The spindle checkpoint

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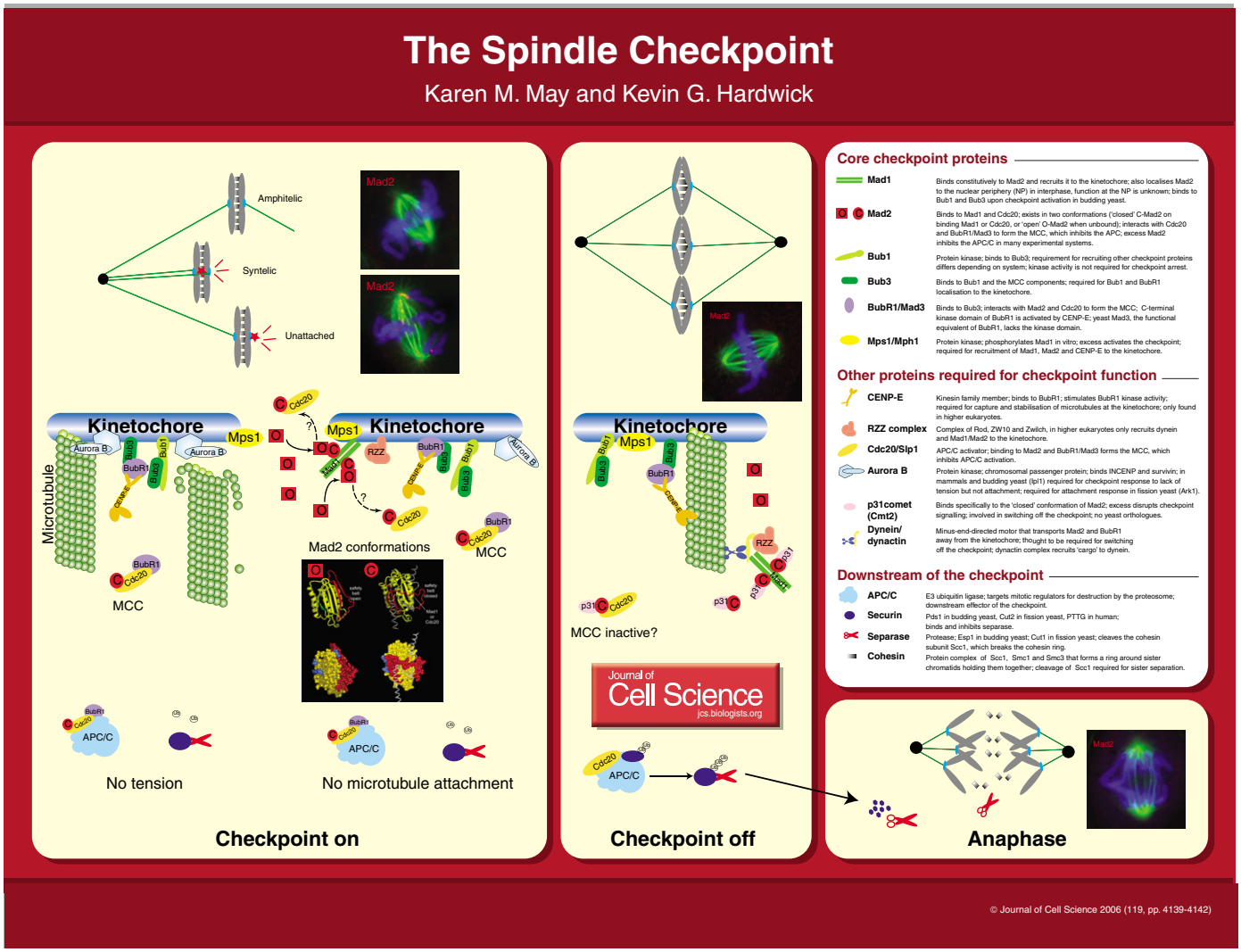
Every mitosis, replicated chromosomes must be accurately segregated into each daughter cell. Pairs of sister chromatids attach to the bipolar mitotic spindle during prometaphase, they are aligned at metaphase, then sisters separate and are pulled to opposite poles during anaphase. Failure to attach correctly to the spindle before anaphase onset results in unequal segregation of chromosomes, which can lead to cell death or disease. The spindle checkpoint is a surveillance mechanism that delays anaphase onset until all chromosomes are correctly attached in a bipolar fashion to the mitotic spindle.

