, A., Regulski, K., Mergaert, P., & Kondorosi, E. (2011). Conserved CDC20 cell cycle functions are carried out by two of the five isoforms in Arabidopsis thaliana. PLOS ONE, 6(6), e20618. doi: 10.1371/journal.pone.0020618. PMID: 21687678; PMCID: PMC3110789.

Khan, Y. A., White, K. I., & Brunger, A. T. (2022). The AAA+ superfamily: A review of the structural and mechanistic principles of these molecular machines. Critical Reviews in Biochemistry and Molecular Biology, 57(2), 156-187. doi: 10.1080/10409238.2021.1979460. PMID: 34632886.

Kopka, J., Schauer, N., Krueger, S., Birkemeyer, C., Usadel, B., Bergmüller, E., Dörmann, P., Weckwerth, W., Gibon, Y., Stitt, M., Willmitzer, L., Fernie, A. R., & Steinhauser, D. (2005). GMD@CSB.DB: The Golm Metabolome Database. Bioinformatics, 21(8), 1635-1638. doi: 10.1093/bioinformatics/bti236. Epub 2004 Dec 21. PMID: 15613389.

Kulkarni, M., & Smith, H. E. (2008). E1 ubiquitin-activating enzyme UBA-1 plays multiple roles throughout C. elegans development. PLoS Genetics, 4(7), e1000131. doi: 10.1371/journal.pgen.1000131. PMID: 18636104; PMCID: PMC2443343.

Kwee HS, Sundaresan V. The NOMEGA gene required for female gametophyte development encodes the putative APC6/CDC16 component of the Anaphase Promoting Complex in Arabidopsis. Plant J. 2003 Dec;36(6):853-66. doi: 10.1046/j.1365-313x.2003.01925.x. PMID: 14675450.

Lee, S. H., Moon, J. H., Yoon, S. K., & Yoon, J. B. (2012). Stable incorporation of ATPase subunits into 19 S regulatory particle of human proteasome requires nucleotide binding and C-terminal tails. Journal of Biological Chemistry, 287(12), 9269-9279. doi: 10.1074/jbc.M111.316208. PMID: 22275368; PMCID: PMC3308751.

Li, Y., Li, S., & Wu, H. (2022). Ubiquitination-proteasome system (UPS) and autophagy two main protein degradation machineries in response to cell stress. Cells, 11(5), 851. doi: 10.3390/cells11050851.

Lim, S., & Kaldis, P. (2013). Cdks, cyclins and CKIs: Roles beyond cell cycle regulation. Development, 140(15), 3079-3093. doi: 10.1242/dev.091744. PMID: 23861057.

Lima, M. D., Eloy, N. B., Pegoraro, C., Sagit, R., Rojas, C., Bretz, T., et al. (2010). Genomic evolution and complexity of the anaphase-promoting complex (APC) in land plants. BMC Plant Biology, 10, 254. doi: 10.1186/1471-2229-10-254.

Lisec, J., Meyer, R. C., Steinfath, M., Redestig, H., Becher, M., Witucka-Wall, H., Fiehn, O., Törjék, O., Selbig, J., Altmann, T., & Willmitzer, L. (2008). Identification of metabolic and biomass QTL in Arabidopsis thaliana in a parallel analysis of RIL and IL populations. The Plant Journal, 53(6), 960-972. doi: 10.1111/j.1365-313X.2007.03383.x. Epub 2007 Nov 28. PMID: 18047556; PMCID: PMC2268983.

Liu, S., Luo, J., Zhen, X., Qiu, J., Ouyang, S., & Luo, Z. Q. (2020). Interplay between bacterial deubiquitinase and ubiquitin E3 ligase regulates ubiquitin dynamics on Legionella phagosomes. eLife, 9, e58114. doi: 10.7554/eLife.58114. PMID: 33136002; PMCID: PMC7669269.

