Title:

APC/C Ubiquitin Ligase: Coupling Cellular Differentiation to G1/G0 Phase in Multicellular Systems

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Abstract

The anaphase promoting complex/cyclosome (APC/C) is an evolutionarily conserved ubiquitin ligase that controls cell cycle progression through spatiotemporally regulated proteolysis. Although recent studies revealed its postmitotic function, our knowledge of the role of APC/C beyond cell cycle regulation in the biology of multicellular organisms is far from complete. Here I review recent advances in the function of APC/C in animal development, specifically focusing on its emerging role in regulating cell differentiation. I describe how APC/C regulates distinct processes during the course of differentiation by deploying diverse molecular machineries in a wide variety of developmental contexts. Also, I discuss the significance and clinical relevance of the unique capacity of APC/C and other cell cycle regulators to couple distinct cellular processes with cell proliferation control.

Introduction

The cell cycle is a series of events through which a cell divides into two cells (**Figure 1A**). In multicellular organisms, proper cell cycle regulation is critical not only for growth but also for development and homeostasis. The last few decades of research, which largely relied upon unicellular organisms and *in vitro* cell culture, have unveiled conserved molecular machineries that control major processes of the cell cycle[1]. However, many questions on cell cycle regulation *in vivo* in metazoan organisms remain unsolved, including how cell cycle regulators are controlled at the tissue, organ and organism levels, and how the cell cycle is orchestrated with other cellular events that are important for development.

Cell cycle progression is ultimately dictated by a set of conserved proteins commonly called 'cell cycle regulators'. The most central among these are **cyclin-dependent kinases** (CDKs, see Glossary), which regulate the cell cycle through protein phosphorylation[2]. In addition, **Cullin-RING ubiquitin ligases** (CRLs) regulate protein abundance in a cell through the **ubiquitin-proteasome pathway** (UPP). Through the irreversible process of proteolysis, CRLs ensure the unidirectionality of cell cycle progresssion[3].

APC/C is a major CRL involved in cell cycle regulation, characterised by its exceptionally large size and intricate architecture composed of 16 distinct proteins[4]. Because of its homology across species, APC/C was assumed to be specialised in cell cycle regulation in multicellular organisms. However, subsequent studies using metazoan models revealed its unanticipated postmitotic roles beyond cell cycle regulation. The first hint of involvement in postmitotic processes was high expression of the APC/C co-activator CDH1 (also known as Fizzy-related or Fzr1) in mammalian neurons[5]. Subsequent analyses established the critical role of APC/C in the terminal differentiation of neurons and ultimately in the function of the nervous system[6–8]. Despite this discovery, whether APC/C is also involved in earlier stages of cell differentiation or if it operates in a wider variety of cell types remains unclear. In this review, I outline novel postmitotic functions of APC/C in cell differentiation in various developmental contexts and highlight multiple context-dependent cellular mechanisms whereby APC/C directly impacts cell differentiation and cell type-specific cell behaviours.

Regulating G1/G0 length

APC/C regulates multiple events during the cell cycle, acting as both an engine and a brake[4,9]. Two structurally homologous accessory subunits, CDC20 (also called Fizzy) and CDH1, commonly referred to as 'APC/C coactivators', mediate substrate recognition and stimulate the ubiquitin ligase activity of APC/C[10,11]. CDC20 binds and activates APC/C in M phase to trigger chromatid separation and mitotic exit through degradation of Securin and mitotic cyclins, respectively. After CDK1 inactivation, CDH1 gets dephosphorylated and binds APC/C to degrade CDC20. APC/C^{CDH1} retains cells in the following G1 phase or allows them to enter G0 **(Figure 1**), making it a key regulator of G1/G0 progression[9]. In yeast and human, CDH1 inactivation shortens G1 phase and thus premature entry into S phase, which causes DNA damage[12,13]. CDH1 is also required for cells to enter G0 when they are deprived of nutrients or mitogens or exposed to anti-mitotic signals[13,14] (**Figure 1)**.

Studies in multicellular models also underpin the critical role of APC/C^{CDH1} in G1/G0 progression *in vivo*. In *Drosophila*, CDH1 is required for G1/G0 arrest of embryonic epidermal cells and photoreceptor progenitor cells before differentiation[15,16]. In mice, conditional knockout of the APC/C subunit, APC2, causes spontaneous proliferation of quiescent hepatocytes, resulting in acute liver failure[17]. However, interestingly, in these animals, many cells remain quiescent or stop dividing after only a few subsequent divisions, indicating that redundant or cooperative mechanisms are present to ensure robust control of proliferation *in vivo*. Human CDH1 has been shown to bind **Retinoblastoma protein** (pRb)**,** another important G1/G0 regulator and tumour suppressor[18], suggesting possible cooperation between APC/C^{CDH1} and pRb in G1/G0 regulation *in vivo*[9]*.*

Notably, a recent single-cell study in human cell culture identified an additional role for APC/C^{CDH1} in the exit from G0[19]; after the **Restriction point** (R-point)[20], cells are able to return to G0 phase upon exposure to various cellular stress, such as DNA damage[19]. In contrast, the re-entry to G0 phase is not observed when APC/C^{CDH1} is irreversibly inactivated by its inhibitor Emi1 subsequent to the R-point[19]. Thus, the full inactivation of APC/C^{CDH1} defines the 'point of no return' before S phase, pointing to a role for APC/C^{CDH1} as the final gatekeeper for mammalian cells to exit from G0 phase. However, the significance of this new 'time point' in the cell cycle with respect to the R-point, and how they control cell proliferation and tissue growth across cell types, remains to be addressed.

As differentiation progresses, cells generally slow down the cell cycle, typically by lengthening G1 phase. Indeed, in the developing mammalian cerebral cortex, the G1 phase of neural progenitors is progressively elongated upon differentiation into intermediate progenitors and then further as they continue differentiation to neurons[21]. Further studies suggest a role for APC/C in regulating this process. In the mouse brain, shortening G1 by over-expressing the G1-specific CDK, CDK4-Cyclin D, inhibits differentiation of the progenitors, whereas elongating G1 by CDK4 depletion promotes their differentiation[22]. This finding has led to 'the cell cycle length hypothesis' for mammalian neurogenesis, in which a longer G1 phase provides sufficient time for cell fate determinants to induce neural differentiation in neural progenitors[21].

