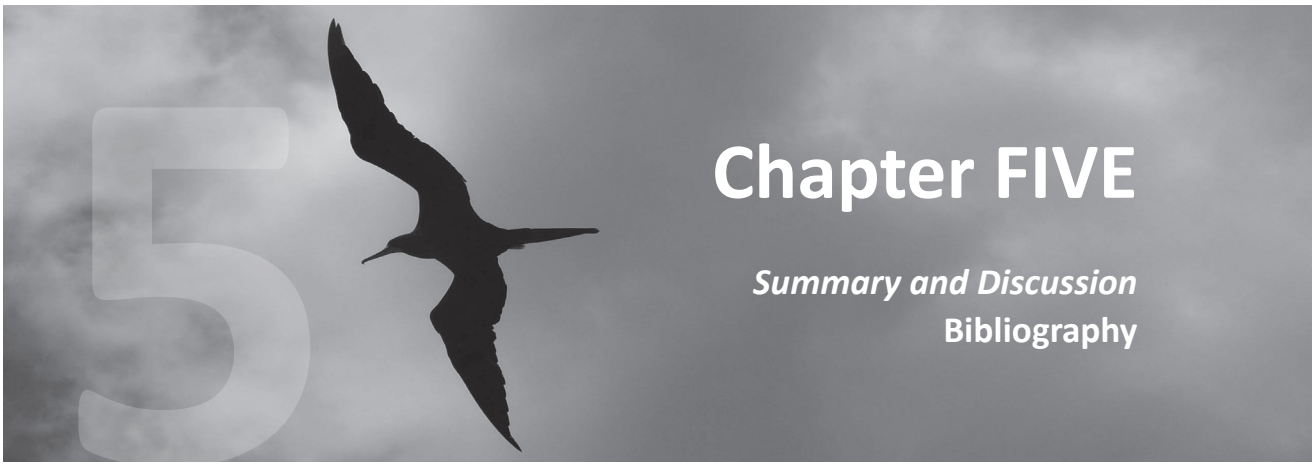

Chapter FIVE

Summary and Discussion
Bibliography

Erik Voets

Division of Cell Biology I (B5), The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

SUMMARY

The decision to enter mitosis is linked to the activation of cyclin B1-Cdk1. Without cyclin B1-Cdk1, cells are not able to complete the cell cycle by producing two daughter cells in mitosis, but instead continue cycling in the absence of mitosis. In this thesis, we investigated the roles and regulation of cyclin B1-Cdk1 and other factors that critically direct mitosis. We identified the human orthologue of Greatwall kinase, MASTL, as an effector of the positive-feedback loop that activates cyclin B1-Cdk1 in G2 phase (C**hapter 2**). The identification of this kinase provided insight into the regulation of the B55 subunit-associated form of PP2A (PP2A-B55), a *bona fide* Cdk1-opposing phosphatase. MASTL suppresses the antagonizing activity of PP2A through its substrates, Arpp19 and Ensa (**Chapter 2**, **addendum**) These two closely related proteins belong to the endosulfine family and act as direct inhibitors of PP2A-B55. The inhibition of PP2A-B55 allows for complete phosphorylation of mitotic substrates by cyclin B1-Cdk1. This is also required in a negative feedback loop at mitotic exit: MASTL-dependent PP2A repression supports the efficient degradation of cyclin B1, which is required for Cdk1 inactivation at metaphase (**Chapter 3**). Using a chemical-genetic screen, we further confirmed the necessity of PP2A inhibition for mitosis (**Chapter 4**). We identified potential effectors of Cdk1 that require tight regulation to allow orderly mitotic progression. Our results indicate that the rise in cyclin B1-Cdk1 activity during mitosis is crucial to the formation of identical daughter cells once cells exit mitosis again. Here, we discuss how the main governer of mitosis, Cdk1, coordinates key steps in both entry into as well as exit out of mitosis.

DISCUSSION

A Cell Cycle Without Mitosis: Lessons Learned From Knockout Studies

Unicellular organisms such as yeast have been very useful to study the roles of cell cycle regulators. In particular, they have proven their effectiveness in the analysis of gene deletions due to the ease of creating knockout collections for screening purposes. However, such model systems often do not resemble multicellular organisms due to the lack of complexity. For instance, the cell cycle of yeast is dependent on only a single Cdk that binds to different cyclins in order to direct different steps in the cell cycle (reviewed in Bloom and Cross, 2007). In contrast, mammalian cells use multiple Cdks (Cdk2, Cdk4, and Cdk6) to drive cells through interphase, as well as Cdk1 to proceed through G2 phase and mitosis. Despite their presence, the interphase Cdks are not strictly essential for the cell cycle or mitosis (Berthet et al., 2006; Malumbres et al., 2004; Santamaría et al., 2007). Apparently, in the absence of other Cdks, Cdk1 can bind to all the different mammalian cyclins, and execute, for instance, the phosphorylation of pRb, to modulate the expression of E2F target genes. In absence of the interphase Cdks, however, cell cycle duration is extended, indicating that Cdk1 may not regulate interphase processes with similar efficiencies as the other Cdks. In addition, mouse embryos lacking *CDK2* and *CDK4/6* do not develop to normally sized mice. So, while Cdk1 may compensate for most interphase Cdk functions, it is not able to sustain the complexity of a mature animal. Accordingly, interphase Cdks probably evolved to support the complexity of multicellular organisms.

Genetic studies demonstrated that cell division only occurs when Cdk1 is present (Diril et al., 2012; Itzhaki et al., 1997; Santamaría et al., 2007; Th'ng et al., 1990). In absence of Cdk1, however, all interphase events are still executed. Cells lacking *CDC2* undergo endoreduplication, a cell cycle consisting of the gap phases G1 and G2, separated by an intervening S phase (G1-S-G2) (Figure 1) (reviewed in Edgar and Orr-Weaver, 2001). So, these cells replicate their entire genome in the absence of mitosis, leading to elevation of the DNA content (also known as polyploidization). Endoreduplication can also occur naturally for instance in the salivary glands of flies (resulting in the formation of polytene chromosomes), but also in trophoblast stem cells or mammalian megakaryocytes, the progenitors of blood platelets (Hammond and Laird, 1985; Ullah et al., 2008; reviewed in Ravid et al., 2002).

In many of these cell types, modulation of Cdk activity by the CKIs is thought to contribute to the observed endoreduplication cycles. For instance, the differentiation process of trophoblast stem cells into trophoblast giant cells requires the temporal expression of p57(Kip2) in order to inhibit Cdk1 activity (Ullah et al., 2008). This CKI inhibits Cdk1 during the Gap phases, while its expression is lost upon S phase entry, allowing for Cdk2-directed DNA replication. Interestingly, endoreduplication may also contribute to regeneration of the liver after its partial surgical resection (Diril et al., 2012). Apparently, liver regeneration can be achieved by cell growth, rather than cell division, which is why it is one of the few tissues that can grow in the absence of Cdk1.

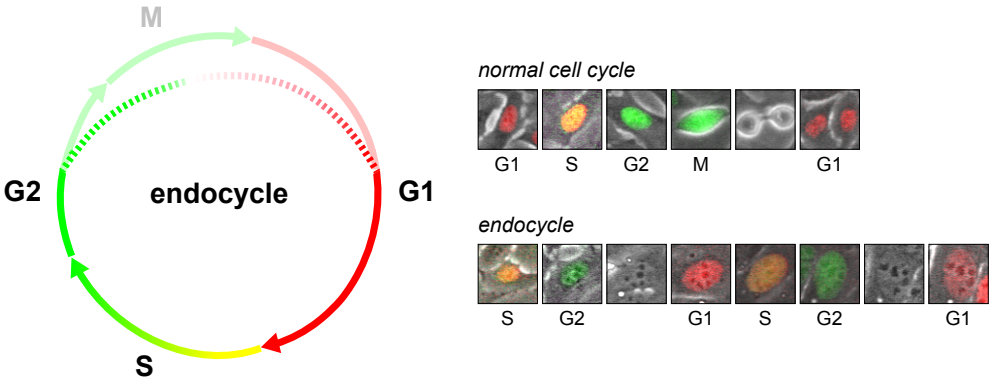

Figure 1 | **The Cell Cycle in Absence of Cdk1 Activity**
A schematic depiction of the cell cycle in cells lacking Cdk1 activity. Without Cdk1, cells are unable to progress from G2 phase to mitosis. Instead, mitosis is bypassed, leading to so-called endocycles consisting of G1-S-G2-G1 etc. As a result, uncontrolled DNA replication will facilitate cellular polyploidization. The filmstrip shows U2OS cells stably expressing the Fucci system to mark the different cell cycle phases. Control cells degrade the geminin fusion protein (in green) specifically in mitosis, whereas the Cdt1 fusion (in red) is degraded in S phase. Cdk1 inhibition induces DNA endoreduplication, demonstrated by the oscillating behaviour of both Cdt1 and geminin fusion proteins, in the absence of mitosis. See text for details.

An essential molecular component driving endoreduplication is the APC/C, together with its substrate binding co-factor Cdh1. In mitosis, Cdk1 phosphorylates Cdh1 to reduce its affinity for the APC/C (Visintin et al., 1998; Zachariae et al., 1998), and suppression of Cdk1, from the exit of mitosis until the end of G1 phase, promotes the formation of APC/C-Cdh1 complexes. Apart from Cdk1, the Emi1 protein is a very prominent inhibitor of the APC/C: *FBXO5* (the gene that encodes Emi1) depletion by RNAi leads to robust APC/C^{Cdh1} activation, which prevents the onset of mitosis by destroying cyclin A and B (Di Fiore and Pines, 2007; Grosskortenhaus and Sprenger, 2002; Ma et al., 2009; Machida and Dutta, 2007). Subsequent oscillations in APC/C^{Cdh1} activity, due to the E2F-dependent re-accumulation of Emi1 in G1 phase, and loss of Emi1 upon cyclin A and Plk1 activation at the end of G2 phase, explain why Emi1 supports completion of the cell cycle. Depletion of *FBXO5*, which leads to loss of the replication inhibitor geminin, or depletion of *GMNN* itself, causes repetitive S phase cycles, known as DNA re-replication (Machida and Dutta, 2007; Melixetian et al., 2004). DNA re-replication differs from endoreduplication in that cells no longer maintain the temporal separation of both licensing and firing of the origins of replication (reviewed in Porter, 2008). Thus, during re-replication, origins can fire more than once within a single S phase leading to a continuous increase in DNA content. A schematic overview of the molecular components driving the oscillations in APC/C activity, necessary for cell cycle progression is summarized in Figure 2.

Cell cycle-dependent oscillations in APC/C activity can be detected by studying the proteolysis of APC/C substrates, which is also part of the so-called Fucci system. This system uses the APC/C degradation motif of geminin and the SCF degradation motif of Cdt1, fused to a green (mAG) or red (mKO2) fluorescent protein, respectively (Sakaue-Sawano et al.,

2008). Geminin is an APC/C substrate that is effectively destroyed in mitosis (Clijsters et al., 2013). It is initially targeted for degradation by APC/C^{Cdc20}, but also APC/C^{Cdh1} may direct its proteolysis. The MKO2-Cdt1 is a substrate of the SCF^{Skp2}, that operates in S and G2 phases. The APC/C^{Cdh1} regulates the stability of the SCF co-factor Skp2, thereby ensuring the inactivation of SCF^{Skp2} in G1 phase (Wei et al., 2004). As a result, the activities of APC/C^{Cdh1} and SCF^{Skp2} oscillate reciprocally during the cell cycle (reviewed in Vodermaier, 2004) (Figure 1). During genome endoreduplication these oscillations occur without any intervening mitosis. Endoreduplication can be induced by the use of small-molecule Cdk1 inhibitors such as RO-3306 (Vassilev et al., 2006). The development of selective Cdk1 inhibitors has gained access to powerful tools in order to study the temporal regulation of cyclin B1-Cdk1–directed processes. Completely inhibiting Cdk1 prevents mitotic commitment, while its partial inhibition allows entry into mitosis (**Chapter 4**).

