

MITOTIC KINASES AS REGULATORS OF CELL DIVISION AND ITS CHECKPOINTS

Erich A. Nigg

Mitosis and cytokinesis are undoubtedly the most spectacular parts of the cell cycle. Errors in the choreography of these processes can lead to aneuploidy or genetic instability, fostering cell death or disease. Here, I give an overview of the many mitotic kinases that regulate cell division and the fidelity of chromosome transmission.

CELL DIVISION

OMNIS CELLULA E CELLULA
All cells are derived from cells.

MITOSIS
The process of nuclear division.

CYTOKINESIS
The process of cytoplasmic division.

SISTER CHROMATIDS
Duplicated chromosomes.

CENTROSOME
The main microtubule-organizing centre of animal cells.

MITOTIC SPINDLE
A highly dynamic bipolar array of microtubules that forms during mitosis or meiosis and serves to move the duplicated chromosomes apart.

*Max-Planck-Institute for Biochemistry, Department of Cell Biology, Am Klopferspitz 18a, D-82152 Martinsried, Germany.
e-mail: nigg@biochem.mpg.de*

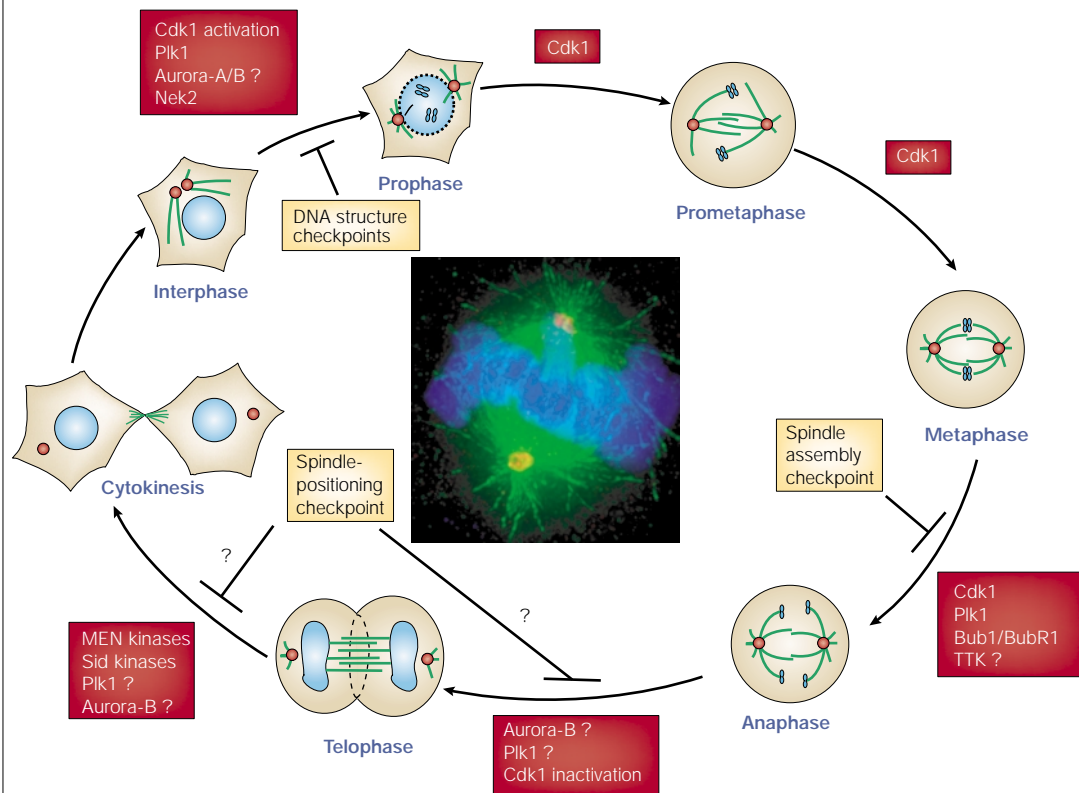
Ever since Rudolf Virchow (1821–1902) proclaimed his famous “OMNIS CELLULA E CELLULA”, the challenge has been to understand how cells divide and how they faithfully transmit genetic information from one cell generation to the next. In a typical somatic cell cycle, M phase comprises MITOSIS and CYTOKINESIS. The main purpose of mitosis is to segregate SISTER CHROMATIDS into two nascent cells, such that each daughter cell inherits one complete set of chromosomes. In addition, each daughter cell must receive one CENTROSOME and the appropriate complements of cytoplasm and organelles. Mitosis is usually divided into five distinct stages: prophase, prometaphase, metaphase, anaphase and telophase (for a brief description of these phases, see BOX 1). Cytokinesis, the process of cell cleavage, occurs at the end of mitosis, and its regulation is linked intimately to mitotic progression. Although the exact temporal and spatial organization of mitosis and cytokinesis differ between animals, plants and fungi, chromosome segregation invariably requires the assembly of the MITOTIC SPINDLE, whereas cytokinesis depends on the concerted action of the spindle, the actomyosin cytoskeleton and the cell cortex.

The regulation of M-phase progression relies predominantly on two post-translational mechanisms: protein phosphorylation and proteolysis. These are intimately intertwined in that the proteolytic machinery is controlled by phosphorylation, whereas several mitotic kinases are downregulated by degradation. The most prominent mitotic kinase is the cyclin-

dependent kinase 1 (Cdk1), the founding member of the Cdk family of cell-cycle regulators. Recent studies have, however, brought to light additional mitotic kinases. These include members of the Polo family, the aurora family and the NIMA (never in mitosis A) family, as well as kinases implicated in mitotic CHECKPOINTS, mitotic exit and cytokinesis (TABLE 1 and BOX 1). Here, I review our current understanding of these new mitotic kinases and their cooperation with Cdk1 in the regulation of mitosis and cytokinesis. Whereas the main text describes progression through M phase in chronological order, separate boxes provide brief summaries on selected kinase families. The review is written mainly from the perspective of cell division in humans, but much of our current thinking reflects extrapolations from pioneering work done in yeast and other genetically tractable organisms.

No correct M phase without proper S phase
The error-free segregation of sister chromatids during mitosis depends on the proper execution of two events during the preceding S phase. These are the replication of chromosomal DNA, with the concomitant establishment of sister-chromatid cohesion, and the duplication of centrosomes. To keep the ploidy of an organism constant, it is essential that both chromosomes and centrosomes are duplicated only once in every cell cycle. Recent work has revealed a first glimpse of how DNA replication and centrosome duplication are coordinated in a somatic cell cycle. Both processes depend on the phos-

Box 1 | A primer on the chronology of M-phase events



The principal events typical of animal cell division can briefly be summarized as follows. During 'prophase', interphase chromatin condenses into well-defined chromosomes and previously duplicated centrosomes migrate apart, thereby defining the poles of the future spindle apparatus. Concomitantly, centrosomes begin nucleating highly dynamic microtubules that probe space in all directions, and the nuclear envelope breaks down. During 'prometaphase', microtubules are captured by kinetochores (specialized proteinaceous structures associated with centromere DNA on mitotic chromosomes). Although monopolar attachments of chromosomes are unstable, the eventual interaction of paired sister chromatids with microtubules emanating from opposite poles results in a stable, bipolar attachment. Chromosomes then congress to an equatorial plane, the metaphase plate, where they continue to oscillate throughout 'metaphase', suggesting that a balance of forces keeps them under tension. After all the chromosomes have undergone a proper bipolar attachment, a sudden loss in sister-chromatid cohesion triggers the onset of 'anaphase'. Sister chromatids are then pulled towards the poles (anaphase A) and the poles themselves separate further towards the cell cortex (anaphase B). Once the chromosomes have arrived at the poles, nuclear envelopes reform around the daughter chromosomes, and chromatin decondensation begins ('telophase'). Finally, an actomyosin-based contractile ring is formed and 'cytokinesis' is completed. The figure summarizes the stages of M phase. It also indicates where the major checkpoints exert quality control over mitotic progression and where mitotic kinases are thought to act. The insert illustrates a Ptk2 cell in metaphase; DNA is shown in blue (DAPI staining), microtubules in green and spindle poles (γ -tubulin) in orange. (Picture kindly provided by P. Meraldi.)

CHECKPOINT
A point where the cell division cycle can be halted until conditions are suitable for the cell to proceed to the next stage.

SPINDLE POLE BODY
The yeast equivalent of the centrosome.

NUCLEAR LAMINA
A nuclear membrane-associated protein structure made up of lamin intermediate-filament proteins.

KINESIN
Microtubule-based molecular motor, most often directed towards the plus end of microtubules.

NUCLEAR ENVELOPE
Double membrane that surrounds the nucleus. The outer membrane is continuous with the endoplasmic reticulum.

phorylation of the *retinoblastoma* gene product and the release of **E2F** transcription factors¹, and both require the activity of **Cdk2**, in association with either **cyclin A** or **cyclin E**¹⁻³. Another kinase, **Mps1p**, has been implicated in the duplication of the **SPINDLE POLE BODY** in *Saccharomyces cerevisiae*⁴. Whether homologues of this kinase control centrosome duplication in metazoan organisms is not known at present.

Cdk1, the maestro of M phase
Of the ensemble of kinases involved in the orchestration of M-phase events, Cdk1 remains the unchallenged conductor. The regulation of Cdk1-cyclin complexes is comparatively well understood (summarized in FIG. 1).

Activation of mammalian Cdk1 depends on dephosphorylation of two neighbouring residues in the ATP-binding site (threonine 14 and tyrosine 15). This occurs at the G2/M transition when the activity of the dual-specificity phosphatase **Cdc25C** towards Cdk1 exceeds that of the opposing kinases **Wee1** and **Myt1**. In turn, these enzymes are controlled by DNA structure checkpoints, which delay the onset of mitosis in the presence of unreplicated or damaged DNA (see below).