In addition to CDK4-Cyclin D, APC/C^{CDH1} also appears to be responsible for G1 progression in mouse neural progenitors as neural progenitor-specific CDH1 knockout cells prevent G1 elongation in the progenitor pool and inhibits the generation of mature neurons in developing mouse brains. This inhibition results in severe reduction of the overall brain size, reminiscent of **microcephaly**[23,24]. Similarly, in an *in vitro* neurogenesis model with primary cortical culture, CDH1 knockout inhibits both G1 elongation and the expression of neural markers in neuronal progenitors, whereas expression of the non-phosphorylatable form of CDH1 (refractory to CDK1-dependent inhibitory phosphorylation) induces G1 elongation and accelerates neuronal differentiation[23]. These data support a model that APC/C^{CDH1} induces the differentiation of neural progenitors by extending G1 phase. However, CDH1 knockout also causes the accumulation of DNA damage in progenitors because of premature entry into S phase. This leads to p53-mediated apoptosis of progenitor cells, which results in a reduction in the number of neurons[23,24]. Thus, the role of APC/C^{CDH1} in the differentiation of neural progenitors is still unclear.

However, despite both APC/C^{CDH1} and CDK4-Cyclin D affecting G1 length and differentiation of neural progenitor differentiation, they appear to regulate the process differently. A detailed cell cycle profiling in the mouse neural progenitors showed that the self-renewing population of the progenitors experience a longer S phase than the differentiating population[25], suggesting that S phase duration may also influence neural differentiation, which might be abrogated by CDH1 depletion, but not by CDK4-Cyclin D. Notably, previous studies in *Drosophila* retinogenesis showed that the loss of G1 arrest does not inhibit the

differentiation of retinal progenitor cells into photoreceptor neurons, indicating that G1 elongation is not a universal requirement for neural differentiation[15,26]. CDK4-Cyclin D has been reported to have various cell cycle-independent functions[27]. Thus, it is possible that CDK4-Cyclin D may induce neural differentiation independent of the cell cycle, which may explain the different effects of CDK4-Cyclin D expression and CDH1 depletion. As discussed later, APC/C also impacts cell differentiation through cell cycle-independent mechanisms. In the case of mouse neural progenitors, APC/C^{CDH1} targets several proteins that are highly expressed in the progenitors, such as KLF4, MCPH1, Radmis and CK1δ[28–31] (**Table 1**). Defining the roles of their degradation may clarify the role of APC/C^{CDH1} in neural differentiation.

Modulating cellular responses to developmental signalling

Transcriptional regulation of gene expression is central to cell fate specification, cellular differentiation, and tissue-specific functions. APC/C influences cellular differentiation by directly targeting various cell type-specific transcription factors (TFs) and their regulators for degradation [32,33](**Table 1**). For example, in an *in vitro* myogenesis system using mouse C2 cells, APC/C^{CDH1}-dependent degradation of a basic helix-loop-helix (bHLH) type TF, Myf5, triggers myogenic fusion during differentiation into multinucleated muscle fibres[34]. In mammalian neurons, APC/C targets the neurogenic bHLH protein, NeuroD2, as well as inhibitors of bHLH TFs, Inhibitor of DNA binding protein 1 and 2 (Id1 and Id2), to regulate neuronal morphology and activity[35–37].

Other major mechanisms that cooperate with TFs in gene regulation are extracellular signalling pathways. By responding to external signals, these pathways transduce intercellular and intracellular signalling cascades, which typically culminate in transcriptional changes within signal-receiving cells. The first evidence for crosstalk between APC/C and signalling pathways was the identification of the SnoN transcriptional repressor as an APC/C substrate[38,39]. SnoN inhibits the ability of receptor-regulated Smad proteins to activate transcription in response to TGF-β family ligands[40]. CDH1 directly binds SnoN and Smad3 to induce APC/C-dependent degradation of SnoN, thereby promoting TGF-β-induced transcription in human cells[38,39] (**Figure 2A)**. Subsequent studies in vertebrate and invertebrate models also support the role of APC/C^{CDH1} in SnoN degradation *in vivo*[32,33,41,42].

However, the cellular levels of SnoN are regulated by multiple transcriptional and posttranscriptional mechanisms, in addition to APC/C-mediated proteolysis[43]. Thus, to what extent APC/C modulates TGF-β signalling pathways remains unclear.

Along with TGF-β signalling, the Wnt signalling pathway is another highly conserved signalling pathway that plays a central role in the regulation of the development and physiology of metazoans. Recently, two genetic screens in *Drosophila* have identified a novel function of APC/C in regulating Wnt signalling[44,45] (**Figure 2B**). In the developing *Drosophila* eye primordium (the eye imaginal disc), Wnt signalling inhibits the specification of undifferentiated progenitors into retinal fate including photoreceptor neurons[46,47]. Partial APC/C inactivation in the progenitors allows their proliferation but blocks their differentiation into photoreceptor neurons, resulting in a severe reduction of adult retinas or the formation of antenna-like tissues in the adult eye, indicative of a failure in cell fate determination[44]. These defects are accompanied by abnormal activation of Wnt-dependent transcription in the progenitors, suggesting that APC/C normally suppresses Wnt signalling responses in these progenitors to allow their specification into retinal fate (**Figure 2B**). Furthermore, APC/C^{CDH1} inactivation late in development in the postmitotic eye and wing epithelia causes mis-orientation of ommatidia and wing hairs in adult flies. As APC/C^{CDH1} inactivation misregulates the non-canonical Wnt/PCP pathway[45], **planar cell polarity** (PCP) is not established in the epithelia, resulting in the above phenotype. These results suggest that APC/C modulates both canonical and non-canonical Wnt pathways to promote cell differentiation and tissue development (**Figure 2B**).

