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Review

# Back to the new beginning: Mitotic exit in space and time

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ABSTRACT

The ultimate goal of cell division is to generate two identical daughter cells that resemble the mother cell from which they derived. Once all the proper attachments to the spindle have occurred, the chromosomes have aligned at the metaphase plate and the spindle assembly checkpoint (a surveillance mechanism that halts cells form progressing in the cell cycle in case of spindle – microtubule attachment errors) has been satisfied, mitotic exit will occur. Mitotic exit has the purpose of completing the separation of the genomic material but also to rebuild the cellular structures necessary for the new cell cycle. This stage of mitosis received little attention until a decade ago, therefore our knowledge is much patchier than the molecular details we now have for the early stages of mitosis. However, it is emerging that mitotic exit is not just the simple reverse of mitotic entry and it is highly regulated in space and time. In this review I will discuss the main advances in the field that provided us with a better understanding on the key role of protein phosphorylation/de-phosphorylation in this transition together with the concept of their spatial regulation. As this field is much younger, I will highlight general consensus, contrasting views together with the outstanding questions awaiting for answers.

Cell division (mitosis) is a fundamental process that is at the basis of our existence. From the single cell of a zygote, about 37.2 trillion cells are generated to make up an adult human being. Divisions need to occur in an extremely accurate manner not only to produce daughter cells that are healthy and viable but also to set up the topological cues for differentiation and organ geometry. Defects in cell division have been shown to be at the basis of several human pathologies. For this reason, the process is highly regulated and surveillance points (checkpoints) are present in order to arrest progression to next phase until the previous one is correctly completed. Lots of research has been conducted to study early mitosis progression (prophase, prometaphase and metaphase) and its checkpoint (the spindle assembly checkpoint) that arrests cells from exiting this stage until all the chromosomes are attached to the mitotic spindle; however, much less is known on the molecular mechanisms that are in place to ensure that the events occurring during the late stages of mitosis (anaphase, telophase) and cytokinesis are coordinated to finally generate a fully functional G1 nucleus ready to support the next cell cycle.

Here, I will review the current knowledge on these late mitotic stages and the molecular bases of their coordination.

## 1. Inactivation/re-activation of the protein phosphatases network

Once the Spindle Assembly Checkpoint (SAC) has been satisfied (for a recent review on this aspect please refer to [1]), the progression of the cell cycle from mitosis to G1 is a no-return process that is highly regulated in space and time. Since the discovery of cyclins we have acknowledged that protein degradation is fundamental to drive this transition [2,3] but only more recently has the need for protein de-phosphorylation become more appreciated.

Before starting to analyse these regulations, we need to understand how the major protein phosphatases involved at this stage of cell division Protein Phosphatase 1 (PP1) and Protein Phosphatase 2A (PP2A) work. Both are serin/threonine phosphatases, and they are present in the cells as multi-subunit enzymes. The catalytic subunit of PP1 assembles into different holoenzyme complexes by binding with other proteins called RIPPOs (Regulatory Interactor of Protein Phosphatase One [4]) and it is estimated to be able to generate around 650 different complexes [5]; each RIPPO contains a distinct combination of PP1 binding motives and presents a specific localization pattern [6–9]. The catalytic subunit itself also exists as different isoforms (PP1α, PP1β two splice variants of PP1γ: PP1γ1 and PP1γ2), thus increasing the plethora of different phosphatase holoenzymes that can be generated.

PP2A is instead a trimeric complex composed of a catalytic subunit

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(PP2ACα), scaffolding A subunits (Aα-β), and one of four regulatory subunits: B (B55), B′ (B56), B″ (PR48/PR70/PR130 and PPP2R3A-C), and B‴ (Striatins or PR93/PR110); PP2A-B55 and PP2A-B56 complexes are the major PP2A complexes regulating mitosis.

From early work conducted using Xenopus egg extracts, it was shown that a calcium/calmodulin-activated phosphatase (calcineurin) was necessary for exit from the meiotic M phase [10] but in mitosis, calcineurin did not play such an important role for mitotic exit. However, another phosphatase activity was required for the process: Protein phosphatase 2A (PP2A)-B55δ. In fact, depletion of PP2A-B55δ led to an increase in mitotic phosphorylation even at a very low level of cyclin B and, after mitosis, led to the hyper-phosphorylation of Cdk substrates even if cyclin B degradation and Cdk inactivation were taking place [11]. These discoveries indicated that, in order to exit mitosis properly, phosphatases must be active to restore the phosphorylation state of proteins to their interphase base of hypophosphorylation. At the same time, other studies conducted on the Xenopus egg extract system showed that Protein phosphatase 1 (PP1) counteracts CDK1-dependent phosphorylation and was also required for mitotic exit [12]. Altogether these findings clearly indicated that the progression from metaphase to anaphase-telophase-cytokinesis does require protein phosphatase activity as well as Cdk inactivation and that PP1 and PP2A are the main phosphatases driving mitotic exit. This concept has a major corollary: these phosphatases must be inactivated during early mitosis otherwise cells will be engaged in futile cycles of a continuum of protein phosphorylation/de-phosphorylation without reaching the threshold necessary to drive a phase transition even at a high kinase activity (Fig. 1).

The identification of the inactivation mechanisms of phosphatases in early mitosis has been crucial to understand how this process is regulated.

PP1 and PP2A are subjected to a major global inactivation but also to some local regulation of activities. In fact, both phosphatases are locally active in some sub-compartments of the mitotic cell.

At mitotic entry, PP1 activity can be globally suppressed through phosphorylation by Cdk1 of the Thr 320 residue at the C terminus of the catalytic subunit [13,14]. This inhibitory effect appears to be due to an indirect mechanism, involving complex protein-protein interactions [15]. Since PP1 auto-dephosphorylates itself, this single mechanism of inactivation would not be sufficient to sustain the high level of phosphorylation required for early mitosis. To maintain PP1 inactivation, it is also necessary that Inhibitor–I binds to PP1. This binding is favoured by PKA phosphorylation of Inhibitor-I at Thr 35 [12]. This combination locks the bulk of PP1 into an inactive state.

PP2A inactivation is also achieved via a Cdk1 mediated phosphorylation but it is mediated by another protein kinase: MASTL/Greatwall. Using Xenopus egg extracts, it was shown that Greatwall depletion promoted exit from mitosis even at high cyclin B/Cdk1 level and caused dephosphorylation of mitotic substrates [16]. Cdk1 activates MASTL/-Greatwall kinase through phosphorylation of Cdk1 sites in MASTL. Upon phosphorylation, MASTL autophosphorylates, resulting in activation of the kinase [13,17,18]. This activated kinase then phosphorylates two small proteins ENSA and ARPP19 which bind PP2A-B55 and act as inhibitors of the phosphatase [19–21].

It therefore emerges that Cdk1 not only triggers the major protein phosphorylation events to execute mitosis but also inactivates (directly or indirectly) the main counteracting phosphatases, thus ensuring that a mitotic status is sustained until the SAC is satisfied.

Although both ENSA and Arpp19 can fulfill the role of inhibiting PP2A-B55, in mammalian embryonic division, they seem to perform different functions. Only Arpp19 is required throughout mouse embryonic development and its ablation leads to aberrant mitosis and premature dephosphorylation of key proteins involved in mitotic exit. On the contrary, ENSA knockout mouse embryos are normal up to stage E8.5 but ENSA appears to be involved in S phase regulation [22]. Interestingly, the levels of Arpp19 are very modest in cells and raise the possibility that a small fraction of this phosphatase is actually fully inactive.

There are also some more subtle and local regulations of the phosphatase activities. The local regulation is more dependent on the phosphorylation of the targeting subunits rather than the catalytic one and could explain local rheostat activities which are necessary for the fine tuning of the error-free mitotic execution. In this review we will not enter into the details of these but provide a few examples. Upon Cdk1 activation, other kinases are fully activated or their substrate recognition depends on a Cdk1 phosphorylated site. Among those, Aurora B, Aurora A, Polo like kinase 1 (Plk1) are of particular interest for this aspect. Some of these kinases are also concentrated at specific sites within the mitotic cell; for example, Aurora B is enriched at the centromeres, Plk1 at the centrosomes and kinetochores and Aurora A at the centrosomes. One of the major PP1 binding motif which is found in several PP1 targeting subunits or RIPPOs (Regulatory Interactor of Protein Phosphatase One) is the RVXF motif with common variants such as RV[S/T]F motives (21% S and 18% T of the RIPPOs). These latter sites are hyper-phosphorylated in mitosis with Aurora B being the primary kinase for their phosphorylation [23]. We and others have shown that phosphorylation of residues outside of the core RVxF region can inhibit PP1 binding [24–26] together with the possibility that the negatively