Activated Cdk1-cyclin complexes then phosphorylate numerous substrates, for example nuclear **lamins**, **KINESIN**-related motors and other microtubule-binding proteins, **condensins** and **Golgi matrix components**, and these events are important for **NUCLEAR ENVELOPE** break-

Table 1 | Mitotic kinases*

	Mammalian members	Founding members	Comments
The Cdk family	Cdk1 (Cdc2)	Cdc2p (Sp)/Cdc28p (Sc)	Mammalian Cdk1 functions in association with both A- and B-type cyclins
The Polo family	Plk1	Polo (Dm)	The vertebrate Polo families comprise additional members (see BOX 2)
The Aurora family	Aurora-A Aurora-B Aurora-C	Aurora (Dm)/Ipl1p (Sc)	The Aurora nomenclature is explained in TABLE 2
The NIMA family	Nek2	NIMA (An)	The vertebrate Nek families comprise additional members (see BOX 3). Whether NIMA and Nek2 represent bona fide functional homologues is not known at present
Mitotic checkpoint	Bub1 BubR1 TTK/Esk	Bub1p (Sc) Mps1p (Sc)	TTK and Esk are the names given to putative human and mouse homologues, respectively, of budding yeast Mps1p
MEN/SIN kinases	?	Multiple yeast kinases (Sc and Sp) (see FIG. 5)	Several metazoan kinases (Ndr/LATS family members) are structurally related to a yeast SIN/MEN kinase (budding yeast Dbf2p/Mob1p and fission yeast Sid2p/Mob1p), but no functional homologies have yet been shown

*This Table is not meant to be exhaustive, and kinases with widely pleiotropic functions, such as MAP kinases and PKA (the cAMP-dependent kinase), are only mentioned in passing. This should not detract from the fact that both MAP kinases and PKA probably have important roles in the regulation of M phase, at least in some cell types. Furthermore, several of the 'mitotic' kinases listed here are highly expressed in the germ line, implying probable functions also in meiosis.

(Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; An, *Aspergillus nidulans*; Dm, *Drosophila melanogaster*.)

down, centrosome separation, spindle assembly, chromosome condensation and Golgi fragmentation, respectively⁵⁻⁸. Furthermore, Cdk1–cyclin complexes contribute to regulate the anaphase-promoting complex/cyclosome (APC/C), the core component of the ubiquitin-dependent proteolytic machinery that controls the timely degradation of critical mitotic regulators, in particular inhibitors of anaphase onset (*securins*) and *cyclins*⁹. On cyclin destruction, Cdk1 is inactivated, setting the stage for mitotic exit and cytokinesis. Cdk1 inactivation also allows the reformation of pre-initiation complexes at origins of replication, thereby licensing cellular chromatin for the next round of replication¹⁰. In the following sections, major M-phase events will be discussed in more detail, with particular emphasis on the intervention of mitotic kinases at various stages.

Early mitotic events

Centrosome separation and activation. In most cell types, duplicated centrosomes remain closely paired and continue to function as a single microtubule-organizing centre during G2. After G2, however, they separate and migrate apart. Concomitantly, they recruit additional γ -TUBULIN RING COMPLEXES, and this maturation event sets the stage for increased microtubule nucleation activity. As inferred from antibody microinjection studies in human cells¹¹ and *Xenopus* embryos¹², centrosome maturation requires the action of Polo-like kinases (Plks; BOX 2). Consistent with this view, *Drosophila* *Polo* is likely to regulate a micro-

tubule-associated protein, termed *Asp* (for 'abnormal spindle'), whose function is to hold γ -tubulin ring complexes at the mitotic centrosome¹³.

The separation of centrosomes seems to be regulated by several kinases. At an early step, the NIMA-family member *Nek2* (BOX 3) is thought to phosphorylate the centrosomal protein, *C-Nap1*, thereby causing the dissolution of a dynamic structure that tethers duplicated centrosomes to each other¹⁴. A type 1 phosphatase interacts with both *Nek2* and *C-Nap1*, and cell-cycle-regulated inhibition of this phosphatase may contribute to cause an abrupt increase in *C-Nap1* phosphorylation at the G2/M transition¹⁵. At a later step, several kinesin-related motor proteins (KRPs) and cytoplasmic DYNEIN are required for centrosome separation. Prominent among these motors is the KRP *Eg5*, whose recruitment to centrosomes depends on the phosphorylation of a highly conserved carboxy-terminal motif by Cdk1–cyclin B^{16,17}.

A role in centrosome separation has also been postulated for aurora-A family members (BOX 4; TABLE 2). This was originally inferred from the phenotype of *aurora* mutants in *Drosophila*¹⁸, and supported by the finding that vertebrate A-type aurora kinases also localize to centrosomes, spindle poles and spindle microtubules^{19,20}. However, RNA-MEDIATED INTERFERENCE (RNAi) with aurora-A (AIR-1) in *Caenorhabditis elegans* did not prevent centrosome separation, although both spindle formation and centrosomal morphology were abnormal²¹. A better understanding of the role of

γ -TUBULIN RING COMPLEXES

Ring-like multiprotein structures implicated in microtubule nucleation.

DYNEIN

Microtubule-based molecular motor that moves towards the minus end of microtubules.

RNA-MEDIATED INTERFERENCE

Process by which an introduced double-stranded RNA specifically silences the expression of genes through degradation of their cognate mRNA.

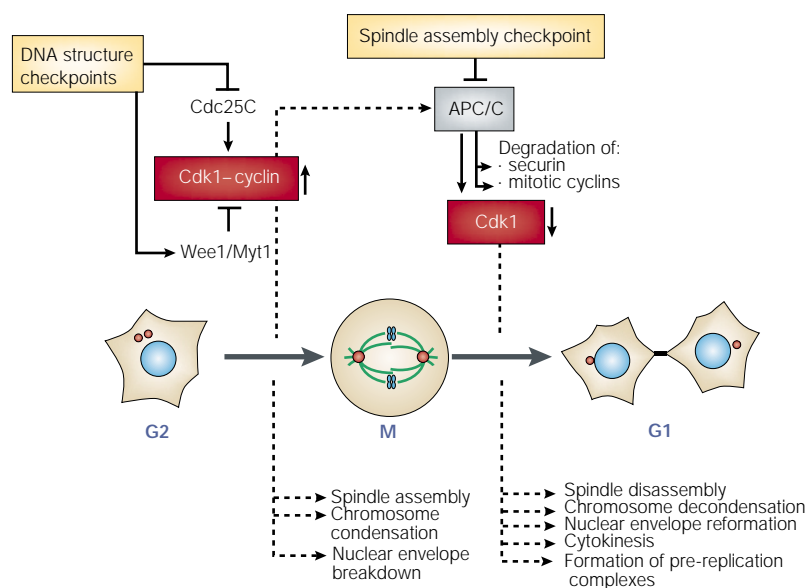


Figure 1 | A narrative of mitotic progression from the Cdk1 perspective. Entry into mitosis results from the activation of Cdk1–cyclin complexes. In mammalian cells, this depends primarily on the dephosphorylation of Cdk1, which occurs when the activity of the phosphatase Cdc25C exceeds that of the kinases Wee1 and Myt1. By contrast, exit from mitosis depends on the inactivation of Cdk1–cyclin complexes. This occurs as a consequence of cyclin destruction, which in turn results from the activation of the APC/C ubiquitin ligase. This simple scheme provides an account of mitotic regulation from the perspective of Cdk1, but it does not consider spatial aspects of mitotic control¹⁰⁹, and it ignores the important contribution of several additional mitotic kinases.

A-type aurora kinases in spindle assembly will require the identification of both physiological substrates and upstream regulators. The *Xenopus* KRP Eg5 is one candidate substrate, but the molecular consequences of this phosphorylation remain unknown²².

Nuclear envelope breakdown. In organisms undergoing open mitosis, nuclear envelope breakdown (NEBD) occurs shortly after centrosome separation. During interphase, the nuclear envelope is stabilized by a karyoskeletal structure known as the nuclear lamina, but at the onset of mitosis, this structure disassembles as a consequence of lamin hyperphosphorylation. Although lamins can be phosphorylated by many kinases *in vitro*, the predominant kinase triggering mitotic lamina depolymerization *in vivo* is almost certainly Cdk1–cyclin B⁵. Lamina disassembly reduces nuclear envelope stability but is not in itself sufficient to cause NEBD. The additional requirements for NEBD remain poorly understood, although phosphorylation probably has an important role.

Chromosome condensation. Chromosome condensation is accompanied by extensive phosphorylation of both histones and non-histone proteins. Histone modifications, including phosphorylation, acetylation and methylation, have long been correlated with changes in chromatin condensation states. The linker histone H1 is an excellent substrate of Cdk1–cyclin B, but despite extensive study, the significance of this phosphorylation remains unknown. More recently, phosphoryla-

tion of the core histone H3 (at serine 10) has also attracted great interest. This modification is highly conserved, correlates with chromosome condensation during mitosis and meiosis, and is required for proper chromosome segregation in at least some organisms (for example, the protozoan *Tetrahymena*).

Of the several histone H3/serine 10 kinases described, two are of particular interest from the perspective of mitosis. Genetic and biochemical data concur to indicate that aurora family members, in particular Ipl1p of *S. cerevisiae* and the B-type aurora AIR-2 of *C. elegans*, can control histone H3 phosphorylation in opposition to a type 1 phosphatase (Glc7p in *S. cerevisiae*)²³. However, studies done in *Aspergillus nidulans* suggest that NIMA is another candidate histone H3 kinase²⁴. This discrepancy illustrates the notorious difficulty in unequivocally assigning kinases to their physiological substrates, and it will be interesting to determine whether aurora and/or NIMA-related kinases (Neks) phosphorylate histone H3 in vertebrates, or whether yet other histone H3 kinases await discovery. Prominent among the *trans*-acting factors involved in chromosome condensation are topoisomerase II and a multi-protein complex known as condensin, and both are regulated by phosphorylation. In the case of the five-member condensin complex, there is evidence that Cdk1–cyclin B regulates its DNA supercoiling activity in *Xenopus* extracts⁶ and its cell-cycle-regulated nuclear accumulation in *Schizosaccharomyces pombe*²⁵.

Spindle dynamics and chromosome movements
Spindle assembly and mitotic movements rely on three parameters: the inherent dynamic properties of microtubule polymers (particularly dynamic instability and treadmilling); a balance of microtubule stabilizing and destabilizing accessory proteins; and the action of microtubule-dependent motors of the dynein and kinesin families. Dynamic instability is particularly important at the onset of mitosis, when the CATASTROPHE RATE increases markedly. This transition can be triggered *in vitro* by several kinases, including Cdk1–cyclin A and mitogen-activated kinase (MAP kinase), but the substrates involved remain unknown⁸. Microtubule dynamics are extensively regulated by microtubule-associated proteins and microtubule-destabilizing proteins, and most of these are controlled by phosphorylation.