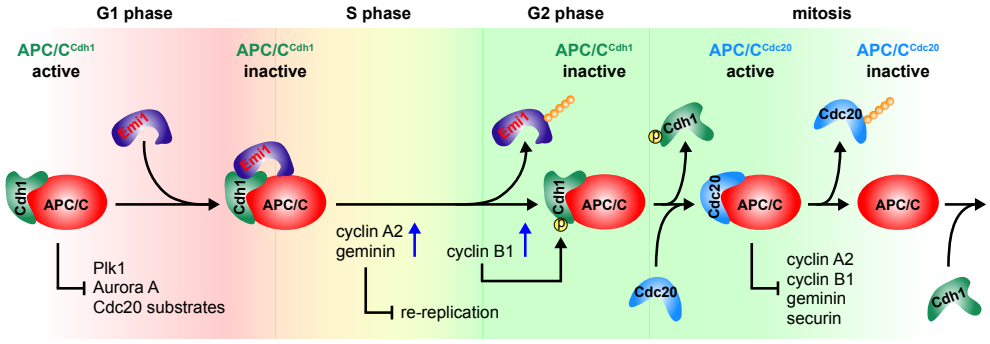

Figure 2 | **Oscillations in APC/C Activity Direct Cell Cycle Progression**
Schematic working model of how the APC/C controls normal cell cycle progression. In early G1 phase, APC/C^{Cdh1} prevents the accumulation of mitotic cyclins by targeting them for proteasomal destruction. The expression of Emi1 at the end of G1 phase ensures APC/C^{Cdh1} inhibition, which is required to establish Cdk2 activity and drive DNA replication. At this point, the APC/C substrates cyclin A and geminin re-accumulate to suppress re-initiation of S phase. Completion of DNA replication is followed by G2 phase, where cyclin B1 starts to be synthesized. Towards the end of G2 phase, Emi1 is destroyed and subsequent phosphorylation of Cdh1 by cyclin B1-Cdk1 safeguards against unscheduled APC/C activation. In mitosis, Cdc20 takes over from Cdh1, and APC/C^{Cdc20} targets a bunch of substrates including mitotic cyclins, securin, and geminin for degradation. The auto-ubiquitylation of Cdc20, together with the destruction of cyclin B1, allows the activation of APC/C^{Cdh1} complexes, thereby completing the cycle.

Checkpoints in Mitosis

The determinants of mitotic entry have been studied extensively. Especially the use of *in vitro* model systems, such as *Xenopus* egg extracts supported progress in the characterization of mitotic modulators. Cyclin B1, which starts to be expressed at the end of S phase, is a limiting factor for the decision to enter mitosis. Only when a certain threshold level is reached, will cyclin B1 starts to direct the activation of Cdk1 (Solomon et al., 1990). The subsequent activation of the double positive-feedback loop makes entry into mitosis an all-or-nothing response (Pomerening et al., 2003; Sha et al., 2003).

The initiation of mitosis is accompanied by the nuclear translocation of cyclin B1-Cdk1, which depends on the presence of active cyclin B1-Cdk1 (Gavet and Pines, 2010a).

One might assume that a sudden drop in cytoplasmic cyclin B1-Cdk1 could, in principle, cease the continuation of its own activation (Lindqvist et al., 2007). Instead, the activation progressively increases, which is now thought to depend on the inhibition cytoplasmic PP2A-B55 (Álvarez-Fernández et al., 2013; Wang et al., 2013).

Prophase, which basically represents the onset of mitosis, is believed to be reversible, dependent on cellular insults (e.g. microtubule poisons) that threaten the proper inheritance of chromosomes. A checkpoint active in early prophase, known as the antephase checkpoint, is able to pull cells back into G2 phase, resulting in a temporal cell cycle block (Matsusaka and Pines, 2004; Rieder and Cole, 1998; Rieder and Cole, 2000). This checkpoint requires the presence of an E3 ubiquitin ligase called checkpoint with FHA and RING finger domains (CHFR) and active p38 stress kinases (Matsusaka and Pines, 2004; Scolnick and Halazonetis, 2000). How exactly CHFR and p38 contribute to the reversal of mitotic commitment is poorly understood, but they are thought to inhibit the activities of several mitotic kinases including Aurora A, Plk1, and cyclin B1-Cdk1 (Matsusaka and Pines, 2004; Summers et al., 2005). The cytoplasmic sequestration of cyclin B1 is likely to be the key event that delays the onset of mitosis. The negative role of p38 kinases in unperturbed mitotic entry has been demonstrated previously (Cha et al., 2007). Live-cell imaging of synchronised cells allows us to study the effects of different variables in the mitotic entry network. For instance, we also find that inhibition of p38 kinases, using the small-molecule inhibitor SB 203580, speeds up entry into mitosis (Figure 3). As expected, inhibiting the Cdc25 phosphatases significantly impairs entry into mitosis. In line with this, the partial inhibition of Cdk1 using roscovitine noticeably delays the G2-to-M transition. Interestingly, the inhibition of Plk1 using BI 2536 (Lénárt et al., 2007; Steegmaier et al., 2007) also strongly prevents the onset of mitosis. This may be explained by the intimate regulation between Plk1 and Cdk1: Cdk1 promotes the activation of Plk1 (Chan et al., 2008), whereas Plk1 in turn is required for the activation of cyclin B1-Cdk1 (Qian et al., 2001; Toyoshima-Morimoto et al., 2001; Toyoshima-Morimoto et al., 2002). Fully activating cyclin B1-Cdk1 is necessary to promote timely entry into prometaphase.

Cells in prophase can still decide to go back to G2 phase, whereas mitotic progression becomes irreversible after the nuclear envelope breaks down and cells enter prometaphase. Apparently, this decision is dependent on the amount of Cdk1 substrates that is phosphorylated: when Cdk1 substrates approach their peak level of phosphorylation, cells become capable of proper M-to-G1 phase transition (Potapova et al., 2011). This so-called "forward M-to-G1 phase transition" requires active APC/C^{Cdc20} in order to process mitotic substrates and promote mitotic exit. Inhibiting Cdk1 kinase activity in prophase, using 10 µM flavopiridol, blocks mitotic progression and even reverts cells into G2 phase (Kaur et al., 1992; Potapova et al., 2011). In **Chapter 4**, we describe that Cdk1 inhibition by 3 µM RO-3306 allows cells to enter mitosis, but subsequently delays spindle checkpoint satisfaction and mitotic exit. The discrepancy between these studies is likely related to the doses of the Cdk inhibitors used. While we found that 3 µM RO-3306 only partially inhibits Cdk1, a dose

of 10 µM flavopiridol is expected to result in near complete Cdk1 inhibition. Consequently, flavopiridol treatment prevents normal Cdk1 substrate phosphorylation needed to promote mitosis. Partial Cdk1 inhibition only allows limited Cdk1 substrate phosphorylation. Consequently, cells will maintain the mitotic state, but delay in the spindle checkpoint since maximal substrate phosphorylation is needed to promote mitotic progression (Lindqvist et al., 2007). We believe that gradually increasing Cdk1 activity in mitosis, like in meiosis, may function as a timer that supports the stabilization of kinetochore-microtubule interactions (Davydenko et al., 2013). Partially blocking Cdk1 activity will delay the timer and therefore postpone satisfaction of the spindle checkpoint.

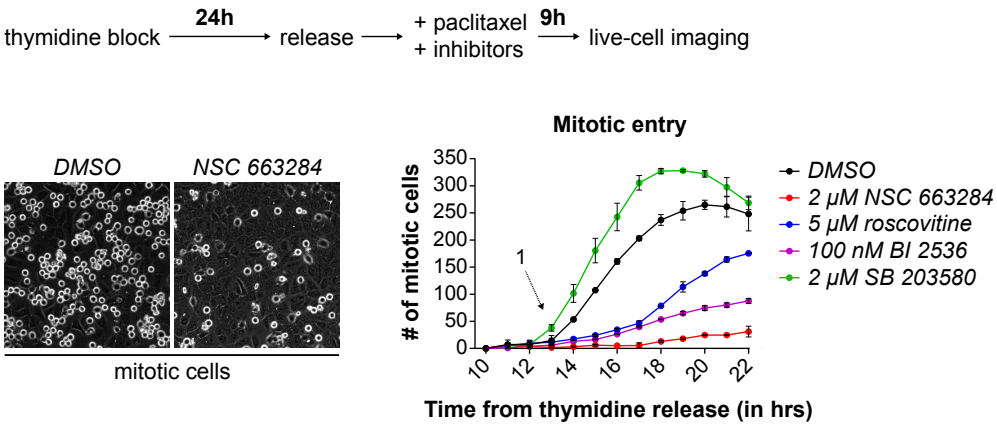

Figure 3 | **Monitoring the Effects of Different Effectors on Mitotic Entry**
U2OS cells were synchronised in mitosis as indicated by the experimental setup. Cells were monitored by phase contrast using a wide-field microscope. Representative images demonstrate the presence of mitotic cells after 18 hours of paclitaxel treatment. The arrow (1) Indicates the moment that cells start to enter mitosis due to activation of the positive-feedback loop. DMSO, control; NSC 663284, Cdc25A/B/C inhibitor; roscovitine, pan-Cdk inhibitor; BI 2536, Plk1 inhibitor; SB 203580, p38α/β inhibitor.

The spindle checkpoint is the major checkpoint that halts mitotic progression until all chromosomes are bi-oriented and correctly attached to microtubules. This checkpoint, composed of the molecular components BubR1, Mad2, and Bub3, prevents premature sister chromatid separation and safeguards genomic stability by inhibiting APC/C^{Cdc20} activity (Sudakin et al., 2001; reviewed in Foley and Kapoor, 2013). The spindle checkpoint is gradually satisfied by the establishment of correct attachments (Collin et al., 2013), triggering the metaphase-anaphase transition and segregation of chromosomes to form two identical daughter cells. During anaphase, certain defects may impact the genomic integrity of the newly formed daughter cells. We have shown in **Chapter 4** that lagging chromosomes segregate erroneously and therefore are a cause of genomic instability. When sister chromatid pairs are not separated properly (e.g. due to the presence of catenated DNA persisting between sister chromatids) anaphase bridges may arise that block the process of abscission (**Chapter 2** and **3**). These chromosome bridges form a physical barrier that

maintains Aurora B activity in order to protect cells against tetraploidization (Steigemann et al., 2009). This Aurora B-dependent abscission checkpoint was initially discovered in budding yeast and named NoCut as it prevents chromosome breakage by the cytokinetic machinery (Norden et al., 2006). The NoCut pathway is thought to monitor the clearance of chromatin from the cell equator to ensure completion of cytokinesis only takes place after all chromosomes have migrated to the poles. Recent findings have, however, contradicted the existence of the NoCut pathway, since non-segregating chromosomes rarely inhibit cell division by cleavage furrow regression. Apparently, chromosome arm segregation requires the combined action of Aurora B and the Condensin I complex (Bembenek et al., 2013; Cuylen et al., 2013). Together, these players prevent chromosome breakage once DNA ends up in the cleavage furrow by coordinating the resolution of chromatin obstructions and delaying abscission. The abscission checkpoint is possibly unable to handle gross segregation errors, which may be a cause of DNA damage and structural chromosome aberrations (Janssen et al., 2011). An overview of the checkpoints that coordinate normal cell division are summarized in Figure 4.

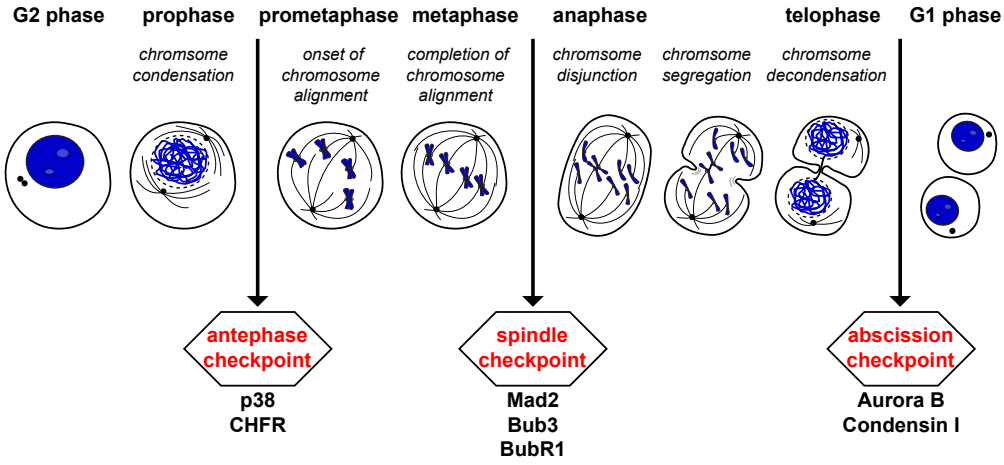

Figure 4 | **The Checkpoints That Function in Mitosis**
A schematic overview of the checkpoints that regulate transition through the different stages of mitosis. During prophase, the antephase checkpoint, dependent on p38 kinase and CHFR, may prevent mitotic progression as a result of certain exogenous stresses. Subsequently, the spindle checkpoint proteins Mad2, Bub3, and BubR1 ensure that anaphase follows metaphase by correctly attaching each sister chromatid to spindle microtubules. In this example, one sister chromatid pair is not completely separated, leading to the formation of an anaphase bridge. This DNA bridge is sensed by Aurora B, which, together with Condensin I, prevents abscission until the bridge is resolved. This checkpoint safeguards the cellular ploidy, resulting in the formation of two identical daughter cells.

