

Retraction Notice

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Title: The RZZ Complex and the Spindle Assembly Checkpoint

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This article has been retracted at the request of the Editor-in-Chief of the Cell Structure and Function. This review article contains a substantial amount of text that had previously appeared in other published articles, including the Elsevier journals Current Biology (Howell *et al.*, Curr. Biol. 2004 Jun 8; 14(11): 953–964), Trends in Cell Biology (Karess, Trends Cell Biol. 2005 Jul; 15(7): 386–392) and Current Opinion in Cell Biology (Yu, Curr. Opin. Cell Biol. 2002 Dec; 14(6): 706–714). In accordance with policies and procedures governing academic publication we concluded that the above-mentioned article published in Cell Struct. Funct. be retracted. We apologize to readers of the journals that this was not detected during the submission and review process.

The RZZ Complex and the Spindle Assembly Checkpoint

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ABSTRACT. The conserved protein Rod is found in various organisms. It is localized on the kinetochores or spindle microtubules during cell division. Rod is required for proper chromosome segregation during both mitosis and meiosis. The effects of *rod* mutations are similar for both equational and reductional divisions, giving rise to anaphases with lagging chromosomes and/or unequal numbers of chromosomes at the two poles. Recent studies have shown that Rod is a significant component of the mitotic checkpoint. It can form the RZZ complex with Zw10 and Zwilch, which plays an important role in maintaining a functional spindle assembly checkpoint.

Key words: Rod/spindle/kinetochore/sister chromatids/SAC

Introduction

Rough Deal (Rod), originally identified in *Drosophila*, is an evolutionarily conserved kinetochore and kinetochore microtubule (KMT)-associated protein among multicellular eukaryotes (Karess and Glover, 1989; Scaërou *et al.*, 1999; Starr *et al.*, 1998; Williams *et al.*, 1992; Williams and Goldberg, 1994; Williams *et al.*, 1996; Scaërou *et al.*, 2001). Cytogenetic analysis places the *rod* gene in the chromosome region 100C4,5-100D1,2 near the tip of chromosome 3R. The gene covers approximately 8.5 kb of the *Drosophila* genome, and is comprised of 14 exons and 13 small introns. The transcript itself is about 6.8 kb in length, and encodes a protein 2098 amino acids long, with a predicted molecular weight of 240 kDa (Scaërou *et al.*, 1999). Rod proteins have yet to be found in any unicellular eukaryote, and the genome of *Saccharomyces cerevisiae* does not contain genes with detectable homologies to *rod* (Williams *et al.*, 2003). Although the *Drosophila* Rod sequence offers no hint of its function, having no identifiable peptide motifs associated with known activities in other proteins, it does have a clear homology to genes in nematodes and humans, two species widely separated by evolution. This protein is likely therefore to be an important kinetochore component in all metazoans (Scaërou *et al.*, 1999). The molecular evolution of Rod and its forms in

different organisms are illustrated by a phylogenetic tree (Fig. 1).

Mutations in the *rod* gene of *Drosophila* greatly increase the missegregation of sister chromatids during mitosis, most noticeably lagging chromatids that remain at the metaphase plate late in anaphase, leading to high levels of aneuploidy among daughter cells, suggesting a role for this gene product in spindle or kinetochore function (Scaërou *et al.*, 1999; Williams *et al.*, 2003). Rod during the course of mitosis changes its location in an unusual and intriguing manner. It is first found on kinetochores at prometaphase, but once the chromosome is properly bi-oriented on the spindle, Rod is found distributed irregularly along the KMT fibers. At the onset of anaphase, Rod is once again found exclusively on the kinetochores, where it remains until the end of telophase. This behavior is in all respects similar to that described for Zw10, and suggests that the proteins function together, which is to assure that chromosomes segregate to daughter cells with a very high degree of fidelity (Scaërou *et al.*, 1999). In mitotic human cells, Rod resides in a complex (RZZ) with Zw10 and Zwilch. The RZZ complex can recruit cytoplasmic dynein-dynactin to the kinetochore; and the complex is essential for the stable binding of a Mad1-Mad2 complex to unattached kinetochores (Scaërou *et al.*, 2001; Kops *et al.*, 2005).

The kinetochores are the sites of microtubule (MT) attachment to eukaryotic chromosomes (Pluta *et al.*, 1995; Rieder and Salmon, 1998). The spindle assembly checkpoint (SAC) monitors events that occur at the kinetochore to verify that all the chromosomes are properly oriented on the mitotic spindle before anaphase is allowed to begin

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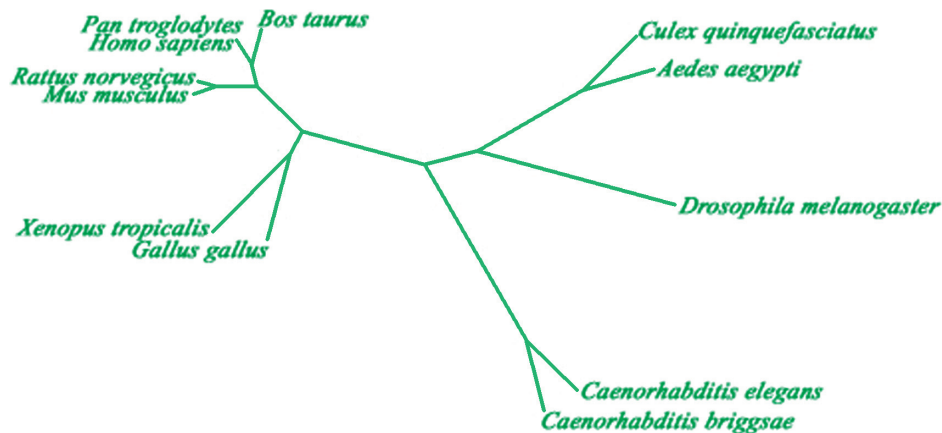


Fig. 1. Graphical phylogenetic tree. The twelve organisms respectively are: *Culex quinquefasciatus*, *Aedes aegypti*, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Caenorhabditis briggsae*, *Xenopus tropicalis*, *Gallus gallus*, *Mus musculus*, *Rattus norvegicus*, *Pan troglodytes*, *Bos taurus* and *Homo sapiens*. This alignment used CLUSTAL W.

(Elledge, 1998; Rieder and Salmon, 1998; Wells, 1996; Scaërrou *et al.*, 1999). The mitotic checkpoint ensures that chromosomes are divided equally between daughter cells and is a primary mechanism preventing the chromosome instability often seen in aneuploid human tumors (Kops *et al.*, 2005). Studies show that human Rod is an important component of the mitotic checkpoint, as cells lacking the protein at kinetochores fail to arrest in mitosis when exposed to MT inhibitors. Checkpoint failure and premature mitotic exit may explain why cells defective for hRod divide with lagging chromosomes, which indicates that metazoans may require an elaborate spindle checkpoint to monitor complex kinetochore functions (Chan *et al.*, 2000).