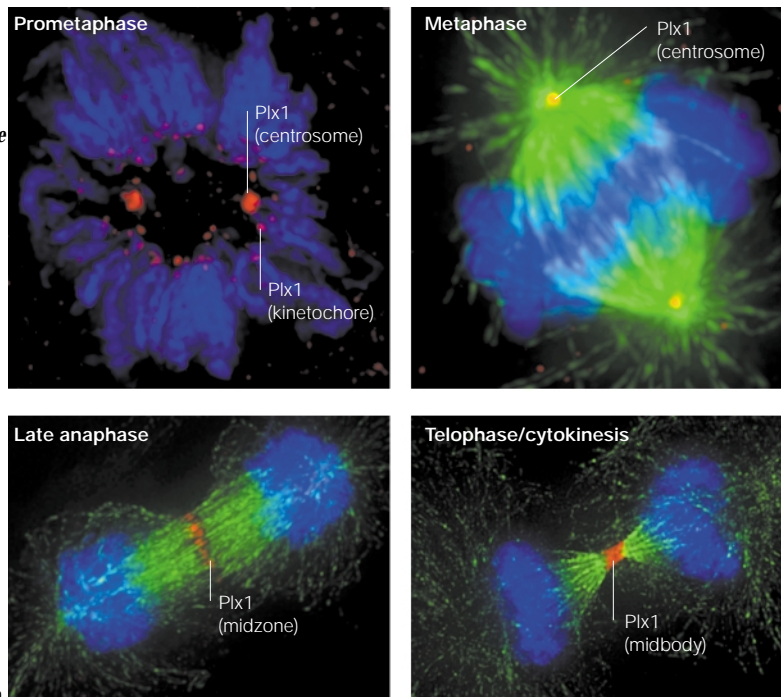
A good example is stathmin (also known as oncoprotein 18), whose microtubule-destabilizing activity is turned off during mitosis by sequential phosphorylation involving Cdk1–cyclin B and an as yet unidentified kinase²⁶. Studies on stathmin phosphorylation also illustrate the importance of spatial regulation in spindle assembly²⁷. In *Xenopus* extracts, chromatin-induced spindle formation seems to depend on gradients of differentially phosphorylated microtubule-associated proteins, and these gradients in turn are likely to arise from the action of immobilized kinases and diffusible phosphatases (or vice versa)²⁷. Although this attractive model awaits rigorous proof, it highlights the importance of the mutual localization of kinases, phosphatases and their

CATASTROPHE RATE
The frequency of transitions between rapid growth and shrinkage of microtubules.

Box 2 | The Polo kinase family

Named after the *Drosophila polo* gene, polo-like kinases (Plks) are conserved in all eukaryotes. Whereas only a single Plk exists in *S. cerevisiae* (Cdc5p), and presumably *S. pombe* (Plo1p), at least three Plks are expressed in mammals. Of these, Plk1 (Plx1 in *Xenopus*) functions during mitosis. Information about the two other mammalian Plks, Snk (Plk2) and Fnk/Prk (Plk3), remains scarce, and there is evidence that their functions may not be limited to cell-cycle control⁹⁴. All known Plks bear the catalytic domain at the amino terminus, and they feature a conspicuously conserved sequence motif, the polo-box, in the carboxy-terminal domain. This motif has been proposed to function

in targeting Plks to subcellular compartments⁹⁵ or to mediate interactions with other proteins⁹⁴. Alternatively, the polo-box may constitute part of an autoregulatory domain⁶². As shown in the figures for *Xenopus* Plx1, Plks associate transiently with several mitotic structures^{96–98}, including spindle poles, kinetochores, the central spindle midzone and the midbody (Plx1 is stained in red, DNA in blue, and microtubules in green; all pictures kindly provided by P. Meraldi). Concomitantly, kinase activity peaks during M phase, and this results from activating phosphorylation(s). With *Xenopus* xPlk1 and the structurally related human kinase, SLK, two candidate Plk-activating kinases have been described recently^{99,100}, but additional regulators of Plks almost certainly await identification. Important issues also remain unresolved with regard to the ubiquitin-dependent degradation of Plks at the end of M phase. Whereas budding yeast Cdc5p features a D-box in the amino terminus and seems to be targeted by APC/C^{Cdc20} (REFS 37,38), this destruction box has not been conserved during evolution. Instead, mammalian Plk1 may constitute a substrate of APC/C^{Cdh1} and thus be degraded primarily during G1 (REF. 39).



substrates in the control of mitotic reactions.

Throughout mitosis, microtubule–kinetochore interactions are highly dynamic. They rely on tethering proteins such as cytoplasmic dynein and CENP-E²⁸, and may be regulated by phosphorylation. This latter point is shown most convincingly in *S. cerevisiae*, where the aurora kinase Ipl1p phosphorylates a KINETOCHORE protein, Ndc10p, thereby reducing its ability to bind microtubules²⁹. Recent findings indicate that metazoan B-type aurora kinases localize to centromeres/kinetochores, presumably through interactions with INCENP proteins³⁰. This raises the possibility that aurora kinases regulate kinetochore function also in multicellular organisms. No mammalian homologue of Ndc10p has yet been identified, but in view of the evidence implicating aurora family members in the phosphorylation of histone H3, the centromere-associated histone H3 variant CENP-A is an attractive candidate substrate.

Spindle assembly and function throughout mitosis depend on several distinct KRPs and cytoplasmic dynein. However, although coordination between different motor activities would seem critical for the cor-

rect execution of chromosome segregation, little is known about how individual motors are targeted to particular structures and how their local activities are controlled. As exemplified by studies on Eg5 (REFS 16,17), phosphorylation is certainly involved in the spatial and temporal control of motor activity, and this field holds considerable promise for future research.

Anaphase onset and mitotic exit

Anaphase begins shortly after all chromosomes have undergone proper bipolar attachment to the spindle. Its onset is characterized by the simultaneous separation of all sister chromatids and results from a loss of sister-chromatid cohesion rather than an increase in forces moving towards the pole. Studies in yeast have revealed that sister-chromatid separation depends on the degradation of an inhibitor, a so-called securin, by ubiquitin-dependent proteolysis^{31,32}. This inhibitor prevents a protease, termed separase, from abolishing sister-chromatid cohesion by cutting a component of a multiprotein complex known as cohesin³³.

Although this mechanism has undoubtedly been conserved during evolution, the situation is more

KINETOCHORE
Specialized assembly of proteins that binds to a region of the chromosome called the centromere.

Box 3 | The NIMA kinase family

NIMA-related kinases (Neks) are named after the NIMA (never in mitosis A) gene product of *Aspergillus nidulans*¹⁰¹. Studies in this filamentous fungus suggested that NIMA cooperates with Cdk1 at the G2/M transition and this prompted extensive searches for NIMA homologues in other organisms. However, whether bona fide functional homologues of NIMA exist outside of filamentous fungi remains unclear. Structural relatives of NIMA have been identified in *S. cerevisiae* and *S. pombe*^{101,102}, but in contrast to NIMA, these genes are not essential for viability. To what extent they functionally resemble NIMA remains to be determined. The mammalian genome carries at least seven NIMA-related kinases, called Nek1–Nek7 (REF. 103). Of these, Nek2 represents the closest structural relative of NIMA, but, except for putative coiled-coil structures, the non-catalytic domains of Nek2 and NIMA bear no resemblance¹⁰⁴. As described in the main text, one important function of Nek2 relates to the control of centrosome structure during the mitotic cell cycle¹⁰⁵. However, Nek2 is also highly expressed in the germ line, suggesting that it may have additional roles. NIMA has been implicated in chromosome condensation and proposed to phosphorylate histone H3 (REF. 24). The available evidence argues against such a function for Nek2, but it remains possible that one of the other mammalian Neks contributes to chromosome condensation. Importantly, there is no reason to assume that all Neks have a role in cell-cycle control. The structural similarity between different NIMA family members is largely confined to the catalytic domain, suggesting that different Neks may function in widely different physiological contexts.

complicated in vertebrates, where different mechanisms seem to destroy cohesin at chromosome arms and CENTROMERES, respectively. The bulk of cohesin is in fact already removed from chromosome arms during prophase, perhaps to permit the extensive chromosome condensation typical of vertebrate mitosis. Importantly, this first wave of cohesin removal does not depend on APC/C and instead requires phosphorylation of cohesin^{34,35}. One kinase able to phosphorylate cohesin is Cdk1, but other mitotic kinases may also be involved. The small amount of cohesin remaining at vertebrate centromeres is then removed at the metaphase–anaphase transition. This second step is dependent on APC/C, and presumably follows the securin–separase pathway described for sister-chromatid separation in yeast³⁶.

APC/C is responsible not only for the destruction of anaphase onset inhibitors but also of other proteins, notably mitotic cyclins and several mitotic kinases (FIG. 2). In addition to Cdk1–cyclin, these include Plks^{37–39}, NIMA family members^{40,41} and auro-kinases⁴². Importantly, however, the degradation of different substrates occurs at different times, implying that there is exquisite regulation of APC/C. In a typical somatic cell, two forms of APC/C are activated sequentially by the association of two distinct WD40 repeat proteins known as Cdc20 and Cdh1, respectively (for alternative names see legend to FIG. 2). Whereas APC/C^{Cdc20} is active at the metaphase–anaphase transition, APC/C^{Cdh1} is turned on later in mitosis but then remains active throughout the subsequent G1 phase⁴³. When assayed *in vitro*, APC/C^{Cdc20} and APC/C^{Cdh1} display partly distinct substrate specificities^{39,41}, but it is important to bear in mind that Cdh1 is not expressed in early *Xenopus* and *Drosophila* embryos, when cell cycles are extremely rapid and essentially comprise alternating S and M phases^{44,45}. The onset of Cdh1 expression during development then correlates with the establishment of G1 phases, suggesting that the sequential activation of APC/C^{Cdc20} and APC/C^{Cdh1} may be more important for temporal aspects of cell-cycle control than substrate selection. Mitotic kinases regulate the two forms of APC/C in opposite fashion

and thus play a key role in establishing the temporal order of APC/C activity: phosphorylation of APC/C core subunits (and perhaps Cdc20) is required for activation of APC/C^{Cdc20}, whereas phosphorylation of Cdh1 prevents the activation of APC/C^{Cdh1} (REF. 9).

