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Review

Cyclin A2: a genuine cell cycle regulator?

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Abstract

Cyclin A2 belongs to the core cell cycle regulators and participates in the control of both S phase and mitosis. However, several observations suggest that it is also endowed with other functions, and our recent data shed light on its involvement in cytoskeleton dynamic and cell motility. From the transcription of its gene to its posttranslational modifications, cyclin A2 regulation reveals the complexity of the regulatory network shaping cell cycle progression. We summarize our current knowledge on this cell cycle regulator and discuss recent findings raising the possibility that cyclin A2 might play a much broader role in epithelial tissues homeostasis.

Keywords: cytoskeleton; degradation; invasion; mitosis; Rho GTPases; S phase; transcription.

Introduction

The cell division cycle, with its two fundamental events, DNA replication and chromosome segregation, requires a complex machinery to ensure that each round of its different steps is completed before the next one takes place. It is, in a large part, regulated by the oscillating activity of cyclin-dependent kinases (CDKs). CDKs function at different phases of the cell cycle and are regulated by their association with specific cofactors, among which the cyclins play a dual role as activators and targeting agents toward specific substrates. Different cyclins exhibit distinct expression and degradation patterns that contribute to the temporal coordination of each cell cycle event. Thus, the activity of the cyclin D-CDK4 and cyclin D-CDK6 is detected during the G1 phase. At the end of it, CDK2 binds to cyclin E and then to cyclin A. Finally, CDC2/CDK1, in association with cyclins A and B, functions at the G2/M transition and in M [for reviews, see (1, 2)]. CDKs are also controlled by transient interactions with inhibitory partners such as p21, p27, p57, as well as reversible phosphorylation reactions (3).

The specific functions of cyclin A protein at different stages of the cell cycle are dependent upon its CDK partners. Cyclin A is essential for at least two critical points in the somatic cell cycle: during the S phase, *via* the activation of CDK2, and during the G2 to M transition, *via* the activation of CDK1 (4). The cyclin A protein localizes predominantly to the nucleus during the S phase, where it regulates the initiation and progression of DNA synthesis (5). As this cyclin has no nuclear localization signal (NLS), it is likely to be addressed to the nucleus *via* its association with partners with genuine NLS, even though several of previously proposed ones (CDK2, p21, p27, and p107) seem to be dispensable (6).

Phosphorylation of components of the DNA replication machinery such as CDC6 by cyclin A-CDK is believed to be important to ensure only one round of DNA replication per cell cycle (7). At the end of G2, cyclin A relocalizes to the centrosomes in the cytoplasm, where it binds to the poles of mitotic spindles. The precise role of cyclin A in mitosis is still not completely understood and is in part related to the control of cyclin B-CDK1 activity (8–10).

This simplistic vision of the cell cycle is complicated by the fact that all cyclin families consist of several members, and cyclin A does not escape this rule. Two forms of cyclin A exist in mammals. Cyclin A2 is ubiquitously expressed and activates CDK2 or CDK1 kinases to promote both S phase progression and G2/M transition, respectively. In contrast, cyclin A1 is present preferentially in germ cells undergoing meiosis and is abundant in the testis as well as in certain myeloid leukemia cells in humans (11–14). Consistently, its targeted expression in the myeloid lineage leads to an altered myelopoiesis and the development of myeloid leukemia in mice (15). Interestingly, whereas there is no detectable cyclin A2 at both the mRNA and the protein levels in the early male meiotic cycle, both cyclins A1 and A2 are present in murine oocytes. The essential role of cyclin A1 in meiosis was confirmed by the observation that cyclin A1-deficient male germ

cells arrest at the G2/M transition of meiotic prophase I and exhibit some apoptotic phenotypes (16–18). Cyclin A2 is essential for embryonic development, with the null embryos dying at around day 5.5 postcoitum (19). A recently published work using mice with conditional *cyclin A2* alleles confirmed its absolute requirement, not only in early embryonic development, but also in the establishment of the hematopoietic lineage, while pointing to the compensatory effect brought about by cyclin E2 in cyclin A2 deficiency (20).

Cyclin A2 expression and regulation: on the road to degradation

In multicellular organisms the activity of cyclin-CDK complexes is controlled at many levels, including for cyclins: cell cycle-regulated transcription, posttranslational modifications, programmed proteolytic destruction, and even subcellular localization.