Liu, Z., Yuan, F., Ren, J., Cao, J., Zhou, Y., Yang, Q., et al. (2012). GPS-ARM: Computational Analysis of the APC/C Recognition Motif by Predicting D-Boxes and KEN-Boxes. PLoS ONE, 7(3), e34370. https://doi.org/10.1371/journal.pone.0034370.

Manohar, S., Jacob, S., Wang, J., Wiechecki, K. A., Koh, H. W. L., Simões, V., Choi, H., Vogel, C., & Silva, G. M. (2019). Polyubiquitin Chains Linked by Lysine Residue 48 (K48) Selectively Target Oxidized Proteins In Vivo. Antioxidants & Redox Signaling, 31(15), 1133-1149. doi: 10.1089/ars.2019.7826. PMID: 31482721; PMCID: PMC6798811.

Marrocco, K., Bergdoll, M., Achard, P., Criqui, M. C., & Genschik, P. (2010). Selective proteolysis sets the tempo of the cell cycle. Current Opinion in Plant Biology, 13(6), 631-639. doi: 10.1016/j.pbi.2010.07.004. PMID: 20810305.

McLean, J. R., Chaix, D., Ohi, M. D., & Gould, K. L. (2011). State of the APC/C: Organization, function, and structure. Critical Reviews in Biochemistry and Molecular Biology, 46, 118-136.

McMaster, G. (2005). Phytomers, phyllochrons, phenology and temperate cereal development. The Journal of Agricultural Science, 143, 137-150. doi: 10.1017/S0021859605005083.

Menges, M., De Jager, S. M., Gruissem, W., & Murray, J. A. H. (2005). Global analysis of the core cell cycle regulators of Arabidopsis identifies novel genes, reveals multiple and highly specific profiles of expression and provides a coherent model for plant cell cycle control. The Plant Journal, 41, 546-566.

Menges, M., Hennig, L., Gruissem, W., & Murray, J. A. (2003). Genome-wide gene expression in an Arabidopsis cell suspension. Plant Molecular Biology, 53(4), 423-442. doi: 10.1023/B:PLAN.0000019059.56489.ca. PMID: 15010610.

Meyer, R. C., Steinfath, M., Lisec, J., Becher, M., Witucka-Wall, H., Törjék, O., et al. (2007). The metabolic signature related to high plant growth rate in Arabidopsis thaliana. Proceedings of the National Academy of Sciences, 104(11), 4759-4764. Available at: https://doi.org/10.1073/pnas.0609709104.

Morgan, D. O. (1995). Principles of CDK regulation. Nature, 374(6518), 131-134. doi: 10.1038/374131a0. PMID: 7877684.

Morgan, D. O. (2013). The D box meets its match. Molecular Cell, 50(5), 609-610. doi: 10.1016/j.molcel.2013.05.023. PMID: 23746347; PMCID: PMC3702177.

Morgan, D. O., & Roberts, J. (2002). Oscillation sensation. Nature, 418, 495-496. doi: 10.1038/418495a.

Morimoto, D., & Shirakawa, M. (2016). The evolving world of ubiquitin: transformed polyubiquitin chains. Biomolecular Concepts, 7(3), 157-167. doi: 10.1515/bmc-2016-0009.

Müller-Xing, R., & Xing, Q. (2022). The plant stem-cell niche and pluripotency: 15 years of an epigenetic perspective. Frontiers in Plant Science, 13, 1018559. doi: 10.3389/fpls.2022.1018559. PMID: 36388540; PMCID: PMC9659954.

Murashige, T., & Skoog, F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Plant Physiology, 15, 473-497.

Nandi, D., Tahiliani, P., Kumar, A., & Chandu, D. (2006). The ubiquitin-proteasome system. Journal of Biosciences, 31(1), 137-155. doi: 10.1007/BF02705243. PMID: 16595883.