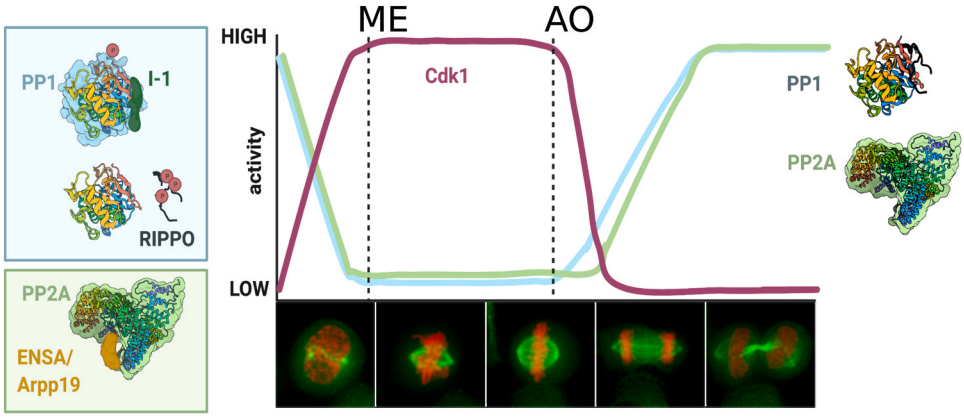


**Fig. 1. Cdk1 activity and protein phosphatase regulation during mitosis.** Left panels: mechanisms of inactivation of PP1 and PP2A. PP1 is inactivated at mitotic entry by multiple mechanisms: phosphorylation of the catalytic subunit C terminal domain, binding of Inhibitor-I (I-1) and holocomplexes are also inactivated by phosphorylation of the RVXF motif and surrounding regions of the PP1 targeting subunits RIPPOs. PP2A is inactivated by the binding of ENSA/Arpp19. PDBs: PP1 4MOV [9], PPP2A/B56 2NPP [91]. To be noted that some of the holocomplexes are still active during early mitosis and play an important role at this stage, hence the activity levels are not be completely suppressed. See text for more details. The graphs indicate the activities of Cdk1, PP1 and PP2A during mitosis. ME=Mitotic Entry, AO=Anaphase onset. The images below show the critical stages during cell division. Live cell imaging of HeLA cells expressing H2B:mRFP and GFP:Tubulin. The panel shows (from left to right): prophase, prometaphase, metaphase, anaphase, late telophase. On the right are represented the fully activate PP1 and PP2A. Figure created in Biorender.

charged surface around the RVxF motif can mediate many electrostatic interactions that could lead to a very high percentage of the PP1 holoenzymes that are phospho-regulated.

The importance of these local inactivations is critical for specific functions. For example, Repo-Man (CDCA2) is a PP1 targeting subunit [27] that is a substrate of several mitotic kinases. Cdk1 mediated phosphorylation (at several positions around the RVTF motif) [24,25] weakens PP1 binding to Repo-Man (this can be considered a global inactivation of the complex that occurs at mitotic entry); however, Aurora B also phosphorylates Repo-Man at the RVTF motif thus further weakening PP1 binding [26]. Because of the local concentration of Aurora B at the centromeres, the proportion of active Repo-Man/PP1 at the centromere would be the lowest, thus ensuring that one of the major Repo-Man/PP1 substrates, H3T3ph is preserved at this chromosome site; this is essential for the error correction mechanisms as it contributes to maintaining the chromosomal passenger complex (CPC) at the site of action [28,29].

Interestingly, some recent work has shown that, in contrast to PP1, the interaction between PP2A-B56 and the LxxIxE motifs can be enhanced by phosphorylation [30–32]. Therefore, PP1 and PP2A-B56 phosphatases seem to have evolved to respond to specific kinases in an opposite manner leading to very distinct signaling roles at least in some specific pathways [33]. These examples show that besides a general regulation of phosphatase activities, local fine tuning of different sub-complexes is essential for a timely regulation of mitotic progression and that the general ON/OFF concept needs to be considered with caution when dealing at molecular scales. How can we get out of this inhibition?

As soon as the SAC is satisfied, degradation of Cyclin B will occur thus leading to a decrease in CDK1 activity. At this point, in order to moveswitch from a low level of phosphatase activity to a high level one, it is necessary that the phosphatase activity prevails; this could be achieved either by tipping the balance between a previously active complex that is no longer counteracted by an active kinase or by a self-activation mechanism. The latter appears to be the case for PP1; in fact, PP1 catalytic activity is required for Thr 320 dephosphorylation (a Cdk1 phosphosite) and pre-phosphorylated PP1 can be dephosphorylated in vitro in the absence of any cofactor, suggesting that PP1 can auto-dephosphorylate. The removal of the second inhibitory lock (Inhibitor-1) is less clear. Some data suggest that PP1 is required for inhibitor-1 Thr 35 dephosphorylation, while others indicate that PP1 may not directly dephosphorylate Thr 35. Then, in terms of re-activating each different holoenzyme, there are few studies that have addressed this aspect. We can envisage that once Cdk1 activity decreases, the dynamic association of PP1c catalytic subunit with the RIPPOs in early mitosis (due to the phosphorylation of these latter) [24,26,34–36] will lead to the binding of the self-reactivated PP1c that, in turn, could de-phosphorylate the targeting subunit and therefore stabilise the re-activated holoenzyme.

In order to reactivate PP2A-B55, MASTL/Greatwall must be de-phosphorylated. This appears to be driven by PP1. PP1 de-phosphorylates the activating site (Ser875) of greatwall. In fact, depletion of PP1 from meiotic Xenopus egg extracts maintains phosphorylation of Ser875 and the kinase activity, thus resulting in a block of mitotic exit [37,38]. As a consequence, PP2A activity is maintained for a slightly longer time after Cyclin B degradation compared to PP1. PP2A-B55 then dephosphorylates and slowly releases ENSA [39]. However, ENSA is in excess over PP2A-B55 therefore this stoichiometry and dependence can lead to an order of substrate de-phosphorylation.

In addition to these mechanisms, another and unexpected PP1/PP2A regulatory cascade was discovered in fission yeast [40]. In early mitosis, PP1 can directly bind to PP2A-B55 and PP2A-B56. This binding is independent from the binding of ENSA/Arpp19. When Cdk1 becomes inactive, the PP2A-B55-associated PP1 self-dephosphorylates and releases from the PP2A-B55. In a second stage, the freed PP2A-B55 removes a phosphate group from the PP1-binding site on PP2A-B56. This latter is a Plk1 phosphosite and Plk1 is active until late mitosis. Only when Plk1 gets degraded and its activity drops, then PP1 will be efficient in conducting a strong de-phosphorylation of the B56 subunit leading to the release of PP1. In this model, PP1 is still upstream of PP2A activation upon exiting mitosis but implies both the existence of an extra mechanism that will put the activation of PP2A-B56 as the last step of the phosphatases activation cascade and that the B subunits of PP2A play an additional role in PP2A inactivation in early mitosis. Built into this model there is also the assumption that some structural conformational changes occur in these phosphatases during mitosis in order to allow for the binding sites for PP1 (normally embedded in the crystal structures available) to be exposed [41].

Just as a matter of comparison, it is important to notice that, contrary to what we have so far described, in *Saccharomyces cerevisiae,* inactivation of CDK1 and de-phosphorylation of its substrates is accomplished by the dual-specificity phosphatase Cdc14. From G1 to metaphase, Cdc14 is sequestered in the nucleolus by Net1/Cfi1 [42], then is released in two steps: in early anaphase it leaves the nucleolus and re-localises in the nucleoplasm and then, in late anaphase, it moves to the cytoplasm [43]. The initial release is supported by the Cdc14 early anaphase (FEAR) pathway, which promotes Net1/Cfi1 phosphorylation by both the polo-like kinase Cdc5 and Cdc28/Cdk1, and the final release is dependent on the mitotic exit network (MEN) pathway, a signalling cascade that is initiated by the GTPase Tem1 and that finally promotes the phosphorylation of the NLS in Cdc14 by the Dbf2-Mob1 kinase, thus favouring its cytoplasmic liberation in the late stages of anaphase [44,45]. Interestingly, although higher eukaryotes do have CDC14 (actually they have 2 genes coding for *CDC14A* and *CDC14B* respectively) the single and double-knockouts of *CDC14A* and *CDC14B* in RPE1 and chicken DT40 cells indicate that both phosphatases are not important for the timing of mitotic phases, cytokinesis and cell proliferation [46,47].

## 2. Temporal and spatial clues in mitotic exit execution: clocks and rulers

### 2.1. Molecular clocks

The phosphatase reactivation models described in the previous section could potentially co-exist in cells and the different and complex phosphatase re-activation mechanisms are in place to allow a gradual and sequential wave of phosphatases thus leading to a specific timing of substrates de-phosphorylation and ordering the cascade of events during mitotic exit. In addition, specific phosphatases are enriched at particular locations of the cells. In early mitosis, PP1 is enriched at kinetochores and on the spindle. PP1α also localises at centrosomes at all cell cycle stages. During the metaphase-to-anaphase transition, there is a dramatic recruitment of PP1γ to chromosomes where the protein remains throughout telophase. At telophase and cytokinesis both PP1α and PP1γ also show accumulations at the cortex and midbody [48].