Cyclin A2 gene transcription

Cyclin A2 is encoded in a 7-Kpb gene (*cyclin A2* or *ccna2*) made up of 8 exons (21), located on human chromosome 4 (mouse chromosome 3), leading to the synthesis of two mRNA, with length of 1.8 and 2.7 Kb, respectively, differing solely in their 3′-untranslated region (3′UTR). Cyclin A2 mRNA level increases at the G1/S transition and peaks during the S phase, resulting from a periodic relief from transcriptional repression when cells enter S phase (22–26). Cyclin A2 transcriptional regulation is modulated by peripheral cues such as growth factors, TGF-β, as well as cell interactions with the extracellular matrix (27–29). The latter certainly plays an important role in mediating the control of cell cycle regulators expression by the cellular niche. Accordingly, *cyclin A2* transcription is down-regulated in the absence of anchorage. Interestingly, the activated forms of Ras and Cdc42 have been shown to synergistically alleviate the anchorage requirement for cell cycle progression, probably through a down-regulation of p38 MAP kinase (27, 30–33).

The *cyclin A2* gene promoter contains two sites, a CRE and a CAAT box, that are constitutively occupied and bind CREB, ATF, and NF-Y family members *in vitro* and *in vivo*, respectively (23). Moreover, the most 5′ CRE element has also been shown to bind transcription factors such as E4F1 and JunB (34, 35). It is worth mentioning that the latter transcription factor, depending on its level of expression, is likely to exert both positive and negative control on the *cyclin A2* promoter. Cyclin A2 is down-regulated in both MEF cells deficient for the *Jun B* gene (35) or cells treated with antisense RNA directed at its mRNA, as well as in cells where JunB expression is forced (our unpublished observation). Whereas a small level of JunB is required for transcription of *cyclin A2*, a high level will induce that of *p16*, and as an indirect consequence, will repress *cyclin A2* transcription through the inhibition of pRb phosphorylation (36). Consistent with this, *Jun B* is a transcriptional target of TGF-β and an important effector of the epithelial to mesenchymal transition induced by this cytokine (37).

In addition, a transcriptional repression element that is constituted of two modules, cell cycle responsive element (CCRE) or cell cycle-dependent element (CDE), and cell cycle homology region (CHR), was identified, with CHR located 3′ of CCRE/CDE (22, 23, 31). These sites are occupied in quiescent cells and in the G1 phase, and their mutation leads to deregulated *cyclin A2* expression. Transcriptional repression of *cyclin A2* involves promoter-bound complexes, which include E2F members, pRb-related proteins, likely p130, and chromatin remodeling factors such as Brahma/SNF2 (26). E2F4 is proposed to be responsible for the inhibitory phase in quiescent cells and early G1 of proliferating cells and replaced in mid/late G1 by E2F3 (38). However, these data are difficult to reconcile with the loss of *in vivo* footprint associated with the activation of *cyclin A2* transcription. As already mentioned, the *cyclin A2* CAAT box is a high affinity-binding site for NF-Y. The latter protein consists of three subunits (NFY-A, NFY-B, and NFY-C) and is likely to organize the binding of nearby factors. In fact, although it was originally considered as a constitutive transcription factor, later data have shown that its activity could be modulated, in particular through NFY-A expression (39, 40). Along these lines, we have shown that TGF-β, through a modulation of the interplay between MAPK pathways, could finely tune the transcriptional regulation of *cyclin A2* (29). Expression of a dominant negative form of NF-Y delays S phase entry and leads to a prolonged cell division time, whereas *nfy-A* knockout leads to early embryonic lethality (41).

Deacetylation plays a central role in repression of many E2F-regulated genes, but chromatin remodeling is essential for *cyclin A2* gene, and relief of its transcription repression results mainly from the loss of two nucleosomes positioned on the promoter (26, 42–44). For example, whereas *cyclin E* gene transcription is induced when cells are treated with inhibitors of deacetylases, this is not the case for cyclin A2 (26, 42–44), and a switch between HDAC-pRb and SWI/SNF-pRb has been proposed to maintain the order of cyclins E and A expression (Figure 1A).

