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REVIEW

Coordination of cell division and differentiation in plants in comparison to animals

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ABSTRACT During animal and plant development all cells are originated from a single fertilized oocyte, the zygote. To generate an adult organism from the single-celled zygote many rounds of cell division are required to be completed. Cell division is manifested through a well-defined series of molecular and cellular events that is often referred as the cell cycle. Studies in various model organisms demonstrated that the eukaryotic cell cycle is regulated in a conserved manner with cyclin-dependent kinases (CDKs) in the centre. It is widely believed that cells must exit the cell cycle for cell differentiation. Accordingly, cell division and differentiation do not happen at the same time. The main questions in developmental biology are how these processes are coordinated during development, how do cells stop division before differentiation, and why and how cells maintain or re-initiate cell division activity? Recent studies indicate direct links between molecular cell cycle and cell differentiation machineries. The basic mechanisms regulating the balance between cell proliferation and differentiation are remarkably similar in plants and animals despite their fundamentally different developmental strategies. There is considerable dissimilarity, however, in the upstream signalling pathways affecting this balance in developmental and environmental contexts. In this chapter we focus our attention on the molecular regulatory mechanism controlling and coordinating cell division and differentiation both in animals and plants with emphasis on the entry and exit points of the cell cycle.
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Introduction

Cells are the building blocks of animal and plant bodies. It is calculated that just one gram of human tissue contains a few billion cells (Bianconi et al. 2013). Even more striking that three hundred million cells are replaced in our bodies every minute. That just indicates that the generation of tissues requires massive cell production, while tissue homeostasis maintains a functional organ by replacing worn and wounded cells. The importance of cell proliferation is even more obvious in plants, these constantly growing and developing organisms. Plant development is mostly post embryonic in contrast to animals where organogenesis occurs mainly during embryogenesis. It means that plants produce new organs throughout their life and every new organ needs a lot of cells. They are capable for this due to their specific regions called meristems, supplying plant development with the required amount of cells. They can produce almost endless number of new cells for a long period of time. The largest organism on the planet is the giant sequoia (*Sequoiadendron giganteum*), which grows to an average height of 70-85 m with 5-7 m in diameter. Even more amazing that it can live for several thousands of years.

In order to grow, an organism has to couple cell division with cell growth, otherwise the proliferating cells would get smaller and smaller, so the total cell mass would not increase. In the case of single-celled yeasts where growth and division depend only on nutrients, the link between cell growth and cell division is obvious. In multicellular organisms, however, these processes have to take place in an organised manner, acting in concert with the regulation of cell differentiation, thus are regulated by extracellular signals produced by other cells (for reviews, Jones and Kazlauskas 2001; Kuijt and Schnittger 2007). These short and long range chemical, molecular or hormonal signals are called mitogens, growth factors or growth hormones. While growth factors and growth hormones are capable of stimulating cellular growth, proliferation, healing, as well as cellular differentiation, the term mitogen is reserved for substances that enforce a cell to commence cell division, triggering mitosis.

Mitosis is the way how somatic cells divide (while meiosis is the division of the reproductive or germ cells). Naturally

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the mitotic cell division is started with interphase (all events prior to M-phase including G1-, S- and G2-phases) and followed by mitosis, which is packed with morphological events subdivided into pro-, meta, ana- and telophases followed by cytokinesis (Magyar et al. 2012). Generally the longest phase of the somatic cell division cycle is the interphase. During this long period cells grow and gather nutrients and energy in Gap1- or G1-phase, make copies of their DNA in the synthesis or S-phase, and in the Gap2- or G2-phase they prepare to share these copies equally between two daughter cells during mitosis or M-phase. There are some variations of mitosis during development, like the rapid embryonic cell cycle in animals lacking both gap phases (G1 and G2), and the endoreduplication or endocycle where the S-phase is not followed by mitosis. In plant organs, cells frequently switch mitosis to endocycle during their differentiation process.

The collective results from studies in various eukaryotes have demonstrated that cell cycle progression is controlled by the activity of cyclin-dependent kinases (CDKs) (Morgan 2007). In multicellular organisms, cell division is tightly regulated in order to avoid uncontrolled cell proliferation as well as to allow cells to leave the cell cycle and differentiate into various cell types in concert with their developmental program. The progression through cell cycle is highly regulated, particularly at the transitions from G1- to S-phase, from G2- to M-phase, and at the exit from M-phase back to G1-phase. These cell cycle transitions represent the main control or checkpoints of cell cycle. The decision to enter or leave the cycle is taken in G1-phase and regulated by complex external and internal signals. According to current animal models, the differentiation of cells correlates with the lengthening of the cell cycle, in particular the G1-phase. In animals it is suggested that the long G1-phase allows the accumulation of factors needed for differentiation. Therefore, the lengthening of G1-phase is rather a cause not a consequence of differentiation (Lang and Calegari 2010). Animal and plant development are significantly different, however the key molecular mechanisms regulating the decision, whether to enter or leave the cell cycle in G1-phase are remarkable similar (see below).

In the G1-phase of the cell cycle, there is a molecular checkpoint (called START in yeast cells or restriction point in animals) after which the cells are committed to divide. This checkpoint is marked by the expression and activation of the so called G1/S-type cyclins and the interphase CDKs (Elledge 1996; Harashima et al. 2013). Most eukaryotic cells divide only in the presence of mitogens triggering cells to pass through this G1/S cell cycle checkpoint. Mitogens act through the release of breaks otherwise blocking the G1/S-type cyclin expression and CDK activation in order to prevent uncontrolled cell division. Although the core cell cycle machinery is rather conserved in both animals and plants (Harashima et al. 2013; see also in the text below and on Fig. 1), the nature of their mitogens as well as the associated signalling pathways are largely different.

There are many functionally and structurally different cell types in animals and plants; however, all these cells could simply be classified as differentiated or undifferentiated. From the cell cycle point of view, the undifferentiated cells could be further divided as cycling cells or quiescent cells. For example, in animals, the embryonic stem cells are fast dividing cells with a very short resting phase (G1, see later) while the adult stem cells are usually in a quiescent phase and rarely divide. However, both of these cells have the ability to regenerate themselves. To understand how the transitions are regulated and coordinated between these cell types during development, and how the decision is made between cell division and differentiation, we can establish that in a multicellular organism cell division activity has to be maintained to allow growth as well as cell replacement. At an exact time and place during development, however, the undifferentiated cells stop dividing and start to specialize in order to become differentiated cells. These cells form tissues and organs. In spite of its importance, it is still not entirely known, why and how cells adopt different cell fates during development. Although during the previous decades our knowledge about the molecular control of the eukaryotic cell cycle increased considerably, we are just starting to face the complexity of its integration with developmental regulation.

In this chapter, the cell cycle machinery governing the eukaryotic cell cycle is briefly introduced with the linked molecular events allowing the coordination of cell division and differentiation. That is followed by a general overview of the distinct mitogenic signalling pathways in animals and plants.

The core of the eukaryotic cell cycle machinery – One for all, all for one?