From the point when mitotic exit starts, the execution of the process requires a very organised series of events that must occur in a sequential fashion but also takes into consideration the topology of the cell. In fact, the sister chromatids released from their cohesin ties will migrate first toward the opposite poles (Anaphase A) then the poles themselves will move further apart and the cell elongates (Anaphase B), the cell membrane will ingress in the middle position, and finally the connection between the two daughter cells will be severed (Cytokinesis) giving rise to the final product of mitosis: two identical daughter cells ready for the new cell cycle. From this picture it emerges the need for some biochemical clues to regulate this temporal progression but also to provide some local control that can delay events until the previous one is completed. In this chapter we will explore the concept of “Clocks” (regulation based on time) and “Rulers” (regulation controlled in space) and why they are important for the progression of mitotic exit.

What are the key characteristics of a molecular clock? First of all, it must contain a trigger event that is not reversible; this will lead to biochemical reactions that are ordered in terms of affinity and

abundance of the substrates: this way there is an established temporal progression of events. The ordered sequence will occur independent of the topology. In contrast, a ruler will monitor reactions locally and delay the next event until the previous one is fully completed. An example of the latter in the mitotic exit context could be delaying the occurrence of cytokinesis until the DNA has successfully been separated in the daughter cells. Let's now examine these concepts separately.

In biochemical terms (and relevant to mitotic exit) the trigger for the clock is the activation of the APC. Therefore, protein degradation is certainly an important component of the clock. However, from what we discussed in the previous section, protein de-phosphorylation is also triggered by the same proteolytic event: this could also constitute another aspect of a molecular clock. Inactivation of Cdk1 is an absolute requirement for segregating the chromosomes. In fact, releasing the cohesin ties without Cdk1 inhibition is not sufficient to achieve a correct chromosome segregation: kinetochore-microtubule attachments are rapidly destabilized. Metaphase chromosomes undergo a bona-fide anaphase only when cohesin cleavage is combined with Cdk1 inhibition [49] that, in normal mitotic progression, occurs via proteolysis of cyclins. The need for Cdk1 inactivation in order to complete mitotic exit correctly is also demonstrated by the fact that, in Drosophila, the overexpression of non-degradable forms of cyclin B1 allows anaphase transition, but chromosome movement is slow and anaphase B spindle elongation does not occur; conversely, stable cyclin B3 overexpression prevents normal spindle maturation and chromosome decondensation but chromosome disjunction, anaphase B, and formation of a cleavage furrow appear to occur normally [50,51]. Similar observations were also reported using rat kidney cells upon expression of a non-destructible form of cyclin B (cyclin BΔ90) [52] and chicken DT40 cells overexpressing cyclin B3 [53]. However, recent experiments in Drosophila S2 cells show that non-degradable cyclin B1 or B3 gives identical anaphase phenotypes and cells remain arrested in anaphase with condensed chromosomes [51]. Collectively, this evidence points to the fact that there may be several triggers for smaller clocks at different transition points: chromosome disjunction, anaphase movements, and cytokinesis that contribute to the full reduction of Cdk1 activity [54].

Although these data could be compatible with a major role of proteolysis in driving progression of mitotic exit, the experiment by Oliveira [49], seems to argue that once cohesin has been removed and Cdk1 inactivated, mitotic exit can progress even in the absence of a functional APC; however, these latter experiments need to be interpreted with caution as, in a normal cell cycle progression, degradation of cyclin B is not acute but more gradual [55].

Decreased levels of Cdk1 leads to the re-activation of protein phosphatases PP1 and PP2A that will initiate the de-phosphoryation process of several mitotic phosphoproteins. Global phosphoproteomic studies have tried to address the sequence of de-phosphorylation events to understand both the specificity for the different phosphatases but also if this process could represent a branch of the clock for mitotic exit. Interestingly, it was shown that the order and extent of protein de-phosphorylation in a "mitotic exit like" situation where prometaphase/metaphase human cells were treated with a Cdk1 inhibitor in the presence of the APC inhibitor MG132, remained unchanged [56]. This strongly suggests that the de-phosphorylation branch of the clock is independent from the protein degradation one and biochemically support the previous observation conducted in Drosophila where a normal mitotic exit progression could be obtained just with Cdk1 inhibition in the absence of APC activity [49]. Therefore, a phosphatase cascade seems to be a major driver for the molecular clock.

Analyses of global de-phosphorylation at high resolution time points during mitotic exit has clearly revealed that PP1 is re-activated before PP2A where the inhibitory pT320 on PPP1c is removed almost 5 min earlier than the PRC1-pT48 which is a PP2A-B55 model substrate [56]. Comparing the timing of de-phosphorylation of several residues during mitotic exit, four major categories could also be identified: early substrates (these are de-phosphorylated within 5–10 min and represents 21% of the phosphosites), middle (15–30 min, 26%), late (40–60 min, 26%) and stable (these phosphosites still maintain phosphorylations after 60', 27%) [56]. This suggests the existence of a clear sequential programme. But what is driving this order?

Several studies have tried to identify if a specificity/affinity for substrates could explain these ordered de-phosphorylation events. Normally, global TP sites are rapidly lost during mitotic exit compared to SP sites [57]. Interestingly, if the phosphoresidue in PRC1, a PP2A-B55 substrate, is converted to serine, this protein is de-phosphorylated less rapidly, but the introduction of an upstream aromatic/acid motif rescues the timely de-phosphorylation [58]. In fact, the amino acids surrounding the phosphosite are also extremely important for the timing of de-phosphorylation where TPP motives are highly stable, whereas TPG ones are more efficiently dephosphorylated [57]. This suggests that combinatorial motives play a key role in ordering the de-phosphorylation events during mitotic exit. The analyses of PP1 substrates provided similar results: for early dephosphorylated sites PP1 has a modest preference for Thr upstream of a basic patch, for the intermediate sites Thr are less preferred with surrounding sequence preferences similar to the early class while the late cluster shows an exclusion of Thr and a minimal sequence selectivity for the surrounding environment [56]. All this evidence therefore indicates that the protein dephosphorylation rate is linked to their sequence characteristics and explains how a dephosphorylation cascade driven by PP1 and PP2A provides a clock mechanism for the order of events during mitotic exit. The identification of the molecular basis for this progression also explains the early studies showing that both yeast and human cells are blocked in anaphase upon okadaic acid treatment [59,60].

Fig. 2 (middle panel) illustrates the concept of molecular clocks.

### 2.2. Topological rulers

At the onset of mitosis, together with CDK1, other mitotic kinases essential for the execution of cell division are activated as well. Among those, we focus our attention on two major players: Aurora B and PLK-1. At anaphase onset these kinases are not subjected to inactivation as it is for CDK1 however they do change spatial localisation while still maintaining full activity; Aurora B translocates from the inner centromere to the spindle midzone where a pool of PLK1 also accumulates. The concentration of these kinases in specific subcellular locations is extremely important for the faithful execution of mitotic exit. For example, by using phosphorylation sensors targeted either to the chromosomes or the centromeres it was shown that an Aurora B mediated phosphorylation gradient is established within the analphase cells, and this is linked to the position of the chromatin relative to the midzone rather than the time elapsed from anaphase onset (clock – independent) [61]. These initial observations have put forward the concept that not only a clock mechanism is important for the timely execution of mitotic exit but also that cells have imbedded "rulers" that can monitor where and when the next step in the mitotic exit cascade can occur. These rulers are important for coordinating several different pathways such as the completion of chromosome segregation, the reformation of the Nuclear Envelope and abscission [62]. In fact, a decreased spindle elongation velocity is compensated by an increase in anaphase duration in both Drosophila S2 and human cells [63]. Such a delay would allow time for fixing problems before abscission and reformation of the nuclear envelope are completed. This phenomenon could be linked to the abscission checkpoint/NoCut checkpoint [64,65], a conserved surveillance mechanism centred around Aurora B [62,66], triggered by DNA bridges, lagging chromatin and nuclear pore defects. The concentration of active kinases in a specific cellular domain will locally maintain this region in a more "early mitotic state" allowing for defects that can normally occur during cell division to be rectified.

However, recent studies have also put forward the idea that the two apparently separated systems (clocks and rulers) are also interlinked. In fact, Aurora B re-localisation to the anaphase spindle is negatively