1. SAC

1.1 An overview of SAC

The metaphase/anaphase transition during mitosis is carefully regulated in order to assure high-fidelity transmission of genetic information to the daughter cells. A surveillance mechanism known as the SAC monitors the attachment of kinetochores to the spindle microtubules, and inhibits anaphase onset until all chromosomes have achieved a proper bipolar orientation on the spindle. Defects in this checkpoint lead to premature anaphase onset, and consequently to greatly increased rates of aneuploidy (Amon, 1999; Nicklas, 1997; Basto *et al.*, 2000; Vallee *et al.*, 2006). The classical definition of a checkpoint component is one whose abrogation leads to mitotic exit even in the presence of such spindle damage. This is precisely how the original Mad and Bub proteins were found in budding yeast. By this definition, however, the checkpoint in metazoan cells has been shown to require additional components not present in

yeast (such as RZZ, Zwint-1, CENP-E, CENP-I, CENP-F). On the other hand, the protein Cdc20 is clearly involved in the checkpoint as it is the principal target of the anaphase inhibitor, and yet it does not fit the classical definition as most mutations in *cdc20* arrest cells in mitosis. It seems simpler and less dogmatic to define a ‘checkpoint protein’ as one participating in the detection of kinetochore attachment status and the transmission of this information to the anaphase promoting complex/cyclosome (APC/C), a definition that would include Cdc20 (Yu, 2002; Cleveland *et al.*, 2003; Chan and Yen, 2003; Musacchio and Hardwick 2002; Karess, 2005). A cell cycle checkpoint typically consists of three essential elements: the sensors that monitor defects; the signal transducers; and the targets or effectors. APC/C-Cdc20 is a critical target of the spindle checkpoint and some of the Mad and Bub proteins are involved in transducing the signals in this system. The spindle checkpoint senses the existence of unattached kinetochores and the lack of tension at kinetochores. It makes sense that the spindle checkpoint monitors both MT occupancy and tension at the kinetochores (Yu, 2002).

1.2 MCC

The activation of the SAC involves the formation of inhibitory complexes between Mad2 and/or Mad3/BubR1 and Cdc20, preventing Cdc20 from activating the APC/C (Hwang *et al.*, 1998; Kim *et al.*, 1998; Wu *et al.*, 2000; Malmanche *et al.*, 2006). The mitotic checkpoint complex (MCC), which contains either (1) BubR1 (Mad3 in yeast), Bub3, Mad2 and Cdc20—or (2) the BubR1-Bub3-Cdc20 and Mad2-Cdc20 subcomplexes (Fang *et al.*, 1998; Hardwick *et al.*, 2000; Fraschini *et al.*, 2001; Millband and Hardwick, 2002; Tang *et al.*, 2001; Sudakin *et al.*, 2001; Fang, 2002). The two views differ in the mechanism of

MCC assembly. In the first model, the unattached kinetochores catalyse the formation of these inhibitory checkpoint protein complexes, which then diffuse away to inhibit the APC/C. The diffusible signal is thus the MCC or its subcomplexes (Skoufias *et al.*, 2001; Taylor *et al.*, 2001). In the second model, the MCC is present throughout the cell cycle and its formation does not occur at the kinetochores. Upon checkpoint activation, a yet unidentified diffusible signal turns over at the kinetochores and sensitises the APC/C for its prolonged inhibition by the MCC (Yu, 2002). The checkpoint complexes might be assembled at the unattached kinetochores (Fang *et al.*, 1998; Kallio *et al.*, 1998). To facilitate the binding of Mad2 to Cdc20, Mad2 is brought to the unattached kinetochores by Mad1 (Luo *et al.*, 2002; Chen *et al.*, 1998; Chung and Chen, 2002). The MCC is present throughout the cell cycle, yet it only associates with mitotic APC/C (Sudakin *et al.*, 2001). The mechanism by which the MCC or its subcomplexes inhibit the APC/C is not clear. These surprising results suggest that the spindle checkpoint and MCC may interfere with the ability of the APC/C to interact with its substrates in a productive way. The co-localization of checkpoint proteins and Cdc20 at the unattached kinetochores appears to be required for the assembly of the MCC and the diffusible APC/C inhibitory signal. Thus, the inactivation of the checkpoint might be initiated by the loss of kinetochore localization of Mad1 and Mad2 and by the partial loss of BubR1, Bub1, Bub3, CENP-E, and Cdc20 at the kinetochores. These checkpoint proteins may be depleted through two mechanisms: free diffusion into the cytosol; and motor-assisted transport to the spindle poles along the MTs (Howell *et al.*, 2001; Yu, 2002). The MCC appears to be evolutionarily conserved as it has been identified in budding yeast (Hardwick *et al.*, 2000), fission yeast (Millband and Hardwick, 2002) and in *Xenopus* (Chung and Chen, 2003). Interestingly, the formation of the yeast MCC was also found to be independent of the kinetochore (Fraschini *et al.*, 2001). By contrast, the formation of the MCC in *Xenopus* egg extracts depends on kinetochores (Chung and Chen, 2003). The reason for this discrepancy is unclear but might be due to inherent differences in the mechanisms that control the somatic and embryonic cell cycles (Chan *et al.*, 2005).

1.3 The models that prevent activation of APC/C

An unattached kinetochore can become the source of the diffusible ‘inhibitor’ of the APC/C, whose ubiquitin ligase activity is required to initiate the proteolytic degradation of cyclin B and securin, which in turn shut off cdc2 kinase (CDK1) activity and allow sister-chromatid separation, respectively (Yu, 2002; Peters, 2002). Although the precise nature of the inhibitor is still incompletely understood, two checkpoint proteins with key functions are Mad2 and BubR1, both of which can bind to the protein Cdc20 and inhibit its ability to activate the APC/C. It was known that

unattached kinetochores have two populations of Mad2 displaying different dynamics (Shah *et al.*, 2004; Howell *et al.*, 2004). One population of kinetochore Mad2 is stably associated with Mad1, while the other very rapidly exchanges with free cytosolic Mad2. It now appears that the kinetochore-bound Mad1-Mad2 complex functions as a catalyst that promotes the binding of free Mad2 to Cdc20, thus rendering it inactive (De Antoni *et al.*, 2005). Kinetochore-associated Mad1-Mad2 levels fall following MT capture, thus disassembling the platform, and shutting off the source of the inhibitor (Karess, 2005). Early studies have shown that kinetochores lacking a full complement of KMTs or tension are checkpoint active, and that a single unattached kinetochore can prevent anaphase onset (Rieder and Salmon, 1998). In the checkpoint-active cytoplasm, Mad2 and BubR1 have key roles in preventing APC/C activity. In yeast, *Xenopus*, and mammalian tissue cells, checkpoint activation induces various Cdc20-containing complexes including Cdc20-Mad2, Cdc20-BubR1-Bub3, and Cdc20-Mad2-BubR1-Bub3, also known as the MCC (Sudakin *et al.*, 2001; Millband and Hardwick, 2002; Chen, 2002). Two current models exist for how unattached kinetochores produce a diffuse signal that prevents activation of APC/C in the cytoplasm. The catalytic model proposes that Cdc20 becomes inactivated in the cytoplasm by transient association of Cdc20 or its inhibitors with unattached kinetochores (Kallio *et al.*, 1998; Kallio *et al.*, 2002; Gorbsky *et al.*, 1998; Howell *et al.*, 2000). If the MCC or its subcomplexes are generated or exchange at kinetochores, then the dynamics of their individual components should share common kinetic features. A second model (the APC/C sensitization model) proposes checkpoint-active kinetochores sensitize the APC/C to inhibition by cytoplasmic MCC, perhaps by phosphorylation (Sudakin *et al.*, 2001). This could be achieved by APC/C rapidly exchanging with the kinetochores (Tugendreich *et al.*, 1995; Jorgensen *et al.*, 1998; Topper *et al.*, 2002; Huang and Raff, 2002). Mps1 also binds the APC/C, and thus it might be the diffusible signal from an unattached kinetochore that sensitizes APC/C to inhibition by MCC (Liu *et al.*, 2003; Howell *et al.*, 2004).