The eukaryotic cell division cycle is centrally governed by members of the class of CDKs (Fig. 1). Their periodic activity regulates the progression through the cell cycle phases. In order to be active, the CDK holoenzymes have to be associated with cyclins, phosphorylated by CDK-activating kinases (CAKs) and dephosphorylated by CDC25-like phosphatases (Morgan 1995). The CDK-cyclin complexes can also be subjected to negative regulation via direct phosphorylation (*e.g.*, by the Wee1 kinase), or steric, or catalytic inhibition by CDK inhibitor proteins (Morgan 1995). Cyclins not only activate the CDKs, but contribute to the substrate specificity and the cellular localisation of the complex (Truman et al. 2001). Therefore cyclins are central determinants of cell-cycle-phase-specific CDK functions. The CDK-based regulatory system is quite complex in mammalian cells; they have many CDK isoforms and multiple families of cyclin partners, which regulate specific cell cycle events. In contrast, the genetic

model organisms of cell cycle studies, the yeast cells, have only a single CDK gene (designated as Cdc2 and CDC28 in the fission and budding yeast, respectively) that is primarily involved in cell cycle control. Progressing through cell cycle the Cdc2/CDC28 kinase forms a complex with different cyclins at different phases. At the START checkpoint, Cdc2/CDC28 forms a complex with G1 cyclins (the fission yeast Puc1 and Cig1 or the budding yeast CLN1, 2, and 3 – Morgan 2007; Bertoli et al. 2013). The entry into the S-phase requires the complex formation between Cdc2/CDC28 and the B-type cyclins Cig1 and Cig2, or CLB5 and CLB6. Subsequently, G2 phase progression and mitosis is governed by the Cdc13- or CLB1-4-containing CDK complexes. All these data indicate that the cell cycle phase specificity is achieved by the interchange of cyclin regulatory subunits of the CDKs (qualitative model – Hochegger et al. 2008). In a previous model, however, it was suggested that the progression through the cell cycle is actually regulated by the different levels of kinase activities (quantitative model – Stern and Nurse 1996). These models were recently tested in the fission yeast (Coudreuse and Nurse 2010). The single Cdc2 and all the important cyclins (Cdc13, Cig1-2, and Puc1) were replaced with a single chimeric gene where the Cdc13 and Cdc2 genes were fused together and expressed under the control of the Cdc13 promoter. Surprisingly, these mutant yeast cells could grow and divide without any G1 cyclins. Coudreuse and Nurse further engineered the Cdc2-Cdc13 fusion molecule by introducing a mutation into the Cdc2 component that became sensitive to inhibition by an ATP analogue (Coudreuse and Nurse 2010). There was a difference in the inhibitor concentration to delay G1/S and G2/M cell cycle phase transitions; while the addition of a small amount of inhibitor in G2 was sufficient to block mitosis, ten times more inhibitor was needed in G1 to stop the cell cycle entry. All these data fully support the quantitative model. The oscillating kinase activity is the only essential feature necessary to drive all of the key cell cycle events. Accordingly, the low level of CDK activity triggers the entry into S-phase; the medium level blocks the repeated S-phase in G2-phase, while a burst of activity is required for the entry into mitosis.

Do interphase CDKs interlink G1 phase length and differentiation?

In contrast to yeast, the cell cycle in metazoa is controlled by several CDK-cyclin complexes (Cross et al. 2011; Harashima et al. 2013) (Fig. 1A). For example, in the early G1 phase, CDK4 and CDK6 pair with D-type cyclins, whereas the entry of cells into S-phase is controlled by CDK2 in complex with CyclinE and CyclinA (Obaya and Sedivy 2002). During mitosis, CDK1 (the functional homolog of the yeast Cdc2) associates with B-type cyclins (CyclinA and CyclinB). Further studies revealed that CDK1 has a regulatory role in G1/S transition as well (Hochegger et al. 2007), therefore it could function at both the G1/S and the G2/M cell cycle phase boundaries just like the yeast Cdc2. Interestingly, mice survive the absence of individual interphase CDKs (iCDKs – CDK2, 3,4 and 6; Berthet et al. 2003). In addition, most cell types in mice could proliferate in the absence of two or even three interphase CDKs (Barriere et al. 2007). Moreover, eliminating the activating subunits of these interphase CDKs (*e.g.*, E- and D-type cyclins) provided similar results (Kozar et al. 2004). When all these interphase CDKs were knocked out, mouse embryos underwent organogenesis and developed to midgestation (Santamaria et al. 2007). In the absence of interphase CDKs, CDK1 was able to bind to all cyclins, and could drive the cells through the whole cell cycle. Accordingly, mouse embryos could not develop in the absence of CDK1. Parallel elimination of its regulatory subunit CyclinB1 caused an early death during embryogenesis (Brandeis et al. 1998). All the above data support that CDK1 is the only essential cyclin-dependent kinase in the mammalian cell cycle. This picture is quite similar to the yeast model, but then why do the mammalian cells have several interphase CDKs? Although the early embryonic development does not suffer from the loss of interphase CDKs, later these embryos die as a result of various failures during organogenesis. So, their functions were found to be necessary for the proliferation of specific cell types (*e.g.*, CDK2 in germ cells, CDK4 in pancreatic beta cells; Santamaria et al. 2007). Further studies indicated regulatory roles for iCDKs in the coordination of proliferation and differentiation (Lange and Calegari 2010; Hindley and Philpott 2012; Kaldis and Richardson 2012; Lim and Kaldis 2012). Pluripotent embryonic stem cells are rapidly dividing cells with remarkably short G1-phase. Short G1-phase is maintained in these cells by the constitutive activity of CDK2 due to the continuous expression of CyclinE and CyclinA throughout the embryonic cell cycle. In contrast, the differentiation of pluripotent embryonic stem cells correlates with lengthening of the G1-phase and the switching of the cell cycle profile back to the canonical one. Namely, the G1/S checkpoint is established to restrict the entry into S-phase, which allows the fine tuning of the proliferation speed. In agreement, the expression of CyclinE becomes restricted again to the G1/S boundary. Manipulating the length of G1-phase by modulating the activities of iCDKs shows a strong connection between cell proliferation and differentiation. The overexpression of CDK4/CyclinD resulted in a short G1-phase and favoured the expansion of stem and progenitor cells while inhibited the differentiation in the nervous system (Artegiani et al. 2011). Decreasing CDK4/CyclinD levels did the opposite; it increased the length of G1-phase and stimulated neurogenesis. The double knock out *cdk2/cdk4* mice (DKO) die during embryogenesis due to organ defects, such as heart failure, but the structure of their brain also shows abnormalities, particularly in the cortical

plate. The embryonic neural stem cells of these DKO mice do not show proliferation problems but they differentiate earlier. The G1 length of these cells was found to be increased.

How interphase CDKs can regulate cell differentiation? Recent studies have indicated that they are able to control the activity of transcription factors involved in differentiation and stem cell maintenance (Lim and Kaldis 2013). For example, during *Xenopus* neurogenesis, CDK2 was found to phosphorylate at multiple sites the neurogenin2 (Ngn2) transcription factor that regulates neuronal differentiation. The highly phosphorylated Ngn2 could not promote the expression of the NeuroD transcription factor, which directly stimulates differentiation. The activity of Ngn2 is gradually elevated in cells containing less active CDK2 and consequently having longer G1 phase (Ali et al. 2011). In myoblasts, CDK activity maintains proliferation by phosphorylating the MyoD transcription factor involved in myogenic differentiation and stimulating its degradation through ubiquitin-mediated proteolysis (Song et al. 1998). Cell cycle regulators can also perform regulatory roles outside the cell cycle. Interestingly, CyclinE for example was found to be highly expressed in post-mitotic neuronal cells (Lim and Kaldis 2013). CyclinE binds to CDK5, a neuronal specific cyclin-dependent kinase and inhibits its activity (Odajima et al. 2011). By modulating the activity of CDK5, CyclinE controls the formation of synapses and participates in the regulation of memory development.

