Dividing the goods: co-ordination of chromosome biorientation and mitotic checkpoint signalling by mitotic kinases

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Abstract
Error-free chromosome segregation during cell division relies on chromosome biorientation and mitotic checkpoint activity. A group of unrelated kinases controls various aspects of both processes. The present short review outlines our current understanding of the roles of these kinases in maintaining chromosomal stability.

Chromosome biorientation: a tale of two sisters
Faithful segregation of chromosomes to resulting daughter cells in mitosis relies on chromosome biorientation and mitotic checkpoint signalling (Figure 1). In early mitosis, the two copies of a duplicated chromosome (i.e. sister chromatids) need to attach via their kinetochores to spindle microtubules emanating from opposite sides of the cell in a process known as biorientation (reviewed in [1]). Crucial for this is efficient microtubule capture by the kinetochores of each sister chromatid (reviewed in [2]). Quite a few duplicated chromosomes, however, initially attach with each sister to microtubules from a single pole rather than opposite poles (a ‘syntelic’ attachment). This error in attachment needs to be corrected for biorientation to occur (reviewed in [3]). Once a duplicated chromosome has bioriented, forces intrinsic to the mitotic spindle move that chromosome to the spindle equator in a process termed chromosome ‘alignment’ or ‘congression’. Biorientation is, however, not an absolute requirement for congression, as non-bioriented chromosomes can congress by using microtubules from bioriented chromosomes as tracks to guide them to the cell equator [4]. On biorientation of the final chromosome, removal of cohesion between the sister chromatids coupled with pulling forces from the opposing spindle poles and possibly molecular motors at the kinetochores ensures that each sister is dragged to opposite sides of the cell.

The mitotic checkpoint
The irreversible event of chromosome segregation needs to be prevented until each and every duplicated chromosome is bioriented. Premature onset of chromosome segregation causes unequal chromosome distributions, or aneuploidy, which is a leading cause of birth defects and the most common genetic alteration among solid tumours. This inspired the hypothesis that aneuploidy could be a contributing factor in carcinogenesis (reviewed in [5]). Attachment of chromosomes to spindle microtubules, a prerequisite for biorientation, is monitored by a cell-cycle checkpoint known as the spindle assembly checkpoint or the mitotic checkpoint, which halts chromosome segregation and subsequent exit from mitosis when as little as one single chromosome has not established attachments (Figure 1) [6,7].

Unattached kinetochores act as scaffolds to recruit and activate components of the checkpoint machinery, culminating in inhibition of the E3-ubiquitin ligase APC/C (anaphase-promoting complex/cyclosome) that controls chromosome segregation and exit from mitosis (reviewed in [8]). Together with its co-activators Cdc20 (cell division cycle 20) and Cdh1, the APC/C promotes anaphase onset and progression through the cell cycle by ensuring the ubiquitin-dependent destruction of securin and cyclin B (reviewed in [9]). The mitotic checkpoint inhibits Cdc20-dependent activation of the APC/C until all kinetochores are properly attached. This ensures an extension of mitosis to allow sufficient time to achieve kinetochore–microtubule attachments and biorientation. The first identification of components of the mitotic checkpoint stems from studies in the baker’s yeast Saccharomyces cerevisiae that screened for mutants that could not survive growth in the presence of a spindle poison [10–12]. Orthologues of these mutants, Bub1, Bub3, Mad1, Mad2, Mad3 and Mps1, were later identified to be crucial for mitotic checkpoint activity in a host of model systems, including human tissue culture cells (reviewed in [13–16]). Many additional components have been suggested to participate in checkpoint signalling in human cells (reviewed in [16]).

Essential for the assembly of an APC/C inhibitor by unattached kinetochores is the activation of Mad2 by dimerization with a Mad1–Mad2 heterodimer that is stably bound to unattached kinetochores. This dimerization converts an inactive Mad2 molecule into an active one that can participate in Cdc20 binding and APC/C inhibition (reviewed in [17]). Two of the current models for checkpoint function are
Figure 1: Two processes essential for error-free chromosome segregation
In early mitosis (prometaphase), the mitotic checkpoint prevents cell-cycle progression while chromosomes attempt to attach with each copy to opposite poles (biorientation). Once productive attachment of every chromosome is reached and chromosomes have bioriented (metaphase), the checkpoint is satisfied and chromosome segregation is allowed.