1.4 Spindle checkpoint protein dynamics

Mad2, BubR1, Bub3, Cdc20, and Mps1 cycle rapidly through unattached kinetochores, whereas most Bub1 and Mad1 are much more stable. Mad1 is known to recruit Mad2, and although a large fraction of Mad1 is bound tightly to Mad2 in the cytoplasm (Chen *et al.*, 1998; Chung and Chen, 2002). Mad1 is much more stable at the kinetochores than Mps1, suggesting Mps1 can leave the kinetochores without Mad1. Therefore, other proteins must be present at the kinetochores (other than Mps1) and provide a binding site for Mad1. The latest studies indicate that a novel protein Spdl-1/Spindly is required to induce the SAC-dependent mitotic delay and localizes the SAC protein Mdf-1/

Mad1 to the kinetochores facing away from the spindle pole in *Caenorhabditis elegans* cells. In addition, Spdl-1/Spindly co-immunoprecipitates with Mdf-1/Mad1 *in vivo*. These results suggest that Spdl-1/Spindly functions in a kinetochore receptor of Mdf-1/Mad1 to induce SAC function (Yamamoto *et al.*, 2008). Mps1 has been shown to phosphorylate Mad1; therefore, phosphorylation of Mad1 may stabilize Mad1 binding to another site on the kinetochore. Bub1, like Mad1, is not likely a component of the diffusible wait-anaphase signal because a large fraction is stable at unattached kinetochores. A major role for Bub1, like Mad1, may be to provide a protein scaffold at the kinetochores to support the binding (Taylor *et al.*, 1998; Chen *et al.*, 1998; Basu *et al.*, 1998; Sharp-Baker and Chen, 2001), and cycling of Bub1 free Bub3 and BubR1. Although BubR1-Bub1 and Bub3-Bub1 are constitutive complexes in the cytoplasm, these complexes are not dynamically exchanging at the kinetochores (Musacchio and Hardwick, 2002). Kinetochore modification of Cdc20, BubR1, or Mps1 is attractive to consider for the diffusible wait-anaphase signal because they all have the components that exhibit rapid cycling through the kinetochore. Kinetochore-modified Cdc20, BubR1, or Mps1 could diffuse into the cytoplasm and promote formation of Cdc20-Mad2, Cdc20-BubR1-Bub3, or the entire MCC from pools of Mad1-Mad2, BubR1-Bub1, or Bub3-Bub1. Mps1 modification may also inhibit APC/C perhaps through phosphorylation (Liu *et al.*, 2003). MCC or its subcomplexes could then provide a positive feedback mechanism by binding the kinetochores that lack MTs (Waters *et al.*, 1998; Shannon *et al.*, 2002; Skoufias *et al.*, 2001), thus stimulating further production of modified Cdc20, BubR1, or Mps1. Both inhibitors of Cdc20, BubR1 and Mad2, cycle rapidly through unattached kinetochores and are substantially depleted on the kinetochores of properly aligned chromosomes. Similarly, Cdc20 also cycles rapidly through kinetochores (Howell *et al.*, 2004).

1.5 Spindle checkpoint mechanisms

The vertebrate kinetochore is a complex structure that specifies the attachments between the chromosomes and MTs of the spindle and is thus essential for accurate chromosome segregation. Kinetochore are assembled on centromeric chromatin through complex pathways that are coordinated with the cell cycle. How proteins assemble onto kinetochores and interact with each other possibly involves the current mitotic checkpoint models: the template model and the two-step model. The template model proposes that Mad1-Mad2 at kinetochores acts as a template to change the conformation of another binding molecule of Mad2. This templated change in conformation is postulated as a mechanism for the amplification of the wait-anaphase signal. While this model fits the *in vitro* data, the existence of such alternative conformers of native Mad2 remains to be dem-

onstrated. Regardless, the mechanism by which the kinetochore generates the wait-anaphase signal is likely to be more complex as it must take into account the roles of other checkpoint proteins. The two-step model proposes that MCC formation and activity does not depend on kinetochores. Importantly, MCC was found to selectively inhibit APC/C. This contrasts with other studies that showed that recombinant Mad2 and BubR1 were only effective against interphase, but not mitotic, APC/C (Tang *et al.*, 2001). The existence of the MCC during interphase provides the cell with a rapid mechanism to inhibit the APC/C when cells enter mitosis. The role of the kinetochore is to provide a signal that maintains the interaction between the MCC and the APC/C. This was supported by reconstitution experiments that suggested that kinetochores might act on the APC/C to sensitize it to prolonged inhibition by the MCC (Sudakin *et al.*, 2001; Chan *et al.*, 2005).

1.6 Metaphase/anaphase transition

In the course of the metaphase/anaphase transition, SAC is switched on in the presence of unattached or improperly attached kinetochores. Faithful transmission of chromosomes during mitosis is ensured by the SAC. Various proteins are involved in this process (Table I). After NEB, Zw10, Rod, Zwilch, CENP-E, and the dynein-dynactin complex, which are in the cytoplasm during interphase, are assembled onto the kinetochore. In addition to the assembly of structural proteins at the kinetochores, a group of checkpoint proteins that include Mad1, Mad2, and Bub1 assemble onto the kinetochore between prophase and prometaphase (Chan *et al.*, 1998; Lu *et al.*, 2008).

Before entering mitosis, the MCC is already formed. After NEB, the checkpoint proteins assemble a platform on unattached kinetochores that promotes the inhibition of Cdc20 by Mad2 and BubR1 (Karess, 2005; Malmanche *et al.*, 2006). Unattached kinetochores bind a collection of mitotic checkpoint components that include the kinases Bub1 and MAPK. Their activities to prevent the overcoming of the checkpoint are very important. A Zw10 partner, Zwint-1, is a part of a separate complex of structural kinetochore components including KNL-1, Mis12, and Ndc80 complex (KMN) in mitotic human cells (Cheeseman *et al.*, 2008). The complex keeps close link with the inner kinetochore (CENP-A, CENP-C). Zwint-1 is critical for recruiting the RZZ complex to unattached kinetochores. The RZZ complex thus acts as a physical bridge between the structural kinetochore proteins and other checkpoint proteins. Furthermore, the RZZ complex is required for the recruitment of two better-known components of the kinetochore: the dynein-dynactin complex and Mad1-Mad2 (Karess, 2005; Lu *et al.*, 2008). Recent studies show that a novel regulator (Spindly) of mitotic dynein is able to function specifically to target dynein to kinetochores (Griffis *et al.*, 2007). Mad2, BubR1, Mps1, and Bub3 are combined at the

Table 1. KINETOCHORE-BINDING PROTEINS

Yeast	<i>Drosophila</i>	Vertebrates	Roles in mitosis
Bub1	Bub1	Bub1	Spindle checkpoint protein, by phosphorylating the mitotic checkpoint complex and activating the spindle checkpoint
Bub3	Bub3	Bub3	Spindle checkpoint protein, this protein involved in spindle checkpoint function
Mad1	Mad1	Mad1	Central component of the spindle assembly checkpoint
Mad2	Mad2	Mad2	Mitotic spindle checkpoint component. Spindle checkpoint function requires Mad2-dependent Cdc20 binding to the Mad3 homology domain of BubR1
Mad3	BubR1	BubR1	Spindle checkpoint protein, it is essential for kinetochore localization of other spindle checkpoint proteins and its phosphorylation requires Mad1
Mps1	Mps1	Mps1	Serine/threonine kinases, involved in the regulation of the onset of mitosis
Cdc20	Cdc20	Cdc20	Subunit of APC/C, active in mitosis
	Zw10	hZw10	Centromere/kinetochore protein, involved in mechanisms to ensure proper chromosome segregation during cell division
	Rod	hRod	Centromere/kinetochore protein, involved in mechanisms to ensure proper chromosome segregation during cell division
	Zwilch	hZwilch	Complexed with Zw10/Rod. Required for the assembly of the dynein-dynactin onto kinetochores
	Spindly	Spindly	A regulator of mitotic dynein, functioning specifically to target dynein to kinetochores
		hZwint-1	Zw10 interactor, another kinetochore protein, possibly regulating the association between Zw10 and kinetochores