CDK activity is also regulated by two classes of CDK inhibitory proteins (CKIs), INK4a-d and the p21 family (CIP1/WAF1, KIP1 and 2). They have different structures and CDK specificities: while the Ink4 proteins specifically target the CDK4/6 kinases, the p21 proteins are more promiscuous (Fig. 1). The elevated expression of CDK inhibitors such as $p27^{Kip1}$ resulted in longer G1-phase that promoted differentiation (Hindley and Philpott 2012).

Plant specific CDKs; why are more CDKs involved in plants to regulate the G2/M-phase transition?

The cell cycle regulation of higher plants exhibits many unique features (Kuijt and Schnittger 2007) (Fig. 1B). First of all, plants have numerous cell cycle regulatory proteins in each class including CDKs, cyclins, and inhibitors. Nevertheless, CDKA seems to be the major regulatory component which, similarly to the yeast CDC28 and animal CDK1, is involved in both the G1/S and the G2/M cell cycle transitions. CDKA is the closest homolog of yeast Cdc2/CDC28 and the only plant CDK that is able to replace the mutant Cdc2 function in dividing yeast cells (Hirt et al. 1991). *Arabidopsis* embryos lacking CDKA contain just 10% of the wild type cells; however, they complete the embryogenesis and produce much smaller but normal looking embryos (Nowack et al. 2010). These data indicate that the function of CDKA is not essential for embryogenesis and patterning but it is required for cell production. In the male germline, microspores go through two rounds of mitotic divisions; the first and asymmetric one results in a bigger vegetative and a smaller generative cell. The generative cell divides further and produces two sperm cells. In the absence of CDKA function, the first formal division is not affected, however the second mitotic division is unsuccessful resulting in mature pollen with only a single sperm cell (Iwakawa et al. 2006; Nowack et al. 2006). As a consequence, the double fertilization fails, because only the egg cell is fertilized, not the central cell, causing seed abortion due to the lack of endosperm (Nowack et al. 2007). Overexpression of CDKA inhibitory proteins, the Kip-related protein 6 and 7 (KRP6-7) resulted in a similar division defect of the generative cell. As it is expected, CDKA pairs both G1/S-phase specific (D- and A-type) and G2/M-phase specific (B-type) cyclins. Based on our current knowledge, CDKA is the only plant CDK functioning in the G1/S-phase transition. Therefore, it is suggested that the CDKA in complex with D- and A-type cyclins is specialized to control the initiation of cell proliferation that is also regulated by the CDK inhibitor KRPs (Magyar et al. 2012).

In contrast to animals, where novel CDKs have evolved to fulfil G1-specific functions, the plant-linage-specific CDKs, the B-type CDKs (two subgroups, the CDKB1 and the CDKB2 both represented by two genes in *Arabidopsis*) are functioning in the G2- and M-phases (Magyar et al. 2012). While the CDKA expresses at constitutive level during the cell cycle, B-type CDKs show cell-cycle-dependent expressions peaking in G2- (CDKB1;1 and 1;2) or M-phases (CDKB2;1 and 2;2; Scofield et al. 2014). Although their transcript and protein levels oscillate during the cell cycle, they require complex formation with cyclins in order to function as other CDKs. As expected, it was found that their most frequent cyclin partners are the B-type mitotic cyclins further supporting their G2/M-phase specific regulatory role (van Leene et al. 2011). Overexpression of a dominant negative CDKB1;1 kinase mutant in *Arabidopsis* disturbed cell division and reduced stomatal density. In addition, leaf cells of this mutant left the cell cycle prematurely, and their ploidy level was increased due to the activation of endocycles that in plants is often associated with differentiation. Therefore, CDKB kinases might have a direct regulatory role in the decision between cell division and differentiation. It is suggested that CDKB1 regulates the switch from mitosis to endocycle in complex with the S-phase specific CyclinA2;3 (Boudolf et al. 2004, 2009). Surprisingly, it was also shown that CDKB1 kinases could pair with D-type cyclins (*e.g.*, CyclinD6;1) and regulate asymmetric cell divisions in the root meristem (Cruz-Ramirez et al. 2012).

Eliminating the functions of the CDKB2 subgroup by an artificial microRNA resulted in arrested shoot apical meristem indicating that CDKB2 kinases are crucial regulators

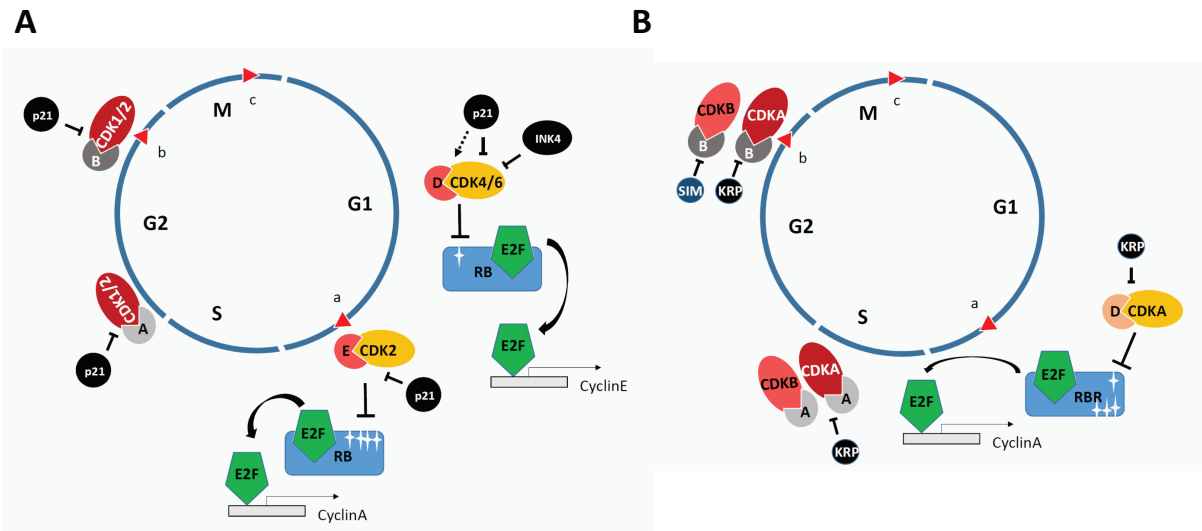

Figure 1. The core regulators of animal (A) and plant (B) cell cycle. The eukaryotic cell cycle is composed of four well defined phases, G1, S, G2 and M. Progression through the cell cycle phases are controlled at checkpoints (red arrowheads) monitoring cell size and the appropriate external conditions including the presence of mitogens (a), the completeness of DNA replication (b), and the correct formation and alignment of chromosomes (c). Animal as well as plant cell cycles are regulated by periodically activated cyclin-dependent kinases (CDK) controlled by various cyclin cofactors (A,B,D,E) as well as CDK inhibitor proteins (INK4, p21, KRP, SIM). In both organisms, overcoming the late G1 (or G1/S) checkpoint requires the CDK-mediated phosphorylation (indicated by asterisks) of the negative regulator retinoblastoma or retinoblastoma-related (RB or RBR) proteins resulting in the release of the E2F transcription factor. The E2F factor initiates the transcription of cyclin genes (among many others) required for the initiation and progression of the S-phase and the rest of the cell cycle. Note, that despite the overall similarity of the regulation, animal and plant cells have different CDK-cyclin complexes. CDK activities are also regulated by phosphorylation both in animals and plants not shown here for simplicity's sake, just as the control of cyclin degradation.

of meristem development and maintenance (Andersen et al. 2008). In contrast to other plant CDKs, the overexpression of these CDKB2 kinases caused developmental defects (growth retardation and organ development problems). Therefore, CDKB2 kinases are not just cell cycle regulators but are also involved in developmental controls.