Intriguingly, this regulation of the Wnt pathways is mediated by the degradation of a conserved kinase, NIMA-related kinase 2 (Nek2)[44,45], which is an established regulator of the centrosome and is also known to regulate various cell cycle processes[48]. In *Drosophila*, Nek2 was recently identified as a positive modulator of the canonical Wnt pathway, which can bind and phosphorylate Dishevelled (Dsh), a component shared between the canonical Wnt and Wnt/PCP pathways[49]. In eye imaginal discs, prior to the specification of photoreceptors, all retinal progenitor cells become synchronously arrested in G1 phase, which requires APC/C^{CDH1} activity[50]. Nek2 is degraded in the cytoplasm in G1 phase through APC/C^{CDH1}–mediated proteolysis, resulting in reduced cellular responsiveness to Wnt signalling in the G1-arrested cell population[44]. Based on these results, it has been proposed that APC/C^{CDH1}

promotes the differentiation of retinal progenitors by inducing G1 arrest and simultaneously suppressing cell responsiveness to Wnt signalling (**Figure 2B)**.

Importantly, the APC/C-dependent regulation of Wnt signalling is likely conserved in vertebrates. The catalytic domain of Nek2 is highly conserved[51]. Human Nek2 is also an APC/C substrate[52] and phosphorylates the human Dsh orthologue[53]. Furthermore, in *Xenopus* embryos, morpholino-mediated depletion of APC/C subunits inhibits axis elongation and neural tube closure and also randomises epidermal ciliary polarity, phenotypes which are commonly observed upon aberrant Wnt signalling activation[54]. Whether these Wnt-related phenotypes in *Xenopus* are mediated by Nek2 needs to be tested. It has been shown that Wnt signalling is regulated during the cell cycle in human cells; the kinase activity of GSK3, a canonical Wnt pathway component downstream of Dsh, is low in G1 and peaks in M phase, which reversely correlates with APC/C activity[55]. During mitosis, Wnt signalling promotes various mitotic processes such as the assembly and orientation of the mitotic spindle and chromosome segregation, through post-transcriptional regulation[56,57]. Notably, three Nek2 isoforms are expressed in human cells and are differentially targeted by APC/C^{CDC20} or APC/C^{CDH1} during the cell cycle[48,58], suggesting that APC/C may differentially regulate multiple Wnt signalling cascades during the cell cycle through different Nek2 isoforms.

Regulating the centrosome

Centrosomes and the cilia are interrelated organelles that share centrioles as core structural components, and through microtubule organisation they regulate a wide range of cellular processes that are crucial for the development of multicellular organisms, including cell division, signal transduction and cell motility[59] (**Figure 3A**). Accordingly, dysfunction of these organelles is tightly associated with various human diseases such as cancer, microcephaly and ciliopathy[60].

In most cells, the copy number of centrioles is strictly controlled (one or two copies) so that centrioles are duplicated only once in a cell cycle by using pre-existing centrioles as templates, analogous to DNA replication. Additionally, centrioles can be converted to a basal body in G1/G0 phase to form the primary cilium. APC/C has emerged as a key

regulator that coordinates duplication and conversion processes of the centriole-based organelles with cell cycle progression[60] (**Figure 3A, Table 1**). During the cell cycle, APC/C targets many structural components and important regulators of the centrosome, such as Sas6, Aurora A and Polo-like kinase 1. The accumulation of any one of these components or regulators results in centrosome overamplification or abnormal microtubule nucleation, highlighting the importance of APC/C-mediated regulation[58,61,62]. Additionally, APC/C^{CDC20} degrades another NEK family member, Nek1, to disassemble primary cilia upon cell cycle reentry[63].

The tight coupling between the structure and function of the centrosome and the cell cycle is likely to be critical for the normal development of metazoan organisms, including humans. Indeed, there is a link between defects in APC/C-dependent destruction of a centrosome component and a pathological developmental disorder. The gene encoding the centriole component STIL has been identified as a causal gene of autosomal recessive primary microcephaly (MCPH)[64]. Some known MCPH mutations in STIL, while maintaining its ability to assemble functional centrosomes, block its APC/C^{CDH1}-dependent degradation and cause its stabilisation, thereby enhancing its ability to induce centrosome amplification in human cells[65]. It has been shown that supernumerary centrosomes cause microcephaly in mice[66]. Moreover, centrosomes conversely regulate the function of APC/C by regulating its activity as a subcellular hub[67,68]. In *Drosophila* neuroblasts, centrosomal localisation of CDH1 promotes APC/C-dependent degradation of Aurora A at the centrosome, which is required to maintain the neuroblast number in the developing adult brain[69]. In human neurons, the centrosome-associated pool of CDC20 is required for APC/C to regulate dendrite morphology[36].

Centrosomes change their organisation and functions to promote cell differentiation and adopt tissue-specific functions during development. Recent studies illustrate how the APC/C impacts cell differentiation through its regulation of the centrosome in different developmental settings (**Figure 3B)**. During the early development of the *Drosophila* tracheal system (the *Drosophila* respiratory system), post-mitotic terminal cells (TC cells) undergo unique unicellular branching, which is initiated with subcellular lumen formation at the apical cell membrane. It was found that during this process, centrosomes localise near the apical membrane in TC cells and form a polarised microtubule network that is necessary

for lumen formation[70]. Abnormal APC/C^{CDH1} activation by mutations in Rca1 (*Drosophila* Emi1) induces centrosome amplification in TC cells and causes lumen bifurcation, leading to excessive TC cell branching[70]. Thus, APC/C^{CDH1} regulates the unicellular morphogenesis of TC cells by restricting centrosome amplification.