kinetochore with Cdc20, where they cycle rapidly through unattached kinetochores (Howell *et al.*, 2004). Mad2 is recruited to the kinetochore by Mad1, and Cdc20 is brought to the kinetochore by BubR1-Bub3. Mad1 also triggers a conformational change of Mad2. Mad2 dissociated from Mad1 might retain a conformation more suitable for binding to Cdc20 (Yu, 2002). As Cdc20 is an essential cofactor for activating the APC/C, inhibiting Cdc20 inactivates the APC/C (Karess, 2005). A preformed Mad1-Mad2 complex can recruit another molecule of Mad2 through Mad2 dimerization (Chan *et al.*, 2005). Mps1 is able to phosphorylate Mad1; therefore, phosphorylation of Mad1 may stabilize Mad1 binding to another site on the kinetochore. Perhaps Spindly can provide such a site (Yamamoto *et al.*, 2008). Unattached kinetochores may also activate and promote the ability of Mps1 to phosphorylate APC/C in the cytoplasm and sensitize the APC/C to inhibition by MCC components (Howell *et al.*, 2004). CENP-E and BubR1 can form a stable complex, in which CENP-E activates BubR1 kinase. BubR1 is postulated to act as a mechanosensor that monitors the activity of CENP-E (Chan *et al.*, 1999). This suggests that the checkpoint activity of BubR1 might be regulated by conformational changes in CENP-E when it interacts with MTs (Basu *et al.*, 1998). Kinetochore binding of Mad2 or BubR1 complexes activates the kinetochore formation of Cdc20, BubR1, or Mps1, which will induce more MCC to be produced (Howell *et al.*, 2004). This MCC, or its subcomplexes, might then diffuse away from the kinetochores to associate with APC/C and block its activity (Yu, 2002). This ensures sufficient timing for chromosome congression

(Malmanche *et al.*, 2006). As a result, APC/C fails to catalyze the ubiquitination of cyclin B, which therefore does not degrade and cyclin-dependent kinase 1 (CDK1/Cdc2) does not lose its activity. For these reasons, downstream proteins cannot be dephosphorylated. Eventually, the spindle checkpoint is turned on, thus preventing the onset of anaphase (Weaver *et al.*, 2003; Johnson *et al.*, 2004; Lu *et al.*, 2008). Once the kinetochore has properly attached to MTs and kinetochore tension has been established, however, the platform is disassembled (Karess, 2005). The kinetochore receptors bind to the checkpoint proteins with reduced affinity. RZZ, Spindly, and Mad1-Mad2 are simultaneously shed. These checkpoint proteins are depleted through two ways: free diffusion into the cytosol; and dynein-dynactin complex assisted transport to the spindle poles along the MTs (Karess, 2005). CENP-E begins to interact with MTs, which leads to the deactivation of BubR1 (Mao *et al.*, 2005). When the checkpoint portents leave the kinetochores, MCC and its subcomplexes might not form efficiently. The dissociation of the critical Mad2-Cdc20 and BubR1-Cdc20 interactions may then lead to the activation of APC/C-Cdc20 (Yu, 2002), thus the spindle checkpoint is turned off (Zhou *et al.*, 2002; Lu *et al.*, 2008). The metaphase/anaphase transition occurs as a result of the Cdc20-dependent activation of the APC/C. APC/C is a multisubunit E3 ubiquitin ligase that triggers ubiquitination of a number of key cell-cycle regulators targeting them for destruction by the 26S proteasome (Malmanche *et al.*, 2006; Yu, 2002). The two chromatids of a chromosome are linked by a protein complex termed cohesin. At anaphase onset,

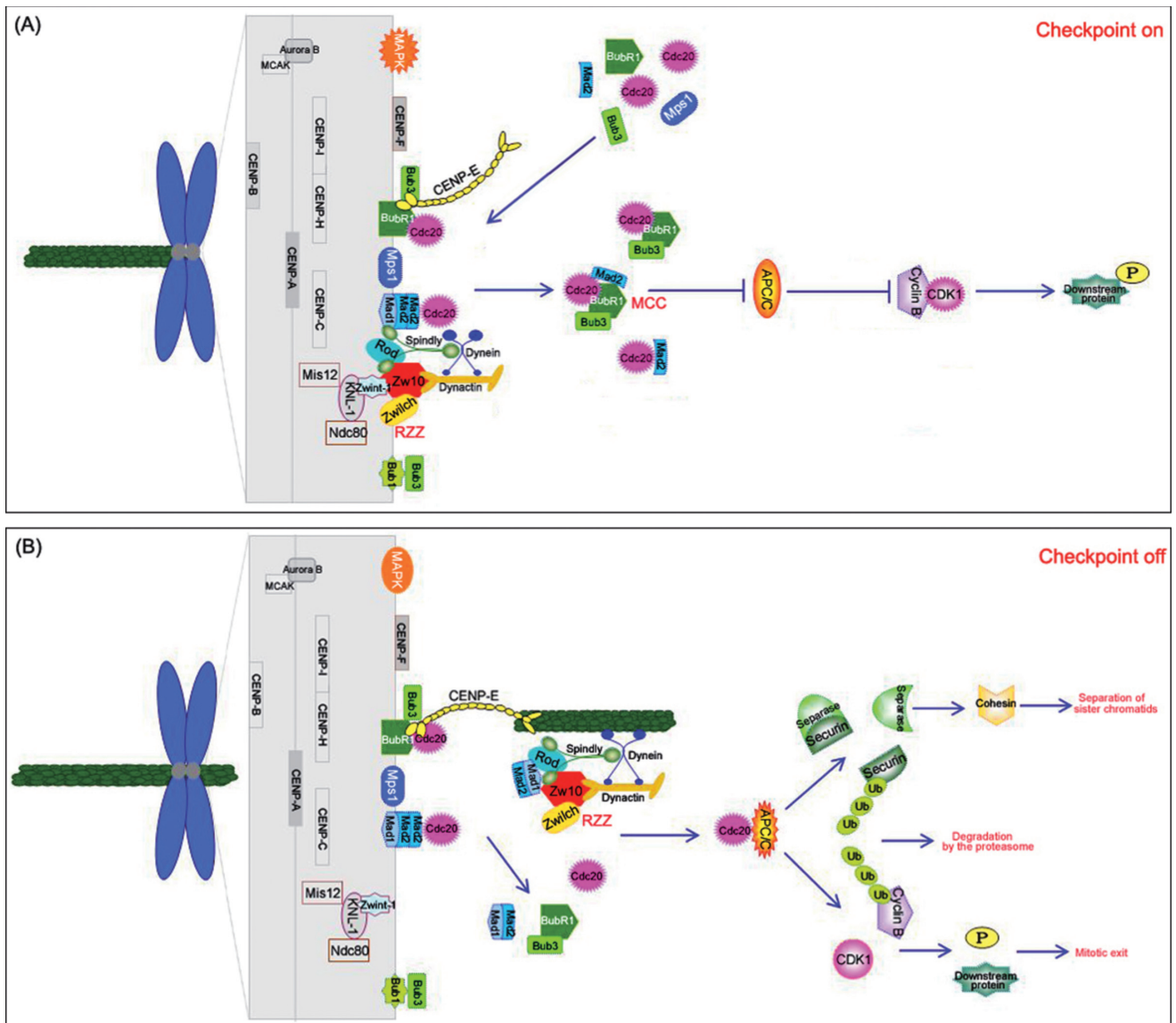


Fig. 2. The relationship between the SAC and cell cycle operation from metaphase to anaphase transition

one of the subunits of that complex, Scc1, is destroyed by a protease called separase. Securins are proteins that bind to and inhibit the activity of separase. Once anaphase is initiated, APC/C targets securin for ubiquitin-mediated proteolysis, thus releasing separase. At about the same time, the APC/C also begins to induce the degradation of cyclin B. At entry to mitosis cyclin B binds and activates CDK1 which is the primary kinase that maintains the mitotic state. Cyclin B is the main target protein of APC/C, and its degradation in turn causes the loss of CDK1 activity. Then the downstream proteins that are phosphorylated by CDK1 begin to dephosphorylate, triggering the onset of anaphase and the mitotic exit (Zhou *et al.*, 2002; Karess, 2005; Lu *et al.*, 2008). The relationship between SAC and cell cycle operation from

metaphase to anaphase transition is shown in Fig. 2.