The kinases Cdk1, Plk1 and BubR1 have all been implicated in the activation of APC/C^{Cdc20} (REFS 9,37,38,45–50), and protein kinase A (PKA) has been described as a negative regulator⁴⁸, but which of these kinases acts directly on APC/C subunits *in vivo* remains to be established. Cdc20 itself is also phosphorylated and has been detected in a complex with auro-A⁵¹, but the role of Cdc20 phosphorylation is not clear⁹. With regard to APC/C^{Cdh1}, it is striking that the inactivity of this complex correlates with Cdh1 phosphorylation from the onset of S phase until late mitosis, suggesting that Cdh1 is sequentially inactivated by cyclin E, cyclin A and cyclin B-dependent Cdk complexes⁹. In budding yeast, the activating dephosphorylation of Cdh1 depends on a phosphatase, Cdc14p^{52,53}, that is activated only after the silencing of a spindle-positioning checkpoint (see below). Once activated, APC/C^{Cdh1} promotes mitotic exit by causing the degradation of B-type cyclins and other ubiquitylation substrates. To what extent this model can be extended to mammalian cells remains to be seen.

G2/M- and M-phase checkpoints
Surveillance mechanisms, the so-called checkpoint pathways, ensure the proper order and correct execution of cell-cycle events⁵⁴. Checkpoints are thought to monitor passage through M phase at several stages^{55,56} (FIG. 3). Some M-phase checkpoints are well established, but others exist primarily in the realm of speculation at present. Best understood are the 'DNA structure checkpoints' that arrest cells at the G2/M transition in response to unreplicated DNA or DNA damage, and the 'spindle assembly checkpoint' that prevents anaphase onset as long as chromosomal kinetochores do not show a correct bipolar attachment. In budding yeast, a third checkpoint, the 'spindle-positioning checkpoint', links Cdk1 inactivation and mitotic exit to the proper orientation of the mitotic spindle. Whether

CENTROMERE
A region of a eukaryotic chromosome that is attached to the mitotic spindle.

Box 4 | The aurora kinase family

The founding members of the aurora kinase family are Ipl1p from *S. cerevisiae* and aurora from *Drosophila melanogaster*^{19,20}. Whereas Ipl1p is the only representative of this family in yeast, two aurora-related kinases are present in *Drosophila* and *C. elegans*, and at least three in mammals (TABLE 2). These kinases share similar catalytic domains located in the carboxyl terminus, but their amino-terminal extensions are of variable lengths with little or no similarity. Unfortunately, the nomenclature used to describe Ipl1/aurora-related kinases is highly confusing^{19,20}. This is particularly true with regard to mammals, where orthologues in man, mouse and rat have been given distinct names. For the sake of clarity, the vertebrate aurora kinases are here referred to as aurora-A, -B and -C (for other names, see TABLE 2).

Both aurora-A and -B are expressed in proliferating cells and overexpressed in tumour cells. During the cell cycle, the activity of aurora-A peaks before that of aurora-B. Furthermore, the two kinases display strikingly distinct subcellular localizations (TABLE 2). Whereas aurora-A is associated predominantly with centrosomes and the spindle apparatus from prophase through telophase, aurora-B is prominent at the midzone during anaphase and in postmitotic bridges during telophase^{19,20}. It is remarkable that all these mitotic structures also carry mitotic Plks (BOX 2), suggesting that a deliberate search for functional interactions between aurora kinases and Plks might be fruitful. Aurora-C has not yet been studied extensively. It is highly expressed in testis, but can also be detected in tumour cell lines, where it localizes to spindle poles from anaphase to cytokinesis¹⁰⁶.

Only very little information is currently available on the regulation and substrates of aurora kinases at present (TABLE 2). Similar to Plks, aurora kinases are regulated by APC/C-dependent proteolysis⁴² and by phosphorylation^{107,108}. However, conflicting data have been reported with regard to the relative timing of activation of aurora and Cdk1 during *Xenopus* oocyte maturation, and the upstream regulatory enzymes (kinases and phosphatases) remain unknown. Similarly, only few candidate substrates have so far been identified (see TABLE 2).

Table 2 | Nomenclature and properties of aurora family kinases

Nomenclature guide*						
Mammals	Other names	<i>Xenopus</i>	<i>C. elegans</i>	<i>Drosophila</i>	<i>S. cerevisiae</i>	
Aurora-A	Aurora-2, HsAIRK1, ARK1, Aik, BTAK, STK-15 (human); ARK1, Ayk1, IAK1 (mouse)	Eg2	AIR-1	aurora	Ipl1p [†]	
Aurora-B	Aurora-1, HsAIRK2, ARK2, Aik2, AIM-1, STK-12 (human); ARK2, STK-1 (mouse); AIM-1 (rat)	AIRK2	AIR-2	IAL		
Aurora-C	Aurora-3, HsAIRK3, AIE2, Aik3, STK-13 (human); AIE1 (mouse)					

Properties			
Family member	Localization	Regulation	Putative substrates
Aurora-A	Centrosome Spindle MTs	Phosphorylation Degradation	Kinesin-related motor Eg5 CPEB [§]
Aurora-B	Kinetochores Spindle midzone	Association with INCENP Phosphorylation? Degradation Functional interaction with Bir-domain proteins (survivin?)	Histone H3?
Aurora-C	Centrosome		
Yeast Ipl1p	?	?	Histone H3 Kinetochores protein Ndc10p

*The aurora-A, -B, -C nomenclature has been approved by many scientists working in the aurora field. For the sake of clarity, the future use of this unifying nomenclature is recommended.

[†]Ipl1p is the only aurora family member in budding yeast and cannot be attributed to the any particular subfamily.

[§]CPEB is involved in regulating polyadenylation and translation of *c-mos* mRNA during *Xenopus* oocyte maturation¹¹⁵. (CPEB, cytoplasmic polyadenylation-element-binding protein.)

^{||}Shown for *C. elegans* AIR-2, which most probably represents a B-type aurora kinase²³.

a corresponding checkpoint also exists in mammalian cells is not known.

The DNA structure checkpoints. The three enzymes that control the activation of mammalian Cdk1, the phosphatase Cdc25C and the kinases Wee1 and Myt1 (FIG. 1), are themselves phosphorylated by multiple kinases, albeit with different consequences. On the one hand, Cdc25C is inhibited by kinases (Chk1, Chk2) that operate in DNA structure checkpoint signalling, whereas Wee1 and Myt1 are upregulated by the same pathways^{54,57}. On the other hand, Cdk1–cyclin B is able to activate Cdc25C and inactivate Wee1, thereby creating a positive feedback loop. Plk1 also activates Cdc25C^{12,58}, and it may downregulate Wee1 and Myt1. Whether this occurs as part of the feedback loop involving Cdk1–cyclin B or, alternatively, contributes to the initial activation of Cdc25C remains a subject of debate^{59–62}. In view of the many roles emerging for Plks in mitotic progression, it is also interesting that mammalian Plk1 is inhibited on DNA damage checkpoint activation⁶³, extending earlier work in yeast^{64,65}.

The spindle assembly checkpoint. Genetic studies in yeast, as well as laser-ablation and micromanipulation studies in animal cells, have identified a checkpoint

that delays sister-chromatid separation until all chromosomes are properly aligned on the spindle (FIG. 4). This checkpoint monitors the attachment of microtubules to kinetochores and/or the generation of tension that results from bipolar attachment of sister chromatids. Hence, it is also referred to as the kinetochore attachment checkpoint^{66,67}.

Yeast mutant screens have identified six gene products involved in this checkpoint, specifically the dual-specificity kinase Mps1p, the kinase Bub1p and its partner Bub3p, and the three proteins Mad1p, Mad2p and Mad3p⁵⁵. Subsequently, homologues of several Mad and Bub proteins have been shown to associate preferentially with unattached kinetochores in animal cells, confirming earlier cytological evidence for a critical role of phosphorylation in the generation of an anaphase-inhibitory signal at kinetochores^{28,66,67}. Studies done in animal cells also indicate that the spindle assembly checkpoint is not merely activated in response to spindle damage, but contributes to the timing of anaphase onset in every cell division^{68,69}.

According to a current model, structural changes induced by microtubule attachment (and/or tension) are translated, through phosphorylation, into a biochemical signal. In vertebrates, this mechano-chemical coupling was proposed to involve a molecular interaction between the KRP CENP-E and the kinase BubR1 (REFS 50,70,71). How this regulates the kinetochore association of Mad proteins is unknown. However, unattached kinetochores are thought to function as sites of continuous assembly and release of Mad2–Cdc20 complexes that prevent the activation of APC/C^{Cdc20}. On attachment of the last kinetochore, the production of inhibitory Mad2–Cdc20 complexes ceases, allowing Cdc20 to dissociate from Mad2 and activate APC/C; as a result, securin is degraded and anaphase ensues (FIG. 4). Although attractive, this model leaves many important questions unresolved. In particular, many kinases are localized to centromeres/kinetochores, and it remains to be explained how these kinases interact with each other. This would seem critical for understanding both checkpoint activation and checkpoint silencing.

It is also not understood at present why mammalian cells express two Bub1 family members, (Bub1 and BubR1). These are unlikely to be redundant, as both Bub1 and BubR1 are required for checkpoint signalling^{50,68,72}. Both kinases are recruited to unattached kinetochores in association with Bub3, a WD-repeat-containing substrate^{73,74}. Whether Bub3 functions as a regulatory subunit or a downstream effector is not known. Similarly, the precise functions of Mps1p family kinases remain to be uncovered. Epistasis experiments suggest that Mps1p and Bub1p cooperate to generate an anaphase-inhibitory signal, and this may involve the phosphorylation of Mad1p⁷⁵. Overexpression of Mps1p imposes an M-phase arrest, thereby mimicking checkpoint activation⁷⁵, and the same is true of the fission yeast homologue Mph1p⁷⁶. However, no functional studies have yet been reported on the putative mammalian members of the Mps1/Mph1 family, human TTK and mouse Esk.