Mitotic Exit: Reversal of Cdk1 Phosphorylation Events

As soon as cells enter mitosis, the activities of Cdk1-antagonizing phosphatases are repressed. In **Chapters 2** and **3** we discussed how MASTL supports cyclin B1-Cdk1 activity by inhibiting PP2A-B55. Also, the activity of PP1, another Cdk1-counteracting phosphatase is, at least in part, regulated by direct cyclin B1-Cdk1 phosphorylation (see **General Introduction**

for more details). How exactly the PP2A-B55 and -B56 phosphatases become active during mitosis is not well understood, but it is thought to involve the inactivation of Cdk1 by APC/C^{Cdc20}-mediated cyclin B1 proteolysis. PP1, which is activated by auto-dephosphorylation (Wu et al., 2009), is thought to stimulate PP2A-B55 activity. Once activated, PP2A-B55 starts dephosphorylating cyclin B1-Cdk1 substrates. MASTL is one of these substrates and is dephosphorylated at the essential Cdk1 site Thr^{194} (Blake-Hodek et al., 2012; Hégarat et al., 2014). As a result, MASTL activity is blocked by PP2A-B55. Interestingly, another phosphatase called Fcp1 is responsible for dephosphorylation of Arpp19 and Ensa (Hégarat et al., 2014). Fcp1 activity is controlled in a proteasome-dependent manner, but the exact mode of regulation is still poorly understood. Fcp1 may become active before PP2A-B55 in order to dephosphorylate Arpp19 and Ensa. Subsequently, PP2A-B55 is relieved from its inhibitors and may mediate the dephosphorylation of MASTL and other crucial cyclin B1-Cdk1 substrates.

The activation state of PP2A-B56 is controlled by a small inhibitory protein, in a way similar to that of PP2A-B55 complexes. This inhibitory protein, termed Biorientation defective 1 (Bod1), directly binds B56 thereby suppressing the phosphatase activity of PP2A-B56 (Porter et al., 2007; Porter et al., 2013). The underlying mechanisms that mediate Bod1 control over PP2A-B56 are unknown, but it is believed that Bod1 is activated by cyclin B1-Cdk1. The crucial Thr^{95} residue is probably a Cdk1 target, which, upon phosphorylation, converts Bod1 into a potent PP2A-B56 inhibitor (Porter et al., 2013). The declining cyclin B1 levels later in mitosis permit the PP1-mediated activation of PP2A-B55, which in turn promotes the dephosphorylation and activation of PP2A-B56 (Grallert et al., 2014). PP2A-B56 activity is thought to promote spindle checkpoint silencing (Espert et al., 2014). Direct substrates of PP2A-B56 are KNL1, a target of the checkpoint kinase Mps1, and probably also other kinetochore proteins. Altogether, the joint action of multiple phosphatases including at least Fcp1, PP1, PP2A-B55 and PP2A-B56, reverses most mitotic phosphorylations in order to promote exit from mitosis.

Cancer Mutations in the Cyclin-Cdk Pathway

Genetic alterations in crucial mitotic regulators may contribute to aneuploidy and facilitate tumorigenesis at a later stage. Stable aneuploidy can occur without chromosomal instability (CIN), the gain and loss of whole chromosomes during cell division, whereas the aneuploidy observed in cancer is often caused by CIN (Lengauer et al., 1997). Most alterations, associated with various cancer types, are found in genes involved in the spindle checkpoint, sister chromatid cohesion factors, and centrosome maturation (reviewed in Kops et al., 2005). None of the cancer-related mutations are found in essential genes, such as *CCNB1* or *CDC2*. However, these genes are appear overexpressed in various cancer types as shown by the CIN signature associated with cancer (Carter et al., 2006). This CIN signature consists of 70 genes and 29 of these are considered mitotic regulators according to their function. Interestingly, also the cytokinesis regulators *KIF4* and *PRC1* are included in the signature. Apart from the

CIN signature, negative cell cycle regulators, appear frequently downregulated, rather than overexpressed. For instance, the expression of CKI family members *CDKN2A* (p16), *CDKN2B* (p15), and *CDKN1B* (p27) often is completely lost by epigenetic inactivation (reviewed in Malumbres and Barbacid, 2001).

Many human tumours harbour defective p53 or pRb pathways. Mutations in the tumour suppressor gene *RB1* generally inactivate pRb and are associated with the development of retinoblastoma and other cancers (Lee et al., 1987; reviewed in Weinberg, 1995). The other tumour suppressor gene, *TP53*, is found mutated in more than 50% of human cancers and p53 loss is directly linked to tumorigenesis (Donehower et al., 1992; Hollstein et al., 1991).

Also mutations in genes other than *RB1* and *TP53* have been causally linked to tumour formation. For instance, the gene encoding for the PP2A scaffold (A) subunit, *PPP2R1A* (a gene we also describe in **Chapter 4**), is found mutated particularly in ovarian carcinoma but is also identified in other cancer subtypes (Calin et al., 2000; Jones et al., 2010). Many of the mutations identified are located in regions near the interface between the A subunit and the regulatory B subunit (Shih et al., 2011). These mutations may alter the binding affinity between PP2A A and B subunits and thereby the catalytic activity of the PP2A complex. It is interesting to note that the interaction between A and B subunits is abolished by the small-t (ST) antigen, a protein originating from a DNA tumour virus called simian virus 40 (SV40) (Ruediger et al., 1992; Ruediger et al., 2001; Yang et al., 1991). The ST antigen is a potent oncogene that inhibits PP2A phosphatase activity. Of all PP2A complexes, there are specific subtypes of the B56 family that may act as true tumour suppressors (Chen et al., 2004). Whether or not the oncogenic properties of PP2A inactivation are related to the activity state of cyclin B1-Cdk1 remains enigmatic. Future work may elucidate the key players involved in tumorigenesis and whether targeting mitosis, with particular attention to cyclin B1-Cdk1, may be a rationale for the treatment of PP2A-mutated cancers.

Concluding remarks

In this thesis, we studied the regulators that contribute to cyclin B1-Cdk1 activation in G2 phase and mitosis as well as the factors that are under direct control of Cdk1. We have uncovered the function of the previously uncharacterized kinase MASTL, and provide evidence that this kinase is the functional human orthologue of Greatwall kinase. The work presented sheds light onto the molecular control of mitosis with emphasis on the positive-feedback loop required to activate cyclin B1-Cdk1. In addition, we identified KIF4 and PRC1 as crucial effectors downstream of cyclin B1-Cdk1. Fine-tuning their activity in mitosis is vital for the formation of microtubule attachments to chromosomes, resulting in the division of equal sets of chromosomes over the newly formed daughter cells. It will be important to gain insight into the substrates whose phosphoregulation confers control of cyclin B1-Cdk1 over the spindle checkpoint and the stability of kinetochore-microtubule attachments. Future work should therefore be directed at identifying the key molecular events that drive spindle checkpoint satisfaction.

We believe our work highlights the importance of proper cyclin B1-Cdk1 activation in the execution of cell division. Any perturbation in this process could affect genome integrity, having disastrous consequences for successive cell cycle events. Our work may help to revisit the use Cdk1 inhibitors in the clinic, starting with the identification of biomarkers that sensitize cells to Cdk1 inhibition. The documentation of these biomarkers will be essential in order to predict tumor responsiveness to Cdk1 inhibitory strategies.

BIBLIOGRAPHY

A

Akopyan, K., Silva Cascales, H., Hukasova, E., Saurin, A. T., Müllers, E., Jaiswal, H., Hollman, D. A. A., Kops, G. J. P. L., Medema, R. H. and Lindqvist, A. (2014). Assessing kinetics from fixed cells reveals activation of the mitotic entry network at the S/G2 transition. *Mol. Cell* **53**, 843–853.

Alvarez, B., Martínez-A., C., Burgering, B. M. T. and Carrera, A. C. (2001). Forkhead transcription factors contribute to execution of the mitotic programme in mammals. *Nature* **413**, 744–747.

Álvarez-Fernández, M., Sánchez-Martínez, R., Sanz-Castillo, B., Pei, P., Sanz-Flores, M., Trakala, M., Ruiz-Torres, M., Lorca, T., Castro, A. and Malumbres, M. (2013). Greatwall is essential to prevent mitotic collapse after nuclear envelope breakdown in mammals. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 17374–17379.

Archambault, V., Zhao, X., White-Cooper, H., Carpenter, A. T. C. and Glover, D. M. (2007). Mutations in Drosophila Greatwall/Scant reveal its roles in mitosis and meiosis and interdependence with Polo kinase. *PLoS Genet.* **3**, e200.

Auer, T. O., Duroure, K., Cian, A. De, Concordet, J.-P. and Bene, F. Del (2014). Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. *Genome Res.* **24**, 142–153.

Baughn, L. B., Di Liberto, M., Wu, K., Toogood, P. L., Louie, T., Gottschalk, R., Niesvizky, R., Cho, H., Ely, S., Moore, M. A. S., et al. (2006). A novel orally active small molecule potently induces G1 arrest in primary myeloma cells and prevents tumor growth by specific inhibition of cyclin-dependent kinase 4/6. *Cancer Res.* **66**, 7661–7667.

Beamish, H., de Boer, L., Giles, N., Stevens, F., Oakes, V. and Gabrielli, B. (2009). Cyclin A/cdk2 regulates adenomatous polyposis coli-dependent mitotic spindle anchoring. *J. Biol. Chem.* **284**, 29015–29023.

Bell, S. P. and Dutta, A. (2002). DNA replication in eukaryotic cells. *Annu. Rev. Biochem.* **71**, 333–374.

Bell, P., Mais, C., McStay, B. and Scheer, U. (1997). Association of the nucleolar transcription factor UBF with the transcriptionally inactive rRNA genes of pronuclei and early Xenopus embryos. *J. Cell Sci.* **110**, 2053–2063.

Bellanger, S., de Gramont, A. and Sobczak-Thépot, J. (2007). Cyclin B2 suppresses mitotic failure and DNA re-replication in human somatic cells knocked down for both cyclins B1 and B2. *Oncogene* **26**, 7175–7184.

Bembenek, J. N., Verbrugghe, K. J. C., Khanikar, J., Csankovszki, G. and Chan, R. C. (2013). Condensin and the spindle midzone prevent cytokinesis failure induced by chromatin bridges in C. elegans embryos. *Curr. Biol.* **23**, 937–946.

Bentley, A. and Normand, G. (2007). Distinct sequence elements of cyclin B1 promote localization to chromatin, centrosomes, and kinetochores during mitosis. *Mol. Biol. Cell* **18**, 4847–4858.

Berns, K., Hijmans, E. M., Mullenders, J., Brummelkamp, T. R., Velds, A., Heimerikx, M., Kerkhoven, R. M., Madiredjo, M., Nijkamp, W., Weigelt, B., et al. (2004). A large-scale RNAi screen in human cells identifies new components of the p53 pathway. *Nature* **428**, 431–437.

Berthet, C., Aleem, E., Coppola, V., Tessarollo, L. and Kaldis, P. (2003). Cdk2 Knockout Mice Are Viable. *Curr. Biol.* **13**, 1775–1785.

Berthet, C., Klarmann, K. D., Hilton, M. B., Suh, H. C., Keller, J. R., Kiyokawa, H. and Kaldis, P. (2006). Combined loss of Cdk2 and Cdk4 results in embryonic lethality and Rb hypophosphorylation. *Dev. Cell* **10**, 563–573.

Bettencourt-Dias, M., Giet, R., Sinka, R., Mazumdar, A., Lock, W. G., Balloux, F., Zafiropoulos, P. J., Yamaguchi, S., Winter, S., Carthew, R. W., et al. (2004). Genome-wide survey of protein kinases required for cell cycle progression. *Nature* **432**, 980–987.

Bhatia, A. and Kumar, Y. (2013). Cancer cell micronucleus: an update on clinical and diagnostic applications. *APMIS* **121**, 569–581.

Bieling, P., Telley, I. A. and Surrey, T. (2010). A minimal midzone protein module controls formation and length of antiparallel microtubule overlaps. *Cell* **142**, 420–432.

Bird, A. W. and Hyman, A. A. (2008). Building a spindle of the correct length in human cells requires the interaction between TPX2 and Aurora A. *J. Cell Biol.* **182**, 289–300.

Blagosklonny, M. and Pardee, A. (2001). Exploiting cancer cell cycling for selective protection of normal cells. *Cancer Res.* **61**, 4301–4305.

Blake-Hodek, K. A., Williams, B. C., Zhao, Y., Castilho, P. V, Chen, W., Mao, Y., Yamamoto, T. M. and Goldberg, M. L. (2012). Determinants for activation of the atypical AGC kinase Greatwall during M phase entry. *Mol. Cell. Biol.* **32**, 1337–1353.

Blethrow, J. D., Glavy, J. S., Morgan, D. O. and Shokat, K. M. (2008). Covalent capture of kinase-specific phosphopeptides reveals Cdk1-cyclin B substrates. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 1442–1447.

Bloom, J. and Cross, F. R. (2007). Multiple levels of cyclin specificity in cell-cycle control. *Nat. Rev. Mol. Cell Biol.* **8**, 149–160.

Bollen, M., Peti, W., Ragusa, M. J. and Beullens, M. (2010). The extended PP1 toolkit: designed to create specificity. *Trends Biochem. Sci.* **35**, 450–458.

Bontron, S., Jaquenoud, M., Vaga, S., Talarek, N., Bodenmiller, B., Aebersold, R. and De Virgilio, C. (2013). Yeast endosulfines control entry into quiescence and chronological life span by inhibiting protein phosphatase 2A. *Cell Rep.* **3**, 16–22.