2. Rod and RZZ Complex

2.1 The rod mutant phenotype

In *Drosophila rod* mutations interfere with the faithful transmission of chromosomes to daughter cells during mitosis. Mutant alleles were isolated, each associated with a similar set of mitotic abnormalities in the dividing neuroblasts of homozygous mutant larvae: high frequencies of aneuploid cells and abnormal anaphase figures, in which chromatids may lag, form bridges, or completely fail to sep-

arate. The nature of the observed abnormalities in mitotic cells suggests that the reduced fidelity of chromosome transmission to the daughter cells is due to a failure in a mechanism involved in assuring the proper release of sister chromatids. The morphological process of spermatogenesis is largely unaffected, but meiotic aneuploidy is common. Surviving females homozygous for any *rod* allele are completely sterile. These results show that *rod* mutations affecting the process of mitosis also affect the behavior of meiotic chromosomes (Karess and Glover, 1989). Like the *zw10* mutant, premature sister chromatid separation (PSCS) occurs when the *rod* mutant is exposed to colchicine. *Rod* mutations severely affect Zw10 protein localization but do not affect the overall accumulation of the Zw10 protein (Scaërou *et al.*, 2001; Lu *et al.*, 2008).

2.2 PSCS

Given that *rod* and *zw10* null mutants have similar mitotic phenotypes (lagging chromatids at anaphase of untreated cells) (Karess and Glover, 1989; Williams *et al.*, 1992) as PSCS in cells treated with taxol or colchicine (Williams *et al.*, 1992), these phenotypes appear at first glance contradictory: the absence of Rod or Zw10 appears to retard chromatid separation in untreated cells, but promotes their premature separation in the presence of the drugs. Although colchicine depolymerizes MTs and taxol stabilizes them, both treatments have the net effect of reducing tension across the kinetochore, which delays anaphase onset by maintaining the activity of the checkpoint (Waters *et al.*, 1998; Scaërou *et al.*, 1999). In wild-type drug-treated cells, the chromosomes might have time to become ready to separate in the above sense, but are arrested by the activity of the SAC. In the absence of the monitoring by Rod and Zw10, however, the cell may prematurely activate the APC/C, overriding the delay imposed by the SAC (Scaërou *et al.*, 1999). The abnormal chromosome segregation typical of *rod* and *zw10* mutants can be related to a dysfunction of the spindle checkpoint machinery and consequently to premature activation of the APC/C. One important source of these defects is premature anaphase onset, before all the chromosomes have had time to congress to the metaphase plate (Starr *et al.*, 1998; Basto *et al.*, 2000). Taken alone, the PSCS phenotype suggests that SAC is slightly defective in *rod* and *zw10* mutants, or can be more easily circumvented, since a failure to arrest in metaphase in response to spindle damage by drugs is a hallmark of mutations affecting components of the SAC (Scaërou *et al.*, 1999). A defective checkpoint implies inappropriate activation of the APC/C, leading to PSCS and premature degradation of cyclin B (Amon, 1999; Zachariae and Nasmyth, 1999; Basto *et al.*, 2000).

2.3 Identification of the RZZ complex

Rod and Zw10 share many important genetic and cytological properties. Null mutations in the *Drosophila* genes encoding either protein cause identical phenotypic syndromes (Williams *et al.*, 1992; Karess and Glover, 1989). Significantly, the mitotic phenotype of the double *zw10; rod* mutant in *Drosophila* is indistinguishable from that of either mutant alone. The two proteins have almost identical behavior during mitosis in both *Drosophila* and human cells, and the mutual dependence of the two proteins for their localization to the mitotic apparatus further emphasizes that neither protein can act independently of the other (Williams and Goldberg, 1994; Williams *et al.*, 1996). These results suggested that the two proteins might associate with each other within the same macromolecular complex. This prediction was verified by the experiments which demonstrated that Zw10 and Rod can be co-immunoprecipitated from both fly and human cell extracts, and that the two *Drosophila* proteins co-elute from gel filtration columns as a complex (Scaërou *et al.*, 2001; Williams *et al.*, 2003; Karess, 2005). There was a relatively weak two-hybrid interaction between Zw10 and Rod, suggesting that these two proteins are in fact in contact with each other within the complex (Scaërou *et al.*, 2001). A third component (Zwilch) of the complex was identified by immune affinity chromatography on Zw10 (Williams *et al.*, 2003). Once again, like Rod and Zw10, Zwilch has neither obvious homologs in yeast nor any structural domains known in other proteins. The same three components from human extracts were identified by affinity tag chromatography on Zw10 (Kops *et al.*, 2005). These three proteins therefore seem to complete the core complex, as no other stable components have been found. The combined mass of Rod, Zw10, and Zwilch (240, 85, 75 kDa, respectively) is about half the apparent mass of the complex. This suggests that the complex contains two copies of each protein, or perhaps that the complex, as isolated, is a stable dimer. However for the moment, little else is known about the biochemistry and assembly of the RZZ complex (Karess, 2005).

2.4 RZZ dynamics

Immunostaining shows that Rod, Zw10, and Zwilch are cytoplasmic during interphase (Williams *et al.*, 1992; Basto *et al.*, 2004), although it is not known whether they are already associated in a complex. In late prophase and during NEB, they enter the nucleus and begin accumulating on kinetochores (Williams *et al.*, 1992; Williams *et al.*, 2003; Basto *et al.*, 2004). Rod and Zw10 levels decline once the KMTs have attached. Strikingly, this reduction in kinetochore association is accompanied by a visible redistribution of Rod and Zw10 along the KMTs towards the poles (Williams *et al.*, 1992; Scaërou *et al.*, 1999). Following anaphase onset, the KMT-associated RZZ rapidly disap-

pears, but substantial amounts remain bound to kinetochores of the migrating chromatids. At this point, the behavior differs slightly in flies and human cells. In *Drosophila*, Rod and Zw10 remain kinetochore-associated until the end of anaphase, whereas in HeLa cells Rod disappears from kinetochores in early anaphase, but Zw10 persists until late anaphase (Scaërou *et al.*, 2001; Chan *et al.*, 2000). The significance of this species difference is unknown. Finally, during telophase and cytokinesis in HeLa cells (Scaërou *et al.*, 2001) and fly spermatocytes (Williams *et al.*, 1996), a small amount of Zw10, but not Rod, associates with the developing central spindle and midbody, and in HeLa cells Rod, but not Zw10, lingers at the poles (Scaërou *et al.*, 2001). The actual amount of Rod and Zw10 in these organelles is far lower than that seen on prometaphase kinetochores. Nevertheless, for Zw10 at least, there is some evidence that it might contribute to the efficacy of cytokinesis (Williams *et al.*, 1996), and its presence in the midbody might reflect this function (Karess, 2005). This spatial separation of Rod and Zw10 further implies that although Rod and Zw10 are sometimes in the same macromolecular complex, the two proteins do not necessarily fulfill identical roles nor do they always have to be associated (Scaërou *et al.*, 2001), since at least some fraction of the RZZ complex dissociates as these cells exit mitosis. The most dramatic aspect of RZZ behavior is its redistribution from kinetochores to KMTs, which occurs following chromosome capture by the spindle (Buffin *et al.*, 2005; Basto *et al.*, 2004). This is an example of kinetochore shedding, a dynein-dynactin-dependent process that removes several outer domain components from the kinetochore and transports them along KMTs towards the poles, where they eventually disperse (Maiato *et al.*, 2004; Howell *et al.*, 2000; Howell *et al.*, 2001; Scaërou *et al.*, 2001; Wojcik *et al.*, 2001; Basto *et al.*, 2004; Karess, 2005).