Ong, J. Y., & Torres, J. Z. (2019). Dissecting the mechanisms of cell division. Journal of Biological Chemistry, 294(30), 11382-11390. doi: 10.1074/jbc.AW119.008149. PMID: 31175154; PMCID: PMC6663871.

Pan, M., Zheng, Q., Wang, T., Liang, L., Mao, J., Zuo, C., Ding, R., Ai, H., Xie, Y., Si, D., Yu, Y., Liu, L., & Zhao, M. (2021). Structural insights into Ubr1-mediated N-degron polyubiquitination. Nature, 600(7888), 334-338. doi: 10.1038/s41586-021-04097-8. PMID: 34789879; PMCID: PMC8798225.

Park, J., Cho, J., & Song, E. J. (2020). Ubiquitin-proteasome system (UPS) as a target for anticancer treatment. Archives of Pharmacal Research, 43(11), 1144-1161. doi: 10.1007/s12272-020-01281-8. PMID: 33165832; PMCID: PMC7651821.

Perianez-Rodriguez, J., Manzano, C., & Moreno-Risueno, M. A. (2014). Post-embryonic organogenesis and plant regeneration from tissues: Two sides of the same coin? Frontiers in Plant Science, 5, 219. doi: 10.3389/fpls.2014.00219. PMID: 24904615; PMCID: PMC4033269.

Petersen, B. O., Wagener, C., Marinoni, F., Kramer, E. R., Melixetian, M., Denchi, E. L., et al. (2000). Cell cycle- and cell growth-regulated proteolysis of mammalian CDC6 is dependent on APC-CDH1. Genes & Development, 14, 2330-2343. doi: 10.1101/gad.832500.

Pfleger, C. M., & Kirschner, M. W. (2000). The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1. Genes & Development, 14(6), 655-665. PMID: 10733526; PMCID: PMC316466.

Qi, F., & Zhang, F. (2020). Cell cycle regulation in the plant response to stress. Frontiers in Plant Science, 10, 1765. doi: 10.3389/fpls.2019.01765. PMID: 32082337; PMCID: PMC7002440.

Qin, L., Guimarães, D. S., Melesse, M., & Hall, M. C. (2016). Substrate Recognition by the Cdh1 Destruction Box Receptor Is a General Requirement for APC/CCdh1-mediated Proteolysis. Journal of Biological Chemistry, 291(30), 15564-15574. doi: 10.1074/jbc.M116.731190. Epub 2016 May 23. Erratum in: Journal of Biological Chemistry, 292(12), 5125-5127. PMID: 27226622; PMCID: PMC4957042.

Radoeva, T., Vaddepalli, P., Zhang, Z., & Weijers, D. (2019). Evolution, Initiation, and Diversity in Early Plant Embryogenesis. Developmental Cell, 50(5), 533-543. doi: 10.1016/j.devcel.2019.07.011. PMID: 31505175.

Robinson, D. O., et al. (2018). Ploidy and size at multiple scales in the Arabidopsis sepal. The Plant Cell, 30(10), 2308-2329. doi: 10.1105/tpc.18.00344.

Scaglione, K. M., Basrur, V., Ashraf, N. S., Konen, J. R., Elenitoba-Johnson, K. S., Todi, S. V., & Paulson, H. L. (2013). The ubiquitin-conjugating enzyme (E2) Ube2w ubiquitinates the N terminus of substrates. Journal of Biological Chemistry, 288(26), 18784-18788. doi: 10.1074/jbc.C113.477596. PMID: 23696636; PMCID: PMC3696654.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671-675. doi: 10.1038/nmeth.2089.

Schwedersky, R. P., Saleme, M. L. S., Rocha, I. A., Montessoro, P. D. F., Hemerly, A. S., Eloy, N. B., et al. (2021). The anaphase promoting Complex/Cyclosome subunit 11 and its role in organ size and plant development. Frontiers in Plant Science, 12. doi: 10.3389/fpls.2021.563760.