Another study revealed a critical role of APC/C in an unconventional mode of centriole biogenesis during mammalian brain development. During the terminal differentiation of mouse ependymal cells, a few hundred centrioles are generated to form motile cilia, which are subsequently required for the generation of the brain fluid stream. In this unique process, many centrioles are formed *de novo* at the deuterostome, the electron-dense subcellular structure unique to multiciliated cells[71,72]. Pharmacological inhibition of APC/C impedes the multiciliation process by inhibiting the detachment of new-born centrioles from the deuterostome and their migration to the apical membrane[73]. Additionally, APC/C inhibition also drives the postmitotic ependymal cells into mitosis[73], suggesting that APC/C regulates the unique centriole amplification process while keeping the ependymal cellcells quiescent. Unlike most postmitotic cells, ependymal cells express the mitotic APC/C coactivator CDC20[73]. Although it is not clear whether CDH1 is co-expressed in epidermal cells, how APC/C^{CDC20} and APC/C^{CDH1} are differentially regulated to enable uncoupling of the centriole amplification cycle from the cell cycle is an area for further research.

Concluding remarks

Initial studies on APC/C in multicellular organisms revealed unanticipated postmitotic roles of APC/C in a subset of cell types, in particular, neurons[6–8]. Building on this discovery, recent studies have established the critical role of APC/C in regulating cell differentiation and cell type-specific functions in a wide variety of cell types by deploying diverse molecular mechanisms (**Figure 4, Key Figure**). Through these studies, several major advances in our understanding of the functions of APC/C, and cell cycle machineries, have been achieved. First, the role of APC/C is not limited to terminal differentiation or specialised functions of a small number of cell types. Instead, APC/C is involved in a wide range of differentiation processes in a large variety of cell types, including progenitors and stem cells. Second, APC/C deploys highly diverse mechanisms to influence cellular differentiation. APC/C targets not only cell type-specific transcriptional regulators but also components of more general

cellular machineries, such as signalling pathways and centriole-based organelles, which participate in a wide spectrum of developmental processes. This suggests that the function of APC/C goes beyond a single cell and can extend to intercellular, tissue-level processes, including cell-cell communication, tissue polarity and morphogenesis. Finally, with APC/C being a ubiquitin ligase, recent findings underscore the importance of ubiquitination in the regulation of cell differentiation. In line with this, growing numbers of UPP components have been identified as regulators of fate determination in various stem cells[74]. The UPP may play a crucial role in differentiation by enabling a rapid change in the cellular proteome through proteolysis, or by expanding a repertoire of protein modifications for signal transduction[75].

Many questions remain to be addressed as to the role of APC/C, thus cell cycle regulators, in metazoan development (see **Outstanding Questions**). An immediate question is how the multiple functions of APC/C are regulated *in vivo* by upstream developmental mechanisms. Several signalling pathways, including Notch and TGF-β, are suggested to transcriptionally or post-translationally regulate the APC/C[38,39,44,76,77]. Additionally, spatial changes in subcellular localisation may further regulate APC/C activity inside each cell[36,69]. A more fundamental question is that of the biological significance of the multitude of functions APC/C has as a single macromolecule. A common theme emerging from recent evidence is that APC/C promotes cell differentiation by employing various cellular machineries to regulate context-dependent differentiation processes while inducing or maintaining G1/G0 arrest in the same cell (**Figure 4**). It is tempting to speculate that there is an intrinsic importance and/or an evolutionary advantage to tightly couple these differentiation processes to the G1 or G0 state. For instance, such coupling may allow a sub-fraction of proliferating cells to respond differentially to the same signal depending on their cell cycle phase. Alternatively, the coupling may contribute to tumour suppression by making quiescent or differentiated cells resistant to mitogenic signals. Lastly, do cell cycle regulators have cell cycle-independent functions that are linked to cancer and other human diseases? The APC/C subunit, CDC27, was identified as a cancer driver gene in multiple human cancers[78,79]. CDH1 heterozygous mice are prone to sporadic tumours[80]. So far, the role of these genetic alterations in cancer development has been considered solely from the perspectives of cell-cycle functions of

APC/C. However, it is conceivable that cell cycle-independent functions of APC/C may contribute to the disease mechanism.

To address these questions, it is crucial to continue exploring the function of APC/C and other cell cycle regulators in a wider variety of developmental contexts. In the past, the biggest challenge in studying the function of cell cycle regulators *in vivo* was their core functions, being essential for cell growth and viability. However, the recent advent of powerful genome editing methods, single-cell analysis technologies, and potent inhibitors that are highly specific to each cell cycle regulator protein enables precise manipulation and dissection of the functions of cell cycle regulators *in vivo*. We can therefore confidently envisage rapid advances in the characterisation of the functions of cell cycle regulators in the coming years.

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Glossary

Cyclin-dependent kinases (CDKs): The family of enzymes composed of a kinase and a regulatory subunit, cyclin, including CDK1-Cyclin A/B, CDK2-Cyclin A/E, and CDK4/6-Cyclin D complexes. Typically, the cellular levels of cyclins oscillate during the cell cycle as the enzymatic activities of their associated kinases.

Cullin-RING ubiquitin ligases (CRLs): ubiquitin ligases are enzymes that catalyse the transfer of ubiquitin molecules onto their substrate proteins. CRLs are a family of multi-subunit ubiquitin ligases containing a Cullin-like protein and a RING finger protein as their catalytic centres, which include APC/C, SCF (Skp1-Cullin1-Fbox), VBC (pVHL-Elongin B/C-Cullin2), Cullin3-BTB and Cullin4 complexes.

Ubiquitin-proteasome pathway (UPP): The highly regulated molecular cascade that mediates targeted protein degradation in an ATP-dependent manner. In the UPP, three types of enzymes: ubiquitin activating enzymes (E1s), ubiquitin conjugating enzymes (E2s) and ubiquitin ligases (E3s), cooperate to covalently link polyubiquitin chains to lysine residues of target proteins. The polyubiquitinated proteins are then recognised by 26S proteasome, a large complex consisting of proteases and ATPase and non-ATPase subunits, and are degraded into small peptides. The highest level of regulation and specificity is conferred by E3s, which directly bind both substrates and E2s in specific spatiotemporal windows and catalyse ubiquitin transfer, and deubiquitinating enzymes (DUBs), a large group of proteases that cleave and modify ubiquitin chains.