2.5 RZZ receptor

The kinetochore recruitment of many checkpoint proteins is interdependent. A novel protein called Zwint-1 was identified by yeast two-hybrid screening of human Zw10 (Starr *et al.*, 2000). This 43 kDa protein, largely coiled-coil, is recruited to kinetochores in early prophase, before the earliest detection of Zw10 (Wang *et al.*, 2004; Starr *et al.*, 2000), and persists into mid-anaphase. Because of these features, Zwint-1 has been considered a good candidate for docking the RZZ complex to vertebrate kinetochores. Unfortunately, no obvious structural homolog of Zwint-1 has yet been identified in the *Drosophila* genome. Recent work supports this prediction. Si-RNA knockdown of Zwint-1 in vertebrate cells blocks recruitment of Zw10 (Kops *et al.*, 2005; Wang *et al.*, 2004). The phenotype of Zwint-1-depleted cells is similar to that of *zw10* and *rod* mutants (defective checkpoint and aberrant anaphases), suggesting that RZZ does not function when it cannot be recruited to kineto-

chores (Wang *et al.*, 2004). By affinity tag chromatography, Zwint-1 has been found in a complex with the key structural kinetochore components KNL-1 and Mis12, as well as in association with the Ndc80 complex (Kops *et al.*, 2005; Cheeseman *et al.*, 2004; Obuse *et al.*, 2004; Cheeseman *et al.*, 2008), an outer kinetochore plate component involved in MT attachment and recruitment of checkpoint proteins. Although initial binding of the RZZ complex is through Zwint-1, subsequent accumulation of RZZ (and its associated proteins of the corona) on kinetochores is possibly through a self-assembly pathway (Karess, 2005).

2.6 RZZ and Aurora B

The Aurora protein kinase family (consisting of Aurora A, B and C) is an important group of serine/threonine protein kinases that controls several aspects of cell division in mammalian cells (Vader and Lens, 2008). Aurora B functions in a complex with two other chromosomal passenger proteins called INCENP and Survivin. Aurora B regulates multiple processes in mitosis, including chromatin condensation, chromosome-MT attachments, chromosome segregation, and cytokinesis through phosphorylation of multiple substrates (Carmena and Earnshaw, 2003; Vagnarelli and Earnshaw, 2004; Zhang *et al.*, 2007). Previous studies have shown that the inhibition of Aurora B kinase activity or disruption of the chromosome passenger complex results in the escape from mitotic-checkpoint arrest induced by the lack of kinetochore tension but not by the lack of kinetochore-MT attachments (Ditchfield *et al.*, 2003; Hauf *et al.*, 2003; Carmena and Earnshaw, 2003; Lens *et al.*, 2003). Aurora B kinase activity has also been shown to be required for the kinetochore recruitment of hBubR1, hCENP-E, and hMad2 (Ditchfield *et al.*, 2003; Hauf *et al.*, 2003). Recent studies show that hZw10 and hRod are tension-sensitive components of the mitotic checkpoint, and that their accumulation at tensionless kinetochores is regulated by their turnover dynamics in an Aurora B kinase-dependent manner. Aurora B phosphorylation of the RZZ complex might reduce its kinetochore turnover rate, therefore leading to the accumulation of hp50 (dynamitin) and the RZZ complex at tensionless kinetochores. Lowering the kinetochore turnover rate of the RZZ complex might involve the modification of the interaction between the RZZ complex and dynein. This could prevent dynein-mediated transport of the RZZ complex, and other essential mitotic-checkpoint components, off the kinetochores. Mitotic-checkpoint arrest in response to the loss of kinetochore tension would thus be maintained by the prevention of the “shedding” of essential checkpoint proteins from kinetochores, even though bipolar attachment of microtubules has been achieved (Famulski and Chan, 2007).

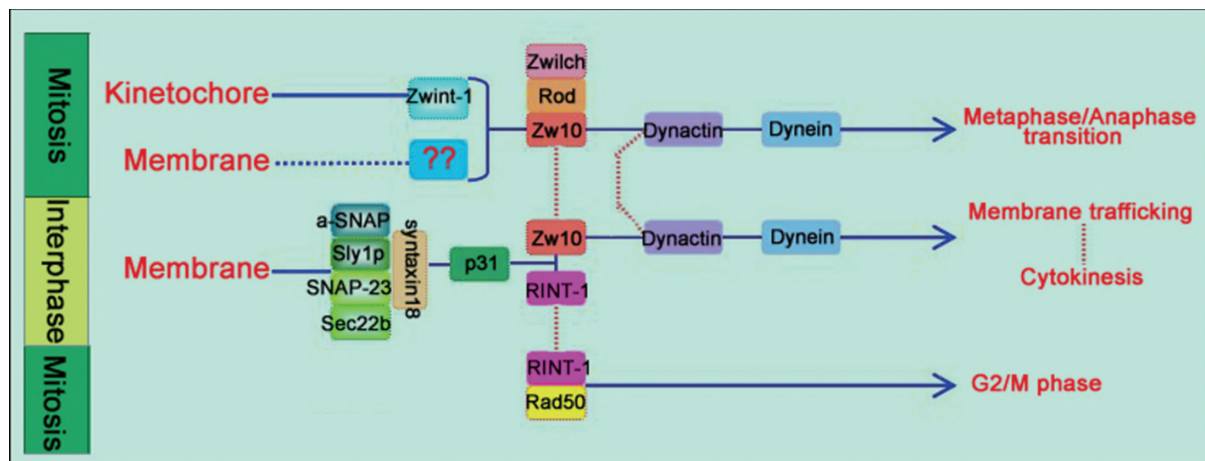


Fig. 3. Relationships of membrane trafficking and cell cycle

2.7 RZZ and membrane trafficking

Recent studies have revealed that Zw10 performs important functions in non-dividing cells as well. These include cytoplasmic dynein targeting to Golgi and other membranes, but also SNARE-mediated ER-Golgi trafficking (Vallee *et al.*, 2006). Zw10 is located in the endoplasmic reticulum (ER), Golgi apparatus, and cytosol during interphase. Zw10 forms a subcomplex with RINT-1 (Rad50-interacting protein) and p31 in a large complex comprising syntaxin 18, an endoplasmic reticulum-localized t-SNARE implicated in membrane trafficking (Hirose *et al.*, 2004; Hatsuzawa *et al.*, 2000). t-SNAREs form a tight complex with vesicle-associated SNAREs (v-SNAREs), which leads to membrane fusion (Wickner and Haas, 2000; Jahn *et al.*, 2003; Bonifacio and Glick, 2004). RINT-1 was originally discovered as a checkpoint protein that interacts with Rad50 only during G2/M phases (Xiao *et al.*, 2001). On the other hand, RINT-1, together with Zw10 and other proteins, forms a complex with syntaxin18 (Hatsuzawa *et al.*, 2000; Hirose *et al.*, 2004). The experiments prove that in interphase cells RINT-1 is located on the ER; in prometaphase cells RINT-1 is not localized on the kinetochores, suggesting that it is not a component of a RZZ complex. These results show that RINT-1 may exist in two distinct complexes, one involved in the G2/M checkpoint and the other in membrane trafficking, as may also be the case for Zw10. Zw10 forms a complex with Rod (Scaërou *et al.*, 2001), and this complex plays a role in turning off the spindle checkpoint (Howell *et al.*, 2001; Wojcik *et al.*, 2001). The present study demonstrates that Rod is not present in isolated syntaxin18 complexes. Zw10 was found to interact in a yeast two-hybrid screen with the 50 kDa dynactin subunit of the dynactin complex (Starr *et al.*, 1998). It is tempting to speculate that the Zw10/RINT-1 complex acts as an anchor for the dynein-dynactin on ER to facilitate membrane transport to Golgi (Hirose *et*

al., 2004). How are the formation and activity of complexes containing Zw10 and/or RINT-1 regulated? Perhaps there could be two models (Fig. 3). One model predicts that all complexes (RZZ, Rad50-RINT-1, Zw10-RINT-1-p31, and possibly others) are constantly present in cells and function independently. In this case, the RZZ and Rad50-RINT-1 complexes must be activated by some mechanisms at the onset of mitosis and G2/M phase, respectively. The alternate model is that Zw10 and RINT-1 are mainly complexed with syntaxin18 during interphase and change their partners depending on the cell cycle. However, the preliminary result shows that the binding of Zw10 and RINT-1 to syntaxin18 is independent of cell cycle, making the former model the more reliable. But there is also the possibility that both models are correct (Arasaki *et al.*, 2006; Lu *et al.*, 2008).