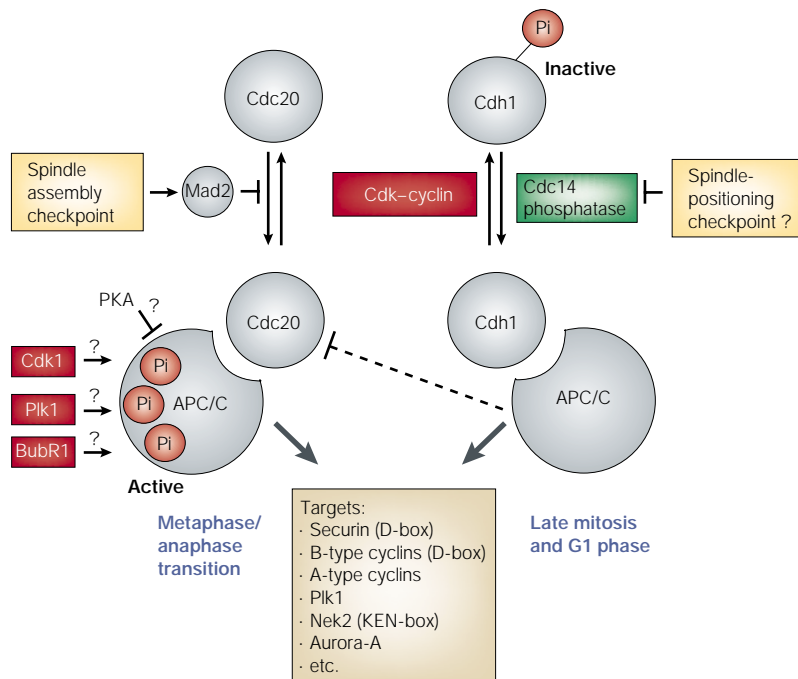


Figure 2 | **Phosphorylation differentially regulates two forms of APC/C.** APC/C acts on different substrates at different times, implying extensive regulation. This involves the binding of adaptor proteins (Cdc20 and Cdh1, respectively), as well as the phosphorylation and dephosphorylation of both APC/C core subunits and adaptor proteins. Note that Cdc20 is also known as Fizzy (*Drosophila*), p55^{CDC} (mammals) and Slp1p (*S. pombe*), whereas synonyms for Cdh1 are Hct1p (*S. cerevisiae*), Fizzy-related (*Drosophila*) and Ste9p/Srw1 (*S. pombe*). Substrates of APC/C can be classified depending on whether they bear D-box or KEN-box consensus motifs, which seem to favour recognition by APC/C^{Cdc20} or APC/C^{Cdh1}, respectively^{39,41}. Much remains to be learned, however, about the precise mechanisms that regulate ubiquitin-dependent degradation of individual substrates. For example, it remains mysterious how cyclin A is degraded during prometaphase — a time when securin degradation by APC/C^{Cdc20} is blocked by the spindle assembly checkpoint.

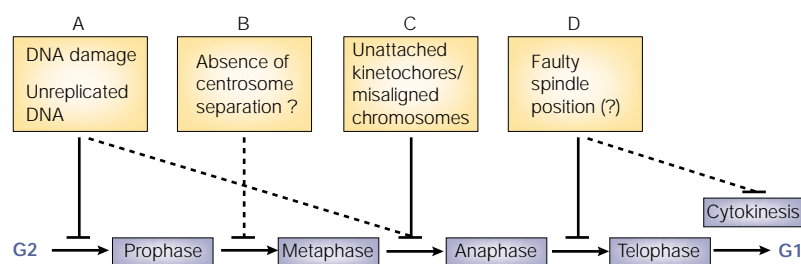


Figure 3 | Checkpoints ordering progression through M phase. The ‘DNA structure checkpoints’ (A) delay the G2/M transition in response to DNA damage or unreplicated DNA^{54,57}, whereas the ‘spindle assembly checkpoint’ (C) monitors microtubule attachment to kinetochores (or tension that results from the proper bipolar attachment of sister chromatids) and delays anaphase onset until all chromosomes are properly aligned⁵⁵. Recent data argue that a ‘spindle-positioning checkpoint’ (D) links M-phase exit to correct spindle orientation, but whether this checkpoint operates in organisms other than *S. cerevisiae* remains to be determined⁷⁷. Additional work will also be required to corroborate the idea that another checkpoint, perhaps monitoring centrosome separation (B), operates at the onset of mitosis in mammalian cells^{11,110}.

The spindle-positioning checkpoint. Intuitively, it seems plausible that a spindle-positioning checkpoint might enforce the correct orientation of the elongating spindle to ensure that cleavage occurs in the right plane and only after complete separation of sister chromatids. Recently, evidence for such a checkpoint has been obtained in *S. cerevisiae*. Its silencing requires that a spindle pole body associates productively with the cortex of the budding cell, thus establishing a dependency between correct spindle positioning and mitotic exit⁷⁷. The first identified component of this pathway was **Bub2p**, a spindle-pole-

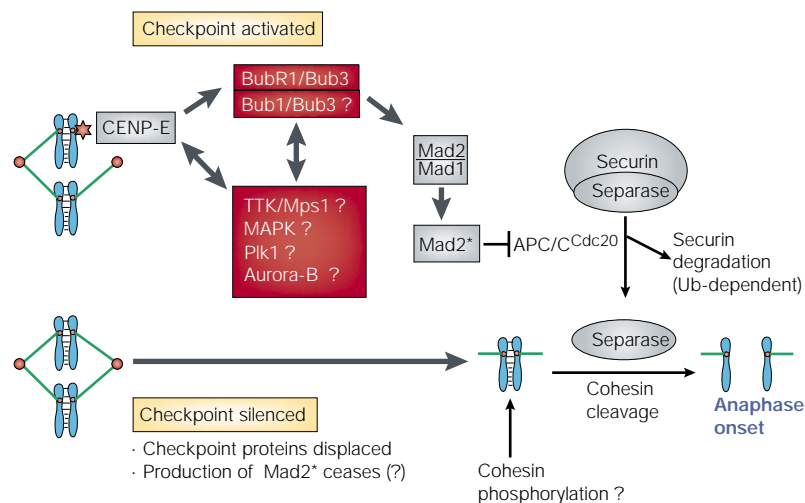


Figure 4 | The spindle assembly checkpoint. One model holds that interactions between the kinesin-related protein CENP-E and BubR1 translate structural information (the presence or absence of appropriate microtubule–kinetochore interactions) into a chemical signal (phosphorylation of as yet unidentified substrates). These events are believed to regulate both the recruitment of Mad1–Mad2 complexes to unattached kinetochores and the release of ‘conformationally altered Mad2’ (represented by Mad2*). Mad2* then blocks a productive interaction between Cdc20 and APC/C, thereby preventing the degradation of securin and the cleavage of cohesin by separase. On attachment of the last kinetochore, the production of Mad2* ceases and activation of APC/C^{Cdc20} ensues. The checkpoint is depicted here as an essentially linear pathway, but this should not detract from the importance of multiprotein complexes and spatial organization in the spindle^{55,111,112}. In addition to Bub1 and BubR1, activated MAP kinase^{113,114}, PIK1 (REFS 96,97) and aurora-B³⁰ have also been detected at kinetochores, suggesting that these enzymes may function in either checkpoint signalling (MAPK ?) or silencing (PIK1 and/or aurora-B ?). (Ub, ubiquitin.)

associated subunit of a two-component GTPase-activating protein (GAP). This GAP downregulates the activity of a small GTPase (**Tem1p**) that in turn functions at an early step in a pathway controlling mitotic exit (FIG. 5). Downstream of active Tem1p, several kinases cooperate in a so-called mitotic exit network (MEN)⁷⁷ to activate the Cdc14p phosphatase. Cdc14p then acts not only as an activator of APC/C^{Cdh1}, but also dephosphorylates the Cdk1-inhibitor **Sic1p** (causing its stabilization) and the transcription factor **Swi5p** (enhancing the production of Sic1p), thereby causing the inactivation of budding yeast Cdk1 by three complementary mechanisms⁵² (FIG. 5).

Interestingly, gene products homologous to most components of the MEN pathway have also been identified in *S. pombe* (FIG. 5). However, as the corresponding genes were identified in studies on septation, a process akin to cytokinesis in animal cells^{78,79}, the term SIN (septation initiation network) was coined. At present, there is no evidence that the SIN is part of a checkpoint controlling Cdk1 inactivation. Taken at face value, this would indicate that apparently homologous gene products control partly distinct processes in the two yeasts. The study of a corresponding pathway in a metazoan organism might help clarify this situation.

Signalling cytokinesis

When considering kinases in relation to cytokinesis, it is useful to distinguish signalling pathways that determine the timing and positioning of contractile ring assembly from mechanical aspects of cleavage furrow ingression. Progress in understanding kinase function in relation to the latter process is beyond the scope of this article. With regard to the coordination of cytokinesis with mitotic progression, it remains unclear whether a kinase cascade similar to the SIN pathway operates in metazoan organisms (see legend to FIG. 5). However, both Plks and B-type aurora kinases have been implicated in the control of cytokinesis. The case for Plk was originally built on data from *S. pombe*, where septation is impaired in the absence of **Plo1p**, whereas overexpression of the kinase triggers additional rounds of septation from any point in the cell cycle⁸⁰. It was concluded that Plo1p functions high up in the SIN pathway^{79,81}, and data from *Drosophila* and mammalian cells support the idea that Plks are upstream regulators of cytokinesis^{62,81,82}. Relevant substrates in Plk mutants have not been identified, but it is possible that the observed cytokinesis defects result from the mislocalization and/or impaired function of the KRP **MKLP-1/Pavarotti**^{83,84} or the cytoskeleton-associated protein **Mid1p**⁸⁵. It should also be emphasized that **Cdc5p** is required for generating a mitotic exit signal through the MEN pathway⁷⁷, which seems to imply a rather indirect link between this budding yeast Plk and cytokinesis.