Restriction point (R-point): the hypothetical time point in G1 phase where mammalian cells are considered to commit to the next round of the cell cycle. The cell that has passed R-point will initiate DNA replication without a delay whether critical amino acids or serums are withdrawn or not. Currently, the stable activation of the transcriptional activity of E2F is considered the defining event of R-point.

Retinoblastoma protein (pRb): the transcriptional repressor important for the regulation of the G1 to S phase transition as well as the exit from G0 phase. pRb directly binds E2F transcription factor to inhibit its transactivation activity. Upon phosphorylation by CDK4-

Cyclin D, pRb releases E2F, which in turn induces transcription of various cell cycle regulator genes including Cyclin E to initiate DNA replication.

Microcephaly: a medical condition characterised by a reduced brain size, which may be present at birth due to abnormal brain development or can develop after birth due to defective brain growth.

Planer cell polarity: The coordinated orientation of cells or cellular structures within the plane of an epithelial tissue. Prime examples are the epithelia of the *Drosophila* eyes and wings where photoreceptor cell clusters and wing hairs are oriented in certain directions across the tissues, which have been used as major model systems to study the mechanism regulating PCP.

Figure Legends

Figure 1 The function of APC/C in cell cycle regulation

(A) The most typical form of the cell cycle consists of four phases: G1, S, G2 and M phases. The cell replicates its genomic DNA in S phase and segregates each copy of their duplicated DNA equally into two daughter cells in M phase. G1 and G2 are preparatory phases before S and M phases and also the main periods during which cells grow. The cell can also exit from the cell cycle to enter 'G0' phase, where the cell has ceased mitotic division and stays in quiescence with unreplicated genome DNA. G0 phase can be reversible or irreversible. In the latter, the cell is often terminally differentiated (ex., neurons and muscle cells) or senescent **(B)** In dividing cells, CDC20 binds and activates APC/C in M phase to trigger chromatid separation and mitotic exit through degradation of Securin and mitotic cyclins, respectively. CDH1 then takes over APC/C during mitotic exit to keep cells in G1 phase or allow them to enter G0 through continuous degradation of cyclins and Skp2 (which degrades CDK inhibitors as a component of SCF ubiquitin ligase. APC/C^{CDC20} and APC/C^{CDH1} are thought to be mutually exclusive and the switch between the two forms is regulated by multiple posttranslational mechanisms, including dephosphorylation of APC/C core subunits and CDH1, and APC/C-dependent degradation of CDC20. However, precise kinetics of this transition and the possible interaction between the two forms of APC/C are unknown.

Figure 2 APC/C^{CDH1} modulates extracellular signalling pathways in G1/G0 cells.

The Wnt and TGF-β pathways are conserved extracellular signalling pathways that regulate diverse developmental and physiological processes including cell proliferation, differentiation, tissue homeostasis, cell polarity, axis formation and neural activity in multicellular organisms. **(A)** TGF-β signalling is induced by the binding of TGF-β superfamily ligands to a Type II receptor, which recruits and phosphorylates a Type I receptor. The Type I receptor then phosphorylates regulatory Smad proteins (R-Smads) to promote the formation of the R-Smads-co-Smad complex, which will be translocated into the nucleus to regulate target gene expression. **(B)** Wnt signalling initiates with the binding of Wnt family ligands to a Frizzled (Fz)-family transmembrane receptor, which passes the signal to the

Dishevelled protein in the cytoplasm. The canonical Wnt pathway leads to the stabilisation of β-catenin, which is translocated into the nucleus to induce transcriptional changes alongside the TCF/LEF family TFs. Wnt signalling can branch off into the noncanonical Wnt/PCP pathway that regulates cell polarity of epithelial tissues and some mesenchymal cells through cytoskeletal reorganisation. In G1 or G0 phase, APC/C^{CDH1} regulates the cellular response to TGF- β and Wnt signals by degrading SnoN and Nek2 proteins, the negative and positive modulators of TGF-β and Wnt signalling, respectively.

Figure 3 APC/C regulates centriole-based organelles in mitotic and postmitotic cells.

(A) A pair of centrioles recruit a protein matrix called pericentriolar material (PCM) to form the centrosome, which acts as a major microtubule organising centre. In G1 phase, a cell normally contains two centrioles connected by a centrosomal linker. The centrioles duplicate in S phase, each forming a procentriole, and become mature centrosomes in G2 phase. As cells enter M phase, the centrosomes start to separate, recruit more PCM and nucleate microtubules to form mitotic spindle. Upon mitotic exit, the mother and daughter centrioles disengage. In many cells that have entered G0 phase, the mother centriole acts as a basal body to form a cilium, which disassembles upon cell cycle re-entry. APC/C ensures this coordination between the centriole behaviour and the cell cycle by targeting numerous regulatory proteins. The inset shows an electron microscopic cross-section image of a centriole in *Drosophila* testis. **(B**) APC/C regulates the formation and functions of centrosomes and cilia in postmitotic cells for cell differentiation. During the *Drosophila* tracheal development, Rca1 inhibits APC/C activity to limit centrosome number and prevent excess unicellular branching of the terminal cell (TC cell). In the differentiating mouse ependymal progenitor cell, APC/C regulates the unique mode of centriole amplification whilst keeping the cell in G0 phase, to enable the formation of multicilia.

Figure 4 A model for APC/C function in cell differentiation in multicellular organisms.

The cell cycle regulator APC/C can regulate various context-dependent mechanisms through ubiquitin-dependent proteolysis. By regulating these processes alongside the cell cycle, APC/C coordinates cell type-specific differentiation processes with elongation of G1 phase or G0 arrest to promote cellular differentiation. How the cell-cycle and cell cycle-

1 independent functions of APC/C are regulated during development remains poorly
2 understood.