Brandeis, M., Rosewell, I., Carrington, M., Crompton, T., Jacobs, M. A., Kirk, J., Gannon, J. and Hunt, T. (1998). Cyclin B2-null mice develop normally and are fertile whereas cyclin B1-null mice die in utero. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 4344–4349.

Bringmann, H., Skiniotis, G., Spilker, A., Kandels-Lewis, S., Vernos, I. and Surrey, T. (2004). A kinesin-like motor inhibits microtubule dynamic instability. *Science* **303**, 1519–1522.

Brummelkamp, T. R., Bernards, R. and Agami, R. (2002). A system for stable expression of short interfering RNAs in mammalian cells. *Science* **296**, 550–553.

Brummelkamp, T. R., Fabius, A. W. M., Mullenders, J., Madiredjo, M., Velds, A., Kerkhoven, R. M., Bernards, R. and Beijersbergen, R. L. (2006). An shRNA barcode screen provides insight into cancer cell vulnerability to MDM2 inhibitors. *Nat. Chem. Biol.* **2**, 202–206.

Buonomo, S. B., Clyne, R. K., Fuchs, J., Loidl, J., Uhlmann, F. and Nasmyth, K. (2000). Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. *Cell* **103**, 387–398.

Burgess, A., Vigneron, S., Brioudes, E., Labbé, J.-C., Lorca, T. and Castro, A. (2010). Loss of human Greatwall results in G2 arrest and multiple mitotic defects due to deregulation of the cyclin B-Cdc2/PP2A balance. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 12564–12569.

Calin, G. A., Iasio, M. G. di, Caprini, E., Vorechovsky, I., Natali, P. G., Sozzi, G., Croce, C. M., Barbanti-Brodano, G., Russo, G. and Negrini, M. (2000). Low frequency of alterations of the alpha (PPP2R1A) and beta (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms. *Oncogene* **19**, 1191–1195.

Carlson, B. A., Dubay, M. M., Sausville, E. A., Brizuela, L. and Worland, P. J. (1996). Flavopiridol induces G1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. *Cancer Res.* **56**, 2973–2978.

Carpenter, A. J. and Porter, A. C. G. (2004). Construction, characterization, and complementation of a conditional-lethal DNA topoisomerase IIα mutant human cell line. *Mol. Biol. Cell* **15**, 5700–5711.

Carroll, C., Enquist-Newman, M. and Morgan, D. (2005). The APC subunit Doc1 promotes recognition of the substrate destruction box. *Curr. Biol.* **15**, 11–18.

C

Carter, S. L., Eklund, A. C., Kohane, I. S., Harris, L. N. and Szallasi, Z. (2006). A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. *Nat. Genet.* **38**, 1043–1048.

Castilho, P. V., Williams, B. C., Mochida, S., Zhao, Y. and Goldberg, M. L. (2009). The M phase kinase Greatwall (Gwl) promotes inactivation of PP2A/B55δ, a phosphatase directed against CDK phosphosites. *Mol. Biol. Cell* **20**, 4777–4789.

Cha, H., Wang, X., Li, H. and Fornace, A. J. (2007). A functional role for p38 MAPK in modulating mitotic transit in the absence of stress. *J. Biol. Chem.* **282**, 22984–22992.

Chan, E. H. Y., Santamaria, A., Silljé, H. H. W. and Nigg, E. A. (2008). Plk1 regulates mitotic Aurora A function through betaTrCP-dependent degradation of hBora. *Chromosoma* **117**, 457–469.

Chang, L., Zhang, Z., Yang, J., McLaughlin, S. H. and Barford, D. (2014). Molecular architecture and mechanism of the anaphase-promoting complex. *Nature* **17**, 13–17.

Chao, S. H., Fujinaga, K., Marion, J. E., Taube, R., Sausville, E. A., Senderowicz, A. M., Peterlin, B. M. and Price, D. H. (2000). Flavopiridol inhibits P-TEFb and blocks HIV-1 replication. *J. Biol. Chem.* **275**, 28345–28348.

Chen, Y. N., Sharma, S. K., Ramsey, T. M., Jiang, L., Martin, M. S., Baker, K., Adams, P. D., Bair, K. W. and Kaelin, W. G. (1999). Selective killing of transformed cells by cyclin/cyclin-dependent kinase 2 antagonists. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 4325–4329.

Chen, W., Possemato, R., Campbell, K. T., Plattner, C. A., Pallas, D. C. and Hahn, W. C. (2004). Identification of specific PP2A complexes involved in human cell transformation. *Cancer Cell* **5**, 127–136.

Chen, F., Archambault, V., Kar, A., Lio', P., D'Avino, P. P., Sinka, R., Lilley, K., Laue, E. D., Deak, P., Capalbo, L., et al. (2007). Multiple protein phosphatases are required for mitosis in Drosophila. *Curr. Biol.* **17**, 293–303.

Chen, Q., Zhang, X., Jiang, Q., Clarke, P. R. and Zhang, C. (2008). Cyclin B1 is localized to unattached kinetochores and contributes to efficient microtubule attachment and proper chromosome alignment during mitosis. *Cell Res.* **18**, 268–280.

Chestukhin, A., Pfeffer, C., Milligan, S., DeCaprio, J. A. and Pellman, D. (2003). Processing, localization, and requirement of human separase for normal anaphase progression. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 4574–4579.

Chow, J. P. H., Poon, R. Y. C. and Ma, H. T. (2011). Inhibitory phosphorylation of cyclin-dependent kinase 1 as a compensatory mechanism for mitosis exit. *Mol. Cell. Biol.* **31**, 1478–1491.

Ciosk, R., Zachariae, W., Michaelis, C., Shevchenko, A., Mann, M. and Nasmyth, K. (1998). An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. *Cell* **93**, 1067–1076.

Clijsters, L., Ogink, J. and Wolthuis, R. (2013). The spindle checkpoint, APC/C(Cdc20), and APC/C(Cdh1) play distinct roles in connecting mitosis to S phase. *J. Cell Biol.* **201**, 1013–1026.

Clijsters, L., van Zon, W., ter Riet, B., Voets, E., Boekhout, M., Ogink, J., Rumpf-Kienzl, C. and Wolthuis, R. M. F. (2014). Inefficient degradation of cyclin B1 re-activates the spindle checkpoint right after sister chromatid disjunction. *Cell Cycle* **13**, 2370–2378.

Collin, P., Nashchekina, O., Walker, R. and Pines, J. (2013). The spindle assembly checkpoint works like a rheostat rather than a toggle switch. *Nat. Cell Biol.* **15**, 1378–1385.

Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A., et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819–823.

Coudreuse, D. and Nurse, P. (2010). Driving the cell cycle with a minimal CDK control network. *Nature* **468**, 1074–1079.

Cukier, I. H., Li, Y. and Lee, J. M. (2007). Cyclin B1/Cdk1 binds and phosphorylates Filamin A and regulates its ability to cross-link actin. *FEBS Lett.* **581**, 1661–1672.

Cundell, M. J., Bastos, R. N., Zhang, T., Holder, J., Gruneberg, U., Novak, B. and Barr, F. A. (2013). The BEG (PP2A-B55/ENSA/Greatwall) pathway ensures cytokinesis follows chromosome separation. *Mol. Cell* **52**, 393–405.

Cuylen, S., Metz, J., Hruby, A. and Haering, C. H. (2013). Entrapment of chromosomes by condensin rings prevents their breakage during cytokinesis. *Dev. Cell* **27**, 469–478.

D'Angiolella, V., Mari, C., Nocera, D., Rametti, L. and Grieco, D. (2003). The spindle checkpoint requires cyclin-dependent kinase activity. *Genes Dev.* **17**, 2520–2525.

Dalton, S. (1992). Cell cycle regulation of the human cdc2 gene. *EMBO J.* **11**, 1797–804.

Daum, J. R., Potapova, T. A., Sivakumar, S., Daniel, J. J., Flynn, J. N., Rankin, S. and Gorbsky, G. J. (2011). Cohesion fatigue induces chromatid separation in cells delayed at metaphase. *Curr. Biol.* **21**, 1018–1024.

Davydenko, O., Schultz, R. M. and Lampson, M. A. (2013). Increased CDK1 activity determines the timing of kinetochore-microtubule attachments in meiosis I. *J. Cell Biol.* **202**, 221–229.

Decordier, I., Cundari, E. and Kirsch-Volders, M. (2008). Survival of aneuploid, micronucleated and/or polyploid cells: crosstalk between ploidy control and apoptosis. *Mutat. Res.* **651**, 30–39.

Den Elzen, N. and Pines, J. (2001). Cyclin A is destroyed in prometaphase and can delay chromosome alignment and anaphase. *J. Cell Biol.* **153**, 121–136.

Dephoure, N., Zhou, C., Villén, J., Beausoleil, S. a, Bakalarski, C. E., Elledge, S. J. and Gygi, S. P. (2008). A quantitative atlas of mitotic phosphorylation. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 10762–10767.

Di Fiore, B. and Pines, J. (2007). Emi1 is needed to couple DNA replication with mitosis but does not regulate activation of the mitotic APC/C. *J. Cell Biol.* **177**, 425–437.

Diril, M. K., Koumar, C., Padmakumar, V. C., Du, T., Wasser, M., Coppola, V., Tessarollo, L. and Kaldis, P. (2012). Cyclin-dependent kinase 1 (Cdk1) is essential for cell division and suppression of DNA re-replication but not for liver regeneration. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 3826–3831.

Dohadwala, M., da Cruz e Silva, E. F., Hall, F. L., Williams, R. T., Carbonaro-Hall, D. A., Nairn, A. C., Greengard, P. and Berndt, N. (1994). Phosphorylation and inactivation of protein phosphatase 1 by cyclin-dependent kinases. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 6408–6412.

Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Jr, C. A. M., Butel, J. S. and Bradley, A. (1992). Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215–221.

Dunsch, A. K., Linnane, E., Barr, F. A. and Gruneberg, U. (2011). The astrin-kinastrin/SKAP complex localizes to microtubule plus ends and facilitates chromosome alignment. *J. Cell Biol.* **192**, 959–968.

Dyson, N. (1998). The regulation of E2F by pRB-family proteins. *Genes Dev.* **12**, 2245–2262.

Easton, J., Wei, T., Lahti, J. and Kidd, V. (1998). Disruption of the cyclin D/cyclin-dependent kinase/INK4/retinoblastoma protein regulatory pathway in human neuroblastoma. *Cancer Res.* **58**, 2624–2632.

Edgar, B. A. and Orr-Weaver, T. L. (2001). Endoreplication cell cycles: more for less. *Cell* **105**, 297–306.

Errico, A., Deshmukh, K., Tanaka, Y., Pozniakovsky, A. and Hunt, T. (2010). Identification of substrates for cyclin dependent kinases. *Adv. Enzyme Regul.* **50**, 375–399.

Espert, A., Uluocak, P., Bastos, R. N., Mangat, D., Graab, P. and Gruneberg, U. (2014). PP2A-B56 opposes Mps1 phosphorylation of Knl1 and thereby promotes spindle assembly checkpoint silencing. *J. Cell Biol.* **206**, 833–842.

F

Farr, C. J., Antoniou-Kourounioti, M., Mimmack, M. L., Volkov, A. and Porter, A. C. G. (2014). The α isoform of topoisomerase II is required for hypercompaction of mitotic chromosomes in human cells. *Nucleic Acids Res.* **42**, 4414–4426.

Ferrigno, P., Langan, T. A. and Cohen, P. (1993). Protein phosphatase 2A1 is the major enzyme in vertebrate cell extracts that dephosphorylates several physiological substrates for cyclin-dependent protein kinases. *Mol. Biol. Cell* **4**, 669–677.

Fischer, P. M. and Gianella-Borradori, A. (2005). Recent progress in the discovery and development of cyclin-dependent kinase inhibitors. *Expert Opin. Investig. Drugs* **14**, 457–477.

Fisher, R. P. and Morgan, D. O. (1994). A novel cyclin associates with M015/CDK7 to form the CDK-activating kinase. *Cell* **78**, 713–724.

Fisher, D. and Nurse, P. (1996). A single fission yeast mitotic cyclin B p34cdc2 kinase promotes both S-phase and mitosis in the absence of G1 cyclins. *EMBO J.* **15**, 850–860.

Foley, E. A. and Kapoor, T. M. (2013). Microtubule attachment and spindle assembly checkpoint signalling at the kinetochore. *Nat. Rev. Mol. Cell Biol.* **14**, 25–37.

Forester, C. M., Maddox, J., Louis, J. V, Goris, J. and Virshup, D. M. (2007). Control of mitotic exit by PP2A regulation of Cdc25C and Cdk1. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 19867–19872.

Fourest-Lieuvin, A., Peris, L., Gache, V., Garcia-Saez, I., Juillan-Binard, C., Lantez, V. and Job, D. (2006). Microtubule regulation in mitosis: tubulin phosphorylation by the cyclin-dependent kinase Cdk1. *Mol. Biol. Cell* **17**, 1041–1050.