Figure 2: Two models of mitotic checkpoint-mediated inhibition of the APC/C
See the text for details.

depicted in Figure 2. In the relay model, activated Mad2 binds Cdc20 and transfers it to BubR1–Bub3 dimer (BubR1 has sequence similarity to yeast Mad3). BubR1–Bub3–Cdc20 complexes can bind the APC/C, where BubR1 functions as a pseudo-substrate inhibitor of the APC/C via its N-terminal destruction [KEN (Lys-Glu-Asn)] motifs [18–22]. In the MCC (mitotic checkpoint complex) model, activated Mad2 and the Bub3–BubR1 dimer bind Cdc20 and form a tetrameric complex. This tetramer, coined the MCC, then inhibits the APC/C as described above [23–25]. One clear difference between these models is that each predicts different amounts of Mad2 to be present in APC/C complexes, but there is currently no consensus on this seemingly straightforward issue. In line with the relay model, some studies have found little Mad2 complexed to BubR1–APC/C in checkpoint-activated cells or have found no need for Mad2 in the direct inhibition of the APC/C by BubR1 [20,22,26]. On the other hand, purifications of inhibited APC/C complexes from mitotic cells contain clearly detectable Mad2, even to near-stoichiometric levels with BubR1 and APC/C subunits [25,27]. Many other issues will need to be addressed before a clear molecular description of the mitotic checkpoint can be formulated. For instance, does BubR1 need to be activated by unattached kinetochores to be able to bind Mad2-bound Cdc20 or inhibit the APC/C? Is dimerization of Mad2 regulated by other factors, such as phosphorylation or protein components? Is there a direct role for Mad2 in APC/C inhibition? How does pseudosubstrate inhibition of the APC/C by BubR1 take effect molecularly? What is the role of the many other proteins identified as important for mitotic checkpoint activity? In vitro insights into how Cdc20 activates APC/C activity towards mitotic substrates might provide clues as to how the inhibitory complex prevents this, and what the roles are of the various components proposed to constitute the inhibitor.

Inhibition of the APC/C is relieved shortly after the final kinetochore has engaged in productive attachments with spindle microtubules. Several mechanisms to shut down production of the inhibitor by unattached kinetochores or to disassemble inhibitory complexes that were produced prior to final attachment have been proposed and are reviewed in [16]. For example, p31comet is a structural mimetic of Mad2 and can thus prevent Mad2 dimerization and may additionally aid disassembly or inhibition of proposed MCC complexes [28–30]. On attachment of the final kinetochore, several or all of these mechanisms combine to rapidly allow activity of APC/C towards cyclin B and securin and promote mitotic progression beyond anaphase. It is not unlikely that unattached kinetochores, besides promoting formation of the inhibitor, also actively prevent disassembly of the inhibitor. Such a tight control of the amount of inhibitor could translate the attachment state of a kinetochore to a switch-like behaviour of the mitotic checkpoint, ensuring rapid activation of the APC/C and commitment to anaphase upon attachment of the final kinetochore. The coming years will probably witness a wealth of insight into the mechanisms of inhibition of the mitotic checkpoint signal and how that is regulated in space and time.

Kinase signalling in checkpoint control and chromosome alignment
The processes of biorientation and checkpoint signalling are highly dynamic. Kinetochore–spindle microtubule connections need to be established and severed rapidly to allow biorientation and alignment. In addition, the checkpoint needs to be switched on as soon as misattachments threaten faithful chromosome segregation and needs to be inactivated equally rapidly once biorientation has been achieved. Not surprisingly therefore, kinases play important roles in these processes. Moreover, as the kinetochore of a single unattached chromatid is sufficient to inhibit all cellular APC/C activity, the signal from each kinetochore needs to be amplified significantly, a process that may be facilitated by kinases.

So far, the kinases Mps1, Bub1, BubR1, NEK2A (never in mitosis A-related kinase 2A), PRP4 (pre-mRNA processing factor 4) and TAO1 (thousand-and-one amino acid protein
1) have been directly implicated in checkpoint signalling in human cells and are also crucial for chromosome biorientation (unreported for PRP4) [31–42]. For practical purposes, this group of enzymes will be referred to here as the subfamily of Bi-MC (biorientation and mitotic checkpoint) kinases. Interestingly, in the three instances in which more details on the role of the Bi-MC kinases were reported, each of them was found to impact biorientation in different ways. In all cases, however, this function is independent of their roles in the mitotic checkpoint, as inactivating the mitotic checkpoint by virtue of Mad2 depletion has no consequence for biorientation [37]. The establishment of stable interactions between kinetochores and spindle microtubules requires BubR1 [32,37]. Cells depleted of BubR1 have unattached kinetochores, and stable attachments can be reinstated when Aurora B kinase activity is additionally inhibited. As Aurora B can destabilize kinetochore–microtubule interactions (reviewed in [3]), this suggests that BubR1 somehow keeps Aurora B activity in check, thus allowing kinetochore–spindle interactions to form. BubR1 further interacts with CENP-E (centromere protein E), a plus-end-directed microtubule motor [31,43]. CENP-E may participate in BubR1’s control over Aurora B since depletion of CENP-E in Drosophila S2 cells was reported to restore stable attachments after BubR1 depletion [44]. It is not known whether BubR1 kinase activity is required for the stabilization of chromosome attachments. Studies in frog extracts, human cells and mouse cells are contradictory with respect to this question [21,45–48]. Careful gene-replacement studies or the identification of a critical substrate will be able to settle this issue. Another aspect of biorientation is regulated by the Bub1 kinase, which ensures conversion of a lateral attachment into an end-on one [36,39]. It is unknown how this conversion takes place, and the role of Bub1 in this is therefore also poorly described. In addition, Bub1 maintains sister chromatid cohesion by ensuring the centromere localization of the cohesin protector shugoshin [49,50]. As shugoshin-depleted cells also have unstable kinetochore–microtubule interactions [51], it is possible that this function of Bub1 is related to its role in the lateral-to-end-on conversion of kinetochore-microtubule attachments. Although Bub1 kinase activity is needed for checkpoint control, at least in fission yeast and frog extracts [32,53], it has not yet been reported whether it is needed for biorientation as well. Finally, we and others have recently shown that Mps1 is dispensable for stable attachments or lateral-to-end-on conversion but instead controls attachment-error correction. In yeast and human cells, Mps1 kinase activity ensures biorientation by facilitating the correction of syntelic and possibly merotelic attachments [35,54]. Although not apparent in yeast cells, Mps1 was shown to promote Aurora B activity via direct phosphorylation of its auxiliary protein Borealin in human cells [35].