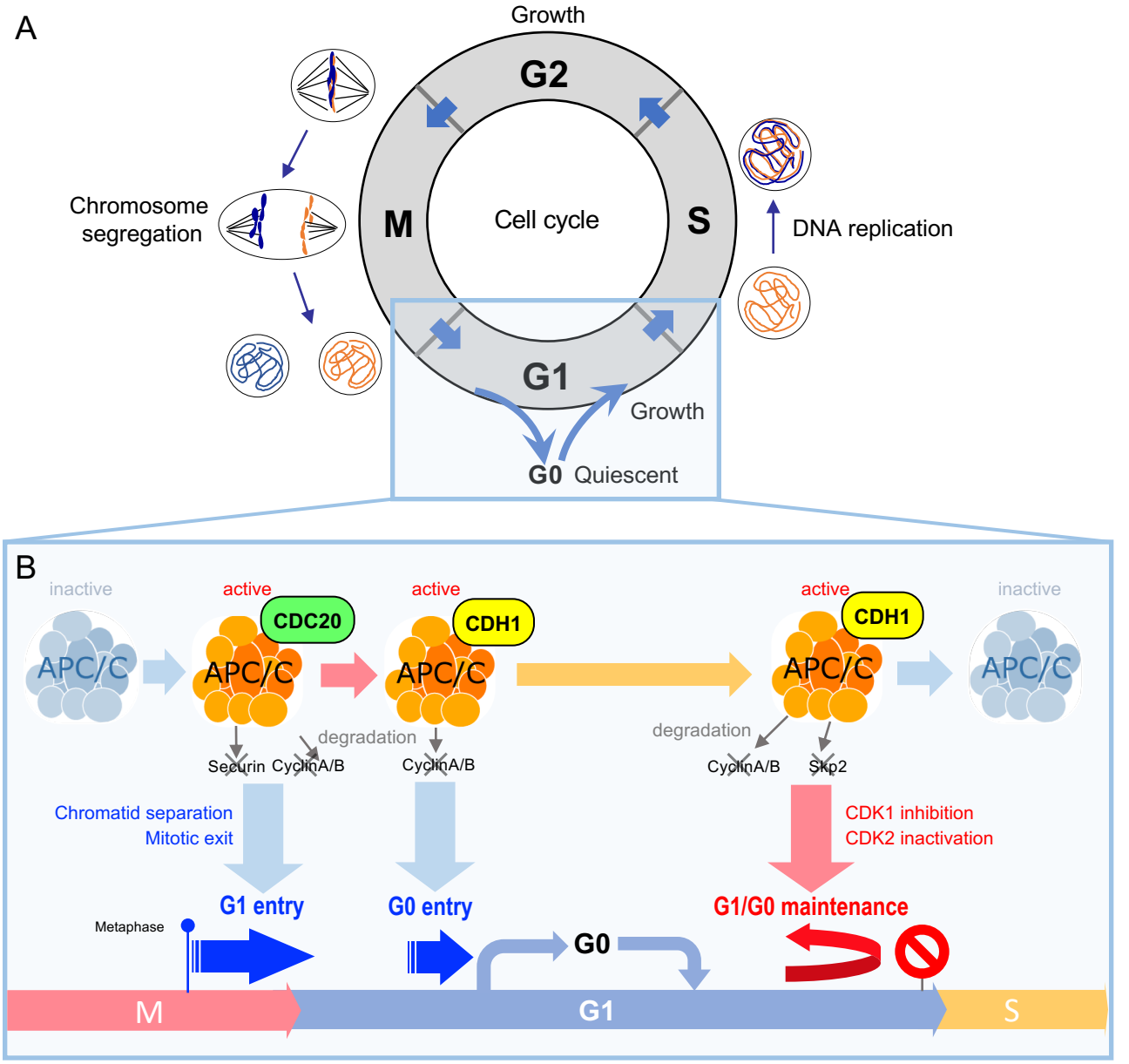
3

4 **Table 1. Known APC/C substrates with cell cycle-independent functions**

Protein name	Function category	Function of degradation	Coactivator	Species**	References
Sas6	centriole biogenesis	centriole number restriction?	CDH1	M	62
STIL/SAS-5	centriole biogenesis	centrosome number restriction?	CDH1	C, M	65,81
Spd2	PCM recruitment	N.D.	CDH1	D	69
Securin	cell cycle regulation, centriole biogenesis	chromatid separation, centriole disengagement	CDC20	Y, D, X, M	82–84
Plk1/Polo	centrosome regulation, mitotic progression	mitotic exit, centrosomal function undetermined	CDH1	Y, D, X, M	61,85
Aurora A	centrosome regulation, mitotic progression	N.D.	CDH1	D, X, M	86,87
Aurora B	chromatin regulation, cell cycle regulation	N.D.	CDH1	Y, D, X, M	88
CKAP2	centrosome and microtubule regulation	spindle formation, cytokinesis?	CDH1	M	89,90
Kif11/Eg5	centrosome and microtubule regulation	centrosome clustering	CDH1	M	91
Nek1	ciliogenesis	cilium resorption	CDC20	X, M	63
Nek2	centrosome and cilium regulation, signal transduction	Wnt signalling suppression, PCP regulation, centrosomal function undetermined	*CDC20, CDH1	D, X, M	44,58
Radmis	microtubule regulation	neural progenitor proliferation?	N.D.	M	30
FOXM1	transcription, cell cycle control	cell cycle regulation of mitotic regulator genes	CDH1	M	92,93
HOXC10	transcription, development	N.D.	CDC20?	M	94
SIX1	transcription, development	N.D.	CDH1	M	95
Ams2	transcription	cell cycle regulation of histone gene expression	CDH1	Y	96
Myf5	transcription, muscle development	muscle differentiation	CDH1	M	34
Duf	muscle development	myoblast fusion	CDH1?	D	97
PAX3	Transcription, Melanocyte regulation	melanocyte differentiation	CDH1	M	98
NeuroD2	transcription, nervous system	presynaptic differentiation	CDC20	M	35
Id1	transcription, nervous system	dendrite growth	CDC20	M	36
Id2	transcription, nervous system	axonal growth	CDH1	M	37
SMAR1	tumour suppression	cell migration, invasion	CDC20	M	99
BRAF	signal transduction	tumour suppression	CDH1	M	100
CK1δ	signal transduction	neurite outgrowth regulation	CDH1	M	101
SnoN	signal transduction	TGF-β signalling activation, axonal growth, lens differentiation	CDH1	D, M	33,38,39
MCPH1	DNA repair, cell cycle control	neural progenitor differentiation	CDH1	D, M	102,103
Liprinα	nervous system	synapse bouton number restriction	N.D.	D	104
AMPA receptor	nervous system	glutamic receptor recycling, synaptic activity regulation	CDH1	C, M	105,106