A role for B-type aurora kinases in cytokinesis is supported by the finding that overexpression of a catalytically inactive aurora-B disrupts cleavage furrow formation in mammalian cells⁸⁶, and by the chromosome segregation and cytokinesis defects observed in *C. elegans* embryos in which the B-type aurora (AIR-

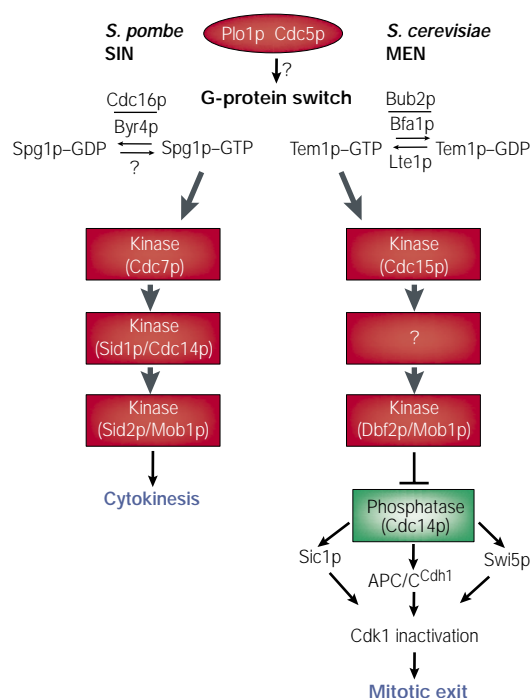


Figure 5 | Mitotic kinases regulating mitotic exit and cytokinesis. Diagram comparing the septation initiation network (SIN) and the mitotic exit network (MEN), two recently described regulatory networks in *S. pombe* and *S. cerevisiae*, respectively. At present, these pathways are based primarily on results from genetic (epistasis) and cytological experiments. For the sake of simplicity, they are depicted in a linear fashion, but biochemical data on direct interactions between the known components remain scarce and additional factors almost certainly await discovery. Interestingly, the two yeast networks comprise several homologous gene products, many of which are kinases or phosphatases. However, although the genomes of *C. elegans*, *Drosophila* and mammals harbour candidate homologues of some of these yeast genes (for example, budding yeast *CDC14* and *DBF2/MOB1*), the existence of homologues of other components (notably the fission yeast Sid1p kinase) is less obvious. Thus, although it is likely that functionally similar networks exist in metazoan organisms, this remains to be proved. (Note that fission yeast Cdc14p is not a phosphatase but a subunit of the Sid1p kinase. The use of the same acronym for the two proteins is purely accidental.)

2) was suppressed by RNAi⁸⁷. A similar phenotype was observed on elimination of the protein Bir1 by RNAi, suggesting that B-type aurora and Bir1 interact either directly or indirectly⁸⁸. This is intriguing because **survivin**, a potential mammalian Bir1 homologue, has been implicated in the protection of cells from apoptosis. If survivin indeed plays such a role, further exploration of a functional interaction between kinetochores, B-type aurora kinases and survivin might provide insight into the important connection between aneuploidy, apoptosis and tumorigenesis.

Mitotic kinases in cancer

Chromosome rearrangements and aneuploidy are hallmarks of most human cancers, and severe karyotypic anomalies generally correlate with poor prognosis. Considering the central role of phosphorylation

in mitotic checkpoints, spindle function and chromosome segregation, it is not surprising that several mitotic kinases have been implicated in tumorigenesis. For example, aurora kinases map to chromosomal regions that are frequently altered in tumours (aurora-A, 20q13.2–q.13.3; aurora-B, 17p13; aurora-C, 19q13.3-ter). Aurora-A is overexpressed in many primary tumours and is able to transform cells in culture⁸⁹, suggesting that it is a relevant gene on the 20q13.2 amplicon²⁰. Furthermore, ectopic expression of aurora-A causes centrosome amplification and aneuploidy in cultured cells, although the underlying mechanism remains to be unravelled⁹⁰.

Similarly, Plk1 can transform rodent cells⁹¹ and is frequently overexpressed in tumours, making it a potential marker for diagnostic or prognostic purposes⁹². Finally, dominantly acting Bub1 mutations were identified in some colorectal tumour cell lines, fuelling the speculation that mitotic checkpoints are commonly inactivated in aneuploid tumours⁷². The known genes for spindle assembly checkpoint are only rarely mutated in tumours⁹³, however, implying that the molecular origins of aneuploidy await discovery and that the search for suspects must go on.

Conclusions and perspectives

The elucidation of mitotic signalling pathways has barely begun. I have focused here on mitotic kinases with serine/threonine specificity; I have not discussed tyrosine kinases, although there are hints that Src family members are also involved in mitotic signalling. Phosphatases have only been addressed in passing, but they most definitely have important roles in mitotic regulation. For future studies on mitotic kinases, the development of activation-state-specific antibodies is eagerly awaited. This would allow more precise determination of when and where each kinase is active during mitosis. Furthermore, the sequencing of phosphorylation sites in a few physiological substrates might facilitate the search for additional candidate substrates. Finally, the most important objective must be to place individual mitotic kinases into functional pathways. This represents a formidable and fascinating challenge for cell biologists, geneticists and biochemists alike. As a reward, it seems legitimate to hope that a better understanding of mitotic signalling will uncover new opportunities for approaching cancer and other proliferation-related diseases.

Links

- DATABASE LINKS [Cdk1](#) | [Polo](#) | [aurora](#) | [NIMA](#) | [retinoblastoma](#) | [E2F](#) | [Cdk2](#) | [cyclin A](#) | [cyclin E](#) | [Mps1p](#) | [Cdc25C](#) | [Wee1](#) | [Myt1](#) | [lamins](#) | [condensins](#) | [Golgi matrix components](#) | [APC/C](#) | [ubiquitin](#) | [securins](#) | [cyclins](#) | [Polo](#) | [Asp](#) | [Nek2](#) | [C-Nap1](#) | [Eg5](#) | [H1](#) | [H3](#) | [Ipl1p](#) | [Glc7p](#) | [stathmin](#) | [CENP-E](#) | [Ndc10p](#) | [ICENP](#) | [CENP-A](#) | [separase](#) | [cohesin](#) | [Cdc20](#) | [Cdh1](#) | [Plk1](#) | [BubR1](#) | [PKA](#) | [Cdc14p](#) | [Chk1](#) | [Bub1p](#) | [Bub3p](#) | [Mad1p](#) | [Mad2p](#) | [Mad3p](#) | [Bub2p](#) | [Tem1p](#) | [Sic1p](#) | [Swi5p](#) | [Plo1p](#) | [MKLP-1/Pavarotti](#) | [Mid1p](#) | [Cdc5p](#) | [survivin](#)
- FURTHER INFORMATION [Nigg lab homepage](#)
- ENCYCLOPEDIA OF LIFE SCIENCES [Mitosis](#)