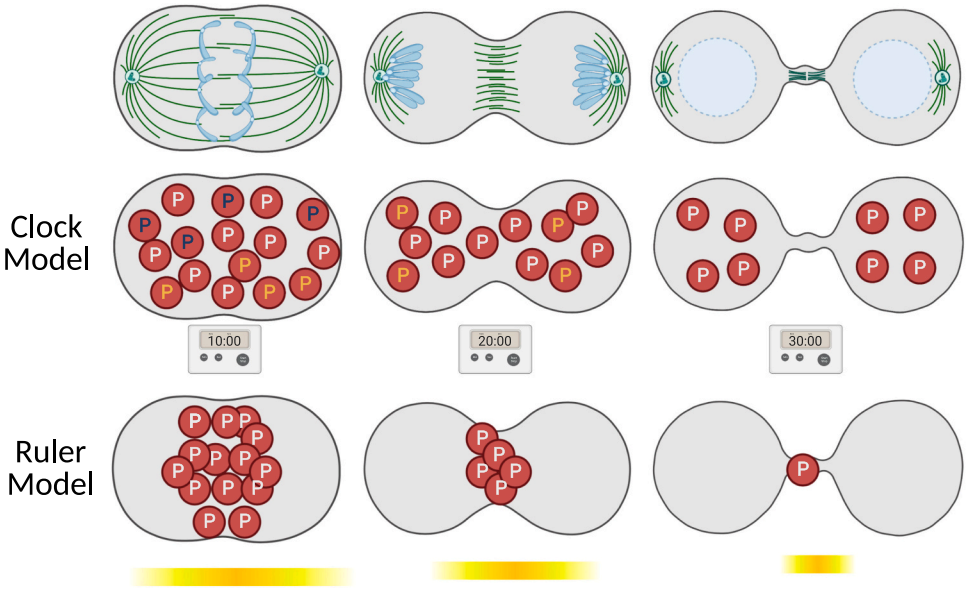


**Fig. 2. Clock and ruler models for the regulation of mitotic exit.** Top panels illustrate the progression of mitotic exit from early anaphase (left panel) to cytokinesis (right panel). Chromosomes are depicted in light blue, and microtubules in green. The middle panels exemplify the progressive dephosphorylation of substrates based on their affinity and/or re-activation of the specific phosphatase where the blue labelled ones will be de-phosphorylated first, followed by orange and then the white. The bottom panel illustrates the ruler model where dephosphorylation of substrates will depend on their relative position within the cell. The yellow bar represents Kinase X activity where darker yellow indicates higher activity and pale-yellow low activity. For more detail, please refer to the text section "Temporal and spatial clues in mitotic exit execution: clocks and rulers". Figure created in Biorender.

regulated by Cdk1 [67] via the kinesin-6/Mklp2 [68]. Therefore, the ruler mechanism appears to be controlled by the clock trigger. Moreover, very detailed quantifications of Cyclin degradation also revealed that, although the majority of Cyclin B is degraded just before the onset of anaphase, some residual levels are maintained and its degradation continues during mitotic exit [55,69,70]. As mentioned in the chapter above, failure to degrade B-type cyclins still allows for some anaphase programme to take place but blocks cells at different stages (depending on the type of cyclin) and prevents chromosome de-condensation. On the contrary, inactivation of Cdk-1 in early mitosis (e.g. in nocodazole arrested cells) leads to some levels of chromosome de-condensation and triggers the reformation of the Nuclear Envelope in a spatially non regulated fashion [63,71] (see next section for more explanations). This seems at least partially mediated by the re-activation of some Protein Phosphatases and also implies that the residual level of Cyclin B1 in anaphase could be important for the "ruler" spatial control. In agreement with this, it was recently shown that some Cyclin B1-Cdk1 persists at the spindle midzone in anaphase cells and its localization is Aurora B dependent [55]. In this model, Aurora B could control either directly (via phosphorylation of Cyclin B1) or indirectly (via regulating the APC activity) Cyclin B degradation rate. This model is quite interesting as it would provide a molecular integration of these two co-existing regulatory models. In a similar manner, we can then envisage that local inactivation of specific phosphatase holoenzymes could be maintained beyond anaphase onset thus allowing timely local dephosphorylation activity to rebuild specific cell compartments. In this respect we should remember that several PP1 holoenzymes assembly are negatively regulated by both Cdk1 and Aurora B [23,24,26,35,36].

The other kinase we mentioned earlier in this section, Plk1 could also play an important part in this anaphase ruler regulation. In fact, Polo is essential for cytokinesis in several organisms [72,73]. Interestingly, Plk1 is activated by Aurora B at the centromeres [74]. The CPC is required for Plk1 activation at kinetochores in human cells and activated Plk1 is also localised at the midbody of human [71] and drosophila [75] cells in an Aurora B dependent manner, thus suggesting that this regulation is conserved in both compartments. If this is the case, some of the effectors of the ruler regulation could be then Plk-1 substrates. More studies will be required to dissect these checkpoints and to unveil to which extent the chromosome separation and no-cut checkpoints are distinct or overlapping. Fig. 2 (bottom panel) illustrates the concept of topological rulers.

## 3. The link between chromosome re-organisation and nuclear envelope re-formation during mitotic exit

The ultimate goal of cell division is to generate two identical daughter cells with the DNA separated from the cytoplasm by a permeable membrane, the Nuclear Envelope (NE), and the chromatin organised and compartmentalised to resume the cellular programme identical to the one of the mother cell. As chromatin is re-organised into the mitotic chromosomes and the NE disassemble upon entry into mitosis to facilitate the separation of sister chromatids, these two structures need to be reset to their respective interphase status in order for the next cell cycle to commence. Therefore, during mitotic exit both chromosome decondensation and NE reformation take place and they are almost completed before cytokinesis occurs. As with the many other aspects occurring within this cell division stage, these latter are also regulated both in space and time during mitotic exit. In this section we are going to review the current knowledge and models for their regulation. Since these aspects have been less studied, there are currently several hypotheses, but more research is still required to really nail down the details of the process.

When sister chromatids migrate to the poles, they need to maintain their compaction in order to allow for the completion of separation to occur before cytokinesis, therefore it is important that condensation is maintained until later in mitotic exit. Chromosome condensation is established and maintained by condensins but careful measurement of chromatin compaction has revealed that the maximum compaction occurs in anaphase [53,76]. Once the chromatids have separated, an axial shortening occurs beginning in mid-anaphase and finishing during late anaphase; this leads to the overall maximal compaction of chromatin in late anaphase. The shortening is independent of the size of the chromosomes and is conserved in mammals. The molecular effectors of this phenomenon are still not fully understood but two elements are important: 1) Aurora B catalytic activity 2) the presence of dynamic microtubules which are essential for the localisation of Aurora B at the central spindle. Interestingly, the axial shortening is not linked to cytokinesis in fact, it can still occur even if the cleavage furrow ingression has been blocked. Therefore, although some similarity with the NoCut pathway could be identified (e.g. presence of lagging chromatin and Aurora B dependence) [64,66], its independence form the furrowing process places this event upstream and maybe more linked to the chromosome separation checkpoint described before [63]. It is also possible that Aurora B could sustain the phosphorylation status of other

not yet identified chromatin components that are de-phosphorylated by a phosphatase which is inhibited by Aurora B; this would allow the compaction to continue in time (and importantly, locally - spatial regulation) into anaphase until the chromatin has been cleared from the midzone. Interfering with this process, leads to abnormally shaped nuclei, thus suggesting that anaphase chromosome shortening is required for the formation of smooth nuclei and therefore could function as a rescue mechanism for chromosome segregation defects [76].

After clearing the central spindle from the segregating chromosome arms, each individual chromosome does not remain as a discrete entity but must participate to the formation of a single nucleus which entraps all the chromosome complement. Using image-based screening of human cells, barrier-to-autointegration factor (BAF) was discovered as key factor guiding membranes to form a single nucleus [77]. BAF is de-phosphorylated by PP2A/LEM4/ANKLE2 during mitotic exit [78]; de-phosphorylated BAF can bind with high-affinity to DNA, mediates DNA bridging and generates a compact and stiff chromatin surface that is essential for both producing a continuum nuclear subphase and a diffusional barrier at the surface of chromatin that prevents access of macromolecular objects in the size-range of membrane tubules. Clearly, avoiding entrapping cytoplasmic structures is an important aspect that the cells need to take care of before resealing the nuclear envelope and this mechanism seems to help in this sense. However, it is still not clear how this process relates to another cytoplasmic exclusion barrier, Ki-67, acting at the end of mitosis. Ki-67 is a master regulator of the mitotic chromosome layer [36], essential for the maintenance of chromosome individuality in early mitosis [79]. A recent study revealed that, during mitotic exit, chromosome tight clustering mediated by Ki-67-regulated surface adhesion contributes also to the nuclear-cytoplasmic compartmentalization in vertebrate cells [80]. The link between these two mechanisms has not been investigated. Why cells need two separate mechanisms for clustering and crosslinking chromatin at the end of mitosis? Are the two systems interconnected? All these questions will require some more probing in order to obtain a clear picture of this important process.