2.8 The functions of RZZ

Information to date suggests that during cell division the RZZ complex fulfills many important functions: recruiting cytoplasmic dynein, Mad1-Mad2 complex and Spindly to the kinetochore; maintaining a functional spindle checkpoint; and participating in the poleward movements of chromosomes during mitosis.

2.8.1 Recruiting dynein-dynactin

The RZZ complex is directly required for the recruitment of cytoplasmic dynein and dynactin to the kinetochores (Starr *et al.*, 1998). The whole RZZ complex is required, as mutations in *rod* or *zwilch* abolish dynein recruitment even though Zw10 is still present in the cell (Williams *et al.*, 2003; Starr *et al.*, 1998; Karess, 2005). The Zw10 protein at least appears to be directly involved, since it is capable of interacting with the p50 subunit of the dynein-dynactin complex in the yeast two-hybrid system (Starr *et al.*, 1998).

Dynein levels are high on unattached kinetochores, and dynein is believed to be implicated in the rapid poleward movement of chromosomes immediately following their initial capture by spindle fibers (Rieder and Alexander, 1990). A more recently identified role for kinetochore dynein is in the shedding and transport of outer domain proteins away from kinetochores following MT capture (Howell *et al.*, 2001; Wojcik *et al.*, 2001). Dynein-depleted cells retain high levels of Mad2 and Rod on their bi-oriented kinetochores. These observations led to the proposal that dynein-mediated shedding might be part of the mechanism for shutting off the checkpoint (Howell *et al.*, 2001; Wojcik *et al.*, 2001), dismantling the platform by removing key checkpoint proteins such as Mad2 from the properly attached kinetochores. Because Mad2 levels fall to zero, the anaphase inhibitor is presumably no longer produced, and the cells will progress into anaphase. Thus, by virtue of its role in recruiting dynein to kinetochores, the RZZ complex sows the seeds of its own removal, the removal of Mad2, and the inactivation of the checkpoint (Karess, 2005). However, the RZZ complex and the dynein-dynactin behavior are not identical. Immunostaining shows that dynein-dynactin, unlike Rod, diminishes rapidly upon MT capture (Hoffman *et al.*, 2001; King *et al.*, 2000). Taken together, these results suggest that the kinetochore RZZ complex and dynein-dynactin can act partially independently of one another (Basto *et al.*, 2004).

2.8.2 Recruiting Spindly

In *Drosophila melanogaster* S2 and human cells, Spindly, that accumulates on unattached kinetochores and is required for silencing the SAC, has been identified. After the depletion of Spindly, dynein cannot target to the kinetochores, and, as a result, cells arrest in metaphase with high levels of kinetochore-bound Mad2 and RZZ. Studies indicate that Spindly is a novel regulator of mitotic dynein, functioning specifically to target dynein to kinetochores. Lis1, another dynein cofactor, also has been proposed to play a role in targeting dynein to kinetochores (Dzhindzhev *et al.*, 2005). Spindly localizes to microtubule plus ends in interphase and to kinetochores during mitosis. These results indicate that Spindly is a part of the corona region of the kinetochore and requires the RZZ complex (but not dynein or dynactin) for its kinetochore localization. A model of Spindly activity thinks the RZZ complex binds to the outer kinetochore region and recruits Mad2, Spindly, and the dynactin complex during mitosis. Spindly and dynactin then work cooperatively to recruit dynein, which then transports the whole complex towards the spindle pole and silences SAC signaling on the kinetochore (Griffis *et al.*, 2007). Another new study finds that Spdl-1 protein (a *Caenorhabditis elegans* homologue of Spindly) localizes to the kinetochore from prometaphase to metaphase, which depends on KNL-1, a highly conserved kinetochore protein, and Czw-1/Zw10, a component of RZZ complex. In two-cell-stage embryos har-

boring abnormal monopolar spindles, Spdl-1 is required to induce the SAC-dependent mitotic delay and localizes the SAC protein Mad1 to the kinetochore facing away from the spindle pole. In addition, Spdl-1 co-immunoprecipitates with Mad1 *in vivo*. These results suggest that Spdl-1 functions in a kinetochore receptor of Mad1 to induce SAC function (Yamamoto *et al.*, 2008). Spdl-1 is recruited to kinetochores by the RZZ complex, indicating that both the localization and cell cycle progression-dependent loss of the RZZ complex from kinetochores are independent of Spdl-1. Spdl-1 localizes downstream from the RZZ complex and is required for all RZZ complex functions established to date: spindle checkpoint activation, kinetochore recruitment of Mad2, and kinetochore recruitment of dynein-dynactin. Spdl-1 targets to the kinetochore immediately downstream from the RZZ complex and is not involved in the assembly of the core kinetochore-MT-binding site constituted by the KMN network. The RZZ complex and Spdl-1 are equivalently required for both dynein-dynactin targeting to kinetochores and spindle checkpoint activation. The physiological function of the regulatory link between the RZZ complex and the KMN network is to ensure a coordinated transition from transient lateral attachments made by dynein, which accelerate formation of end-coupled attachments of correct geometry, to stable load-bearing end-coupled attachments that do the job of chromosome segregation. In this model, the RZZ complex inhibition of the KMN network is modulated by the MT minus-end-directed motility of dynein-dynactin, which is linked to the outer kinetochore via Spdl-1 and the RZZ complex. When dynein-dynactin is laterally attached to a microtubule that extends past the kinetochore, the RZZ complex is under low tension and negatively regulates the MT-binding activity of the KMN network. This prevents the KMN network from tightly binding to a MT extending past the kinetochore that has been captured by dynein-dynactin. When dynein-dynactin translocation towards the MT minus end is met with resistance due to the MT plus end being embedded in the kinetochore outer plate, the RZZ complex is placed under tension and the inhibition of the KMN network is relieved. So this activity of the RZZ complex is normally controlled by dynein-dynactin localized via Spdl-1 (Gassmann *et al.*, 2008).

2.8.3 Participating in the poleward movements of chromosomes

One finding shows that the Rod protein, together with Zw10, is involved in production and/or regulation of the force responsible for poleward chromosome motion. Poleward chromosome motion is mediated by kinetochores, which can move on the surface of MTs or on their disassembling plus ends. Although the MT motor cytoplasmic dynein is thought to be involved in this motion (Karki and Holzbaur, 1999), studies have found that the rate of poleward chromosome motion in *zw10* mutants is greatly attenuated throughout the division process, and that chromosome

disjunction at anaphase is highly asynchronous. Similar behaviours have also been observed in spermatocytes lacking Rod (Starr *et al.*, 1998; Scaërou *et al.*, 1999). As in *zw10* mutants, chromosome disjunction in cells lacking Rod was highly asynchronous, and during anaphase the dyads either failed to undergo poleward motion or moved polewards at greatly attenuated rates (Savoian *et al.*, 2000).