More recently, a multimolecular complex named DREAM, initially isolated in *Drosophila* [*Drosophila* RBF, E2F, and Myb (45)], has been proposed to be instrumental in controlling the transcription of CCRE/CDE-CHR-containing cell cycle-regulated genes [(46, 47); reviewed in (48)]. Homologues of all subunits of this complex have been found in the human genome and highlighted a family of Myb-interacting proteins that cooperate with pRb in tumor suppression. The core complex contains E2F4/5, DP1/2, and five human homologues of *Caenorhabditis elegans* proteins encoded by the synMuvG group of genes. DREAM switches from a transcriptionally repressive state in G0 and G1 by binding to p130 or p107, but not pRb, and in association with E2F4/5, to a transcriptionally active state in S and G2 *via* the interaction with B-Myb (49–52) (Figure 1B). The presence of B-Myb, itself a product of an E2F-responsive gene, would suggest that *cyclin A2* belongs to a subset of E2F secondary response genes that relies on non-E2F transcription factors for their up-regulation. Interestingly enough, B-Myb is a target of CDK2-cyclin A2 (49–59), thus revealing a positive feedback loop necessary for

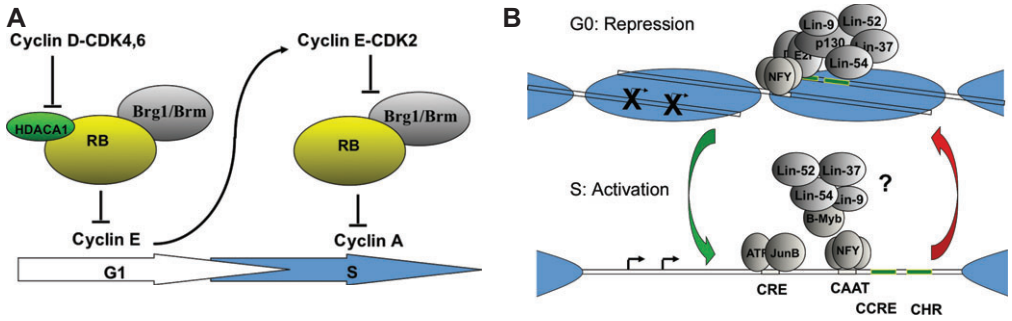

Figure 1 Transcriptional regulation of *cyclin A2*.
(A) HDAC-pRb and SWI/SNF-pRb maintain the order of cyclins E and A expression. (B) Transition from a repressed to an active *cyclin A2* transcriptional state results from both chromatin remodeling and a switch in the composition of bound transcription factors.

genes transcribed in the S and G2 phases, such as *cyclin A2*, *cyclin B*, *survivin*, or *cdk1*.

Finally, cyclin A2 mRNA accumulation is also likely to result from the modulation of its stability during the cell cycle, with a clear stabilization in the S phase and a decrease afterward when cells exit G2. Among the usual suspects, two potential candidates, HuR (human antigen R, or ELAV-like protein 1) and WTAP (Wilms tumor 1-associated protein) have been found to impinge upon cyclin A2 mRNA stability (60–62). The former was first characterized as a specific tumor antigen, and afterward, as the major interactor of AU-rich sequences present in the 3′UTR of unstable mRNAs [for a review, see (63)]. The latter protein was shown to interact both *in vitro* and *in vivo* with WT1 (64, 65) and was identified as the mammalian homologue of the *Drosophila* female-lethal-2-D, a protein involved in alternative splicing. Both proteins have been found to associate with cyclin A2 mRNA 3′UTR, and accordingly, their knockdown resulted in a significant reduction of cyclin A2 mRNA stability. Interestingly, WTAP-deficient cells accumulate in G2, and *wtap*-null mice die at day 6.5 postcoitum. Both phenotypes are reminiscent of what is observed in cyclin A2-deficient cells as well as in *cyclin A2*-null mice (62).