The core spindle checkpoint proteins are Mad1, Mad2, BubR1 (Mad3 in yeast), Bub1, Bub3 and Mps1. The Mad and Bub proteins were first identified in budding yeast by genetic screens for mutants that failed to arrest in mitosis when the spindle was destroyed (Taylor et al., 2004). These proteins are conserved in all eukaryotes. Several other checkpoint components, such as Rod, Zw10 and CENP-E, have since been identified in higher eukaryotes but have no yeast orthologues (Karess, 2005; Mao et al., 2003). This reflects a more complex checkpoint regulation in higher eukaryotes where, unlike in yeasts, checkpoint proteins are essential and regulate normal mitotic timing (Meraldi et al., 2004; Taylor et al., 2004). Here, we highlight current understanding of how the spindle checkpoint is activated, how it delays anaphase onset, and how it is silenced.

Activation of the checkpoint

During mitosis spindle microtubules bind to complex protein structures called kinetochores, which assemble on the centromere of each chromosome. The Mad and Bub proteins localise to the outer kinetochore early in mitosis, before proper attachments are established, and accumulate on unattached kinetochores. When spindle microtubules make contact with the outer kinetochore a number of complex molecular interactions take place that regulate both attachment and microtubule dynamics (Maiato et al., 2004). The checkpoint proteins are therefore ideally placed to monitor these interactions.



(See poster insert)

When a chromosome is attached to microtubules from opposite poles, tension is generated across the sister kinetochores by the pulling forces of the spindle. Laser ablation of the last unattached kinetochore relieves the checkpoint-dependent arrest and the cell enters anaphase even though the remaining sister kinetochore is not under tension (Rieder et al., 1995). This indicates that lack of microtubule attachment elicits the checkpoint response. Conversely, kinetochores lacking tension because both sisters are attached to microtubules from the same pole (syntelic attachment) activate the checkpoint even though kinetochore-microtubule attachments are made, indicating that lack of tension can be sufficient for checkpoint activation. Chemical inhibition of spindle dynamics, which relieves tension but does not destroy kinetochore-microtubule attachments, also activates the checkpoint (Clute and Pines, 1999; Skoufias et al., 2001). However, interpreting such experiments is complicated because microtubule attachment is stabilised by tension (Nicklas et al., 2001).

One clear difference between the checkpoint response to lack of tension and that to lack of attachment is the recruitment of the Mad and Bub proteins. Mad1 and Mad2 localise to unattached kinetochores but not to attached kinetochores that lack tension (Waters et al., 1998), but Bub1 and BubR1/Mad3 localise to kinetochores lacking either tension or microtubule attachment (Skoufias et al., 2001; Taylor et al., 2001). However, because Mad1 and Mad2 are required for checkpoint activation in response to tension, these differences are unlikely to reflect distinct checkpoint signalling pathways (Shannon et al., 2002).

The protein kinase Aurora B, a component of the chromosomal passenger complex (Vagnarelli and Earnshaw, 2004), is thought to promote bipolar attachment by destabilising kinetochore microtubule interactions that are not under tension (Pinsky et al., 2006). This may explain the requirement of Aurora B kinase for the checkpoint response to lack of tension, because by breaking inappropriate attachments Aurora B kinase produces unattached kinetochores that could then be sensed by the Mad/Bub machinery. However, the response to lack of tension appears to be more complicated than this, and Aurora B kinase has additional checkpoint roles in some systems (Kallio et al., 2002; Petersen and Hagan, 2003). Thus, whether the core checkpoint simply detects lack of attachment or is also capable of sensing a lack of tension remains controversial (Pinsky and Biggins, 2005).