Further specificities of cell cycle regulators in plants

In summary, plants and animals have specific CDKs that are structurally different, but appear to play similar regulatory functions by controlling the proliferation of specific cell types and coordinating cell proliferation with differentiation. In addition to the CDKs, the other components of the core cell cycle regulation also exhibit specificities in plants. For example, the number of cyclins is especially high in plants (*e.g.*, more than 50 in *Arabidopsis* as well as in rice). While the structure and the role of B-type mitotic cyclins is very similar in animal and plant cells, the D-type cyclins of these organisms share hardly any similarities; moreover certain plant D-type cyclins may play a role not only at the G1/S but at the G2/M transition as well (Kono et al. 2003). Nevertheless *cln* mutant yeast cells could divide in the presence of plant CyclinD genes demonstrating functional homology between yeast and plant CyclinD family members (Dahl et al. 1995). In contrast to yeast, plant and animal CyclinD proteins have a specific motif called LxCxE used for binding to the retinoblastoma tumour suppressor protein (see below). On the other hand, CyclinE homologs are missing from plants. Considering the CDK inhibitor proteins plants do not have INK4 homologs, but several KIP-class proteins named ICKs (interactor of CDKs) or KRPs (KIP-related proteins), although the structural similarity of the animal and plant proteins is very limited (De Veylder et al. 2001). Plants also have unique CDK inhibitory proteins called SIAMESE (SIM – Peres et al. 2007). It is suggested that they have a function to regulate the mitosis to endocycle switch (Kasili et al. 2010), but it is not yet clear whether they control the CDKB kinases or not. The regulation of CDKA by phosphorylation at the G2/M-phase boundary seems to be also different or even non-existent in plants seemingly lacking the homolog of the CDC25 phosphatase (Francis 2011).

The transcriptional module regulating the cell cycle entry

As it was previously emphasized, the decision to enter or leave the cell division cycle is taken in the G1-phase of the cell cycle (although it is widely believed that certain plant cells can leave the cycle in the G2 phase). It is well established that G1 specific CDK-cyclin complexes are activated by dis-

tinct growth stimuli and can drive cells into the cell cycle. In animals and in plants as well, the major target of G1/S CDKs is the retinoblastoma protein (RB) or the retinoblastoma-related protein (RBR), respectively (Fig. 1AB). However, the RB protein such as its targets, the E2F transcription factors (see further) are missing from yeast cells indicating in these single-celled eukaryotes a different transcriptional control mechanism of S-phase entry (Cross et al. 2011). RB was the first tumour suppressor gene identified in mammalian cells and its function as a central regulator of cell cycle progression has been studied extensively. Initially, the tumour suppressor function of RB was thought to be largely due to its capacity to arrest cells in G1 by inhibiting the activity of E2F transcription factors. E2F binds to target promoter sequences in a heterodimeric form in complex with its dimerization partner (DP) and activates the expression of genes required for entering the cell cycle. RB has to bind E2F to inhibit its activity. Interphase CDKs inactivate RB by phosphorylation resulting in the release of the E2F factor that activates the expression of cell cycle genes and promotes the cell cycle entry (Harbour and Dean 2000). However, further studies revealed that RB could achieve G1 arrest in a number of different ways including the regulation of differentiation, chromosomal stability, and protein turnover and some of these actions are actually E2F independent (Burkhart and Sage 2008). In addition, mammalian cells contain a family of RB and E2F proteins; they have two additional RB relatives (p107 and p130), while the E2F family consist of eight members (E2F1-8). Moreover, E2Fs can either activate (E2F1-3) or repress (E2F4-8) transcription and some of them function in RB-dependent (E2F1-5) while others in RB-independent manner (E2F6-8). Recent experiments suggest that activator E2Fs can also function as repressors in a tissue specific manner if they are in complex with RB indicating that the functional classification of E2Fs into activators and repressors is not as rigid as previously thought (Chong et al. 2009).

Drosophila provides a simple E2F-RB system since in this organism there are only two E2F factors, the dE2F1 and dE2F2. The dE2F1 is an activator, while the dE2F2 is a repressor (Frolov et al. 2001). Eliminating the dE2F1 function inhibits cell proliferation restored by the simultaneous loss of repressing dE2F2. Accordingly, dE2F1 activates transcription by replacing the repressor dE2F2 from target sequences. Further studies also revealed that dE2F2 and RBF (the fly homolog of RB) form complexes with MyB and MyB-interacting transcription factors in actively dividing cells and repress the genes involved in differentiation (*D*rosophila, RBF, E2F2 And MyB - DREAM complex; Dimova et al. 2003). DREAM is regulated differently than the traditional E2F-RB complexes since it is insensitive to the traditional RB-kinases consisting of CDK and CyclinD proteins. Similar multiprotein complexes have been purified from other model organisms like *Caenorhabditis elegans*, mouse and human cells (van den Heuvel and Dyson 2008). Although the composition of these complexes is very similar, they have different functions from repressing the G1/S transition to the activation of G2/M transition. However, the exact mechanisms of how DREAM complexes regulate transcription are not entirely identified. It was suggested that plant E2F and RBR proteins could function in DREAM-related complexes, since MyB and certain type of MyB-interacting proteins are also present in plant cells (Magyar et al. 2012). Accordingly, protein complexes related to animal DREAM complexes were recently identified from *Arabidopsis* (Kobayashi et al. 2015). Interestingly, *Arabidopsis* has at least two different DREAM-like complexes during proliferating and post-mitotic stages of organ development (Fischer and DeCaprio 2015).

Although plant and animal developments are significantly different, the transcriptional mechanism regulating the cell cycle entry is remarkably conserved. E2F- and RB-related genes have been identified from the unicellular *Clamydomonas reinhardtii* to higher plants, including the model plant *Arabidopsis thaliana* (Magyar et al. 2008). In *Arabidopsis*, there is a single RB-related gene (RBR) controlling the functions of three E2F transcription factors (E2FA, E2FB and E2FC). Functionally the three E2Fs are classified as activator E2Fs (E2FA and E2FB) and a repressor (E2FC). The RBR function is essential since the *rbr* knock out plant is gametophytic lethal (Ebel et al. 2004), while later, during plant development RBR silencing can cause overproliferation defects leading to growth arrested plants. Repressing the RBR function in root meristems increased the size of stem cell pools, indicating that RBR controls meristem maintenance. The overexpression of RBR causes the opposite, decreases stem cells and stimulates differentiation. Overexpressing E2FA or E2FB resulted in delayed differentiation due to an extended period of proliferation. However, these factors participate in different molecular mechanisms; E2FA forms a complex with RBR to repress genes involved in the regulation of endocycle, while E2FB activates cell cycle genes and its function is repressed by RBR (Magyar et al. 2012). Plant RBR kinases that consist of CDKA and CyclinD proteins phosphorylate RBR and the phosphorylated RBR releases E2FB. In contrast, the E2FA-RBR complex was suggested to be insensitive to these RBR-kinases and its regulation is still unclear (Magyar et al. 2012). In addition, E2F and RB have clear role in stem cell fate decisions as it was first demonstrated in *Arabidopsis*; the repression of RBR or the overexpression of E2FA leads to increase the numbers of stem cells in the root meristem, while ectopic RBR decreases the pool of stem cells by stimulating differentiation (Wildwater et al. 2005). Animal studies clearly demonstrate that E2F and RB can regulate cell fate decisions in various tissues and influence stem cell maintenance and differentiation (Julian and Blais 2015).