Cyclin A2 protein degradation

The name cyclins comes from the fact that, along with many other cell cycle regulators, they are periodically degraded in cycling cells by the ATP-dependent proteasome machinery, following their ubiquitinylation by two ubiquitin ligases, the SKP1-CUL1-F box protein complex (SCF) and the anaphase-promoting complex, also known as the cyclosome (APC/C) [for reviews, see (66, 67)]. The latter is an E3 ubiquitin ligase that contains a dozen of core subunits and associates with activator proteins such as Cdc20/Fizzy (Fzy) and Cdh1/Fizzy-related (Fzr), which play important roles in substrate recruitment. It is also a target of the spindle assembly checkpoint through proteins such as Mad2, Bub3, and BubR1 (68–70). This occurs when some chromosomes are not yet attached to the mitotic apparatus and thus prevents the APC/C from prematurely degrading securin, the inhibitor of separase, the enzyme that cleaves cohesins, the proteins responsible for sister chromatid attachment. Cyclin A2 is destroyed at the onset of prometaphase when the nuclear membrane breaks down and requires the transient association of Cdc20/Fizzy to APC/C (71, 72). Substrate recognition is ensured by a motif called the destruction box (D-box) localized in the amino terminus of the protein (amino acids 57–72), and this direct binding of Cdc20 allows cyclin A2 to be degraded regardless of the activation state of the spindle assembly checkpoint. The destruction box is necessary but not sufficient, and a more extended region, with a participation of its CDK partner, is necessary for an efficient degradation. Recent data suggest that, in fact, Cks1 (Cdk subunits; Cks1 and Cks2) is a major contributor to cyclin A2 direct binding to phosphorylated mitotic APC/C (73). Whether this involves a modulation of substrate affinity for the degradation machinery through a cooperation between Cdc20 and Cks1 remains to be established. Cdh1 also seems to play a role in cyclin A2 degradation at the G1/S transition because in its absence early cyclin A2 accumulation is associated with premature DNA replication (74).

Cyclin A2 was also shown to bind acetyl transferases such as PCAF (p300/CREB binding protein-associated factor) or GCN5 (general control nonderepressible 5). Acetylation occurs on four lysines present in the amino-terminal half of the protein (Lys 54, 68, 95, 112), two of which (Lys 54, 68) are also targets for ubiquitinylation and concurs to the subsequent ubiquitinylation of cyclin A2. Consistently, the quadruple mutant is expected to be much more stable than its wild-type counterpart, which has a decreased stability in the presence of deacetylase inhibitors such as trichostatin A. However, this does not seem to be the case in all experimental settings examined so far [(75); our unpublished observations].

From cell cycle to motility control: a new role in cytoskeleton dynamic?

Cyclin A2 and cell cycle transitions

As already mentioned, and according to a widely accepted view, whereas cyclin E-CDK2 is necessary for S phase initiation, cyclin A2 works in relay, and in association with CDK2,

is necessary for S phase progression. Later on, a new exchange with CDK1 occurs, and mitosis is triggered following nuclear envelope breakdown that signs the destruction of cyclin A2 and the full activation of cyclin B-CDK1. This switch from CDK2 to CDK1 is proposed to be instrumental in our understanding of the dual role of cyclin A2. When quiescent cells are committed to proliferate, they enter the cell division cycle through activation of cyclin D-CDK4/CDK6 complexes that are, again, according to the classical view, seen as sensors of external cues. Once the restriction point is passed, cells are believed to obey a cell autonomous mechanism, independent of further stimulation by cell growth factors, and then enter an exponentially dividing state during which G1 phase is devoted to the resetting of replication origins. Then, new cyclin synthesis is required to activate CDK2, first with cyclin E, then cyclin A2, and afterward cyclin B, with now the participation of CDK1. Cyclin A2-CDK2 will finish the work of cyclin E-CDK2 at the beginning of the S phase by fully phosphorylating pRB and its related proteins, as well as the proteins of the SWI/SNF complex, thus allowing a new round of transcription of genes coding for proteins required in S and G2/M (Figure 1A). Meanwhile, proteins involved in replication origin firing and licensing such as Cdc6 constitute essential S phase targets (7). When cells progress to mitosis, and while phosphorylating several transcription factors such as B-Myb, NF-Y, or FoxM1 (76), cyclin A2 targets many components of the APC/C to down-regulate its activity in concert with Emi1, whose destruction is a prerequisite to entry into prometaphase (67). Two major phosphorylation events characterize this transition: the massive phosphorylation of histone H3, which is associated with chromosomes condensation, and that of lamins and components of the nuclear pore complexes, which results in loss of nuclear envelope integrity. Cyclin A2 has been proposed to participate in both events (77).