Anaphase delay

The downstream target of the spindle checkpoint is the anaphase-promoting complex/cyclosome (APC/C), a multiprotein E3 ubiquitin ligase that ubiquitylates a range of cell-cycle regulators, targeting them for degradation by the 26S proteasome (Castro et al., 2005). Securin is the key regulator of anaphase onset and a substrate for the APC/C: its destruction releases separase, which in turn destroys cohesin (the molecular glue holding sister chromatids together) and thus allows chromatids to be pulled to opposite poles. APC/C activity is regulated by the accessory proteins Cdc20 and Cdh1, which are thought to interact with specific substrates and present them to the APC/C for ubiquitylation (Peters, 2002). Cdc20 (Slp1 in fission yeast) is required for the destruction of securin and anaphase onset and is the key target of the spindle checkpoint (Hwang et al., 1998; Kim et al., 1998).

The precise localisation of the APC/C is still unclear. APC/C subunits have been reported to localise to the kinetochores in a checkpoint-dependent manner (Acquaviva et al., 2004) and also to centrosomes and the mitotic spindle (Tugendreich et al., 1995). Because a single unattached chromosome is sufficient to activate the checkpoint (Rieder et al., 1995), the signal that inhibits the APC/C must be amplified and conveyed to the APC/C present on other mitotic structures. The nature of this signal and its mode of transmission remain enigmatic.

FRAP experiments have shown that Bub1 and Mad1 are stably associated with unattached kinetochores, suggesting that they function as scaffolds recruiting the dynamic BubR1/Mad3 and Mad2 proteins, which are candidates for the inhibitory signal (Howell et al., 2004; Shah et al., 2004). Mad2 binds to Cdc20; this interaction is essential for checkpoint-dependent inhibition of the APC/C (Hwang et al., 1998; Kim et al., 1998). In solution, free Mad2 adopts an open conformation (O-Mad2) but, on binding to Mad1 or Cdc20, this changes to a stable closed conformation (C-Mad2) (De Antoni et al., 2005). Because Mad1 and Cdc20 compete for the same binding site on Mad2, it was initially thought that kinetochore recruitment stimulated exchange of inactive Mad2 from Mad1 to an active form that binds Cdc20. However, this has recently been challenged by two new models in which kinetochore-bound Mad1–C-Mad2 is a stable complex that acts as a template recruiting O-Mad2, which is then able to bind Cdc20 (De Antoni et al., 2005; Yu, 2006). Consistent with this idea is the finding that Mad1 and a proportion of Mad2 are stably localized to the kinetochore whereas the remaining Mad2 and a pool of Cdc20 rapidly cycle on and off the kinetochore with similar dynamics (Howell et al., 2004; Shah et al., 2004; Vink et al., 2006).

Although Mad2 is a good in vitro APC/C inhibitor, formation of Mad2-Cdc20 is unlikely to be sufficient to inhibit the APC in vivo. BubR1/Mad3 appears to be the other crucial player. How many distinct anaphase inhibitors exist in vivo, and their molecular mechanisms of action, are still matters for debate. Mad2 and Cdc20 are found in a complex with BubR1/Mad3 and Bub3 called the mitotic checkpoint complex (MCC), which even in vitro is a more potent inhibitor of the APC/C than Mad2-Cdc20 alone (Sudakin et al., 2001). The BubR1-Cdc20 complex can inhibit the APC/C independently of Mad2, but Mad2 and BubR1/Mad3 act synergistically, which indicates that both Mad2 and BubR1/Mad3 are required to inhibit APC/C activity fully (Fang, 2002; Tang et al., 2001). Consistent with this is the finding that in fission yeast only Mad3 is essential for the metaphase arrest caused by Mad2 overexpression (Millband and Hardwick, 2002). In most models the anaphase inhibitors sequester Cdc20 or otherwise prevent efficient interaction of substrates with the APC/C. However, in budding yeast the Mad

proteins have also been shown to regulate the levels of Cdc20 protein in the cell, through an APC-dependent mechanism (Pan and Chen, 2004).