Fowler, V. M. and Adam, E. J. H. (1992). Spectrin redistributes to the cytosol and is phosphorylated during mitosis in cultured cells. *J. Cell Biol.* **119**, 1559–1572.

Francisco, L., Wang, W. and Chan, C. S. M. (1994). Type 1 protein phosphatase acts in opposition to IpL1 protein kinase in regulating yeast chromosome segregation. *Mol. Cell. Biol.* **14**, 4731–4740.

Fry, D. W., Harvey, P. J., Keller, P. R., Elliott, W. L., Meade, M., Trachet, E., Albassam, M., Zheng, X., Leopold, W. R., Pryer, N. K., et al. (2004). Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. *Mol. Cancer Ther.* **3**, 1427–1438.

Furuno, N., den Elzen, N. and Pines, J. (1999). Human cyclin A is required for mitosis until mid prophase. *J. Cell Biol.* **147**, 295–306.

Gandhi, M. J., Cummings, C. L. and Drachman, J. G. (2003). *FLJ14813* Missense Mutation: A Candidate for Autosomal Dominant Thrombocytopenia on Human Chromosome 10. *Hum. Hered.* **55**, 66–70.

Gascoigne, K. E. and Taylor, S. S. (2008). Cancer cells display profound intra- and interline variation following prolonged exposure to antimitotic drugs. *Cancer Cell* **14**, 111–122.

Gavet, O. and Pines, J. (2010a). Activation of cyclin B1-Cdk1 synchronizes events in the nucleus and the cytoplasm at mitosis. *J. Cell Biol.* **189**, 247–259.

Gavet, O. and Pines, J. (2010b). Progressive activation of CyclinB1-Cdk1 coordinates entry to mitosis. *Dev. Cell* **18**, 533–543.

Gharbi-Ayachi, A., Labbé, J.-C., Burgess, A., Vigneron, S., Strub, J.-M., Brioudes, E., Van-Dorsselaer, A., Castro, A. and Lorca, T. (2010). The substrate of Greatwall kinase, Arpp19, controls mitosis by inhibiting protein phosphatase 2A. *Science* **330**, 1673–1677.

Goga, A., Yang, D., Tward, A. D., Morgan, D. O. and Bishop, J. M. (2007). Inhibition of CDK1 as a potential therapy for tumors over-expressing MYC. *Nat. Med.* **13**, 820–827.

Gong, D. and Ferrell, J. (2010). The roles of cyclin A2, B1, and B2 in early and late mitotic events. *Mol. Biol. Cell* **21**, 3149–3161.

Gong, D., Pomerening, J. R., Myers, J. W., Gustavsson, C., Jones, J. T., Hahn, A. T., Meyer, T. and Ferrell, J. E. (2007). Cyclin A2 regulates nuclear-envelope breakdown and the nuclear accumulation of cyclin B1. *Curr. Biol.* **17**, 85–91.

Gorr, I. H., Boos, D. and Stemmann, O. (2005). Mutual inhibition of separase and Cdk1 by two-step complex formation. *Mol. Cell* **19**, 135–141.

Gould, K. L. and Nurse, P. (1989). Tyrosine phosphorylation of the fission yeast cdc2+ protein kinase regulates entry into mitosis. *Nature* **342**, 39–45.

Gowdy, P. M., Anderson, H. J. and Roberge, M. (1998). Entry into mitosis without Cdc2 kinase activation. *J. Cell Sci.* **111**, 3401–3410.

Grallert, A., Boke, E., Hagting, A., Hodgson, B., Connolly, Y., Griffiths, J. R., Smith, D. L., Pines, J. and Hagan, I. M. (2014). A PP1-PP2A phosphatase relay controls mitotic progression. *Nature* **517**, 94–98.

Grosskortenhaus, R. and Sprenger, F. (2002). Rca1 inhibits APC-Cdh1(Fzr) and is required to prevent cyclin degradation in G2. *Dev. Cell* **2**, 29–40.

Hagting, A., Karlsson, C., Clute, P., Jackman, M. and Pines, J. (1998). MPF localization is controlled by nuclear export. *EMBO J.* **17**, 4127–4138.

Hagting, A., Den Elzen, N., Vodermaier, H. C., Waizenegger, I. C., Peters, J.-M. and Pines, J. (2002). Human securin proteolysis is controlled by the spindle checkpoint and reveals when the APC/C switches from activation by Cdc20 to Cdh1. *J. Cell Biol.* **157**, 1125–1137.

Hames, R. S., Wattam, S. L., Yamano, H., Bacchieri, R. and Fry, A. M. (2001). APC/C-mediated destruction of the centrosomal kinase Nek2A occurs in early mitosis and depends upon a cyclin A-type D-box. *EMBO J.* **20**, 7117–7127.

Hammond, M. and Laird, C. (1985). Chromosome structure and DNA replication in nurse and follicle cells of Drosophila melanogaster. *Chromosoma* **91**, 267–278.

Harbour, J. W., Luo, R. X., Santi, A. D., Postigo, A. A. and Dean, D. C. (1999). Intramolecular Interactions that Progressively Block Rb Functions as Cells Move through G1. *Cell* **98**, 859–869.

Heald, R. and McKeon, F. (1990). Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. *Cell* **61**, 579–589.

Hégarat, N., Vesely, C., Vinod, P. K., Ocasio, C., Peter, N., Gannon, J., Oliver, A. W., Novák, B. and Hochegger, H. (2014). PP2A/B55 and Fcp1 regulate greatwall and Ensa dephosphorylation during mitotic exit. *PLoS Genet.* **10**, e1004004.

Hellmuth, S., Böttger, F., Pan, C., Mann, M. and Stemmann, O. (2014). PP2A delays APC/C-dependent degradation of separase-associated but not free securin. *EMBO J.* **33**, 1134–1148.

Hinds, P. W., Mittnacht, S., Dulic, V., Arnold, A., Reed, S. I. and Weinberg, R. A. (1992). Regulation of retinoblastoma protein functions by ectopic expression of human cyclins. *Cell* **70**, 993–1006.

Hochegger, H., Dejsuphong, D., Sonoda, E., Saberi, A., Rajendra, E., Kirk, J., Hunt, T. and Takeda, S. (2007). An essential role for Cdk1 in S phase control is revealed via chemical genetics in vertebrate cells. *J. Cell Biol.* **178**, 257–268.

Holland, A. J. and Taylor, S. S. (2006). Cyclin-B1-mediated inhibition of excess separase is required for timely chromosome disjunction. *J. Cell Sci.* **119**, 3325–3336.

Holland, A. J., Böttger, F., Stemmann, O. and Taylor, S. S. (2007). Protein phosphatase 2A and separase form a complex regulated by separase autocleavage. *J. Biol. Chem.* **282**, 24623–24632.

Hollstein, M., Sidransky, D., Vogelstein, B. and Harris, C. C. (1991). p53 mutations in human cancers. *Sci.* **253** , 49–53.

Hsu, J. Y., Sun, Z. W., Li, X., Reuben, M., Tatchell, K., Bishop, D. K., Grushcow, J. M., Brame, C. J., Caldwell, J. A., Hunt, D. F., et al. (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell* **102**, 279–291.

H

Hu, B., Mitra, J., Heuvel, S. van den and Enders, G. H. (2001). S and G2 phase roles for Cdk2 revealed by inducible expression of a dominant-negative mutant in human cells. *Mol. Cell. Biol.* **21**, 2755–2766.

Hu, C.-K., Coughlin, M., Field, C. M. and Mitchison, T. J. (2011). KIF4 regulates midzone length during cytokinesis. *Curr. Biol.* **21**, 815–824.

Iorns, E., Lord, C. J., Grigoriadis, A., McDonald, S., Fenwick, K., Mackay, A., Mein, C. A., Natrajan, R., Savage, K., Tamber, N., et al. (2009). Integrated functional, gene expression and genomic analysis for the identification of cancer targets. *PLoS One* **4**, e5120.

Itzhaki, J., Gilbert, C. and Porter, A. (1997). Construction by gene targeting in human cells of a "conditional" CDC2 mutant that rereplicates its DNA. *Nat. Genet.* **15**, 258–265.

Izawa, D. and Pines, J. (2011). How APC/C-Cdc20 changes its substrate specificity in mitosis. *Nat. Cell Biol.* **13**, 223–233.

Janssen, A., van der Burg, M., Szuhai, K., Kops, G. J. P. L. and Medema, R. H. (2011). Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. *Science* **333**, 1895–1898.

Janssens, V. and Goris, J. (2001). Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem. J* **439**, 417–439.

Jiang, W., Jimenez, G., Wells, N. J., Hope, T. J., Wahl, G. M., Hunter, T. and Fukunaga, R. (1998). PRC1: a human mitotic spindle-associated CDK substrate protein required for cytokinesis. *Mol. Cell* **2**, 877–885.

Johnson, H. J., Gandhi, M. J., Shafizadeh, E., Langer, N. B., Pierce, E. L., Paw, B. H., Gilligan, D. M. and Drachman, J. G. (2009). In vivo inactivation of MASTL kinase results in thrombocytopenia. *Exp. Hematol.* **37**, 901–908.

Johnson, N., Li, Y.-C., Walton, Z. E., Cheng, K. A., Li, D., Rodig, S. J., Moreau, L. A., Unitt, C., Bronson, R. T., Thomas, H. D., et al. (2011). Compromised CDK1 activity sensitizes BRCA-proficient cancers to PARP inhibition. *Nat. Med.* **17**, 875–882.

Jones, S., Wang, T.-L., Shih, I.-M., Mao, T.-L., Nakayama, K., Roden, R., Glas, R., Slamon, D., Diaz, L. A., Vogelstein, B., et al. (2010). Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. *Science* **330**, 228–231.

Juanes, M. A., Khoueiry, R., Kupka, T., Castro, A., Mudrak, I., Ogris, E., Lorca, T. and Piatti, S. (2013). Budding Yeast Greatwall and Endosulfines Control Activity and Spatial Regulation of PP2ACdc55 for Timely Mitotic Progression. *PLoS Genet.* **9**, e1003575.

Kabeche, L. and Compton, D. A. (2013). Cyclin A regulates kinetochore microtubules to promote faithful chromosome segregation. *Nature* **502**, 110–113.

Kamenz, J. and Hauf, S. (2014). Slow checkpoint activation kinetics as a safety device in anaphase. *Curr. Biol.* **24**, 646–651.

Kanda, T., Sullivan, K. F. and Wahl, G. M. (1998). Histone-GFP fusion protein enables sensitive analysis of chromosome dynamics in living mammalian cells. *Curr. Biol.* **8**, 377–385.

Katsumi, Y., Iehara, T., Miyachi, M., Yagyu, S., Tsubai-Shimizu, S., Kikuchi, K., Tamura, S., Kuwahara, Y., Tsuchiya, K., Kuroda, H., et al. (2011). Sensitivity of malignant rhabdoid tumor cell lines to PD 0332991 is inversely correlated with p16 expression. *Biochem. Biophys. Res. Commun.* **413**, 62–68.

Kaur, G., Stetler-Stevenson, M., Sebers, S., Worland, P., Sedlacek, H., Myers, C., Czech, J., Naik, R. and Sausville, E. (1992). Growth Inhibition With Reversible Cell Cycle Arrest of Carcinoma Cells by Flavone L86-8275. *J. Natl. Cancer Inst.* **84**, 1736–1740.

Kim, M.-Y., Bucciarelli, E., Morton, D. G., Williams, B. C., Blake-Hodek, K., Pellacani, C., Von Stetina, J. R., Hu, X., Somma, M. P., Drummond-Barbosa, D., et al. (2012). Bypassing the

Greatwall-Endosulfine pathway: plasticity of a pivotal cell-cycle regulatory module in Drosophila melanogaster and Caenorhabditis elegans. *Genetics* **191**, 1181–1197.

Kitajima, T. S., Kawashima, S. A. and Watanabe, Y. (2004). The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. *Nature* **427**, 510–517.

Kitajima, T. S., Sakuno, T., Ishiguro, K., Iemura, S., Natsume, T., Kawashima, S. A. and Watanabe, Y. (2006). Shugoshin collaborates with protein phosphatase 2A to protect cohesin. *Nature* **441**, 46–52.

Kops, G. J. P. L., Weaver, B. A. A. and Cleveland, D. W. (2005). On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat. Rev. Cancer* **5**, 773–785.

Kozar, K., Ciemerych, M. A., Rebel, V. I., Shigematsu, H., Zagozdzon, A., Sicinska, E., Geng, Y., Yu, Q., Bhattacharya, S., Bronson, R. T., et al. (2004). Mouse development and cell proliferation in the absence of D-cyclins. *Cell* **118**, 477–491.

Kraft, C., Herzog, F., Gieffers, C., Mechtler, K., Hagting, A., Pines, J. and Peters, J.-M. (2003). Mitotic regulation of the human anaphase-promoting complex by phosphorylation. *EMBO J.* **22**, 6598–6609.