Similarly to chromosomes, centrosomes must be duplicated, and this takes place at the onset of the S phase to allow the faithfully duplicated organelles to move to the poles of the duplicating cell and then to be distributed to the daughter cells. Both cyclins E and A2 associated with CDK2 have been implicated in this phenomenon, with nucleophosmin as an important target (78, 79). Interestingly, cyclin A2 has also been shown to localize to the centrosome in a CDK-independent manner, and through the binding of MCM5 and Orc1, prevents the reduplication of this organelle (80–82). Knockdown of cyclin A2 and inhibition of CDK2 prevent cells from forming stable attachments of their mitotic spindle to the cell cortex (9, 83). This results in the mislocalization of the spindle and an unleashed rotation of the nucleus. Moreover, cyclin A2-CDK2 specifically associates with adenomatous polyposis coli in the late G2 phase and phosphorylates it on Ser1360, whose mutation results in identical off-centered mitotic spindles (83). Thus, astral microtubule attachment to the cortical surface in mitosis relies on the phosphorylation by cyclin A2-CDK2 of critical amino acids within the mutation cluster region of adenomatous polyposis coli.

By now, there are only a few described S phase targets, whereas mitotic ones are much more numerous. However, the full comprehensive list of such substrates remains to be established since the redundancy highlighted by the various knockout models is reflected by the large overlapping *in vitro* substrates when the various complexes are compared. In that respect, cyclin A2 deficiency is compensated for by cyclin E in most adult tissues with the exception of the hematopoietic system (20). Subcellular localization is also likely to play a primeval role because cyclin A2-CDK shuttles between the nucleus and the cytoplasm (84), a situation that is dramatically changed after nuclear envelope breakdown.

Cyclin A2, cytoskeleton, and cell morphology

More recently, our group uncovered a novel aspect of cyclin A2 function that does not seem to require its association to CDKs. Its depletion in fibroblasts leads to an increase in cell motility in wound healing assays and cooperates with oncogenic transformation to increase their invasiveness in collagen matrices (85). Cyclin A2-deficient fibroblasts contain a perturbed cytoskeleton, harboring a cortical localization of actin filaments and a redistribution of focal adhesions. Interestingly, a cyclin A2 mutant unable to bind CDK1 and CDK2 corrects these defects. As cyclin A2 has no NLS, this mutant, which is no longer able to interact with all known partners of the cyclin, remains cytoplasmic. Moreover, redirecting the mutant to the nucleus through the addition of an SV40 NLS abolishes its capacity to restore a wild-type phenotype, strongly suggesting that this novel function takes place in the cytoplasm. Previous studies have already reported the retention of cyclin A2 in the cytoplasm either *via* a complex with the cellular protein SCAPER (86) or under the form of a fusion with a viral protein, consecutive to the insertion of HBV in the *cyclin A2* locus (87, 88). Curiously, in both instances, cyclin A2 was addressed to the endoplasmic reticulum, and in the latter case, this was associated with cell transformation in cooperation with activated Ras. Whether SCAPER is part of a core control of cytoplasmic cyclin A2 functions remains to be established. These data have led us to check whether decreased levels of cyclin A2 might be linked to metastasis. Indeed, cyclin A2 levels are much higher in SW480 cells with respect to SW620 cells, which are derived from a primary colon carcinoma and a distant lymph node metastasis, respectively, in the same patient. Moreover, the same is true in primary colon adenocarcinoma relative to hepatic metastases in matched human tumors (85).

Cyclin A2 deficiency is associated with a down-regulation of the RhoA-ROCK pathway and decreased phosphorylation of cofilin, which is involved in the reorganization of actin filaments, consecutively leading to an increased cell migration and invasion. Importantly, pharmacological inhibition of ROCK in control fibroblasts leads to an increase in migration velocity similar to that of cyclin A2-depleted cells (our unpublished results). Consistent with this, cyclin A2 and RhoA proteins interact both *in vivo* and *in vitro* and recombinant cyclin A2 enhances *in vitro* the exchange activity of RhoA-specific GEFs. As RhoA, RhoB, and RhoC share more than 95% sequence similarity, it is not surprising that cyclin A2 interacts with all three GTPases (85). Indeed, when cyclin A2 is knocked down in epithelial cells, such as normal mouse

mammary epithelial cells (NMuMG), they exhibit a strong down-regulation of RhoA activity and an increase in that of RhoC (our unpublished observation). No interaction or any variation in its activity was detected with Rac1, suggesting that the balance usually observed between the activities of Rho and Rac GTPases is disrupted in cyclin A2-deficient cells. These data complete earlier observations on other cell cycle regulators [reviewed in (89); commented in (90)] and are summarized in Figure 2. Whereas p57, p21, and cyclin D1, *via* p27, are inhibitors of the RhoA-ROCK signaling pathway, cyclin A2 leads to its activation. This is likely at the expense of RhoC, as the two GTPases share probably the same GEFs, but this remains to be established.