In summary, the plant RBR protein can potentially affect all E2F functions and by modifying the activities of the

various E2F factors it can regulate the balance between cell proliferation and differentiation. RBR also can influence the function of other transcription factors involved in cell fate determination (Cruz-Ramirez et al. 2012) and differentiation (Matos et al. 2014). The plant RBR function therefore resembles its animal counterpart and can also be regarded as a transcriptional co-factor able to associate with a number of transcription factors in order to regulate their activities (Burkhart and Sage 2008).

Divide and rule - mitogen signalling in animals and plants

As discussed in the previous sections, cell division activity has to be firmly balanced with cell differentiation to ensure proper tissue and organ development in multicellular organisms. The progression through the cell division cycle therefore is controlled by breaks (e.g. CKIs, RB) and engines (CDK-cyclin complexes). Whenever cell division is required during development these breaks have to be released and the engine has to be started. This is what mitogens or cell division-activating substances do in animals as well as in plants. The molecular pathways linking mitogens to cell cycle regulation in these organisms are overviewed and compared below.

Mitogen signalling in metazoa

Due to historical reasons, mitogens in animals are frequently referred as growth factors, therefore these two terms are used interchangeably in the literature. The majority of natural mitogens are secreted proteins, such as the platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and fibroblastic growth factor (FGF) (for details see http://themedicalbiochemistrypage.org/growth-factors.php).

These mitogens act through cell surface receptors which are in many cases receptor tyrosine kinases (RTKs) (Jones and Kazlauskas 2001) (Fig. 2). These receptors have extracellular ligand-binding domains, transmembrane domains, and intracellular protein kinase domains. Ligand binding causes homo- or heterodimerization of the receptors and subsequent trans-phosphorylation of their cytoplasmic region on a specific tyrosine residue. This phospho-tyrosine is recognised and bound by scaffold proteins having PTB (phospho-tyrosine-binding) or SH2 (SRC Homology 2) domains. The scaffold proteins include the GRB2 (Growth factor receptor-bound protein 2) and SHC1 (SRC homology 2 domain containing transforming protein 1). These adaptor proteins binding the activated receptor alone or in complex with each other recruit cytoplasmic regulatory proteins to the plasma membrane. One of these proteins is the SOS (Son of sevenless homolog) protein. SOS is a RAS guanine nucleotid exchange factor or RASGEF that promotes the nucleotide exchange on the plasma membrane-localised small GTP-binding protein called RAS (abbreviated from „rat sarcoma" reflecting the effect of the mutation leading to the discovery of the first RAS protein).

RAS belongs to a large protein superfamily of small (mostly app. 21 kiloDalton) GTP-binding (or G-) proteins named after the founding member as "RAS superfamily" (Wennerberg et al. 2005). G-proteins serve as two-state molecular switches: they are switched on and transduce signals in their GTP-bound conformation and are switched off when are bound to GDP. They have intrinsic GDP-to-GTP exchange as well as GTP-hydrolysis activities, therefore can cycle between their active and inactive states. However, in order to fine tune their signalling activity, their biochemical cycle is regulated by several proteins falling mainly into three types (Wennerberg et al. 2005). They are activated by guanine nucleotide exchange factors (GEFs) promoting the GDP-to-GTP exchange as mentioned above, while inactivated by the GTPase accelerating proteins (GAPs) that enhance the hydrolysis rate of the bound GTP and therefore result in the accumulation of GDP-bound proteins. The third family is that of the guanine nucleotide inhibitors (GDIs) blocking the GDP to GTP exchange, therefore the activation of G-proteins and, in parallel, preventing the membrane association of proteins. The G-proteins in the GTP-bound concentration can interact with a plethora of effector proteins transmitting the signals to further cellular targets. The RAS superfamily can be subdivided into several families of G-proteins with a similar structure, biochemical activity, and regulation, but with characteristically different cellular functions and, consequently, with various effectors (Wennerberg et al. 2005). The five main families are the ARF/SAR1 family involved in membrane vesicle formation, the RAB family devoted to vesicular membrane targeting, the RAN family regulating nucleus/cytoplasm protein transport, the RHO family mainly controlling cytoskeletal dynamics, and the RAS family having a role in mitogenic signalling.

As ligand binding to the growth factor receptor activates the SOS protein, GTP-bound RAS accumulates at the cell cortex (Wilkinson and Millar 2000; Jones and Kazlauskas 2001). The activated RAS turns on signalling through a kinase cascade in which protein kinases activate each other in a sequential manner (Wilkinson and Millar 2000) (Fig. 2). The first member of the cascade is the RAS effector kinase RAF (Rapidly accelerated fibrosarcoma) kinase that phosphorylates MEK/ERK (Mitogen-activated protein/Extracellular signal-regulated kinase kinase) that in turn phosphorylates the MAP kinase (Mitogen activated protein kinase or MAPK). This phosphorylation chain is referred as the MAP kinase cascade. The final targets of the cascade are nuclear transcriptional factors that regulate the transcription of genes associated with cell division. One of these transcription factors is the c-MYC (the name coming from the similarity to the