### Bi-MC kinases in pathologies

All human aneuploidies during development result in embryonic lethality, except certain combinations of sex chromosomes and trisomies 13, 18 and 21, which lead to severe birth defects [55]. In addition, comprehensive analysis of over 20000 tumour samples revealed that aneuploidy is the characteristic most commonly shared by all tumours [56]. Various cancer cell lines show CIN (chromosomal instability), meaning they frequently lose and gain whole chromosomes during divisions. A number of observations have lent support to the hypothesis that aneuploidy, despite providing cells with significant proliferative disadvantages in vitro [57,58], can contribute to or even drive carcinogenesis. Mice in which aneuploidy is induced have a significant increase in sensitivity to carcinogens and can co-operate in a synergistic fashion with tumour suppressor mutations for the outgrowth of tumours (reviewed in [59]). Normal diploid cells can become aneuploid when any of various mechanisms that all centre around ensuring fidelity of chromosome segregation during mitosis are compromised. It has thus been proposed that mitotic checkpoint activity may be weakened in tumour cells, allowing for occasional chromosome mis-segregation events. Although various studies using suboptimal assays (reviewed in [5]) have claimed weakened or absent mitotic checkpoints in tumour cell lines or tumour samples, recent careful analyses of a total of five CIN cancer cell lines showed that chromosome mis-segregations in vitro are not caused by potential defects in the mitotic checkpoint in these cell lines [60,61].

Correlations between expression/mutations in the Bi-MC kinases and aneuploidy-related pathologies have been reported, but causal links have not been established. For instance, Mps1 mRNA expression in a panel of 12 breast cancer cell lines and 9 primary tumour samples was found to be increased up to 500 times relative to normal cells [62]. This may influence stability of kinetochore–spindle interactions and thereby increase the frequency of chromosome mis-segregations. Of course, such overexpression of a kinase may alternatively influence processes that are unrelated to its endogenous functions and thereby contribute to the transformed phenotype. In an example of a very strong correlation between alterations of a Bi-MC kinase and a disease, germline mutations in the BUB1B gene encoding BubR1 were recently reported in a recessive condition called MVA (mosaic variegated aneuploidy), a characteristic of which is the development of childhood cancer [63,64]. In a British cohort of MVA patients, biallelic mutations in BUB1B were found that combined a predicted protein truncation (removing the C-terminal kinase domain) with a missense mutation, often located in or near the kinase domain [63]. In a Japanese cohort, monoallelic mutations were found, mostly, but not exclusively, predicted to result in a truncated protein from which the kinase domain is removed [64]. In these patients, expression from the second, non-mutated allele was reduced and this correlated significantly with a certain haplotype. Although not assayed entirely correctly, fibroblasts from a Japanese MVA patient appeared to have a weakened mitotic checkpoint response to spindle microtubules and displayed elevated amounts of micronuclei, an indication of chromosomes missegregations. A thorough analysis of the effect of the MVA mutations on BubR1 function and...
Figure 3 | Bi-MC kinases integrate distinct processes required for proper chromosome segregation

Bi-MC kinases promote biorientation and prevent premature chromosome segregation by activating the mitotic checkpoint. A molecular description of their role in these processes (question marks) will provide valuable insight into how cells ensure correct chromosome segregation.

Concluding remarks

A general principle is emerging in which kinases that set up the requirements for faithful chromosome segregation also signal to the cell-cycle machinery to halt until those requirements are met. These kinases are therefore crucial in the maintenance of chromosomal stability, and molecular insights into their activities will shed light on how chromosome segregation is regulated at the molecular level (Figure 3).

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