Fas2	cell migration, nervous system	neuron-glia interaction	CDH1	D	107
Dlc90F	cell migration, eye development	neuron-glia interaction	CDH1	D	108
Drp1	mitochondria regulation	mitochondrial fusion	CDH1	M	109
PFKFB3	glycolysis	glycolysis inhibition	CDH1	M	110,111
GLS1	glutaminolysis	glutaminolysis inhibition	CDH1	M	112
TK1	dTTP production	dNTP pool maintenance	CDH1	M	113
SPOP	protein degradation	N.D.	CDH1	M	114

****Abbreviations: N.D.: Not determined; M: Mammals; D: *Drosophila melanogaster* C: *Caenorhabditis elegans*; X: *Xenopus*; Y: Yeast**


A
Growth
G2
Chromosome segregation
M
Cell cycle
S
DNA replication
G1
Growth
G0 Quiescent
B
inactive
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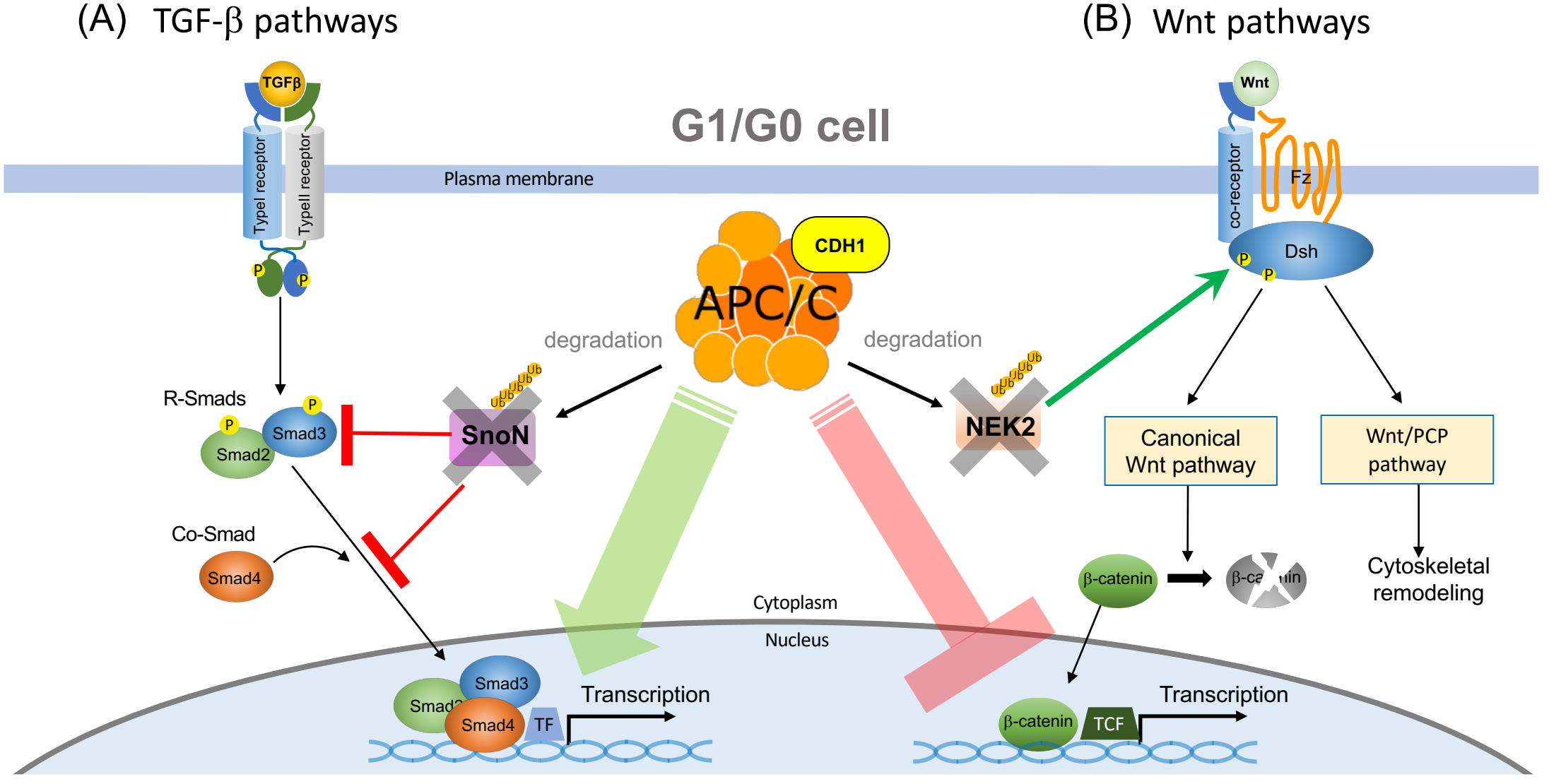
CDH1
active
CDH1
inactive
APC/C
APC/C
APC/C
APC/C
APC/C
degradation
Securin
CyclinA/B
CyclinA/B
degradation
CyclinA/B
Skp2
Chromatid separation
Mitotic exit
CDK1 inhibition
CDK2 inactivation
G1 entry
G0 entry
G1/G0 maintenance
Metaphase
G0
M
G1
S


(A) TGF-β pathways
(B) Wnt pathways
G1/G0 cell
Plasma membrane
TGFβ
TypeI receptor
TypeII receptor
P
CDH1
APC/C
degradation
degradation
R-Smads
Smad2
Smad3
SnoN
Co-Smad
Smad4
NEK2
Wnt
co-receptor
Fz
Dsh
Canonical Wnt pathway
Wnt/PCP pathway
β-catenin
Cytoskeletal remodeling
Cytoplasm
Nucleus
Smad3
Smad4
TF
Transcription
β-catenin
TCF
Transcription