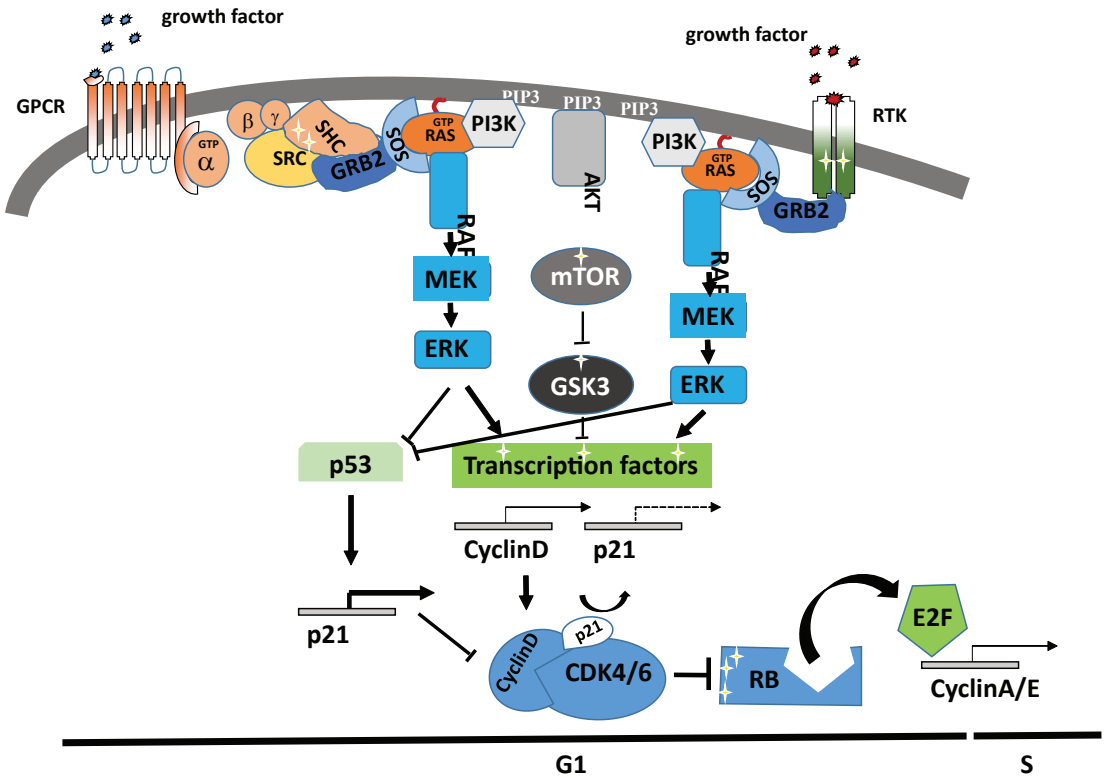

Figure 2. Simplified scheme of mitogen signalling in animals. See details in the text.

product of the myelocytomatosis viral oncogene). Phosphorylated c-MYC among others promotes the transcription of the G1 cell cycle phase specific CyclinD gene. Growth factor signalling contributes not only to CyclinD accumulation, but also to the assembly of the CyclinD-CDK4/6 complex (reviewed in Wilkinson and Millar 2000). This is due to the MEK/ERK kinase cascade-mediated transient accumulation of the p21-type CDK-inhibitor proteins which at this stage act as assembly factors promoting the complex formation between the kinase and its cyclin partner. The accumulation of the CyclinD-CDK4/6 complex results in RB phosphorylation and the release of the E2F transcription factor that initiates the events required to irreversibly overcome the G1/S restriction point as described earlier.

The activated RTKs also recruit and tightly bind the SH2-domain-containing multi-subunit phosphoinositide 3 kinase (PI3K) (Wilkinson and Millar 2000) (Fig. 2). PI3K is a RAS effector and gets active only if associated with GTP-bound RAS initiating a second signalling pathway promoting cell cycle entry. The PI3K-mediated local accumulation of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) in the membrane results in the membrane association of Protein kinase B (PKB; also called as Akt kinase) that activates further downstream kinases including mTOR (mammalian Target of rapamycin), a serine/threonine protein kinase (Wilkinson and Millar 2000; Hemmings and Restuccia 2012). The mTOR pathway inhibits the Glycogen synthase kinase-3 (GSK3) that results in the stabilization of transcription factors activating CyclinD expression. mTOR also blocks the p21 CDK-inhibitor protein that contributes to the release of cells from G1 arrest. In addition to mitogen signalling, the PI3K-mTOR pathway is associated with a high number of regulatory networks including those controlling cellular metabolism. Therefore, mTOR is considered to be the central integrator of cell growth and proliferation (Fingar and Blenis 2004).

In addition to RTKs, members of another class of cell surface receptors, the G-protein-coupled receptors (GPCRs) were also demonstrated to signal towards the mitogenic MAP kinase cascade in a RAS-dependent way (Gutkind 2000) (Fig. 2). GPCRs, with more than 1000 members, are the largest and most diverse group of mammalian cell membrane receptors. Thus GPCRs are involved in a variety of cellular responses which include cell proliferation. The cytoplasmic domain of agonist-activated GPCRs functions as a GDP-to-GTP exchange factor (GEF) for the α subunit (Gα) of heterotrimeric GTP-binding proteins. The GTP-bound activated Gα protein may directly interact with effector proteins but also releases the Gβγ dimer that in many cases has a central role

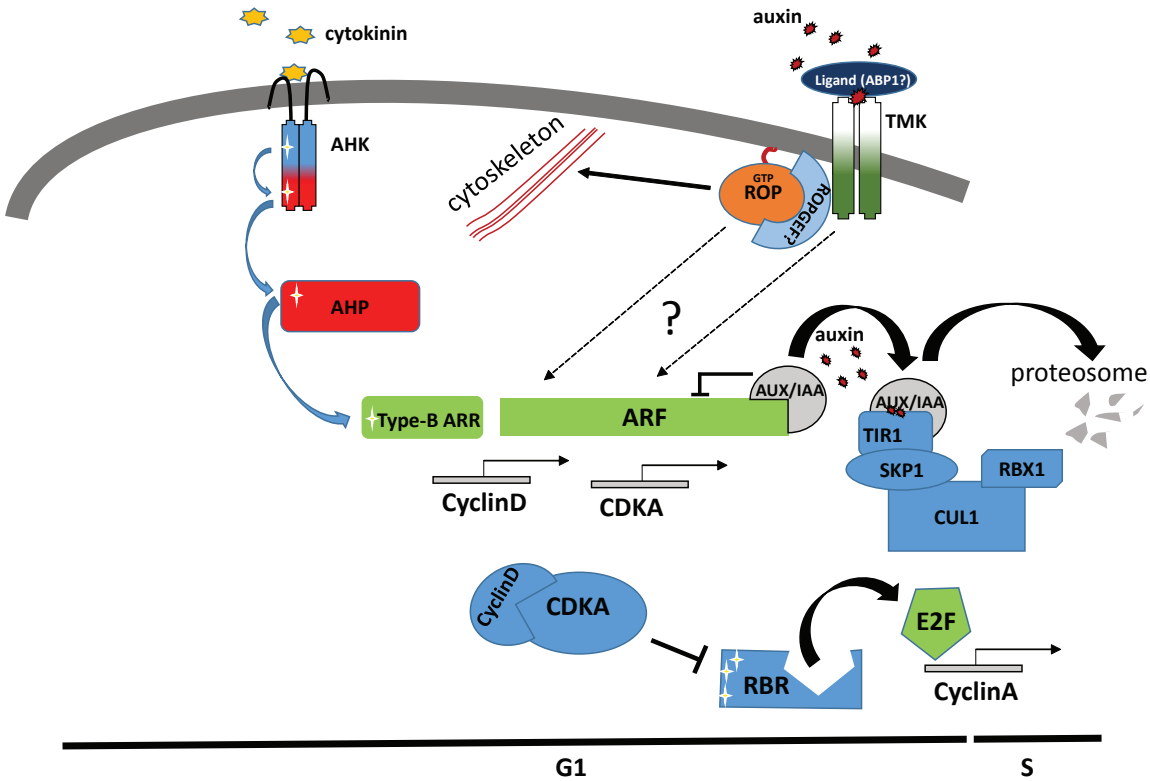

Figure 3. Simplified scheme of mitogen signalling in plants. See details in the text.

in the signal transmission towards cellular targets. In the case of mitogenesis, for example, the Gβγ-mediated activation of the non-receptor SRC tyrosine kinase takes place (the name comes from its homology to the Rous sarcoma virus oncogene V-SRC). SRC phosphorylates an adaptor protein recruiting the RASGEF protein SOS to the membrane that initiates the activation of the MAPK cascade via the GTP-bound RAS protein as described above.