When mitosis is completed, a sealed but permeable nuclear membrane separates the G1 chromatin from the cytoplasm. However, this complex barrier is not just appearing at the surface of the chromatin after mitotic exit is complete, but it is actually built during this stage in a progressive manner and its assembly starts as early as anaphase A. The first detectable sign of deposition of NE components around the segregating chromosomes seems also to be quite asymmetric (Fig. 2 A). Some Nucleoporins and Importin-β are deposited first at the side of chromatin towards the spindle poles; then they extend laterally, finally invading the chromatin region facing the midzone. Other components are less so: for example, Lamin A is deposited later but in a more homogeneous manner in both chicken and human cells (Fig. 3 A). Its appearance around the segregating chromatin is more dependent on the time from anaphase onset rather than other spatial clues. On the contrary, some NPC components and Importin-β are highly biased in their distribution thus responding somehow to some topological constrains. The nature of these constrains is still an open debate and different hypotheses have been put forward. All these models are based on the analyses of either chromatin bridges in anaphase/telophase or lagging chromosome/chromosome fragments. Although a recent analysis has shown that anaphase lagging chromosomes are often transient and rarely form

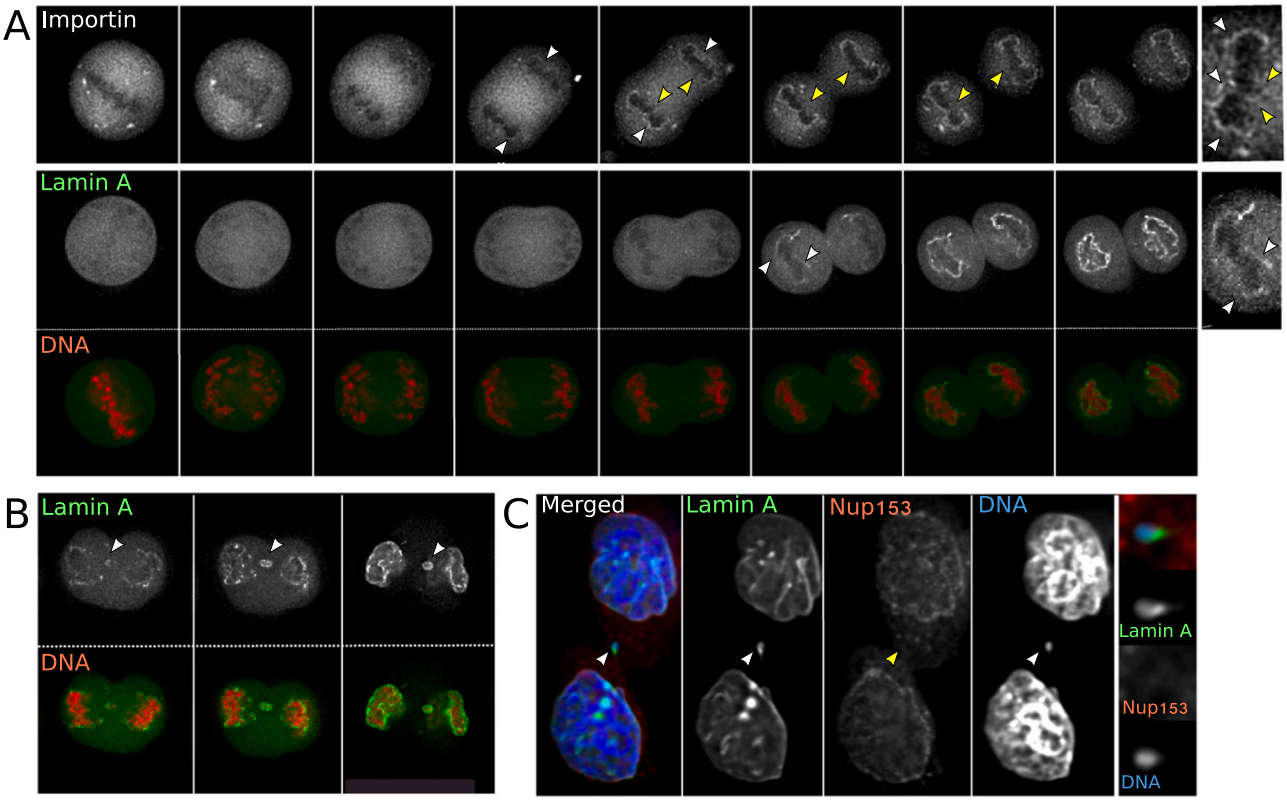


**Fig. 3. Link between Nuclear Envelope reformation and chromosome segregation.** A) Top panel: live cell imaging of HeLa cells expressing mRFP:Importin-β. Importin-β starts assembling at the chromosome periphery in the regions towards the poles (image 4 from the left – white arrowheads), then it expands laterally still leaving a gap at the chromosome region towards the central spindle (images 5–7 yellow arrowheads). Middle and bottom panels: live cell imaging of HeLa cells expressing H2B:mRFP and GFP:Lamin A. Lamin A assembles on the chromosomes later than Importin-β and does not follow a preferential deposition site (image 6 from the left – white arrowheads). B) Anaphase cell with a lagging chromosome (white arrowhead): GFP:Lamin A assembles around the lagging chromosome and the main segregated chromatin at the same time. C) Cells in cytokinesis with a mis-segregated chromosome in the middle (arrowheads). This chromosome shows NE defects as it contains Lamin A (green) but not the nucleoporin NUP153 (red) (yellow arrowhead).

micronuclei [81], occasionally they end up becoming a micronucleus in G1 and do not assemble a functional NE [63,71,82]. These laggards can efficiently recruit Lamin A, Emerin, LAP2α, LAP2β, BAF at the correct time as the main segregating nuclei (Fig. 3 B, C) [71,82], but they cannot properly assemble all the FG-NUPS recognised by mAb414, Importin-β [71], Nup133, LBR, ELYS, TPR [82] (Fig. 3 C). The altered NE of the micronuclei will have major consequences as the chromatin within these structures will encounter replication/repair errors, will become a source of genomic instability leading to the phenomenon of chromothripsis thus playing a major role in cancer evolution [83]. However, in normal condition, a p53-dependent and cGAS-STING-dependent mechanism eliminates micronucleated cells [84], thus safeguarding cells from these potentially hazardous situations. Interestingly, the recent finding that Aurora B-mediated phosphorylation of chromatin-associated cGAS prevents its premature activation during mitosis [85], could potentially link the Aurora B-dependent 'chromosome separation checkpoint' to the clearance of micronucleated cells.

Why are these aberrant NE assemblies occurring? One model is based on a midzone surveillance mechanism that is sustained by Aurora B kinase [86]. This model implies that chromatin lagging in the midzone would be recognised by the chromosome segregation checkpoint that delays the anaphase to telophase transition thus providing an opportunity for the laggard to rejoin the main nucleus. According to this model, Aurora B at the spindle midzone establishes a phosphorylation gradient that locally delays the degradation of residual Cyclin B1 (and possibly B3) at the spindle midzone [55]. Consequently, as chromosomes separate and move away from the spindle midzone, Cdk1 activity decreases, allowing the PP1/PP2A-mediated dephosphorylation of Cdk1 and Aurora B substrates necessary for mitotic exit. This model would explain the differential loading of NUPs and Lamin B according to the position of chromatin. However, this model links the chromatin organisation to the NE reformation. This link does not seem so clear as it is possible to recruit NE components on condensed chromatin also in early mitosis by targeting to chromosomes a hyperactive phosphatase complex (without de-condensing chromatin [24]); moreover, in MCPH1 patients cells, chromosomes fail to de-condense until G1 but nevertheless the NE is correctly reformed [87]. In line with this, a similar model also includes the role of Plk1 [71]. Active Plk-1 is also present at the midzone and its activity depends on Aurora B [71]. It was recently shown that Plk1 inhibition delays Nuclear Pore dissolution at mitotic entry and that Plk1 phosphorylation of NUP98 [88] and NUP53 [89] are essential for the process. Interestingly, Plk1 was shown to be recruited to DNA tethers of acentric chromatin during cell division [90] and the overexpression of Plk1 in HeLa cells, produced a significant reduction of NPC at micronuclei [71]. We can then hypothesise that maintaining Plk1 activity (via Aurora B [71,74]) at the midzone also delays the process of NPC re-assembly. At the same time, the local activity of CDK-1 and Aurora B will maintain at least PP1 key holoenzymes in a local inactive status (see the section "Inactivation/re-activation of the protein phosphatases network" on how the regulation of the homocomplexes is achieved), thus ensuring that the NE reassembly is delayed at the midzone.

In agreement with this, a global phosphoproteomic mapping of early mitotic exit in human cells revealed that the amount of dephosphorylation during mitotic exit tightly corresponds with the order of recruitment of POM121, NUP53, NUP93, and NUP188 to the reforming nuclear pore [57].

On the contrary, some components such as Lamin A can load onto the chromatin in a timely fashion independent of topological clues; this is more reminiscent of a control that exclusively is dependent on molecular clocks. In fact, upon Cdk-1 decrease in activity, when the overall threshold is achieved, Lamin A will load onto chromatin. Of course, Lamin A needs to be de-phosphorylated but there are hundreds of phosphatase holocomplexes that can be differentially regulated. For example, we can explain these differences considering that the phosphatase responsible for Lamin A de-phosphorylation is only regulated by Cdk-1 (and not Aurora B or Plk1); this complex will re-activate earlier during mitotic exit and will not be subjected to the local inhibition of high level of Aurora B or Plk1.

This model will explain both the normal progression of NE reformation and accounts for the defects seen on the lagging chromatin.

One open question is still if this system indeed represents a checkpoint and how robust this checkpoint is; the SAC can arrest cells for up to 20 h, but it is not known how long the chromosome separation checkpoint will last for in physiological condition. The fact that there are several cancer cells with many micronuclei and NE defects would suggest that the system is not very robust or that it depends on proteins commonly mutated in several cancers. Therefore, there is still the need to understand what is the nature of the signal that is detected by the checkpoint, which are the targets and why the system appears to be more error-prone than the SAC.