A

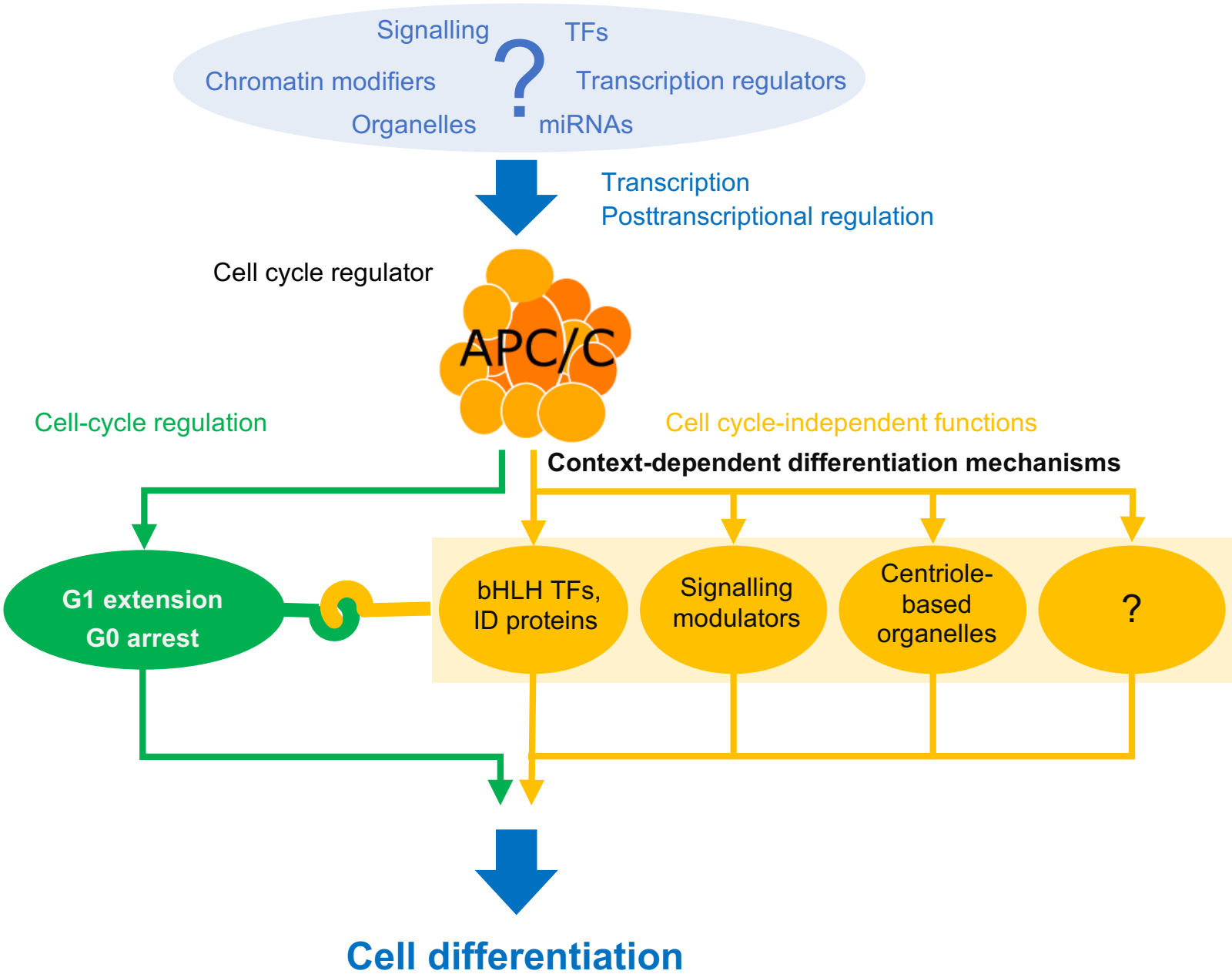
PLK1
CDK1-CycB
Centrosome maturation
NEK2
Centrosome disjunction
Pericentriolar material
Kif11
Centrosome separation
PLK1
CDK1-CycB
AurA
Spindle formation
Microtubules
Chromosomes
PLK1
Securin
Centriole disengagement
Centrosomal linker
G2
M
S
G1
G0
Cell cycle
The centriole structure (electron microscopy)
100 nm
Procentriole
Centriole duplication
Sas6
STIL
Mother centriole
Daughter centriole
Cilium formation
NEK1
Cilium disassembly
AurA
Cilium
Microtubules
Basal body (mother centriole)
: APC/C substrates

B

Drosophila tracheal development

Lumen
Centrosome
G0?
Microtubule
TC cell
Rca1
Centrosome amplification
Excess branching
Rca1 mutation
APC/C
Ependymal cell
CDK1-CycB
Motile cilia
Daughter centriole
Procentriole
Deuterosome
Basal body (mother centriole)
G0
Centriole amplification & growth
Centriole detachment
Apical migration
G0

Mouse brain development


Signalling
TFs
Chromatin modifiers
?
Transcription regulators
Organelles
miRNAs
Transcription
Posttranscriptional regulation
Cell cycle regulator
APC/C
Cell-cycle regulation
Cell cycle-independent functions
Context-dependent differentiation mechanisms
G1 extension
G0 arrest
bHLH TFs,
ID proteins
Signalling
modulators
Centriole-
based
organelles
?
Cell differentiation

Highlights

- The cell cycle regulator APC/C regulates cell differentiation through cell cycle-independent functions in multicellular organisms.
- APC/C regulates a wide range of differentiation processes, from cell fate specification in unspecified progenitor cells to terminal differentiation of specific cell types, via ubiquitin-dependent proteolysis.
- APC/C influences cell differentiation by exerting at least three types of context-dependent mechanisms: (1) regulating the expression levels of cell type-specific transcriptional regulators, (2) modulating cellular responses to signalling pathways, (3) regulating the organisation and functions of centrosomes.
- APC/C is able to spatiotemporally coordinate these context-dependent differentiation processes with G1/G0 progression in the same cell.