Mitogen signalling in higher plants

As it was discussed above, although with characteristic differences, the core cell cycle machinery is well conserved between plants and animals. It is just the opposite, if one considers the mitogenic signalling pathways. MAPK cascades exist in plants and they are involved in a variety of plant developmental and environmental signalling networks but not in mitogenic signalling per se. Moreover, plants neither have receptor tyrosine kinases, nor functional GPCR receptors, nor RAS GTPases, nor other associated proteins including the growth factors.

In plants, the phytohormone auxin is the central regulator of plant growth and development (Teale et al. 2006). Accumulating pieces of evidence support the primary role of auxin to control cell division activity in plants. Among others, the removal of the auxin from plant cell culture media results in cell cycle arrest after a lag period indicating that auxin is required to maintain cell division activity.

There are at least two different auxin perception pathways (Fig. 3). Receptor-like transmembrane kinase 1 (TMKs) have recently been shown to be required for auxin-dependent epidermal cell shape development in *Arabidopsis* (Dai et al. 2013; Xu et al. 2014). Although TMK function seems to be primarily important for cell elongation, cell proliferation was also affected in certain *tmk* mutants (Dai et al. 2013). TMKs are therefore potentially be involved in mitogen signalling, however, the pathway linking these receptors to the cell cycle machinery is still unrevealed (Fig. 3). As TMKs can't bind auxin directly, it is also unknown how they are activated in an auxin-dependent way. Auxin-binding protein1 (ABP1) (Sauer and Kleine-Vehn 2011) has recently been proposed to serve as a potential ligand for TMKs (Xu et al. 2014). ABP1 was shown to interact with TMK in vitro dependent on auxin bound to ABP1 (Xu et al. 2014). Although ABP1 mostly resides within the endoplasmic reticulum, a small portion is secreted and can be found in association with inner and outer surfaces of the plasma membrane (Sauer and Kleine-Vehn 2011). ABP1 has long been considered as a potential

auxin receptor as it was shown to have important roles in many auxin-dependent cellular processes including rapid electrophysiological responses, endocytosis, cell elongation as well as cell division (Sauer and Kleine-Vehn 2011; David et al. 2007; Perrot-Rechenmann 2010). Recent investigations, however, questioned the role of ABP1 in auxin signalling and raised the possibility that previous approaches were inappropriate to alter and investigate ABP1 functions. The *abp1-5* TILLING line that had been widely used in functional studies was shown to carry several background mutations at other loci (Enders et al. 2015). Moreover, novel *abp1*null mutants were generated that did not exhibit auxin-dependent phenotypes (Gao et al. 2015). In contrast, gain-of-function roles of ABP1 have been found to be dependent on its intact auxin-binding pocket supporting its role as an auxin receptor (Grones et al. 2015). These contrasting findings requires the re-evaluation of the potential role of ABP1 in mitogen signalling leaving still open the question how TMK receptor kinases are activated by auxin.

Auxin is not a proteinaceous substance and can cross the plasma membrane unlike many animal growth factors. Intracellular auxin receptors include the auxin-related F-box (AFB) proteins with the founding member TIR1 (Transport inhibitor response 1) (Tan et al. 2007). AFB proteins are E3 ubiquitin ligases. Auxin-binding alters AFB conformation allowing the docking of the AUX/IAA corepressor proteins, which are degraded by the ubiquitin proteolytic machinery in response to auxin. Degradation of their AUX/IAA corepressors deliberates the ARF (auxin response factor)-type transcriptional coactivators resulting in the rapid activation of gene expression. The AFB-AUX/IAA-ARF pathway is clearly involved in cell division regulation as some of the mutants disrupting this signalling chain exhibit cell division defects (Okushima et al. 2005; Perrot-Rechenmann 2010). Many core cell cycle genes have auxin responsive elements in their promoter regions indicating the regulation by ARFs. However, the significance of this regulation still awaits experimental validation in most cases. During lateral root formation, the indirect effect of ARFs via the downstream Lateral organ boundary (LBD) transcription factors has been reported (Ikeuchi et al. 2013). The LBD factors having a role in this process were shown to activate the expression of the E2FA transcription factor required for S-phase progression. The overexpression of LBD genes induce the over proliferation of root tissues in a greater extent than that of E2FA. This indicates that LBDs may also regulate additional cell division factors (Ikeuchi et al. 2013).

TMK-dependent cell membrane and AFB-AUX/IAA-ARF-dependent nuclear auxin perception and signalling pathways might hypothetically be interlinked and therefore they might contribute together to mitogen signalling.

Cytokinin can also be considered as a mitogenic plant hormone, although it may also serve as an anti-mitogenic agent depending on the target tissue (Schaller et al. 2014). Cytokinin signalling follows a special route as this plant hormone is perceived by histidine kinases (AHKs, for *Arabidopsis* histidine kinases). These receptors activate two-component phospho-relay systems including *Arabidopsis* histidine phosphotransferase proteins or AHPs and the Type-B *Arabidopsis* response regulators or ARRs that are transcriptional activators (Fig. 3). This type of receptor signalling is missing from animal organisms (for a comprehensive review on cytokine signalling see Hwang et al. 2012).

Although the mitogenic plant hormones and animal growth factors mostly target similar cell cycle components (see further for details), their signalling pathways are fundamentally different. Multicellularity evolved in plants and animals independently, therefore it is not surprising that in these organisms intercellular signalling is based on different molecular components. Most of plant cell surface receptors belong to receptor serine/threonine kinases (termed as receptor-like kinases or RLKs) with no evolutionary relation to animal receptor tyrosine kinases (RTKs) (Shiu and Bleecker 2001). Based on their kinase domain, plant receptor kinases are relatives of the non-receptor animal PELLE kinases but evolved to have receptor kinase configuration (with extracellular ligand-binding and transmembrane domains) and went through huge expansion in plants with more than 600 members in *Arabidopsis* (Li and Tax 2013). Despite of their different evolutionary origin, animal RTKs and plant RLKs function in a similar way: they get activated through binding of an extracellular ligand that is often followed by their homo- or hetero-dimerization and auto- or transphosphorylation on their intracellular kinase domain (Afzal et al. 2008). When phosphorylated, they recruit to the membrane and may or may not phosphorylate diverse signalling molecules.

The other class of cell surface receptors, the G-protein coupled receptors (GPCRs) regulating mitogenic signalling in animal cells is missing from plants (Urano and Jones 2014). Although plants have structurally more or less similar transmembrane proteins, those do not possess the nucleotide-exchange factor (GEF) activity, a characteristic feature of many animal GPCRs. Consequently, the few members of the plant heterotrimeric G-protein family are self-activating and their signalling activity is regulated in different ways than in animals (Urano and Jones 2014). Nevertheless, plant heterotrimeric G-proteins are involved in a variety of cellular processes, including hormone and nutritional responses that may influence cell division.

Plants do not have cognate RAS proteins either (Vernoud et al. 2003). Moreover, the RAS-homologous, RHO, small GTPase families are also underrepresented in plants (Vernoud et al. 2003). Plants possess only a single signalling-type small GTPase family designated as Rho-of-plants or ROP. In the absence of RAS, it is hypothesized that ROPs might be involved in MAP kinase cascade activation in plants. This hypothesis

is best supported by the experiments which showed that the OsRAC1 ROP protein and the OsMAPK6 kinase are in the same complex and act together during rice pathogen defence reactions (Kawano et al. 2010). Although the role of ROPs in auxin-activated gene expression could also be experimentally demonstrated in *Arabidopsis* and tobacco (Tao et al. 2002, 2005), and auxin also seems to affect MAP kinase signalling (Colcombet and Hirt 2008), the existence of a classical mitogenic MAP kinase cascade is yet to be revealed in plants if exist. Interestingly, MAPK signalling was found, however, to regulate plant cell division during the G2/M cell cycle phase transition and cytokinesis (Zhang 2008).