How can we link subcellular localization and degradation of cyclin A2 to the completion of mitosis? Future challenges

The reciprocal alteration of RhoA and RhoC activities observed in cyclin A2-deficient epithelial cells is reminiscent of what is observed during the epithelial to mesenchymal transition (EMT) (91). Interestingly, whereas RhoC is dispensable for embryonic and postnatal mouse development (92), its loss leads to a drastic inhibition of metastasis without affecting tumor development at the primary site. These observations call for more work on the molecular mechanisms through which cyclin A2 impinges upon cellular morphology, most particularly in the context of EMT.

As already mentioned, cyclin A2 shuttles between the nucleus and the cytoplasm, a compartmentalization that is obviously abolished once the nuclear envelope breaks down. The targeting of the centrosomes by mitotic kinases raises the question of its potential influence on the relationships entertained by this organelle with other structures. In this respect, the Golgi apparatus is a good candidate because the close proximity of these two structures suggests that they are engaged in functional interactions (93). Indeed, mitotic spindle orientation and cell polarization are likely to be the two sides of the same coin. The Golgi is fragmented during mitosis when the nuclear envelope is disrupted, and both processes contribute to the accumulation of lipidic membranes and vesicles in the cytoplasm. Later, a constriction forms in a plane perpendicular to the spindle axis in preparation for the last phase of mitosis, which culminates with the separation of the two daughter cells. Specifying the orientation of the mitotic spindle, and therefore the plane of the contractile ring, plays a central role during the development of epithelia and wound healing. Basal progenitor cells may undergo symmetric or asymmetric divisions, depending on the orientation of the mitotic spindle with respect to the basement membrane. The outcome for the tissue is twofold: an increase in surface or in volume, on the one hand, and morphogenesis through a change in cell fate, on the other.

Cyclin A2-deficient fibroblasts are depolarized, as visualized through Golgi staining with snail lectin (our unpublished observation). Recently, *Drosophila* cyclin A localization was proposed to be a downstream target of Par-1 in male germ cells through a currently unclear mechanism (94). PAR proteins are conserved and form complexes containing, among other proteins, Par-3, Par-6, and aPKC (atypical protein kinase C), which are dedicated to the control of cell polarity [for a review, see (95)]. One important insight is that Cdc42 has been implicated in the control of cell polarization through the formation of a complex with Par-3, Par-6, and aPKC. In mammalian cells, the orientation of the cell division axis is strongly influenced by the extracellular matrix that interacts at focal adhesion sites with integrins (96, 97). Among their known cytoplasmic effectors, the integrin-linked kinase localizes to the centrosome and participates on the mitotic spindle organization (98), a situation reminiscent of a previous work linking Cdc42 activation and integrin engagement to the establishment of polarity in migrating cells (99). An interesting challenge is now to establish the possible functional interactions between this GTPase and cyclin A2 during epithelial morphogenesis (100, 101) (Figure 3).

More puzzling is the direct binding of cyclin A2 to RhoA that appears, at least *in vitro*, to facilitate its GTP loading by GEFs. This is consistent with the involvement of RhoA in early mitosis, when its increased activity leads to cortical retraction and cell rounding *via* its downstream effectors ROCK and mDia. In addition, the formation of the contractile ring during cytokinesis depends upon RhoA activation in a precise zone at the cell equator (102) (Figure 3).