A completely different mechanism has also been proposed [82]. In this latter one, the polarised assembly of NE components around chromatin during mitotic exit is explained by spatial restriction where the spindle would form a selective barrier to certain populations of ER membranes. Although very simple and potentially very effective, the molecular details are not very clear. It also does not explain why some components (such as the FG NUPs) are prevented from loading onto the chromatin facing the midzone but they can at the chromatin towards the poles: both regions are highly dense in microtubules. Moreover, targeting a hyperactive phosphatase to the mitotic chromosomes allows the recruitment of some NUPs even in regions of high density of spindle microtubules [24]. It is however possible that this mechanism is important for a subset of membranes that assemble first laterally on the segregating chromatin.

Clearly, it emerges that major details on these proposed models are still lacking and the links between dynamic microtubules, Aurora B, Plk1 and phosphatases needs to be separated in order to obtain a clear picture of how this process is regulated in space and time.

### 3.1. Future perspective

In the last few years, the analysis of mitotic exit execution has revealed some very interesting aspects of cell biology and has brought together several disciplines. We have just started the journey towards understanding how it is executed and its key regulatory steps. However, several questions are still outstanding. The identification of pathways and regulation of the de-phoshorylation events during this transition is of paramount importance. It would also be necessary to employ strategies that allow the separation between specific topological problems and kinase/phosphatase activities. Therefore, approaches such as rapid protein degradation, knock-sideways and optogenetics together with specific mutants will need to be employed as preferred tools over the use of inhibitors. The conservation and divergence (if any) in different cell types would also be an essential aspect to address that may explain some of the discrepancies currently in the arena. Finally, more computational studies should be advocated to generate useful models allowing prediction and intervention on this important last step of cell division.

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## References

[1] D. Hayward, T. Alfonso-Perez, U. Gruneberg, Orchestration of the spindle assembly checkpoint by CDK1-cyclin B1, FEBS Lett. 593 (20) (2019) 2889–2907.
[2] L. Meijer, D. Arion, R. Golsteyn, J. Pines, L. Brizuela, T. Hunt, D. Beach, Cyclin is a component of the sea urchin egg M-phase specific histone H1 kinase, EMBO J. 8 (8) (1989) 2275–2282.