Kurasawa, Y., Earnshaw, W. C., Mochizuki, Y., Dohmae, N. and Todokoro, K. (2004). Essential roles of KIF4 and its binding partner PRC1 in organized central spindle midzone formation. *EMBO J.* **23**, 3237–3248.

Kutay, U. and Hetzer, M. W. (2008). Reorganization of the nuclear envelope during open mitosis. *Curr. Opin. Cell Biol.* **20**, 669–677.

Kwon, Y. G., Lee, S. Y., Choi, Y., Greengard, P. and Nairn, A. C. (1997). Cell cycle-dependent phosphorylation of mammalian protein phosphatase 1 by cdc2 kinase. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 2168–2173.

Laoukili, J., Kooistra, M. R. H., Brás, A., Kauw, J., Kerkhoven, R. M., Morrison, A., Clevers, H. and Medema, R. H. (2005). FoxM1 is required for execution of the mitotic programme and chromosome stability. *Nat. Cell Biol.* **7**, 126–136.

Laoukili, J., Alvarez, M., Meijer, L. A. T., Stahl, M., Mohammed, S., Kleij, L., Heck, A. J. R. and Medema, R. H. (2008). Activation of FoxM1 during G2 requires cyclin A/Cdk-dependent relief of autorepression by the FoxM1 N-terminal domain. *Mol. Cell. Biol.* **28**, 3076–3087.

Lee, W. H., Bookstein, R., Hong, F., Young, L. J., Shew, J. Y. and Lee, E. Y. (1987). Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science* **235**, 1394–1399.

Lénárt, P., Petronczki, M., Steegmaier, M., Di Fiore, B., Lipp, J. J., Hoffmann, M., Rettig, W. J., Kraut, N. and Peters, J.-M. (2007). The small-molecule inhibitor BI 2536 reveals novel insights into mitotic roles of polo-like kinase 1. *Curr. Biol.* **17**, 304–315.

Lengauer, C., Kinzler, K. and Vogelstein, B. (1997). Genetic instability in colorectal cancers. *Nature* **386**, 623–627.

Li, C., Andrake, M., Dunbrack, R. and Enders, G. H. (2010). A bifunctional regulatory element in human somatic Wee1 mediates cyclin A/Cdk2 binding and Crm1-dependent nuclear export. *Mol. Cell. Biol.* **30**, 116–130.

Lindqvist, A., van Zon, W., Karlsson Rosenthal, C. and Wolthuis, R. M. F. (2007). Cyclin B1-Cdk1 activation continues after centrosome separation to control mitotic progression. *PLoS Biol.* **5**, e123.

Lindqvist, A., Rodríguez-Bravo, V. and Medema, R. H. (2009). The decision to enter mitosis: feedback and redundancy in the mitotic entry network. *J. Cell Biol.* **185**, 193–202.

Loog, M. and Morgan, D. (2005). Cyclin specificity in the phosphorylation of cyclin-dependent kinase substrates. *Nature* **434**, 104–108.

Lukas, C., Sørensen, C. S., Kramer, E., Santoni-Rugiu, E., Lindeneg, C., Peters, J. M., Bartek, J. and Lukas, J. (1999). Accumulation of cyclin B1 requires E2F and cyclin-A-dependent rearrangement of the anaphase-promoting complex. *Nature* **401**, 815–818.

Lundberg, A. S. and Weinberg, R. A. (1998). Functional Inactivation of the Retinoblastoma Protein Requires Sequential Modification by at Least Two Distinct Cyclin-cdk Complexes Functional Inactivation of the Retinoblastoma Protein Requires Sequential Modification by at Least Two Distinct Cyclin-cdk. *Mol. Cell. Biol.* **18**, 753–761.

Luzhna, L., Kathiria, P. and Kovalchuk, O. (2013). Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. *Front. Genet.* **4**, 1–17.

Ma, H. T., Tsang, Y. H., Marxer, M. and Poon, R. Y. C. (2009). Cyclin A2-cyclin-dependent kinase 2 cooperates with the PLK1-SCFbeta-TrCP1-EMI1-anaphase-promoting complex/cyclosome axis to promote genome reduplication in the absence of mitosis. *Mol. Cell. Biol.* **29**, 6500–6514.

Machida, Y. J. and Dutta, A. (2007). The APC/C inhibitor, Emi1, is essential for prevention of rereplication. *Genes Dev.* **21**, 184–194.

Macůrek, L., Lindqvist, A., Lim, D., Lampson, M. A., Klompmaker, R., Freire, R., Clouin, C., Taylor, S. S., Yaffe, M. B. and Medema, R. H. (2008). Polo-like kinase-1 is activated by aurora A to promote checkpoint recovery. *Nature* **455**, 119–123.

Major, M. L., Lepe, R. and Costa, R. H. (2004). Forkhead box M1B transcriptional activity requires binding of Cdk-cyclin complexes for phosphorylation-dependent recruitment of p300/CBP coactivators. *Mol. Cell. Biol.* **24**, 2649–2661.

Makela, T. P., Tassan, J.-P., Nigg, E. A., Frutiger, S., Hughes, G. J. and Weinberg, R. A. (1994). A cyclin associated with the CDK-activating kinase MO15. *Nature* **371**, 254–257.

Malumbres, M. and Barbacid, M. (2001). To cycle or not to cycle: a critical decision in cancer. *Nat. Rev. Cancer* **1**, 222–231.

Malumbres, M. and Barbacid, M. (2009). Cell cycle, CDKs and cancer: a changing paradigm. *Nat. Rev. Cancer* **9**, 153–166.

Malumbres, M., Sotillo, R., Santamaría, D., Galán, J., Cerezo, A., Ortega, S., Dubus, P. and Barbacid, M. (2004). Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. *Cell* **118**, 493–504.

Manchado, E., Guillamot, M., de Cárcer, G., Eguren, M., Trickey, M., García-Higuera, I., Moreno, S., Yamano, H., Cañamero, M. and Malumbres, M. (2010). Targeting mitotic exit leads to tumor regression in vivo: Modulation by Cdk1, Mastl, and the PP2A/B55α,δ phosphatase. *Cancer Cell* **18**, 641–654.

Manning, A. L., Bakhoum, S. F., Maffini, S., Correia-Melo, C., Maiato, H. and Compton, D. A. (2010). CLASP1, astrin and Kif2b form a molecular switch that regulates kinetochore-microtubule dynamics to promote mitotic progression and fidelity. *EMBO J.* **29**, 3531–3543.

Marston, A. L., Tham, W.-H., Shah, H. and Amon, A. (2004). A genome-wide screen identifies genes required for centromeric cohesion. *Science* **303**, 1367–1370.

Matsusaka, T. and Pines, J. (2004). Chfr acts with the p38 stress kinases to block entry to mitosis in mammalian cells. *J. Cell Biol.* **166**, 507–516.

Mayer-Jaekel, R. E., Ohkura, H., Gomes, R., Sunkel, C. E., Baumgartner, S., Hemmings, B. A. and Glover, D. M. (1993). The 55 kd regulatory subunit of Drosophila protein phosphatase 2A is required for anaphase. *Cell* **72**, 621–633.

McCloy, R. A., Rogers, S., Caldon, C. E., Lorca, T., Castro, A. and Burgess, A. (2014). Partial inhibition of Cdk1 in G2 phase overrides the SAC and decouples mitotic events. *Cell Cycle* **13**, 1400–1412.

Melixetian, M., Ballabeni, A., Masiero, L., Gasparini, P., Zamponi, R., Bartek, J., Lukas, J. and Helin, K. (2004). Loss of Geminin induces rereplication in the presence of functional p53. *J. Cell Biol.* **165**, 473–482.

Mochida, S. (2014). Regulation of α–endosulfine, an inhibitor of protein phosphatase 2A, by multisite phosphorylation. *FEBS J.* **281**, 1159–1169.

Mochida, S. and Hunt, T. (2007). Calcineurin is required to release Xenopus egg extracts from meiotic M phase. *Nature* **449**, 336–340.

Mochida, S. and Hunt, T. (2012). Protein phosphatases and their regulation in the control of mitosis. *EMBO Rep.* **13**, 197–203.

Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. (2009). Regulated activity of PP2A-B55 delta is crucial for controlling entry into and exit from mitosis in Xenopus egg extracts. *EMBO J.* **28**, 2777–2785.

Mochida, S., Maslen, S. L., Skehel, M. and Hunt, T. (2010). Greatwall phosphorylates an inhibitor of protein phosphatase 2A that is essential for mitosis. *Science* **330**, 1670–1673.

Mollinari, C., Kleman, J.-P., Jiang, W., Schoehn, G., Hunter, T. and Margolis, R. L. (2002). PRC1 is a microtubule binding and bundling protein essential to maintain the mitotic spindle midzone. *J. Cell Biol.* **157**, 1175–1186.

Morgan, D. O. (2007). The Cell Cycle: Principles of Control. New Science Press. ISBN-13: 98-0199206100.

Mueller, P. R., Coleman, T. R., Kumagai, A. and Dunphy, W. G. (1995). Myt1: A Membrane-Associated Inhibitory Kinase That Phosphorylates Cdc2 on Both Threonine-14 and Tyrosine-15. *Science* **270**, 86–90.

Murphy, M., Stinnakre, M.-G., Senamaud-Beaufort, C., Winston, N. J., Sweeney, C., Kubelka, M., Carrington, M., Bréchot, C. and Sobczak-Thépot, J. (1997). Delayed early embryonic lethality following disruption of the murine cyclin A2 gene. *Nat. Genet.* **15**, 83–86.

Neef, R., Gruneberg, U., Kopajtich, R., Li, X., Nigg, E. A., Sillje, H. and Barr, F. A. (2007). Choice of Plk1 docking partners during mitosis and cytokinesis is controlled by the activation state of Cdk1. *Nat. Cell Biol.* **9**, 436–444.

Norden, C., Mendoza, M., Dobbelaere, J., Kotwaliwale, C. V, Biggins, S. and Barral, Y. (2006). The NoCut pathway links completion of cytokinesis to spindle midzone function to prevent chromosome breakage. *Cell* **125**, 85–98.

Nunes Bastos, R., Gandhi, S. R., Baron, R. D., Gruneberg, U., Nigg, E. A. and Barr, F. A. (2013). Aurora B suppresses microtubule dynamics and limits central spindle size by locally activating KIF4A. *J. Cell Biol.* **202**, 605–621.

Nurse, P. and Thuriaux, P. (1980). Regulatory genes controlling mitosis in the fission yeast Schizosaccharomyces pombe. *Genetics* **96**, 627–637.

O'Connor, D. S., Grossman, D., Plescia, J., Li, F., Zhang, H., Villa, A., Tognin, S., Carlo, P. and Altieri, D. C. (2000). Regulation of apoptosis at cell division by p34 cdc2 phosphorylation of survivin. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 10–14.

O'Farrell, P. H. (2001). Triggering the all-or-nothing switch into mitosis. *Trends Cell Biol.* **11**, 512–519.

Ohkura, R. E. M.-J. H., Ferrigno, P., Andjelkovic, N., Shiomi, K., Uemura, T., Glover, D. M. and Hemmings, B. A. (1994). Drosophila mutants in the 55 kDa regulatory subunit of protein phosphatase 2A show strongly reduced ability to dephosphorylate substrates of p34cdc2. *J. Cell Sci.* **107**, 2609–2616.

Ohtani, K., DeGregori, J. and Nevins, J. R. (1995). Regulation of the cyclin E gene by transcription factor E2F1. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 12146–12150.

Ohtani, K., Gregori, J. D. E., Leone, G., Herendeen, D. R., Kelly, T. J. and Nevins, J. R. (1996). Expression of the HsOrc1 gene, a human ORC1 homolog, is regulated by cell proliferation via the E2F transcription factor. *Mol. Cell. Biol.* **16**, 6977–6984.

O

Okumura, E., Morita, A., Wakai, M., Mochida, S., Hara, M. and Kishimoto, T. (2014). Cyclin B–Cdk1 inhibits protein phosphatase PP2A-B55 via a Greatwall kinase–independent mechanism. *J. Cell Biol.* **204**, 881–889.

Olsen, J. V, Vermeulen, M., Santamaria, A., Kumar, C., Miller, M. L., Jensen, L. J., Gnad, F., Cox, J., Jensen, T. S., Nigg, E. A., et al. (2010). Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. *Sci. Signal.* **3**, ra3.

Ortega, S., Prieto, I., Odajima, J., Martín, A., Dubus, P., Sotillo, R., Barbero, J. L., Malumbres, M. and Barbacid, M. (2003). Cyclin-dependent kinase 2 is essential for meiosis but not for mitotic cell division in mice. *Nat. Genet.* **35**, 25–31.

Pagliuca, F. W., Collins, M. O., Lichawska, A., Zegerman, P., Choudhary, J. S. and Pines, J. (2011). Quantitative proteomics reveals the basis for the biochemical specificity of the cell-cycle machinery. *Mol. Cell* **43**, 406–417.