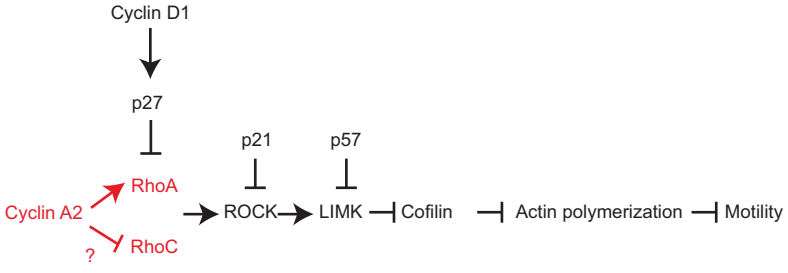

Figure 2 Cell cycle regulators and the Rho/ROCK signaling pathway.
Although cyclin D1, p27, p21, and p57 have been shown to inhibit RhoA or its downstream effectors, cyclin A2 appears to be required for its full activation. Cyclin D1 binds directly to p27 and thereby blocks RhoA activation by inhibiting interaction with its GEF (105–108). Cytoplasmic p21 has been shown to bind and inhibit ROCK1, whereas p57 was shown to sequester LIMK.

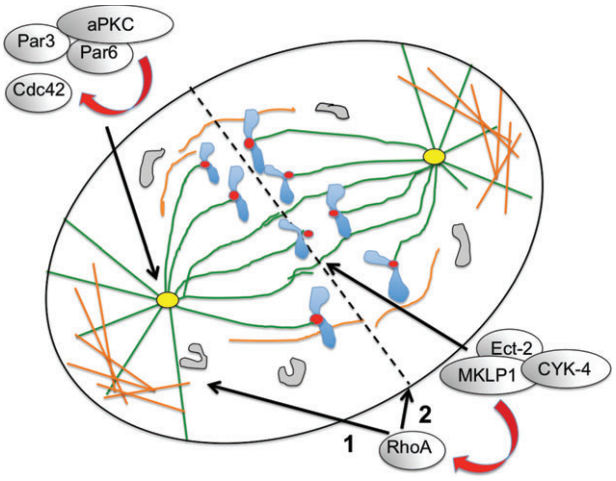

Figure 3 Rho GTPases, cell polarity, and mitosis.
RhoA is involved early in mitosis (1) to induce cortical retraction and cell rounding, probably through mobilization of actin (orange), then to orientate the mitotic furrow, and later, to induce the formation of the contractile ring (2) at the cell equator (dashed line). A complex called central spindlin, localized at the spindle midzone and catalyzes microtubule bundling (green), activates RhoA. This contains a kinesin-like protein (MKLP1), a Rho GTPase-activating protein (CYK-4/MgcRacGAP) and the ECT2 Rho GEF, likely responsible for the translocation of active RhoA to the equatorial cortex. RhoA recruits locally citron kinase, which phosphorylates MRLC, thus promoting actomyosin assembly. Actin localizes also to the mitotic spindle in cables, wrapping it and extending to the cortex. Cdc42 interacts with Par3, Par6, and aPKC, which are parts of the polarity complex, and is involved in the interaction of radial asters with the cortex to position the apical pole of epithelial cells. It is also likely to participate to functional interactions between the centrosome and the Golgi apparatus, which is fragmented during mitosis (gray vesicles).

This occurs at a time when, according to the simplistic model presented in the first chapter, there should not be enough cyclin A2 remaining to activate CDK1. A functional role of cyclin A2 at that time would imply its localization to specific subcellular structures that have yet to be described. Moreover, the consequences would be twofold: first, its diversion from the usual degradation pathway, and second, its targeting to the periphery of the dividing cell, where it could participate, perhaps synergistically, with the spindle poles in the definition of the apical-basal axis. Remarkably enough, autophagy, initially described as a process of nonselective recycling of bulk cytoplasmic structures, has already been shown to participate in RhoA-mediated cortical remodeling and cell spreading of macrophages (103). This addresses the relevance of this observation with the morphological transition occurring at mitosis when the cell switches from a round shape to a more flat one during its respreading.

Conclusion

If these observations complete our vision of the intricate relationships entertained by cell cycle regulators and small GTPases, they point to the putative involvement of cyclin A2 in the mechanisms governing the orientation of the mitotic spindle within an epithelial structure. Most particularly, this addresses its potential role as a mediator between external cues and cell autonomous mechanisms in the establishment of cell polarization, i.e., whether the candidate proliferating cell will undergo a symmetric or an asymmetric mode of division. Moreover, cyclin A2 deficiency appears to be also linked to metastatic spreading. Consistent with this, cyclin A2 seems to be down-regulated in some metastatic cells compared with cells from the primary site (85, 104). Even though cyclin A2 interaction with Rho GTPases unveils some new avenues, the nature of the mechanisms at play are still an open question, and going further will certainly require the use of animal models.

Acknowledgments

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