[3] V. Simanis, P. Nurse, The cell cycle control gene cdc2+ of fission yeast encodes a protein kinase potentially regulated by phosphorylation, Cell 45 (2) (1986) 261–268.
[4] D. Wu, V. De Wever, R. Derua, C. Winkler, M. Beullens, A. Van Eynde, M. Bollen, A substrate-trapping strategy for protein phosphatase PP1 holoenzymes using hypoactive subunit fusions, J. Biol. Chem. 293 (39) (2018) 15152–15162.
[5] M. Bollen, W. Peti, M.J. Ragusa, M. Beullens, The extended PP1 toolkit: designed to create specificity, Trends Biochem. Sci. 35 (8) (2010) 450–458.
[6] G.B. Moorhead, L. Trinkle-Mulcahy, M. Nimick, V. De Wever, D.G. Campbell, R. Gourlay, Y.W. Lam, A.I. Lamond, Displacement affinity chromatography of protein phosphatase one (PP1) complexes, BMC Biochem. 9 (2008) 28.
[7] A. Hendrickx, M. Beullens, H. Ceulemans, T. Den Abt, A. Van Eynde, E. Nicolaescu, B. Lesage, M. Bollen, Docking motif-guided mapping of the interactome of protein phosphatase-1, Chem. Biol. 16 (4) (2009) 365–371.
[8] E. Heroes, B. Lesage, J. Gornemann, M. Beullens, L. Van Meervelt, M. Bollen, The PP1 binding code: a molecular-lego strategy that governs specificity, FEBS J. 280 (2) (2013) 584–595.
[9] M.S. Choy, M. Hieke, G.S. Kumar, G.R. Lewis, K.R. Gonzalez-DeWhitt, R.P. Kessler, B.J. Stein, M. Hessenberger, A.C. Nairn, W. Peti, R. Page, Understanding the antagonism of retinoblastoma protein dephosphorylation by PNUTS provides insights into the PP1 regulatory code, Proc. Natl. Acad. Sci. U.S.A. 111 (11) (2014) 4097–4102.
[10] S. Mochida, T. Hunt, Calcineurin is required to release Xenopus egg extracts from meiotic M phase, Nature 449 (7160) (2007) 336–340.
[11] S. Mochida, S. Ikeo, J. Gannon, T. Hunt, Regulated activity of PP2A-B55 delta is crucial for controlling entry into and exit from mitosis in Xenopus egg extracts, EMBO J. 28 (18) (2009) 2777–2785.
[12] J.Q. Wu, J.Y. Guo, W. Tang, C.S. Yang, C.D. Freel, C. Chen, A.C. Nairn, S. Kornbluth, PP1-mediated dephosphorylation of phosphoproteins at mitotic exit is controlled by inhibitor-1 and PP1 phosphorylation, Nat. Cell Biol. 11 (5) (2009) 644–651.
[13] M. Dohadwala, E.F. da Cruz e Silva, F.L. Hall, R.T. Williams, D.A. Carbonaro-Hall, A.C. Nairn, P. Greengard, N. Berndt, Phosphorylation and inactivation of protein phosphatase 1 by cyclin-dependent kinases, Proc. Natl. Acad. Sci. U.S.A. 91 (14) (1994) 6408–6412.
[14] Y.G. Kwon, S.Y. Lee, Y. Choi, P. Greengard, A.C. Nairn, Cell cycle-dependent phosphorylation of mammalian protein phosphatase 1 by cdc2 kinase, Proc. Natl. Acad. Sci. U.S.A. 94 (6) (1997) 2168–2173.
[15] F. Salvi, B. Hoermann, J. Del Pino Garcia, M. Fontanillo, R. Derua, M. Beullens, M. Bollen, O. Barabas, M. Kohn, Towards dissecting the mechanism of protein phosphatase-1 Inhibition by Its C-terminal phosphorylation, Chembiochem 22 (2020) 834–838.
[16] S. Vigneron, E. Brioudes, A. Burgess, J.C. Labbe, T. Lorca, A. Castro, Greatwall maintains mitosis through regulation of PP2A, EMBO J. 28 (18) (2009) 2786–2793.
[17] K.A. Blake-Hodek, B.C. Williams, Y. Zhao, P.V. Castilho, W. Chen, Y. Mao, T. M. Yamamoto, M.L. Goldberg, Determinants for activation of the atypical AGC kinase Greatwall during M phase entry, Mol. Cell Biol. 32 (8) (2012) 1337–1353.
[18] P.V. Castilho, B.C. Williams, S. Mochida, Y. Zhao, M.L. Goldberg, The M phase kinase Greatwall (Gwl) promotes inactivation of PP2A/B55delta, a phosphatase directed against CDK phosphosites, Mol. Biol. Cell 20 (22) (2009) 4777–4789.
[19] A. Gharbi-Ayachi, J.C. Labbe, A. Burgess, S. Vigneron, J.M. Strub, E. Brioudes, A. Van-Dorsselaer, A. Castro, T. Lorca, The substrate of Greatwall kinase, Arpp19, controls mitosis by inhibiting protein phosphatase 2A, Science 330 (6011) (2010) 1673–1677.
[20] S. Mochida, S.L. Maslen, M. Skehel, T. Hunt, Greatwall phosphorylates an inhibitor of protein phosphatase 2A that is essential for mitosis, Science 330 (6011) (2010) 1670–1673.
[21] T. Lorca, C. Bernis, S. Vigneron, A. Burgess, E. Brioudes, J.C. Labbe, A. Castro, Constant regulation of both the MPF amplification loop and the Greatwall-PP2A pathway is required for metaphase II arrest and correct entry into the first embryonic cell cycle, J. Cell Sci. 123 (Pt 13) (2010) 2281–2291.
[22] K. Hached, P. Goguet, S. Charrasse, S. Vigneron, M.P. Sacristan, T. Lorca, A. Castro, ENSA and ARPP19 differentially control cell cycle progression and development, J. Cell Biol. 218 (2) (2019) 541–558.
[23] I. Nasa, S.F. Rusin, A.N. Kettenbach, G.B. Moorhead, Aurora B opposes PP1 function in mitosis by phosphorylating the conserved PP1-binding RVxF motif in PP1 regulatory proteins, Sci. Signal 11 (530) (2018), eaai8669.
[24] P. Vagnarelli, S. Ribeiro, L. Sennels, L. Sanchez-Pulido, F. de Lima Alves, T. Verheyen, D.A. Kelly, C.P. Ponting, J. Rappsilber, W.C. Earnshaw, Repo-Man coordinates chromosomal reorganization with nuclear envelope reassembly during mitotic exit, Dev. Cell 21 (2) (2011) 328–342.
[25] J. Qian, B. Lesage, M. Beullens, A. Van Eynde, M. Bollen, PP1/Repo-man dephosphorylates mitotic histone H3 at T3 and regulates chromosomal aurora B targeting, Curr. Biol. 21 (9) (2011) 766–773.
[26] G.S. Kumar, E. Gokhan, S. De Munter, M. Bollen, P. Vagnarelli, W. Peti, R. Page, The Ki-67 and RepoMan mitotic phosphatases assemble via an identical, yet novel mechanism, Elife 5 (2016).
[27] L. Trinkle-Mulcahy, J. Andersen, Y.W. Lam, G. Moorhead, M. Mann, A.I. Lamond, Repo-Man recruits PP1 gamma to chromatin and is essential for cell viability, J. Cell Biol. 172 (5) (2006) 679–692.
[28] A.E. Kelly, C. Ghenoiu, J.Z. Xue, C. Zierhut, H. Kimura, H. Funabiki, Survivin reads phosphorylated histone H3 threonine 3 to activate the mitotic kinase Aurora B, Science 330 (6001) (2010) 235–239.
[29] A.A. Jeyaprakash, C. Basquin, U. Jayachandran, E. Conti, Structural basis for the recognition of phosphorylated histone h3 by the survivin subunit of the chromosomal passenger complex, Structure 19 (11) (2011) 1625–1634.
[30] E.P.T. Hertz, T. Kruse, N.E. Davey, B. Lopez-Mendez, J.O. Sigurethsson, G. Montoya, J.V. Olsen, J. Nilsson, A conserved motif provides binding specificity to the PP2A-B56 phosphatase, Mol. Cell 63 (4) (2016) 686–695.
[31] X. Wang, R. Bajaj, M. Bollen, W. Peti, R. Page, Expanding the PP2A interactome by defining a B56-specific SLiM, Structure 24 (12) (2016) 2174–2181.
[32] J. Wang, Z. Wang, T. Yu, H. Yang, D.M. Virshup, G.J. Kops, S.H. Lee, W. Zhou, X. Li, W. Xu, Z. Rao, Crystal structure of a PP2A B56-BubR1 complex and its implications for PP2A substrate recruitment and localization, Protein Cell 7 (7) (2016) 516–526.
[33] R.J. Smith, M.H. Cordeiro, N.E. Davey, G. Vallardi, A. Ciliberto, F. Gross, A. T. Saurin, PP1 and PP2A use opposite phospho-dependencies to control distinct processes at the kinetochore, Cell Rep. 28 (8) (2019) 2206–2219 e8.
[34] P. Vagnarelli, W.C. Earnshaw, Repo-Man-PP1: a link between chromatin remodelling and nuclear envelope reassembly, Nucleus 3 (2) (2012) 138–142.
[35] J. Qian, M. Beullens, J. Huang, S. De Munter, B. Lesage, M. Bollen, Cdk1 orders mitotic events through coordination of a chromosome-associated phosphatase switch, Nat. Commun. 6 (2015), 10215.
[36] D.G. Booth, M. Takagi, L. Sanchez-Pulido, E. Petfalski, G. Vargiu, K. Samejima, N. Imamoto, C.P. Ponting, D. Tollervey, W.C. Earnshaw, P. Vagnarelli, Ki-67 is a PP1-interacting protein that organises the mitotic chromosome periphery, Elife 3 (2014), e01641.
[37] S. Ma, S. Vigneron, P. Robert, J.M. Strub, S. Cianferani, A. Castro, T. Lorca, Greatwall dephosphorylation and inactivation upon mitotic exit is triggered by PP1, J. Cell Sci. 129 (7) (2016) 1329–1339.
[38] A. Heim, A. Konietzny, T.U. Mayer, Protein phosphatase 1 is essential for Greatwall inactivation at mitotic exit, EMBO Rep. 16 (11) (2015) 1501–1510.
[39] B.C. Williams, J.J. Filter, K.A. Blake-Hodek, B.E. Wadzinski, N.J. Fuda, D. Shalloway, M.L. Goldberg, Greatwall-phosphorylated Endosulfine is both an inhibitor and a substrate of PP2A-B55 heterotrimers, Elife 3 (2014), e01695.
[40] A. Grallert, E. Boke, A. Hagting, B. Hodgson, Y. Connolly, J.R. Griffiths, D.L. Smith, J. Pines, I.M. Hagan, A PP1-PP2A phosphatase relay controls mitotic progression, Nature 517 (7532) (2015) 94–98.
[41] M. Bollen, Cell cycle: It takes three to find the exit, Nature 517 (7532) (2015) 29–30.
[42] R. Visintin, E.S. Hwang, A. Amon, Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus, Nature 398 (6730) (1999) 818–823.
[43] F. Stegmeier, A. Amon, Closing mitosis: the functions of the Cdc14 phosphatase and its regulation, Annu. Rev. Genet. 38 (2004) 203–232.
[44] W. Shou, J.H. Seol, A. Shevchenko, C. Baskerville, D. Moazed, Z.W. Chen, J. Jang, A. Shevchenko, H. Charbonneau, R.J. Deshaies, Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex, Cell 97 (2) (1999) 233–244.
[45] A.J. Bardin, R. Visintin, A. Amon, A mechanism for coupling exit from mitosis to partitioning of the nucleus, Cell 102 (1) (2000) 21–31.
[46] A. Mocciaro, E. Berdougo, K. Zeng, E. Black, P. Vagnarelli, W. Earnshaw, D. Gillespie, P. Jallepalli, E. Schiebel, Vertebrate cells genetically deficient for Cdc14A or Cdc14B retain DNA damage checkpoint proficiency but are impaired in DNA repair, J. Cell Biol. 189 (4) (2010) 631–639.
[47] P. Partscht, B. Uddin, E. Schiebel, Human cells lacking CDC14A and CDC14B show differences in ciliogenesis but not in mitotic progression, J. Cell Sci. 134 (2) (2021), jcs255950.
[48] L. Trinkle-Mulcahy, A.I. Lamond, Mitotic phosphatases: no longer silent partners, Curr. Opin. Cell Biol. 18 (6) (2006) 623–631.
[49] R.A. Oliveira, R.S. Hamilton, A. Pauli, I. Davis, K. Nasmyth, Cohesin cleavage and Cdk inhibition trigger formation of daughter nuclei, Nat. Cell Biol. 12 (2) (2010) 185–192.
[50] S. Sigrist, H. Jacobs, R. Stratmann, C.F. Lehner, Exit from mitosis is regulated by Drosophila fizzy and the sequential destruction of cyclins A, B and B3, EMBO J. 14 (19) (1995) 4827–4838.
[51] D.H. Parry, P.H. O'Farrell, The schedule of destruction of three mitotic cyclins can dictate the timing of events during exit from mitosis, Curr. Biol. 11 (9) (2001) 671–683.
[52] S.P. Wheatley, E.H. Hinchcliffe, M. Glotzer, A.A. Hyman, G. Sluder, Y. Wang, CDK1 inactivation regulates anaphase spindle dynamics and cytokinesis in vivo, J. Cell Biol. 138 (2) (1997) 385–393.
[53] P. Vagnarelli, D.F. Hudson, S.A. Ribeiro, L. Trinkle-Mulcahy, J.M. Spence, F. Lai, C. J. Farr, A.I. Lamond, W.C. Earnshaw, Condensin and Repo-Man-PP1 co-operate in the regulation of chromosome architecture during mitosis, Nat. Cell Biol. 8 (10) (2006) 1133–1142.
[54] F. Wolf, C. Wandke, N. Isenberg, S. Geley, Dose-dependent effects of stable cyclin B1 on progression through mitosis in human cells, EMBO J. 25 (12) (2006) 2802–2813.
[55] O. Afonso, C.M. Castellani, L.P. Cheeseman, J.G. Ferreira, B. Orr, L.T. Ferreira, J. J. Chambers, E. Morais-de-Sa, T.J. Maresca, H. Maiato, Spatiotemporal control of mitotic exit during anaphase by an aurora B-Cdk1 crosstalk, Elife 8 (2019).
[56] J. Holder, S. Mohammed, F.A. Barr, Ordered dephosphorylation initiated by the selective proteolysis of cyclin B drives mitotic exit, Elife 9 (2020).
[57] R.A. McCloy, B.L. Parker, S. Rogers, R. Chaudhuri, V. Gayevskiy, N.J. Hoffman, N. Ali, D.N. Watkins, R.J. Daly, D.E. James, T. Lorca, A. Castro, A. Burgess, Global phosphoproteomic mapping of early mitotic exit in human cells identifies novel substrate dephosphorylation motifs, Mol. Cell Proteom. 14 (8) (2015) 2194–2212.