1. Meraldi, P., Lukas, J., Fry, A. M., Bartek, J. & Nigg, E. A. Centrosome duplication in mammalian somatic cells requires E2F and Cdk2-cyclin A. *Nature Cell Biol.* **1**, 88–93 (1999).
2. Matsumoto, Y., Hayashi, K. & Nishida, E. Cyclin-dependent kinase 2 (Cdk2) is required for centrosome duplication in mammalian cells. *Curr. Biol.* **9**, 429–432 (1999).
3. Hinchcliffe, E. H., Li, C., Thompson, E. A., Maller, J. L. & Sluder, G. Requirement of Cdk2-cyclin E activity for repeated centrosome reproduction in *Xenopus* egg extracts. *Science* **283**, 851–854 (1999).
4. Lauze, E. *et al.* Yeast spindle pole body duplication gene MPS1 encodes an essential dual specificity protein kinase. *EMBO J.* **14**, 1655–1663 (1995).
5. Nigg, E. A. Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle. *BioEssays* **17**, 471–480 (1995).
6. Kimura, K., Hirano, M., Kobayashi, R. & Hirano, T. Phosphorylation and activation of 13S condensin by Cdc2 *in vitro*. *Science* **282**, 487–490 (1998).
7. Lowe, M. *et al.* Cdc2 kinase directly phosphorylates the cis-Golgi matrix protein GM130 and is required for Golgi fragmentation in mitosis. *Cell* **94**, 783–793 (1998).
8. Andersen, S. S. Balanced regulation of microtubule dynamics during the cell cycle: a contemporary view. *BioEssays* **21**, 53–60 (1999).
9. Kramer, E. R., Scheuringer, N., Podtelejnikov, A. V., Mann, M. & Peters, J. M. Mitotic regulation of the APC activator proteins CDC20 and CDH1. *Mol. Biol. Cell* **11**, 1555–1569 (2000).
- A careful biochemical study on the role of phosphorylation in controlling the activities of the Cdc20 and Cdh1 forms of APC/C.**
10. Noton, E. & Diffley, J. F. CDK inactivation is the only essential function of the APC/C and the mitotic exit network proteins for origin resetting during mitosis. *Mol. Cell* **5**, 85–95 (2000).
11. Lane, H. A. & Nigg, E. A. Antibody microinjection reveals an essential role for human polo-like kinase 1 (Plk1) in the functional maturation of mitotic centrosomes. *J. Cell Biol.* **135**, 1701–1713 (1996).
12. Qian, Y. W., Erikson, E., Li, C. & Maller, J. L. Activated polo-like kinase Plx1 is required at multiple points during mitosis in *Xenopus laevis*. *Mol. Cell Biol.* **18**, 4262–4271 (1998).
13. do Carmo-Avides M. & Glover, D. M. Abnormal spindle protein, Asp, and the integrity of mitotic centrosomal microtubule organizing centers. *Science* **283**, 1733–1735 (1999).
14. Fry, A. M. *et al.* C-Nap1, a novel centrosomal coiled-coil protein and candidate substrate of the cell cycle-regulated protein kinase Nek2. *J. Cell Biol.* **141**, 1563–1574 (1998).
15. Helps, N. R., Luo, X., Barker, H. M. & Cohen, P. T. NIMA-related kinase 2 (Nek2), a cell-cycle-regulated protein kinase localized to centrosomes, is complexed to protein phosphatase 1. *Biochem. J.* **349**, 509–518 (2000).
16. Sawin, K. E. & Mitchison, T. J. Mutations in the kinesin-like protein Eg5 disrupting localization to the mitotic spindle. *Proc. Natl Acad. Sci. USA* **92**, 4289–4293 (1995).
17. Blangy, A. *et al.* Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation *in vivo*. *Cell* **83**, 1159–1169 (1995).
18. Glover, D. M., Leibowitz, M. H., McLean, D. A. & Parry, H. Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. *Cell* **81**, 95–105 (1995).
19. Giet, R. & Prigent, C. Aurora/lpl1-related kinases, a new oncogenic family of mitotic serine-threonine kinases. *J. Cell Sci.* **112**, 3591–3601 (1999).
20. Bischoff, J. R. & Plozman, G. D. The Aurora/lpl1p kinase family: regulators of chromosome segregation and cytokinesis. *Trends Cell Biol.* **9**, 454–459 (1999).
21. Schumacher, J. M., Ashcroft, N., Donovan, P. J. & Golden, A. A highly conserved centrosomal kinase, AIR-1, is required for accurate cell cycle progression and segregation of developmental factors in *Caenorhabditis elegans* embryos. *Development* **125**, 4391–4402 (1998).
22. Giet, R., Uzbekov, R., Cubizolles, F., Le Guellec, K. & Prigent, C. The *Xenopus laevis* aurora-related protein kinase pEg2 associates with and phosphorylates the kinesin-related protein XIg5. *J. Biol. Chem.* **274**, 15005–15013 (1999).
23. Hsu, J. Y. *et al.* Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Gic7/PP1 phosphatase in budding yeast and nematodes. *Cell* **102**, 279–291 (2000).
24. De Souza, C. P., Osmani, A. H., Wu, L. P., Spotts, J. L. & Osmani, S. A. Mitotic histone H3 phosphorylation by the NIMA kinase in *Aspergillus nidulans*. *Cell* **102**, 293–302 (2000).
25. Sutani, T. *et al.* Fission yeast condensin complex: essential roles of non-SMC subunits for condensation and Cdc2 phosphorylation of Cut3/SMC4. *Genes Dev.* **13**, 2271–2283 (1999).
26. Larsson, N., Marklund, U., Gradin, H. M., Brattsand, G. & Gullberg, M. Control of microtubule dynamics by oncoprotein 18: dissection of the regulatory role of multisite phosphorylation during mitosis. *Mol. Cell Biol.* **17**, 5530–5539 (1997).
27. Andersen, S. S. *et al.* Mitotic chromatin regulates phosphorylation of Stathmin/Op18. *Nature* **389**, 640–643 (1997).
28. Rieder, C. L. & Salmon, E. D. The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* **8**, 310–318 (1998).
29. Biggins, S. *et al.* The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. *Genes Dev.* **13**, 532–544 (1999).
30. Adams, R. R. *et al.* INCENP binds the aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow. *Curr. Biol.* **10**, 1075–1078 (2000).
- This paper identifies the 'chromosomal passenger protein' INCENP as a partner of aurora-B, and shows that INCENP is required for correct targeting of the kinase.**
31. Nasmyth, K., Peters, J. M. & Uhlmann, F. Splitting the chromosome: cutting the ties that bind sister chromatids. *Science* **288**, 1379–1385 (2000).
32. Yanagida, M. Cell cycle mechanisms of sister chromatid separation: roles of Cut1/separin and Cut2/securin. *Genes Cells* **5**, 1–8 (2000).
33. Uhlmann, F., Wernic, D., Poupart, M. A., Koonin, E. V. & Nasmyth, K. Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. *Cell* **103**, 375–386 (2000).
- This important paper identifies the Esp1p gene product as a protease (separase) and elegantly shows that cohesin cleavage by separase is sufficient for sister chromatid separation in budding yeast.**
34. Sumara, I., Vorlauffer, E., Geffers, C., Peters, B. H. & Peters, J. M. Characterization of vertebrate cohesin complexes and their regulation in prophase. *J. Cell Biol.* **151**, 749–762 (2000).
35. Losada, A., Yokochi, T., Kobayashi, R. & Hirano, T. Identification and characterization of SA/Scp3 subunits in the *Xenopus* and human cohesin complexes. *J. Cell Biol.* **150**, 405–416 (2000).
36. Waizenegger, I. C., Hauf, S., Meinke, A. & Peters, J. M. Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. *Cell* **103**, 399–410 (2000).
37. Shirayama, M., Zachariae, W., Ciosk, R. & Nasmyth, K. The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in *Saccharomyces cerevisiae*. *EMBO J.* **17**, 1336–1349 (1998).
38. Charles, J. F. *et al.* The Polo-related kinase Cdc5 activates and is destroyed by the mitotic cyclin destruction machinery in *S. cerevisiae*. *Curr. Biol.* **8**, 497–507 (1998).
39. Fang, G., Yu, H. & Kirschner, M. W. Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G1. *Mol. Cell* **2**, 163–171 (1998).
40. Ye, X. S., Fincher, R. R., Tang, A., Osmani, A. H. & Osmani, S. A. Regulation of the anaphase-promoting complex/cyclosome by bimAAPC3 and proteolysis of NIMA. *Mol. Biol. Cell* **9**, 3019–3030 (1998).
41. Pflieger, C. M. & Kirschner, M. W. The KEN box: an APC recognition signal distinct from the D box targeted by cdh1. *Genes Dev.* **14**, 655–665 (2000).
42. Honda, K. *et al.* Degradation of human Aurora2 protein kinase by the anaphase-promoting complex-ubiquitin-proteasome pathway. *Oncogene* **19**, 2812–2819 (2000).
43. Morgan, D. O. Regulation of the APC and the exit from mitosis. *Nature Cell Biol.* **1**, E47–E53 (1999).
44. Sigrist, S. J. & Lehner, C. F. *Drosophila* fizzy-related downregulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles. *Cell* **90**, 671–681 (1997).
45. Lorca, T. *et al.* Fizzy is required for activation of the APC/cyclosome in *Xenopus* egg extracts. *EMBO J.* **17**, 3565–3575 (1998).
46. Descombes, P. & Nigg, E. A. The polo-like kinase Plx1 is required for M phase exit and destruction of mitotic regulators in *Xenopus* egg extracts. *EMBO J.* **17**, 1328–1335 (1998).
47. Patra, D. & Dunphy, W. G. 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 (1998).
48. Kotani, S. *et al.* PKA and MPF-activated polo-like kinase regulate anaphase-promoting complex activity and mitosis progression. *Mol. Cell* **1**, 371–380 (1998).
49. Kotani, S., Tanaka, H., Yasuda, H. & Todokoro, K. Regulation of APC activity by phosphorylation and regulatory factors. *J. Cell Biol.* **146**, 791–800 (1999).
50. Chan, G. K., Jablonski, S. A., Sudakin, V., Hittle, J. C. & Yen, T. J. Human BUBR1 is a mitotic checkpoint kinase that monitors CENP-E functions at kinetochores and binds the cyclosome/APC. *J. Cell Biol.* **146**, 941–954 (1999).
51. Farruggio, D. C., Townsley, F. M. & Ruderman, J. V. Cdc20 associates with the kinase aurora2/Aik. *Proc. Natl Acad. Sci. USA* **96**, 7306–7311 (1999).
52. Visintin, R. *et al.* The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. *Mol. Cell* **2**, 709–718 (1998).
53. Jaspersen, S. L., Charles, J. F. & Morgan, D. O. Inhibitory phosphorylation of the APC regulator Hct1 is controlled by the kinase Cdc28 and the phosphatase Cdc14. *Curr. Biol.* **9**, 227–236 (1999).
54. Elledge, S. J. Cell cycle checkpoints: preventing an identity crisis. *Science* **274**, 1664–1672 (1996).
55. Hardwick, K. G. The spindle checkpoint. *Trends Genet.* **14**, 1–4 (1998).
56. Burke, D. J. Complexity in the spindle checkpoint. *Curr. Opin. Genet. Dev.* **10**, 26–31 (2000).
57. Russell, P. Checkpoints on the road to mitosis. *Trends Biochem. Sci.* **23**, 399–402 (1998).
58. Kumagai, A. & Dunphy, W. G. Purification and molecular cloning of Plx1, a Cdc25-regulatory kinase from *Xenopus* egg extracts. *Science* **273**, 1377–1380 (1996).
- This paper identifies the Cdc25 phosphatase as a likely physiological substrate of Plx1 and thereby implicates Plks in controlling Cdk1 activity.**
59. Abrieu, A. *et al.* The Polo-like kinase Plx1 is a component of the MPF amplification loop at the G2/M-phase transition of the cell cycle in *Xenopus* eggs. *J. Cell Sci.* **111**, 1751–1757 (1998).
60. Karaiskou, A., Jessus, C., Brassac, T. & Ozon, R. Phosphatase 2A and polo kinase, two antagonistic regulators of cdc25 activation and MPF auto-amplification. *J. Cell Sci.* **112**, 3747–3756 (1999).
61. Qian, Y. W., Erikson, E. & Maller, J. L. Mitotic effects of a constitutively active mutant of the *Xenopus* polo-like kinase Plx1. *Mol. Cell Biol.* **19**, 8625–8632 (1999).
62. Nigg, E. A. Polo-like kinases: positive regulators of cell division from start to finish. *Curr. Opin. Cell Biol.* **10**, 776–783 (1998).
63. Smits, V. A. *et al.* Polo-like kinase-1 is a target of the DNA damage checkpoint. *Nature Cell Biol.* **2**, 672–676 (2000).
64. Sanchez, Y. *et al.* Control of the DNA damage checkpoint by chk1 and rad53 protein kinases through distinct mechanisms. *Science* **286**, 1166–1171 (1999).
65. Toczyski, D. P., Galgoczy, D. J. & Hartwell, L. H. CDC5 and CKII control adaptation to the yeast DNA damage checkpoint. *Cell* **90**, 1097–1106 (1997).
66. Gorbisky, G. J. Cell cycle checkpoints: arresting progress in mitosis. *BioEssays* **19**, 193–197 (1997).
67. Nicklas, R. B. How cells get the right chromosomes. *Science* **275**, 632–637 (1997).
68. Taylor, S. S. & McKeon, F. Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. *Cell* **89**, 727–735 (1997).
- This paper implicates the murine Bub1 kinase in the timing of anaphase onset during normal mitosis.**
69. Basu, J. *et al.* Mutations in the essential spindle checkpoint gene bub1 cause chromosome missegregation and fail to block apoptosis in *Drosophila*. *J. Cell Biol.* **146**, 13–28 (1999).
70. Yao, X., Abrieu, A., Zheng, Y., Sullivan, K. F. & Cleveland, D. W. CENP-E forms a link between attachment of spindle microtubules to kinetochores and the mitotic checkpoint. *Nature Cell Biol.* **2**, 484–491 (2000).
71. Abrieu, A., Kahana, J. A., Wood, K. W. & Cleveland, D. W. CENP-E as an essential component of the mitotic checkpoint *in vitro*. *Cell* **102**, 817–826 (2000).
72. Cahill, D. P. *et al.* Mutations of mitotic checkpoint genes in human cancers. *Nature* **392**, 300–303 (1998).
73. Basu, J. *et al.* Localization of the *Drosophila* checkpoint control protein Bub3 to the kinetochore requires Bub1 but not Zw10 or Rod. *Chromosoma* **107**, 376–385 (1998).
74. Taylor, S. S., Ha, E. & McKeon, F. The human homologue of Bub3 is required for kinetochore localization of Bub1 and a Mad3/Bub1-related protein kinase. *J. Cell Biol.* **142**, 1–11 (1998).