In all systems, the kinetochore is the apparent source of the checkpoint signal, but in humans and yeast kinetochores are not required for Mad2-Cdc20 or MCC formation (Fraschini et al., 2001; Poddar et al., 2005; Sudakin et al., 2001). MCC isolated from interphase cells is active in vitro, although it can only inhibit mitotic APC/C (Sudakin et al., 2001). Such findings suggest that a primary kinetochore checkpoint function is to propagate a signal that either renders the APC/C more susceptible to checkpoint inhibition and/or increases the levels or potency of anaphase inhibitors. The levels of MCC increase on checkpoint activation, and both the APC/C and the MCC are phosphorylated during mitosis and upon checkpoint activation (Kraft et al., 2003). Bub1 kinase can phosphorylate human Cdc20 (Tang et al., 2004), and Cdc20 phosphorylation is necessary for its inhibition (Chung and Chen, 2003), but whether there is a role for kinetochores in transducing such signals is not known. In most systems Bub1 kinase activity is not required for checkpoint activation, although it may play a more subtle role in amplifying inhibitory signals (Tang et al., 2004; Vanoosthuyse and Hardwick, 2005). The Mps1 kinase is an upstream regulator of the spindle checkpoint, and its overexpression activates the checkpoint in the absence of spindle defects (Hardwick et al., 1996). The kinase activity of Mps1 peaks in metaphase and is essential for checkpoint activation, (Jones et al., 2005; Winey and Huneycutt, 2002). Human Mps1 interacts with the APC/C, but as yet we do not know whether it phosphorylates APC/C subunits (Liu et al., 2003). In mammalian cells, BubR1 kinase activity is required for checkpoint activation (Mao et al., 2003), but yeast Mad3 lacks the kinase domain. Thus, many aspects of checkpoint signalling and Cdc20-APC/C inhibition remain to be clarified, and despite the conservation of many components, some diversity in the mechanisms clearly exists.

Silencing the checkpoint

Once all kinetochores have bipolar attachments the checkpoint must be switched off, and several mechanisms have been proposed. Mad1/Mad2 and BubR1 are transported away from the kinetochore along microtubules by dynein, preventing further inhibitory signalling (Howell et al., 2001). In mammalian cells, the binding of microtubules to CENP-E downregulates BubR1 kinase activity, resulting in checkpoint silencing (Mao et al., 2003), and phosphorylation of Mad2 disrupts MCC formation by preventing the interaction of Mad2 with Mad1 and Cdc20. (Wassmann et al., 2003). The checkpoint inhibitor p31^{comet} (Cmt2) binds to C-Mad2, but does not disrupt Mad1–C-Mad2 or Cdc20–C-Mad2 complexes. It remains to be determined whether p31^{comet} prevents interactions between Mad1, C-Mad2 and O-Mad2, thus inhibiting the formation of new MCCs, or inactivates existing MCCs by forming a p31^{comet}-Mad2-Cdc20 ternary complex (Vink et al., 2006; Xia et al., 2004). The silencing mechanism in yeast was completely unclear: functional equivalents of p31^{comet} have not been found, Mad2 phosphorylation has yet to be reported, and CENP-E and BubR1 kinases are not present. However, a recent report suggests that APC/C-dependent degradation of yeast Mps1 provides a feedback loop that inactivates the checkpoint as the APC/C becomes active (Palframan et al., 2006). Although great strides have been taken in our understanding of spindle-checkpoint mechanics since the Mad and Bub proteins were discovered, many of the key questions remain unanswered.

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