In plants, similarly to animals, the evolutionarily conserved TOR kinase is implicated as a central integrator of metabolism, cell growth, and proliferation including mitogen (auxin) signalling (Bögre et al. 2013). Although, the signalling pathways upstream as well as downstream of TOR are yet to be fully revealed in plants, it is obvious that they also have many plant-specific components. For example, recently it was shown, that S6K1, one of the downstream targets of TOR kinase forms a complex with RBR and potentiates its nuclear localization and repression of E2FB (Henriques et al. 2010; Bögre et al. 2013). In addition, TOR kinase was found to phosphorylate E2FA in response to the production of photosynthesis-derived glucose resulting in E2FA activation and cell cycle entry in the non-dividing root meristem (Xiong et al. 2013).

The functional similarity between auxin and animal growth factors is emphasized by the fact that auxin exerts its effect on the same molecular components of the cell cycle, namely on the G1-specific CyclinD-CDK complex (Del Pozo et al. 2005; Perrot-Rechenmann 2010) (Fig. 3). Auxin was reported to activate CDK and cyclin genes as well as to down-regulate the production of CDK inhibitor proteins.

However, the hormonal regulation of plant cell division is rather complex (Del Pozo et al. 2005). Among others, although auxin is capable to induce the expression and assembly of the G1-specific CDKA complex, cytokinin is required for its full activation (Pasternak et al. 2000; Del Pozo et al. 2005). In *Arabidopsis*, CyclinD3 was found to be the rate limiting CDKA partner triggering the progression through the G1/S checkpoint (Kuijt and Schnittger 2007). CyclinD3 expression is induced by auxin as well as by cytokinin and sugar availability (Kuijt and Schnittger 2007; Schaller et al. 2014) (Fig. 3), and therefore is capable to integrate various signals monitoring the cells' nutritional and developmental status.

Similarly as described for animal cells, the activated CyclinD-CDK complex of plants is capable to bind and phosphorylate the plant RBR (for Retinoblastoma-related) protein that similarly to its animal counterpart (the RB protein) is a negative regulator of cell proliferation (see before and Kuijt and Schnittger 2007) (Fig. 3). Phosphorylation of RBR results in the derepression of the activity of plant E2F transcription factors that can switch on the genes required for S-phase entry and cell cycle progression. Auxin was also shown to stabilise one of the activator E2Fs, the E2FB protein (Magyar et al. 2005). Moreover, co-overexpression of E2FB with its dimerization partner DPA could maintain proliferation in the absence of auxin, which is analogous to what was found for mammalian E2F1 stimulating S-phase entry in cells that would otherwise arrest in the absence of growth factors (Johnson et al. 1993). Interestingly, elevated E2FB/DPA levels resulted in a higher number of cells but with a smaller cell size. Therefore it was suggested that E2FB does not merely uncouple cell growth from the cell cycle, but also actively represses growth. Recently it was discovered that E2FB negatively regulates the 40S ribosomal protein S6 kinase 1 (S6K1), the central regulator of cell growth providing a molecular mechanism how E2FB can control this process (Henriques et al. 2010, 2013).

Our knowledge on the exact molecular steps involved in mitogenic plant signalling is rather limited at present, but it obviously is rather complex and follows different ways than learned in animal systems. This might be the consequence of the life strategy of plants that requires strong but at the same time flexible integration of environmental and developmental signals into the regulation of cell division.

Conclusions and perspectives

In summary, cell production is regulated by the activity of cyclin-dependent kinases (CDKs) both in animals and plants. Cell cycle progression is controlled by structurally and functionally conserved CDKs found in every eukaryotic organism from plants to humans including yeasts. Further studies show that specialized CDKs developed independently to regulate cell differentiation in multicellular organisms, which are therefore characteristically different for animals and plants. In animals, CDKs called interphase CDKs or iCDKs evolved to determine whether cells commit themselves to division or differentiation in the G1-phase of the cell cycle. They program cells by modulating the activity of transcriptional regulators such as the retinoblastoma tumour suppressor (RB) and cell-type-specific transcription factors involved in differentiation. In plant cells, specific interphase CDKs called B-type CDKs emerged to regulate the molecular mechanisms involved in the switch from the mitotic to the endocycle in the G2-phase of the cell cycle, and also to determine whether cells halt or continue proliferation. Curiously, B-type CDKs can additionally regulate the asymmetric divisions of plant stem cells throughout plant development. Both in animals and plants, cell division activity is dependent on mitogenic signals that serve to release the breaks that otherwise prevent uncontrolled cell divisions. These signals mostly, but not exclusively, converge on CDK inhibitor levels as well as

on the RB- or RBR-mediated transcriptional control of cell division genes at the G1/S cell cycle phase boundary. Despite their similar targets, the chemical nature of mitogens as well as the associated signal transduction cascades is different in animals and plants. Many details of these signalling cascades are still hidden, especially in plants, where even the perception of auxin, the main mitogenic plant hormone, is in debate at present.

For the above reasons, it can be stated that although the basic cell cycle machinery is well conserved in eukaryotes, the independent evolution of multicellularity in plants and animals resulted in largely different pathways to control and coordinate cell division activity and cell differentiation.

It is clear that cell cycle regulation and cell fate decisions are closely interconnected both in animals and plants. The cell cycle regulatory proteins have cell cycle independent regulatory functions in other cellular processes and in cell fate decisions. However, it is still not entirely clear how these basic cell cycle regulatory proteins influence the acquisition of new cell fates. These are direct or indirect processes? Do cell cycle proteins only slow down the cell cycle in G1 phase allowing morphogens to change the fate of cells or do they directly regulate genes involved in cell fate decisions? How the stem cell division is regulated? For example, lengthening the cell cycle in G1 is thought to be permissive for differentiation in human embryonic stem cells, while it correlates with the maintenance of stemness in adult stem cells. It is still not fully understood what the difference is between embryonic and adult stem cells. Pluripotent stem cells can divide and have self-renewal potential and they also have the ability to differentiate into different cell types. On the other hand, these stem cells can be extremely tumorigenic, which is a major concern of their use in targeted cellular therapies. The contribution of mitogens and related signalling cascades in the malignant transformation of stem cells requires special attention. In conclusion, the understanding of the molecular mechanisms regulating the switch between cell division and differentiation is a basic prerequisite to successfully manipulate cell stemness, both in plants and animals. The control of stemness in animals, and particularly in humans, is strongly linked to hopes for future therapeutic benefits including the use of stem cells as regenerative cellular therapies as well as to identify molecular targets for cancer treatments. In contrast, stem cells can easily be induced to form and function in the case of plants. This allows plants to regenerate full organs or even the whole plant from a few or even only one differentiated cell. Furthermore, plant growth is mainly regulated on the level of cell division and cell elongation. Therefore, understanding the molecular mechanisms coupling cell division and differentiation in plants provide the opportunity for further growth improvement in economically important species. In addition, due to this improved knowledge the strong regeneration ability of plants could be better exploited for clonal propagation of individuals with superior characteristics.

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