75. Hardwick, K. G., Weiss, E., Luca, F. C., Winey, M. & Murray, A. W. Activation of the budding yeast spindle assembly checkpoint without mitotic spindle disruption. *Science* **273**, 953–956 (1996).
76. He, X., Jones, M. H., Winey, M. & Sazer, S. Mph1, a member of the Mps1-like family of dual specificity protein kinases, is required for the spindle checkpoint in *S. pombe*. *J. Cell Sci.* **111**, 1635–1647 (1998).
77. Hoyt, M. A. Exit from mitosis: spindle pole power. *Cell* **102**, 267–270 (2000).
78. Le, G., X, Utzig, S. & Simanis, V. Controlling septation in fission yeast: finding the middle, and timing it right. *Curr. Genet.* **35**, 571–584 (1999).
79. Balasubramanian, M. K., McCollum, D. & Surana, U. Tying the knot: linking cytokinesis to the nuclear cycle. *J. Cell Sci.* **113**, 1503–1513 (2000).
80. Ohkura, H., Hagan, I. M. & Glover, D. M. The conserved kinase *plp1*, required to form a bipolar spindle, the actin ring, and septum, can drive septum formation in G1 and G2 cells. *Genes Dev.* **9**, 1059–1073 (1995).
81. Glover, D. M., Hagan, I. M. & Tavares, A. A. Polo-like kinases: a team that plays throughout mitosis. *Genes Dev.* **12**, 3777–3787 (1998).
82. Carmona, M. *et al.* *Drosophila* polo kinase is required for cytokinesis. *J. Cell Biol.* **143**, 659–671 (1998).
83. Lee, K. S., Yuan, Y. L., Kuriyama, R. & Erikson, R. L. Plk is an M-phase-specific protein kinase and interacts with a kinesin-like protein, CHO1/MKLP-1. *Mol. Cell Biol.* **15**, 7143–7151 (1995).
84. Adams, R. R., Tavares, A. A., Salzberg, A., Bellen, H. J. & Glover, D. M. *pavarotti* encodes a kinesin-like protein required to organize the central spindle and contractile ring for cytokinesis. *Genes Dev.* **12**, 1483–1494 (1998).
85. Bahler, J. *et al.* Role of polo kinase and Mid1p in determining the site of cell division in fission yeast. *J. Cell Biol.* **143**, 1603–1616 (1998).
86. Terada, Y. *et al.* AIM-1: a mammalian midbody-associated protein required for cytokinesis. *EMBO J.* **17**, 667–676 (1998).
- This study characterizes mammalian aurora-B and provides evidence that this kinase has a role in cytokinesis.**
87. Schumacher, J. M., Golden, A. & Donovan, P. J. AIR-2: An Aurora/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in *Caenorhabditis elegans* embryos. *J. Cell Biol.* **143**, 1635–1646 (1998).
88. Spiliotes, E. K., Uren, A., Vaux, D. & Horvitz, H. R. The survivin-like *C. elegans* BIR-1 protein acts with the Aurora-like kinase AIR-2 to affect chromosomes and the spindle midzone. *Mol. Cell* **6**, 211–223 (2000).
89. Bischoff, J. R. *et al.* A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers. *EMBO J.* **17**, 3052–3065 (1998).
- This paper provides evidence for a causal relationship between aurora-A overexpression and tumorigenesis.**
90. Zhou, H. *et al.* Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nature Genet.* **20**, 189–193 (1998).
91. Smith, M. R. *et al.* Malignant transformation of mammalian cells initiated by constitutive expression of the polo-like kinase. *Biochem. Biophys. Res. Commun.* **234**, 397–405 (1997).
92. Knecht, R. *et al.* Prognostic significance of polo-like kinase (PLK) expression in squamous cell carcinomas of the head and neck. *Cancer Res.* **59**, 2794–2797 (1999).
93. Cahill, D. P. *et al.* Characterization of MAD2B and other mitotic spindle checkpoint genes. *Genomics* **58**, 181–187 (1999).
94. Kauselmann, G. *et al.* The polo-like protein kinases Fnk and Snk associate with a Ca²⁺- and integrin-binding protein and are regulated dynamically with synaptic plasticity. *EMBO J.* **18**, 5528–5539 (1999).
95. Lee, K. S., Grenfell, T. Z., Yarm, F. R. & Erikson, R. L. Mutation of the polo-box disrupts localization and mitotic functions of the mammalian polo kinase Plk. *Proc. Natl Acad. Sci. USA* **95**, 9301–9306 (1998).
96. Arnaud, L., Pines, J. & Nigg, E. A. GFP tagging reveals human Polo-like kinase 1 at the kinetochore/centromere region of mitotic chromosomes. *Chromosoma* **107**, 424–429 (1998).
97. Wianny, F., Tavares, A., Evans, M. J., Glover, D. M. & Zernicka-Goetz, M. Mouse polo-like kinase 1 associates with the acentriolar spindle poles, meiotic chromosomes and spindle midzone during oocyte maturation. *Chromosoma* **107**, 430–439 (1998).
98. Golsteyn, R. M., Mundt, K. E., Fry, A. M. & Nigg, E. A. Cell cycle regulation of the activity and subcellular localization of Plk1, a human protein kinase implicated in mitotic spindle function. *J. Cell Biol.* **129**, 1617–1628 (1995).
99. Qian, Y. W., Erikson, E. & Maller, J. L. Purification and cloning of a protein kinase that phosphorylates and activates the polo-like kinase Plx1. *Science* **282**, 1701–1704 (1998).
- An impressive biochemical study that identifies the first candidate upstream regulator of a Plk.**
100. Ellinger-Ziegelbauer, H. *et al.* Ste20-like kinase (SLK), a regulatory kinase for polo-like kinase (Plk) during the G2/M transition in somatic cells. *Genes Cells* **5**, 491–498 (2000).
101. Osmani, S. A. & Ye, X. S. Cell cycle regulation in *Aspergillus* by two protein kinases. *Biochem. J.* **317**, 633–641 (1996).
102. Krien, M. J. *et al.* A NIMA homologue promotes chromatin condensation in fission yeast. *J. Cell Sci.* **111**, 967–976 (1998).
103. Kandil, M., Feige, E., Chen, A., Kilfin, G. & Motro, B. Isolation and characterization of two evolutionarily conserved murine kinases (Nek6 and nek7) related to the fungal mitotic regulator, NIMA. *Genomics* **68**, 187–196 (2000).
104. Fry, A. M., Schultz, S. J., Bartek, J. & Nigg, E. A. Substrate specificity and cell cycle regulation of the Nek2 protein kinase, a potential human homolog of the mitotic regulator NIMA of *Aspergillus nidulans*. *J. Biol. Chem.* **270**, 12899–12905 (1995).
105. Fry, A. M., Meraldi, P. & Nigg, E. A. A centrosomal function for the human Nek2 protein kinase, a member of the NIMA family of cell cycle regulators. *EMBO J.* **17**, 470–481 (1998).
- This study implicates Nek2 in the regulation of the centrosome, thereby providing first insights into the role of a mammalian NIMA kinase family member.**
106. Kimura, M., Matsuda, Y., Yoshioka, T. & Okano, Y. Cell cycle-dependent expression and centrosome localization of a third human aurora/Ipl1-related protein kinase, AIK3. *J. Biol. Chem.* **274**, 7334–7340 (1999).
107. Andresson, T. & Ruderman, J. V. The kinase Eg2 is a component of the *Xenopus* oocyte progesterone-activated signaling pathway. *EMBO J.* **17**, 5627–5637 (1998).
108. Frank-Vaillant, M. *et al.* Progesterone regulates the accumulation and the activation of Eg2 kinase in *Xenopus* oocytes. *J. Cell Sci.* **113**, 1127–1138 (2000).
109. Pines, J. Four-dimensional control of the cell cycle. *Nature Cell Biol.* **1**, E73–E79 (1999).
110. Scolnick, D. M. & Halazonetis, T. D. Chfr defines a mitotic stress checkpoint that delays entry into metaphase. *Nature* **406**, 430–435 (2000).
111. Rieder, C. L. *et al.* Mitosis in vertebrate somatic cells with two spindles: implications for the metaphase/anaphase transition checkpoint and cleavage. *Proc. Natl Acad. Sci. USA* **94**, 5107–5112 (1997).
112. Hardwick, K. G., Johnston, R. C., Smith, D. L. & Murray, A. W. MAD3 encodes a novel component of the spindle checkpoint which interacts with bub3p, cdc20p, and mad2p. *J. Cell Biol.* **148**, 871–882 (2000).
113. Shapiro, P. S. *et al.* Activation of the MKK/ERK pathway during somatic cell mitosis: direct interactions of active ERK with kinetochores and regulation of the mitotic 3F3/2 phosphoantigen. *J. Cell Biol.* **142**, 1533–1545 (1998).
114. Zecevic, M. *et al.* Active MAP kinase in mitosis: localization at kinetochores and association with the motor protein CENP-E. *J. Cell Biol.* **142**, 1547–1558 (1998).
115. Mendez, R. *et al.* Phosphorylation of CPE binding factor by Eg2 regulates translation of c-mos mRNA. *Nature* **404**, 302–307 (2000).

Acknowledgements

I thank F. Barr, P. Duncan, W. Earnshaw, A. Fry, P. Meraldi, J. Pines, H. Silljé and S. Wheatley for helpful comments on the manuscript, and P. Meraldi for generously providing immunofluorescence figures. My apologies go to all authors whose primary work could not be cited because of space constraints.