2.8.4 The SAC function of the RZZ complex

2.8.4.1 The SAC defective phenotype of the *rzz* mutants

Mutations in the *Drosophila* *zw10* or *rod* genes cause similar defects, most noticeably in lagging chromatids that remain at the metaphase plate late in anaphase, leading to high levels of aneuploidy among daughter cells (Wang *et al.*, 2004). Like *rod* and *zw10* mutants, *zwilch* mutations produce the same mitotic phenotype (Kops *et al.*, 2005). The chromosome segregation defects in *zw10* and *rod* mutants might also involve another activity of RZZ: it is required for the function of SAC (Amon, 1999; Williams *et al.*, 2003; Gatti and Baker, 1989; Scaërou *et al.*, 1999; Williams *et al.*, 1992; Scaërou *et al.*, 2001). Recently, both Rod and Zw10 have been shown to fit the paradigm of true spindle checkpoint components in *Drosophila* and human cells (Basto *et al.*, 2000; Chan *et al.*, 2000; Savoian *et al.*, 2000). In their absence, cells no longer arrest in response to spindle damage, but rather go on to separate sister chromatids, degrade cyclin B, and exit mitosis, indicating a defect in the checkpoint apparatus (Scaërou *et al.*, 2001), which can potentially explain the high incidence of aneuploidy (Chan *et al.*, 2000).

2.8.4.2 Recruiting Mad1-Mad2

How then does RZZ contribute to a functional spindle checkpoint? Very recent work has at last shed some light on this question. Two groups have now shown that depleting Rod or Zw10, either by si-RNA, immunodepletion from extracts, or with classical mutations, blocks Mad1 and Mad2 recruitment to unattached kinetochores (Kops *et al.*, 2005; Buffin *et al.*, 2005). These observations provide the first plausible explanation for the checkpoint defect in *rod* and *zw10* mutants: without the Mad1-Mad2 complex assembled on kinetochores, the checkpoint should be inactive (Karess, 2005). Mad2 and Rod colocalize to the outermost kinetochore region (the corona). Moreover, Mad2 requires RZZ for its accumulation on unattached kinetochores. RZZ thus contributes to checkpoint activation by promoting Mad2 recruitment and to checkpoint inactivation by recruiting dynein-dynactin that subsequently removes Mad2 from attached kinetochores. Mad2 recruitment is severely impaired in *rod* and *zw10* mutants. Prometaphase or colchicine-treated wild-type neuroblasts have bright GFP-Mad2 signals on their kinetochores. In *rod* or *zw10* mutant cells, little or no Mad2 is seen on the kinetochores or spindle, although Mad2 is still present in interphase nuclei. Colchicine treatment does not increase Mad2 kinetochore accumulation (Buffin *et al.*, 2005).

2.8.4.3 KMN network and RZZ

Using *in vivo* assays to monitor chromosome segregation, kinetochore assembly, and the mechanical stability of chromosome, the results show that the conserved KNL-1/Mis12 complex/Ndc80 complex, also known as the KMN network, is essential for KMT interactions (Cheeseman *et al.*, 2004; Cheeseman *et al.*, 2006). The KMN network constitutes the core MT-binding site of the kinetochore. The KNL-1 and Mis12 complex make important contributions to kinetochore assembly and chromosome segregation (Cheeseman *et al.*, 2008; Cheeseman *et al.*, 2004; Obuse *et al.*, 2004). A 4-subunit Ndc80 complex (Ndc80, Nuf2, Spc24, and Spc25) exists in fungi and vertebrates (Cheeseman *et al.*, 2004; Obuse *et al.*, 2004). *In vitro* analysis suggests that KNL-1 and the Mis12 complex together generate a binding site for the Ndc80 complex (Cheeseman *et al.*, 2006; Przewlaka *et al.*, 2007). Vertebrate KNL-1 is expected to function upstream of the Ndc80 complex (Cheeseman *et al.*, 2008). The KMN network is thus a key structural component of the kinetochore, but also directly binds to MTs via the Ndc80 subunit of the Ndc80 complex and KNL-1 (Cheeseman *et al.*, 2006; Cheeseman *et al.*, 2008). The intact KMN network is incorporated into the outer kinetochore plate to form the repeating MT-binding sites of eukaryotic kinetochores (Cheeseman *et al.*, 2006; Przewlaka *et al.*, 2007).

One study shows that Zwint-1 as an important Zw10-binding partner that links RZZ to the kinetochore and mitotic checkpoint (Wang *et al.*, 2004). Zwint-1 contains an extended coiled-coil motif, interacts with Zw10, and localizes to an outerplate kinetochore during mitosis. Mis12 co-immunoprecipitates with Zwint-1 (Chan *et al.*, 2005), therefore it can be assumed that the Mis12 complex might be connected to the outer kinetochore through interaction with Zwint-1 (Obuse *et al.*, 2004). However, the newest study demonstrated that the depletion of KNL-1 abolished the localization of the outer kinetochore proteins Zwint-1 and reduced the localization of Dsn1, a component of the Mis12 complex (Cheeseman *et al.*, 2004; Cheeseman *et al.*, 2008). So it is possible that Zwint-1 interacts with Mis12 through KNL-1. In addition, the Ndc80 complex physically associates with KNL-1 and the Mis12 complex within the KMN network (Cheeseman *et al.*, 2004; Obuse *et al.*, 2004). Thus, the RZZ complex is physically connected to the Ndc80 complex via interaction with Zwint-1, suggesting that the RZZ complex plays a key role to transmit the checkpoint signal generated from the unattached kinetochore to the SAC.

3. Conclusions and Perspectives

In the past few years, significant progress has been made in understanding Rod function, primarily from genetic and cell biological studies in *Drosophila*, where they were first iden-

tified, but also from studies in vertebrate cells. Existing data on Rod indicate that it participates in multiple physiological processes, but a unifying molecular function has yet to be determined (Karess, 2005; Vallee *et al.*, 2006). RZZ plays an important part in bringing at least two crucial proteins to kinetochores—dynein and Mad2—and so contributes to both the activation of the SAC and to its subsequent inactivation. However, many outstanding questions remain. How does RZZ promote recruitment of Mad1-Mad2? Precisely what features of MT capture and bi-orientation of kinetochores promote the dynein-mediated shedding of RZZ and Mad1-Mad2? Nothing is known about the biochemistry of the RZZ complex. Does it exist in interphase, does it form in M phase, or is it assembled only on kinetochores? Is it modified by mitotic kinases? Does it indeed have self-assembly properties? Could the RZZ complex serve a role in recruiting cytoplasmic dynein to membranes? How it may bind to membranes remain important issues for future investigation (Karess, 2005; Vallee *et al.*, 2006). The SAC is activated by kinetochores not yet attached by MTs and not under tension. These kinetochores mediate the assembly of checkpoint complexes, which are responsible for the inhibition of APC/C-Cdc20. Despite the progress made, many significant questions remain, such as how the MCC is assembled and how it inhibits APC/C-Cdc20. In particular, the mechanism by which Mad2 is relayed from Mad1 to Cdc20 remains to be established. It will be crucial to understand the mechanism of the regulated kinetochore localization of various checkpoint proteins. This is the key for the activation and inactivation of the checkpoint (Yu, 2002). More recently, interphase Zw10 has been shown to form an ATP-sensitive complex with syntaxin18 and several other proteins involved in vesicle transport between Golgi and ER (Hirose *et al.*, 2004). Moreover, given the emerging role for membrane vesicle dynamics in cytokinesis (Albertson *et al.*, 2005), this work could well provide the explanation for the cytokinesis phenotype (Karess, 2005). The present results may shed light on possible crosstalk between membrane trafficking and the cell cycle checkpoint. The reason why proteins involved in membrane trafficking also function in mitosis remains an enigma (Arasaki *et al.*, 2006; Lu *et al.*, 2008).

Rod and Zw10 proteins behave similarly in the process of cell division, but their behaviors have shown great difference in interphase. Future research may resolve the problem of what led to the discrepancy. Among different multicellular animals, the activity of Rod is not exactly the same, and its homologue is not found in multicellular plants. In the plant cells, which protein replaces its function is also an interesting issue.

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