Shi, B., Zhang, C., Tian, C., Wang, J., Wang, Q., Xu, T., et al. (2016). Two-Step Regulation of a Meristematic Cell Population Acting in Shoot Branching in Arabidopsis. PLoS Genetics, 12(7), e1006168. doi: 10.1371/journal.pgen.1006168.

Soss, S. E., Yue, Y., Dhe-Paganon, S., & Chazin, W. J. (2011). E2 conjugating enzyme selectivity and requirements for function of the E3 ubiquitin ligase CHIP. Journal of Biological Chemistry, 286(24), 21277-21286. doi: 10.1074/jbc.M111.224006. PMID: 21518764; PMCID: PMC3122187.

Takeda, S., & Aida, M. (2011). Establishment of the embryonic shoot apical meristem in Arabidopsis thaliana. Journal of Plant Research, 124(2), 211-219. doi: 10.1007/s10265-010-0390-x. PMID: 21104289.

Tanaka, K. (2009). The proteasome: Overview of structure and functions. Proceedings of the Japan Academy. Series B, Physical and Biological Sciences, 85(1), 12-36. doi: 10.2183/pjab.85.12. PMID: 19145068; PMCID: PMC3524306.

Thompson, S. J., Loftus, L. T., Ashley, M. D., & Meller, R. (2008). Ubiquitin-proteasome system as a modulator of cell fate. Current Opinion in Pharmacology, 8(1), 90-95. doi: 10.1016/j.coph.2007.09.010. PMID: 17981502; PMCID: PMC2265078.

Ting Pan, Shan Gao, Xiaoyu Cui, Lili Wang, Shunping Yan (2023). APC/CCDC20 targets SCFFBL17 to activate replication stress responses in Arabidopsis. The Plant Cell, 35(2), 910-923. https://doi.org/10.1093/plcell/koac360.

Tsukaya, H. (2013). Does ploidy level directly control cell size? Counterevidence from Arabidopsis genetics. PLoS One, 8(12), e83729. doi: 10.1371/journal.pone.0083729. PMID: 24349549; PMCID: PMC3861520.

Tyson, J. J., Csikasz-Nagy, A., & Novak, B. (2002). The dynamics of Cell Cycle Regulation. BioEssays, 24(12), 1095-1109. doi: 10.1002/bies.10191.

Van Voorhis, V. A., & Morgan, D. O. (2014). Activation of the APC/C ubiquitin ligase by enhanced E2 efficiency. Current Biology, 24(13), 1556-1562. doi: 10.1016/j.cub.2014.05.052.

Voorhis, V., & Morgan, D. (2014). Activation of the APC/C Ubiquitin Ligase by Enhanced E2 Efficiency. Current Biology, 24. doi: 10.1016/j.cub.2014.05.052.

Wang, Y., Hou, Y., Gu, H., Kang, D., Chen, Z., Liu, J., & Qu, L. J. (2012). The Arabidopsis APC4 subunit of the anaphase-promoting complex/cyclosome (APC/C) is critical for both female gametogenesis and embryogenesis. The Plant Journal, 69(2), 227-240. doi: 10.1111/j.1365-313X.2011.04785.x. Epub 2011 Oct 21. PMID: 21910774.

Watanabe, Y., Taguchi, K., & Tanaka, M. (2020). Ubiquitin, autophagy, and neurodegenerative diseases. Cells, 9(9), 2022. doi: 10.3390/cells9092022. PMID: 32887381; PMCID: PMC7563958.

Willems, A., & De Veylder, L. (2022). The Plant Anaphase-Promoting Complex/Cyclosome. Annual Review of Cell and Developmental Biology, 38, 25-48. doi: 10.1146/annurev-cellbio-120420-092421. PMID: 35395166.

Xu, R. Y., Xu, J., Wang, L., Niu, B., Copenhaver, G. P., Ma, H., Zheng, B., & Wang, Y. (2019). The Arabidopsis anaphase-promoting complex/cyclosome subunit 8 is required for male meiosis. New Phytologist, 224(1), 229-241. doi: 10.1111/nph.16014. Epub 2019 Jul 24. PMID: 31230348; PMCID: PMC6771777.