[58] M.J. Cundell, L.H. Hutter, R. Nunes Bastos, E. Poser, J. Holder, S. Mohammed, B. Novak, F.A. Barr, A PP2A-B55 recognition signal controls substrate dephosphorylation kinetics during mitotic exit, J. Cell Biol. 214 (5) (2016) 539–554.
[59] K. Yamashita, H. Yasuda, J. Pines, K. Yasumoto, H. Nishitani, M. Ohtsubo, T. Hunter, T. Sugimura, T. Nishimoto, Okadaic acid, a potent inhibitor of type 1 and type 2A protein phosphatases, activates cdc2/H1 kinase and transiently induces a premature mitosis-like state in BHK21 cells, EMBO J. 9 (13) (1990) 4331–4338.
[60] N. Kinoshita, H. Ohkura, M. Yanagida, Distinct, essential roles of type 1 and 2A protein phosphatases in the control of the fission yeast cell division cycle, Cell 63 (2) (1990) 405–415.
[61] B.G. Fuller, M.A. Lampson, E.A. Foley, S. Rosasco-Nitcher, K.V. Le, P. Tobelmann, D.L. Brautigan, P.T. Stukenberg, T.M. Kapoor, Midzone activation of aurora B in anaphase produces an intracellular phosphorylation gradient, Nature 453 (7198) (2008) 1132–1136.
[62] G. Neurohr, A. Naegeli, I. Titos, D. Theler, B. Greber, J. Diez, T. Gabaldon, M. Mendoza, Y. Barral, A midzone-based ruler adjusts chromosome compaction to anaphase spindle length, Science 332 (6028) (2011) 465–468.
[63] O. Afonso, I. Matos, A.J. Pereira, P. Aguiar, M.A. Lampson, H. Maiato, Feedback control of chromosome separation by a midzone Aurora B gradient, Science 345 (6194) (2014) 332–336.
[64] C. Norden, M. Mendoza, J. Dobbelaere, C.V. Kotwaliwale, S. Biggins, Y. Barral, The NoCut pathway links completion of cytokinesis to spindle midzone function to prevent chromosome breakage, Cell 125 (1) (2006) 85–98.
[65] M. Mendoza, C. Norden, K. Durrer, H. Rauter, F. Uhlmann, Y. Barral, A mechanism for chromosome segregation sensing by the NoCut checkpoint, Nat. Cell Biol. 11 (4) (2009) 477–483.
[66] P. Steigemann, C. Wurzenberger, M.H. Schmitz, M. Held, J. Guizetti, S. Maar, D. W. Gerlich, Aurora B-mediated abscission checkpoint protects against tetraploidization, Cell 136 (3) (2009) 473–484.
[67] S. Hummer, T.U. Mayer, Cdk1 negatively regulates midzone localization of the mitotic kinesin Mklp2 and the chromosomal passenger complex, Curr. Biol. 19 (7) (2009) 607–612.
[68] U. Gruneberg, R. Neef, R. Honda, E.A. Nigg, F.A. Barr, Relocation of Aurora B from centromeres to the central spindle at the metaphase to anaphase transition requires MKlp2, J. Cell Biol. 166 (2) (2004) 167–172.
[69] P. Clute, J. Pines, Temporal and spatial control of cyclin B1 destruction in metaphase, Nat. Cell Biol. 1 (2) (1999) 82–87.
[70] K.E. Gascoigne, S.S. Taylor, Cancer cells display profound intra- and interline variation following prolonged exposure to antimitotic drugs, Cancer Cell 14 (2) (2008) 111–122.
[71] I.J. de Castro, R.S. Gil, L. Ligammari, M.L. Di Giacinto, P. Vagnarelli, CDK1 and PLK1 coordinate the disassembly and reassembly of the nuclear envelope in vertebrate mitosis, Oncotarget 9 (8) (2018) 7763–7773.
[72] M. Petronczki, P. Lenart, J.M. Peters, Polo on the rise-from mitotic entry to cytokinesis with Plk1, Dev. Cell 14 (5) (2008) 646–659.
[73] M.E. Burkard, J. Maciejowski, V. Rodriguez-Bravo, M. Repka, D.M. Lowery, K. R. Clauser, C. Zhang, K.M. Shokat, S.A. Carr, M.B. Yaffe, P.V. Jallepalli, Plk1 self-organization and priming phosphorylation of HsCYK-4 at the spindle midzone regulate the onset of division in human cells, PLoS Biol. 7 (5) (2009), e1000111.
[74] M. Carmena, X. Pinson, M. Platani, Z. Salloum, Z. Xu, A. Clark, F. Macisaac, H. Ogawa, U. Eggert, D.M. Glover, V. Archambault, W.C. Earnshaw, The chromosomal passenger complex activates Polo kinase at centromeres, PLoS Biol. 10 (1) (2012), e1001250.
[75] D. Kachaner, X. Pinson, K.B. El Kadhi, K. Normandin, L. Talje, H. Lavoie, G. Lepine, S. Carreno, B.H. Kwok, G.R. Hickson, V. Archambault, Interdomain allosteric regulation of Polo kinase by Aurora B and Map205 is required for cytokinesis, J. Cell Biol. 207 (2) (2014) 201–211.
[76] F. Mora-Bermudez, D. Gerlich, J. Ellenberg, Maximal chromosome compaction occurs by axial shortening in anaphase and depends on Aurora kinase, Nat. Cell Biol. 9 (7) (2007) 822–831.
[77] M. Samwer, M.W.G. Schneider, R. Hoefler, P.S. Schmalhorst, J.G. Jude, J. Zuber, D. W. Gerlich, DNA cross-bridging shapes a single nucleus from a set of mitotic chromosomes, Cell 170 (5) (2017) 956–972 e23.
[78] C. Asencio, I.F. Davidson, R. Santarella-Mellwig, T.B. Ly-Hartig, M. Mall, M. R. Wallenfang, I.W. Mattaj, M. Gorjanacz, Coordination of kinase and phosphatase activities by Lem4 enables nuclear envelope reassembly during mitosis, Cell 150 (1) (2012) 122–135.
[79] S. Cuylen, C. Blaukopf, A.Z. Politi, T. Muller-Reichert, B. Neumann, I. Poser, J. Ellenberg, A.A. Hyman, D.W. Gerlich, Ki-67 acts as a biological surfactant to disperse mitotic chromosomes, Nature 535 (7611) (2016) 308–312.
[80] S. Cuylen-Haering, M. Petrovic, A. Hernandez-Armendariz, M.W.G. Schneider, M. Samwer, C. Blaukopf, L.J. Holt, D.W. Gerlich, Chromosome clustering by Ki-67 excludes cytoplasm during nuclear assembly, Nature 587 (7833) (2020) 285–290.
[81] B. Orr, A.M. Gomes, L. Ferreira, A. Figueiredo, H. Maiato, An anaphase surveillance mechanism prevents micronuclei formation from mitotic errors, 2002.2026.433009, bioRxiv (2021), https://doi.org/10.1101/2021.02.26.433009.
[82] S. Liu, M. Kwon, M. Mannino, N. Yang, F. Renda, A. Khodjakov, D. Pellman, Nuclear envelope assembly defects link mitotic errors to chromothripsis, Nature 561 (7724) (2018) 551–555.
[83] N.T. Umbreit, C.Z. Zhang, L.D. Lynch, L.J. Blaine, A.M. Cheng, R. Tourdot, L. Sun, H.F. Almubarak, K. Judge, T.J. Mitchell, A. Spektor, D. Pellman, Mechanisms generating cancer genome complexity from a single cell division error, Science 368 (6488) (2020), eaba0712.
[84] K.J. Mackenzie, P. Carroll, C.A. Martin, O. Murina, A. Fluteau, D.J. Simpson, N. Olova, H. Sutcliffe, J.K. Rainger, A. Leitch, R.T. Osborn, A.P. Wheeler, M. Nowotny, N. Gilbert, T. Chandra, M.A.M. Reijns, A.P. Jackson, cGAS surveillance of micronuclei links genome instability to innate immunity, Nature 548 (7668) (2017) 461–465.
[85] T. Li, T. Huang, M. Du, X. Chen, F. Du, J. Ren, Z.J. Chen, Phosphorylation and chromatin tethering prevent cGAS activation during mitosis, Science (2021), eabc5386.
[86] H. Maiato, O. Afonso, I. Matos, A chromosome separation checkpoint: A midzone Aurora B gradient mediates a chromosome separation checkpoint that regulates the anaphase-telophase transition, Bioessays 37 (3) (2015) 257–266.
[87] A.P. Jackson, H. Eastwood, S.M. Bell, J. Adu, C. Toomes, I.M. Carr, E. Roberts, D. J. Hampshire, Y.J. Crow, A.J. Mighell, G. Karbani, H. Jafri, Y. Rashid, R.F. Mueller, A.F. Markham, C.G. Woods, Identification of microcephalin, a protein implicated in determining the size of the human brain, Am. J. Hum. Genet. 71 (1) (2002) 136–142.
[88] E. Laurell, K. Beck, K. Krupina, G. Theerthagiri, B. Bodenmiller, P. Horvath, R. Aebersold, W. Antonin, U. Kutay, Phosphorylation of Nup98 by multiple kinases is crucial for NPC disassembly during mitotic entry, Cell 144 (4) (2011) 539–550.
[89] M.I. Linder, M. Kohler, P. Boersema, M. Weberruss, C. Wandke, J. Marino, C. Ashiono, P. Picotti, W. Antonin, U. Kutay, Mitotic disassembly of nuclear pore complexes involves CDK1- and PLK1-mediated phosphorylation of key interconnecting nucleoporins, Dev. Cell 43 (2) (2017) 141–156 e7.
[90] A. Royou, M.E. Gagou, R. Karess, W. Sullivan, BubR1- and Polo-coated DNA tethers facilitate poleward segregation of acentric chromatids, Cell 140 (2) (2010) 235–245.
[91] Y. Xu, Y. Xing, Y. Chen, Y. Chao, Z. Lin, E. Fan, J.W. Yu, S. Strack, P.D. Jeffrey, Y. Shi, Structure of the protein phosphatase 2A holoenzyme, Cell 127 (6) (2006) 1239–1251.