Patra, D. and Dunphy, W. G. (1998). Xe-p9, a Xenopus Suc1/Cks protein, is essential for the Cdc2-dependent phosphorylation of the anaphase-promoting complex at mitosis. *Genes Dev.* **12**, 2549–2559.

Pearson, B. E., Nasheuer, H.-P. and Wang, T. S.-F. (1991). Human DNA polymerase alpha gene: sequences controlling expression in cycling and serum-stimulated cells. *Mol. Cell. Biol.* **11**, 2081–2095.

Pelkmans, L., Fava, E., Grabner, H., Hannus, M., Habermann, B., Krausz, E. and Zerial, M. (2005). Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis. *Nature* **436**, 78–86.

Peters, J.-M. (2002). The anaphase-promoting complex: proteolysis in mitosis and beyond. *Mol. Cell* **9**, 931–943.

Petronczki, M., Lénárt, P. and Peters, J.-M. (2008). Polo on the Rise-from Mitotic Entry to Cytokinesis with Plk1. *Dev. Cell* **14**, 646–659.

Pines, J. (2006). Mitosis: a matter of getting rid of the right protein at the right time. *Trends Cell Biol.* **16**, 55–63.

Pines, J. (2011). Cubism and the cell cycle: the many faces of the APC/C. *Nat. Rev. Mol. Cell Biol.* **12**, 427–438.

Pomerening, J. R., Sontag, E. D. and Ferrell, J. E. (2003). Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. *Nat. Cell Biol.* **5**, 346–351.

Porter, A. C. G. (2008). Preventing DNA over-replication: a Cdk perspective. *Cell Div.* **3**, 3.

Porter, I. M., McClelland, S. E., Khoudoli, G. A., Hunter, C. J., Andersen, J. S., McAinsh, A. D., Blow, J. J. and Swedlow, J. R. (2007). Bod1, a novel kinetochore protein required for chromosome biorientation. *J. Cell Biol.* **179**, 187–197.

Porter, I. M., Schleicher, K., Porter, M. and Swedlow, J. R. (2013). Bod1 regulates protein phosphatase 2A at mitotic kinetochores. *Nat. Commun.* **4**, 2677.

Potapova, T. A., Daum, J. R., Pittman, B. D., Hudson, J. R., Jones, T. N., Satinover, D. L., Stukenberg, P. T. and Gorbsky, G. J. (2006). The reversibility of mitotic exit in vertebrate cells. *Nature* **440**, 954–958.

Potapova, T. A., Daum, J. R., Byrd, K. S. and Gorbsky, G. J. (2009). Fine tuning the cell cycle: activation of the Cdk1 inhibitory phosphorylation pathway during mitotic exit. *Mol. Biol. Cell* **20**, 1737–1748.

Potapova, T. A., Sivakumar, S., Flynn, J. N., Li, R. and Gorbsky, G. J. (2011). Mitotic progression becomes irreversible in prometaphase and collapses when Wee1 and Cdc25 are inhibited. *Mol. Biol. Cell* **22**, 1191–1206.

Qian, Y.-W., Erikson, E., Taieb, F. E. and Maller, J. L. (2001). The polo-like kinase Plx1 is required for activation of the phosphatase Cdc25C and cyclin B-Cdc2 in Xenopus oocytes. *Mol. Biol. Cell* **12**, 1791–1799.

Rane, S. G., Dubus, P., Mettus, R. V, Galbreath, E. J., Boden, G., Reddy, E. P. and Barbacid, M. (1999). Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in beta-islet cell hyperplasia. *Nat. Genet.* **22**, 44–52.

Rangone, H., Wegel, E., Gatt, M. K., Yeung, E., Flowers, A., Debski, J., Dadlez, M., Janssens, V., Carpenter, A. T. C. and Glover, D. M. (2011). Suppression of scant identifies Endos as a substrate of greatwall kinase and a negative regulator of protein phosphatase 2A in mitosis. *PLoS Genet.* **7**, e1002225.

Rattani, A., Vinod, P. K., Godwin, J., Tachibana-Konwalski, K., Wolna, M., Malumbres, M., Novák, B. and Nasmyth, K. (2014). Dependency of the spindle assembly checkpoint on Cdk1 renders the anaphase transition irreversible. *Curr. Biol.* **24**, 630–637.

Ravid, K., Lu, J., Zimmet, J. M. and Jones, M. R. (2002). Roads to polyploidy: the megakaryocyte example. *J. Cell. Physiol.* **190**, 7–20.

Riedel, C. G., Katis, V. L., Katou, Y., Mori, S., Itoh, T., Helmhart, W., Gálová, M., Petronczki, M., Gregan, J., Cetin, B., et al. (2006). Protein phosphatase 2A protects centromeric sister chromatid cohesion during meiosis I. *Nature* **441**, 53–61.

Rieder, C. L. and Cole, R. W. (1998). Entry into mitosis in vertebrate somatic cells is guarded by a chromosome damage checkpoint that reverses the cell cycle when triggered during early but not late prophase. *J. Cell Biol.* **142**, 1013–1022.

Rieder, C. L. and Cole, R. (2000). Microtubule disassembly delays the G2-M transition in vertebrates. *Curr. Biol.* **10**, 1067–1070.

Rocco, J. W. and Sidransky, D. (2001). p16(MTS-1/CDKN2/INK4a) in Cancer Progression. *Exp. Cell Res.* **264**, 42–55.

Ruchaud, S., Carmena, M. and Earnshaw, W. C. (2007). Chromosomal passengers: conducting cell division. *Nat. Rev. Mol. Cell Biol.* **8**, 798–812.

Ruediger, R., Roeckel, D., Fait, J., Bergqvist, A., Magnusson, G. and Walter, G. (1992). Identification of binding sites on the regulatory A subunit of protein phosphatase 2A for the catalytic C subunit and for tumor antigens of simian virus 40 and polyomavirus. *Mol. Cell. Biol.* **12**, 4872–4882.

Ruediger, R., Pham, H. and Walter, G. (2001). Disruption of protein phosphatase 2A subunit interaction in human cancers with mutations in the A alpha subunit gene. *Oncogene* **20**, 10–15.

Russo, A. A., Jeffrey, P. D., Patten, A. K., Massague, J. and Pavletich, N. P. (1996). Crystal structure of the p27Kip1 cyclin-dependent-kinase inhibitor bound to the cyclin A-Cdk2 complex. *Nature* **382**, 325–331.

Sackton, K. L., Dimova, N., Zeng, X., Tian, W., Zhang, M., Sackton, T. B., Meaders, J., Pfaff, K. L., Sigoillot, F., Yu, H., et al. (2014). Synergistic blockade of mitotic exit by two chemical inhibitors of the APC/C. *Nature* **514**, 646–649.

Sakaue-Sawano, A., Kurokawa, H., Morimura, T., Hanyu, A., Hama, H., Osawa, H., Kashiwagi, S., Fukami, K., Miyata, T., Miyoshi, H., et al. (2008). Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. *Cell* **132**, 487–498.

Santaguida, S., Tighe, A., D'Alise, A. M., Taylor, S. S. and Musacchio, A. (2010). Dissecting the role of MPS1 in chromosome biorientation and the spindle checkpoint through the small molecule inhibitor reversine. *J. Cell Biol.* **190**, 73–87.

Santamaría, D., Barrière, C., Cerqueira, A., Hunt, S., Tardy, C., Newton, K., Cáceres, J. F., Dubus, P., Malumbres, M. and Barbacid, M. (2007). Cdk1 is sufficient to drive the mammalian cell cycle. *Nature* **448**, 811–815.

Santos, S. D. M., Wollman, R., Meyer, T. and Ferrell, J. E. (2012). Spatial positive feedback at the onset of mitosis. *Cell* **149**, 1500–1513.

Sassoon, I., Severin, F. F., Andrews, P. D., Taba, M.-R., Kaplan, K. B., Ashford, A. J., Stark, M. J. R., Sorger, P. K. and Hyman, A. A. (1999). Regulation of Saccharomyces cerevisiae kinetochores by the type 1 phosphatase Glc7p. *Genes Dev.* **13**, 545–555.

Schmidt, J. C., Kiyomitsu, T., Hori, T., Backer, C. B., Fukagawa, T. and Cheeseman, I. M. (2010). Aurora B kinase controls the targeting of the Astrin-SKAP complex to bioriented kinetochores. *J. Cell Biol.* **191**, 269–280.

Schmitz, M. H. A., Held, M., Janssens, V., Hutchins, J. R. A., Hudecz, O., Ivanova, E., Goris, J., Trinkle-Mulcahy, L., Lamond, A. I., Poser, I., et al. (2010). Live-cell imaging RNAi screen identifies PP2A-B55alpha and importin-beta1 as key mitotic exit regulators in human cells. *Nat. Cell Biol.* **12**, 886–893.

Schulze, A., Zerfass, K., Spitkovsky, D., Middendorp, S., Bergès, J., Helin, K., Jansen-Dürr, P. and Henglein, B. (1995). Cell cycle regulation of the cyclin A gene promoter is mediated by a variant E2F site. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 11264–11268.

Scolnick, D. M. and Halazonetis, T. D. (2000). Chfr defines a mitotic stress checkpoint that delays entry into metaphase. *Nature* **406**, 430–435.

Seki, A., Coppinger, J. A., Jang, C.-Y., Yates, J. R. and Fang, G. (2008). Bora and the kinase Aurora a cooperatively activate the kinase Plk1 and control mitotic entry. *Science* **320**, 1655–1658.

Sha, W., Moore, J., Chen, K., Lassaletta, A. D., Yi, C., Tyson, J. J. and Sible, J. C. (2003). Hysteresis drives cell-cycle transitions in Xenopus laevis egg extracts. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 975–980.

Shih, I.-M., Panuganti, P. K., Kuo, K.-T., Mao, T.-L., Kuhn, E., Jones, S., Velculescu, V. E., Kurman, R. J. and Wang, T.-L. (2011). Somatic mutations of PPP2R1A in ovarian and uterine carcinomas. *Am. J. Pathol.* **178**, 1442–1447.

Shindo, N., Kumada, K. and Hirota, T. (2012). Separase sensor reveals dual roles for separase coordinating cohesin cleavage and cdk1 inhibition. *Dev. Cell* **23**, 112–123.

Slangy, A., Lane, H. A., D'Hérin, P., Harper, M., Kress, M. and Nigg, E. A. (1995). Phosphorylation by p34 cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo. *Cell* **63**, 1159–1169.

Solomon, M. J., Glotzer, M., Lee, T. H., Philippe, M. and Kirschner, M. W. (1990). Cyclin activation of p34cdc2. *Cell* **63**, 1013–1024.

Soni, D. V., Sramkoski, R. M., Lam, M., Stefan, T. and Jacobberger, J. W. (2008). Cyclin B1 is rate limiting but not essential for mitotic entry and progression in mammalian somatic cells. *Cell Cycle* **7**, 1285–1300.

Steegmaier, M., Hoffmann, M., Baum, A., Lénárt, P., Petronczki, M., Krssák, M., Gürtler, U., Garin-Chesa, P., Lieb, S., Quant, J., et al. (2007). BI 2536, a potent and selective inhibitor of polo-like kinase 1, inhibits tumor growth in vivo. *Curr. Biol.* **17**, 316–322.

Steffensen, S., Coelho, P. A., Cobbe, N., Vass, S., Costa, M., Hassan, B., Prokopenko, S. N., Bellen, H., Heck, M. M. S. and Sunkel, C. E. (2001). A role for Drosophila SMC4 in the resolution of sister chromatids in mitosis. *Curr. Biol.* 295–307.

Steigemann, P., Wurzenberger, C., Schmitz, M. H. A., Held, M., Guizetti, J., Maar, S. and Gerlich, D. W. (2009). Aurora B-mediated abscission checkpoint protects against tetraploidization. *Cell* **136**, 473–484.

Stemmann, O., Zou, H., Gerber, S. A., Gygi, S. P. and Kirschner, M. W. (2001). Dual inhibition of sister chromatid separation at metaphase. *Cell* **107**, 715–726.

Stevens, D., Gassmann, R., Oegema, K. and Desai, A. (2011). Uncoordinated loss of chromatid cohesion is a common outcome of extended metaphase arrest. *PLoS One* **6**, e22969.

Strohmaier, H., Spruck, C. H., Kaiser, P., Won, K. A., Sangfelt, O. and Reed, S. I. (2001). Human F-box protein hCdc4 targets cyclin E for proteolysis and is mutated in a breast cancer cell line. *Nature* **413**, 316–322.

Stumpff, J., Wagenbach, M., Franck, A., Asbury, C. L. and Wordeman, L. (2012). Kif18A and chromokinesins confine centromere movements via microtubule growth suppression and spatial control of kinetochore tension. *Dev. Cell* **22**, 1017–1029.

Subramanian, R., Wilson-Kubalek, E. M., Arthur, C. P., Bick, M. J., Campbell, E. A., Darst, S. A., Milligan, R. A. and Kapoor, T. M. (2010). Insights into antiparallel microtubule crosslinking by PRC1, a conserved nonmotor microtubule binding protein. *Cell* **142**, 433–443.