Xu, R.-Y., Xu, J., Wang, L., Niu, B., Copenhaver, G. P., Ma, H., Zheng, B., & Wang, Y. (2019). The Arabidopsis anaphase-promoting complex/cyclosome subunit 8 is required for male meiosis. New Phytologist, 224(1), 229-241. doi: 10.1111/nph.16014.

Xue, Z., Liu, L., & Zhang, C. (2020). Regulation of shoot apical meristem and axillary meristem development in plants. International Journal of Molecular Sciences, 21(8), 2917. doi: 10.3390/ijms21082917. PMID: 32326368; PMCID: PMC7216077.

Yang, Q., Zhao, J., Chen, D., & Wang, Y. (2021). E3 ubiquitin ligases: styles, structures and functions. Molecular Biomedicine, 2(1), 23. doi: 10.1186/s43556-021-00043-2. PMID: 35006464; PMCID: PMC8607428.

Yang, Q., Zhao, J., Chen, D., & Wang, Y. (2021). E3 ubiquitin ligases: Styles, structures, and functions. Molecular Biomedicine, 2(1), 23. doi: 10.1186/s43556-021-00043-2. PMID: 35006464; PMCID: PMC8607428.

Yuan, L., Lv, Z., Adams, M. J., & Olsen, S. K. (2021). Crystal structures of an E1-E2-ubiquitin thioester mimetic reveal molecular mechanisms of transthioesterification. Nature Communications, 12(1), 2370. doi: 10.1038/s41467-021-22598-y. PMID: 33888705; PMCID: PMC8062481.

Zhang, S., & Mao, Y. (2020). AAA+ ATPases in Protein Degradation: Structures, Functions and Mechanisms. Biomolecules, 10(4), 629. doi: 10.3390/biom10040629.

Zhen, Y., Qin, G., Luo, C., Jiang, H., Yu, K., et al. (2014). Exploring the RING-catalyzed ubiquitin transfer mechanism by MD and QM/MM calculations. PLOS ONE, 9(7), e101663. doi: 10.1371/journal.pone.0101663.

Zheng, B., Chen, X., & McCormick, S. (2011). The anaphase-promoting complex is a dual integrator that regulates both MicroRNA-mediated transcriptional regulation of cyclin B1 and degradation of Cyclin B1 during Arabidopsis male gametophyte development. The Plant Cell, 23(3), 1033-1046. doi: 10.1105/tpc.111.083980. Epub 2011 Mar 25. PMID: 21441434; PMCID: PMC3082252.

Zheng, B., Chen, X., & McCormick, S. (2011). The anaphase-promoting complex is a dual integrator that regulates both microRNA-mediated transcriptional regulation of cyclin B1 and degradation of Cyclin B1 during Arabidopsis male gametophyte development. The Plant Cell, 23(3), 1033-1046. doi: 10.1105/tpc.111.083980. PMID: 21441434; PMCID: PMC3082252.

Zheng, N., Schulman, B. A., Song, L., Miller, J. J., Jeffrey, P. D., Wang, P., Chu, C., Koepp, D. M., Elledge, S. J., Pagano, M., Conaway, R. C., Conaway, J. W., Harper, J. W., & Pavletich, N. P. (2002). Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. Nature, 416(6882), 703-709. doi: 10.1038/416703a. PMID: 11961546.

Zheng, N., Schulman, B. A., Song, L., Miller, J. J., Jeffrey, P. D., Wang, P., Chu, C., Koepp, D. M., Elledge, S. J., Pagano, M., Conaway, R. C., Conaway, J. W., Harper, J. W., & Pavletich, N. P. (2002). Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. Nature, 416(6882), 703-709. doi: 10.1038/416703a. PMID: 11961546.