Sudakin, V., Chan, G. K. T. and Yen, T. J. (2001). Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. *J. Cell Biol.* **154**, 925–936.

Sumara, I., Vorlaufer, E., Stukenberg, P. T., Kelm, O., Redemann, N., Nigg, E. A. and Peters, J.-M. (2002). The Dissociation of Cohesin from Chromosomes in Prophase Is Regulated by Polo-like Kinase. *Mol. Cell* **9**, 515–525.

Summers, M. K., Bothos, J. and Halazonetis, T. D. (2005). The CHFR mitotic checkpoint protein delays cell cycle progression by excluding Cyclin B1 from the nucleus. *Oncogene* **24**, 2589–2598.

Swanton, C., Marani, M., Pardo, O., Warne, P. H., Kelly, G., Sahai, E., Elustondo, F., Chang, J., Temple, J., Ahmed, A. A., et al. (2007). Regulators of mitotic arrest and ceramide metabolism are determinants of sensitivity to paclitaxel and other chemotherapeutic drugs. *Cancer Cell* **11**, 498–512.

Talarek, N., Cameroni, E., Jaquenoud, M., Luo, X., Bontron, S., Lippman, S., Devgan, G., Snyder, M., Broach, J. R. and De Virgilio, C. (2010). Initiation of the TORC1-regulated G0 program requires Igo1/2, which license specific mRNAs to evade degradation via the 5′-3′ mRNA decay pathway. *Mol. Cell* **38**, 345–355.

Tang, Z., Shu, H., Qi, W., Mahmood, N. A., Mumby, M. C. and Yu, H. (2006). PP2A is required for centromeric localization of Sgo1 and proper chromosome segregation. *Dev. Cell* **10**, 575–585.

Tetsu, O. and McCormick, F. (2003). Proliferation of cancer cells despite CDK2 inhibition. *Cancer Cell* **3**, 233–245.

Th'ng, J. P., Wright, P. S., Hamaguchi, J., Lee, M. G., Norbury, C. J., Nurse, P. and Bradbury, E. M. (1990). The FT210 cell line is a mouse G2 phase mutant with a temperature-sensitive CDC2 gene product. *Cell* **63**, 313–324.

Thornton, B. R. and Toczyski, D. P. (2003). Securin and B-cyclin/CDK are the only essential targets of the APC. *Nat. Cell Biol.* **5**, 1090–1094.

Toyoshima, F., Moriguchi, T., Wada, A., Fukuda, M. and Nishida, E. (1998). Nuclear export of cyclin B1 and its possible role in the DNA damage-induced G2 checkpoint. *EMBO J.* **17**, 2728–2735.

Toyoshima-Morimoto, F., Taniguchi, E., Shinya, N., Iwamatsu, A. and Nishida, E. (2001). Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase. *Nature* **410**, 215–220.

Toyoshima-Morimoto, F., Taniguchi, E. and Nishida, E. (2002). Plk1 promotes nuclear translocation of human Cdc25C during prophase. *EMBO Rep.* **3**, 341–348.

Tsutsui, T., Hesabi, B., Moons, D. S., Pandolfi, P. P., Hansel, K. S., Koff, A. and Kiyokawa, H. (1999). Targeted disruption of CDK4 delays cell cycle entry with enhanced p27Kip1 activity. *Mol. Cell. Biol.* **19**, 7011–7019.

Ullah, Z., Kohn, M. J., Yagi, R., Vassilev, L. T. and DePamphilis, M. L. (2008). Differentiation of trophoblast stem cells into giant cells is triggered by p57/Kip2 inhibition of CDK1 activity. *Genes Dev.* **22**, 3024–3036.

V

Van Zon, W., Ogink, J., ter Riet, B., Medema, R. H., te Riele, H. and Wolthuis, R. M. F. (2010). The APC/C recruits cyclin B1-Cdk1-Cks in prometaphase before D box recognition to control mitotic exit. *J. Cell Biol.* **190**, 587–602.

Vandré, D. D. and Wills, V. L. (1992). Inhibition of mitosis by okadaic acid: possible involvement of a protein phosphatase 2A in the transition from metaphase to anaphase. *J. Cell Sci.* **101**, 79–91.

Vassilev, L. T., Tovar, C., Chen, S., Knezevic, D., Zhao, X., Sun, H., Heimbrook, D. C. and Chen, L. (2006). Selective small-molecule inhibitor reveals critical mitotic functions of human CDK1. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 10660–10665.

Vázquez-Novelle, M. D., Sansregret, L., Dick, A. E., Smith, C. a, McAinsh, A. D., Gerlich, D. W. and Petronczki, M. (2014). Cdk1 inactivation terminates mitotic checkpoint surveillance and stabilizes kinetochore attachments in anaphase. *Curr. Biol.* **24**, 638–645.

Vigneron, S., Brioudes, E., Burgess, A., Labbé, J.-C., Lorca, T. and Castro, A. (2009). Greatwall maintains mitosis through regulation of PP2A. *EMBO J.* **28**, 2786–2793.

Vigneron, S., Gharbi-Ayachi, A., Raymond, A.-A., Burgess, A., Labbé, J.-C., Labesse, G., Monsarrat, B., Lorca, T. and Castro, A. (2011). Characterization of the mechanisms controlling Greatwall activity. *Mol. Cell. Biol.* **31**, 2262–2275.

Villerbu, N., Gaben, A.-M., Redeuilh, G. and Mester, J. (2002). Cellular effects of purvalanol A: a specific inhibitor of cyclin-dependent kinase activities. *Int. J. Cancer* **97**, 761–769.

Visconti, R., Palazzo, L., Della Monica, R. and Grieco, D. (2012). Fcp1-dependent dephosphorylation is required for M-phase-promoting factor inactivation at mitosis exit. *Nat. Commun.* **3**, 894.

Visintin, R., Craig, K., Hwang, E. S., Prinz, S., Tyers, M. and Amon, A. (1998). The Phosphatase Cdc14 Triggers Mitotic Exit by Reversal of Cdk-Dependent Phosphorylation. *Mol. Cell* **2**, 709–718.

Vodermaier, H. C. (2004). APC/C and SCF: controlling each other and the cell cycle. *Curr. Biol.* **14**, R787–R796.

Voets, E. and Wolthuis, R. M. F. (2010). MASTL is the human orthologue of greatwall kinase that facilitates mitotic entry, anaphase and cytokinesis. *Cell Cycle* **9**, 3591–3601.

Von Stetina, J. R., Tranguch, S., Dey, S. K., Lee, L. A., Cha, B. and Drummond-Barbosa, D. (2008). alpha-Endosulfine is a conserved protein required for oocyte meiotic maturation in Drosophila. *Development* **135**, 3697–3706.

Wandke, C., Barisic, M., Sigl, R., Rauch, V., Wolf, F., Amaro, A. C., Tan, C. H., Pereira, A. J., Kutay, U., Maiato, H., et al. (2012). Human chromokinesins promote chromosome congression and spindle microtubule dynamics during mitosis. *J. Cell Biol.* **198**, 847–863.

Wang, P., Pinson, X. and Archambault, V. (2011). PP2A-twins is antagonized by greatwall and collaborates with polo for cell cycle progression and centrosome attachment to nuclei in Drosophila embryos. *PLoS Genet.* **7**, e1002227.

Wang, P., Galan, J. A., Normandin, K., Bonneil, É., Hickson, G. R., Roux, P. P., Thibault, P. and Archambault, V. (2013). Cell cycle regulation of Greatwall kinase nuclear localization facilitates mitotic progression. *J. Cell Biol.* **202**, 277–293.

Ward, G. E. and Kirschner, M. W. (1990). Identification of cell cycle-regulated phosphorylation sites on nuclear lamin C. *Cell* **61**, 561–577.

Wei, W., Ayad, N. G., Wan, Y., Zhang, G.-J., Kirschner, M. W. and Kaelin, W. G. (2004). Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. *Nature* **428**, 2–6.

Weinberg, R. A. (1995). The retinoblastoma protein and cell cycle control. *Cell* **81**, 323–30.

Wolf, F., Wandke, C., Isenberg, N. and Geley, S. (2006). Dose-dependent effects of stable cyclin B1 on progression through mitosis in human cells. *EMBO J.* **25**, 2802–2813.

Wölfel, T., Hauer, M., Schneider, J., Serrano, M., Wölfel, C., Klehmann-Hieb, E., De Plaen, E., Hankeln, T., Meyer zum Büschenfelde, K. H. and Beach, D. (1995). A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science* **269**, 1281–1284.

Wolthuis, R., Clay-Farrace, L., van Zon, W., Yekezare, M., Koop, L., Ogink, J., Medema, R. and Pines, J. (2008). Cdc20 and Cks direct the spindle checkpoint-independent destruction of cyclin A. *Mol. Cell* **30**, 290–302.

Wong, O. K. and Fang, G. (2007). Cdk1 phosphorylation of BubR1 controls spindle checkpoint arrest and Plk1-mediated formation of the 3F3/2 epitope. *J. Cell Biol.* **179**, 611–617.

Wu, J. Q., Guo, J. Y., Tang, W., Yang, C.-S., Freel, C. D., Chen, C., Nairn, A. C. and Kornbluth, S. (2009). PP1-mediated dephosphorylation of phosphoproteins at mitotic exit is controlled by inhibitor-1 and PP1 phosphorylation. *Nat. Cell Biol.* **11**, 644–651.

Yamashita, K., Yasuda, H., Pines, J., Yasumoto, K., Nishitani, H., Ohtsubo, M., Hunter, T., Sugimura, T. and Nishimoto, T. (1990). 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**, 4331–4338.

Yan, Z., DeGregori, J., Shohet, R., Leone, G., Stillman, B., Nevins, J. R. and Williams, R. S. (1998). Cdc6 is regulated by E2F and is essential for DNA replication in mammalian cells. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 3603–3608.

Yanagida, M. (2009). Clearing the way for mitosis: is cohesin a target? *Nat. Rev. Mol. Cell Biol.* **10**, 489–496.

Yang, S.-I., Lickteig, R. L., Estes, R., Rundell, K., Walter, G. and Mumby, M. C. (1991). Control of protein phosphatase 2A by simian virus 40 small-t antigen. *Mol. Cell. Biol.* **11**, 1988–1995.

Yang, J., Bardes, E. S. G., Moore, J. D., Brennan, J., Powers, M. A. and Kornbluth, S. (1998). Control of Cyclin B1 localization through regulated binding of the nuclear export factor CRM1. *Genes Dev.* **12**, 2131–2143.

Yu, H. (2007). Cdc20: a WD40 activator for a cell cycle degradation machine. *Mol. Cell* **27**, 3–16.

Yu, J., Fleming, S. L., Williams, B., Williams, E. V, Li, Z., Somma, P., Rieder, C. L. and Goldberg, M. L. (2004). Greatwall kinase: a nuclear protein required for proper chromosome condensation and mitotic progression in Drosophila. *J. Cell Biol.* **164**, 487–492.

Yu, J., Zhao, Y., Li, Z., Galas, S. and Goldberg, M. L. (2006). Greatwall kinase participates in the Cdc2 autoregulatory loop in Xenopus egg extracts. *Mol. Cell* **22**, 83–91.

Yuan, J., Yan, R., Krämer, A., Eckerdt, F., Roller, M., Kaufmann, M. and Strebhardt, K. (2004). Cyclin B1 depletion inhibits proliferation and induces apoptosis in human tumor cells. *Oncogene* **23**, 5843–5852.

Yuan, J., Krämer, A., Matthess, Y., Yan, R., Spänkuch, B., Gätje, R., Knecht, R., Kaufmann, M. and Strebhardt, K. (2006). Stable gene silencing of cyclin B1 in tumor cells increases susceptibility to taxol and leads to growth arrest in vivo. *Oncogene* **25**, 1753–1762.

Zachariae, W., Schwab, M., Nasmyth, K. and Seufert, W. (1998). Control of Cyclin Ubiquitination by CDK-Regulated Binding of Hct1 to the Anaphase Promoting Complex. *Science* **282**, 1721–1724.

Zhao, Y., Haccard, O., Wang, R., Yu, J., Kuang, J., Jessus, C. and Goldberg, M. L. (2008). Roles of Greatwall kinase in the regulation of cdc25 phosphatase. *Mol. Biol. Cell* **19**, 1317–1327.

Zheng, B., Woo, C. F. and Kuo, J. F. (1991). Mitotic arrest and enhanced nuclear protein phosphorylation in human leukemia K562 cells by okadaic acid, a potent protein phosphatase inhibitor and tumor promoter. *J. Biol. Chem.* **266**, 10031–10034.

Zhu, C. and Jiang, W. (2005). Cell cycle-dependent translocation of PRC1 on the spindle by Kif4 is essential for midzone formation and cytokinesis. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 343–348.

Z

Zhu, C., Lau, E., Schwarzenbacher, R., Bossy-Wetzel, E. and Jiang, W. (2006). Spatiotemporal control of spindle midzone formation by PRC1 in human cells. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 6196–6201.