the proteasome interior? And to what extent are alternative approaches to accessing the activities of the proteasome exploited *in vivo*? The proteasome is a remarkable machine, and there is still much to learn.

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Ran in the spindle checkpoint: a new function for a versatile GTPase

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The small GTPase Ran has a well-established role in nucleocytoplasmic trafficking. In recent years, the repertoire of Ran has expanded to include regulation of spindle assembly, formation of the nuclear envelope and DNA replication. Now, new studies further extend the role of Ran to regulating the spindle checkpoint during mitosis.

Extensive studies in nuclear trafficking have shown that Ran acts as a molecular switch to regulate the assembly and disassembly of nuclear transport receptor–cargo complexes, depending on the guanine-nucleotide-bound state of Ran [1,2]. The nucleotide exchange factor RCC1 catalyzes formation of RanGTP, whereas the hydrolysis of RanGTP is stimulated by RanGAP1 and RanBP1. Because RCC1 is chromatin-bound whereas RanGAP1 and RanBP1 are cytoplasmic, the concentration of RanGTP is high in the nucleus. This distribution of RanGTP ensures the directionality of nuclear import and export during interphase (Figure 1).

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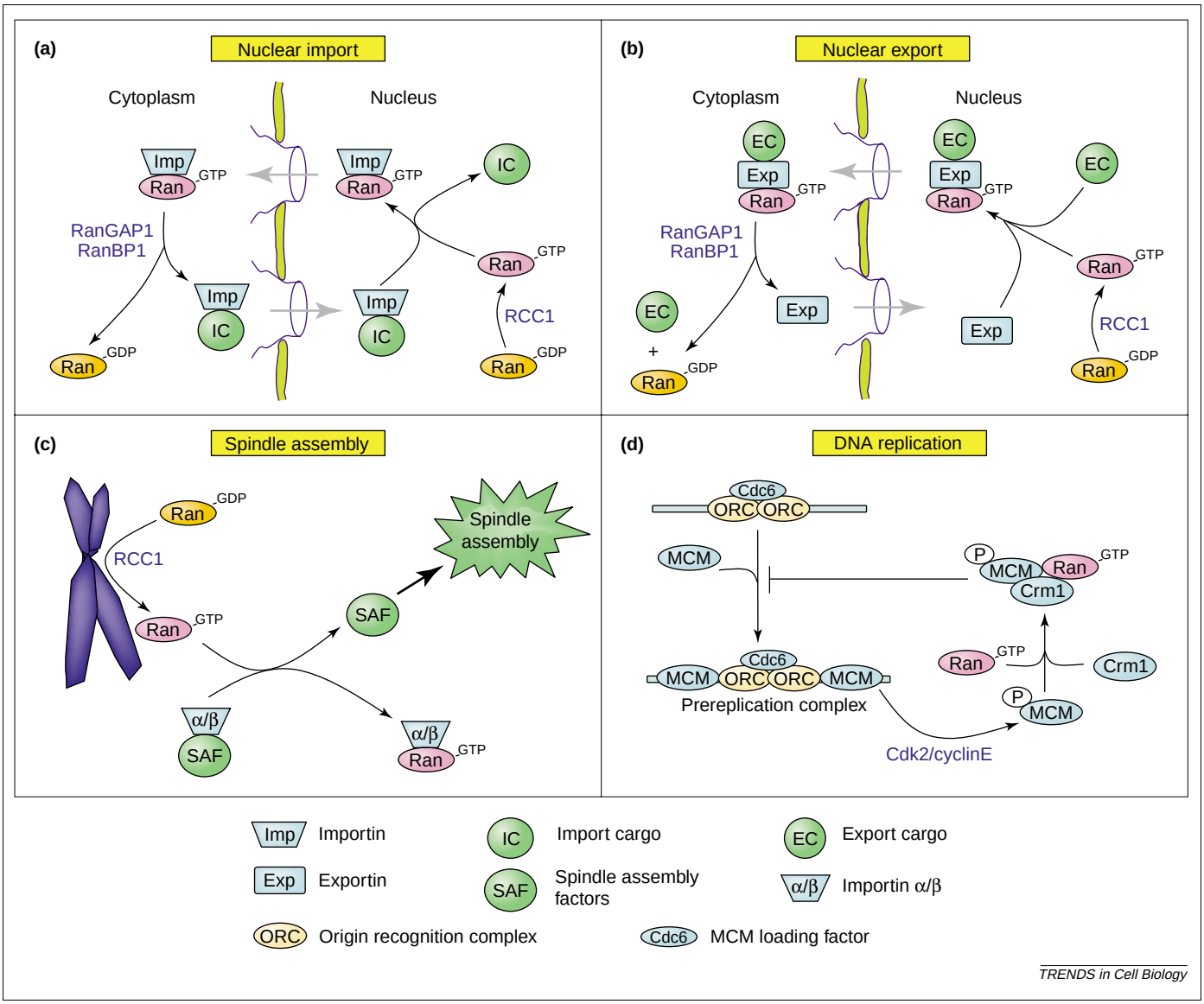

Figure 1. Schematic models of Ran function in nucleocytoplasmic transport, spindle assembly and DNA replication. **(a, b)** Ran-regulated nuclear import (a) and export (b). In the import cycle (a), import receptors, such as importin β and its adaptor importin α, bind to their cargo in the cytoplasm and transit into the nucleus. Nuclear RanGTP binds to importin and stimulates cargo release. Importin–RanGTP returns to the cytoplasm. Hydrolysis of RanGTP to RanGDP (stimulated by RanGAP1 and RanBP1) releases importin from Ran, facilitating the recycling of importin. In the export cycle (b), export receptors exportin, such as Crm1, bind to their cargo and RanGTP in the nucleus and transit into the cytoplasm. In the cytoplasm, RanGTP hydrolysis leads to the release of cargo and the recycling of exportin. (c) RanGTP stimulates spindle assembly during mitosis. A high RanGTP concentration is generated on mitotic chromosomes by chromosome-bound RCC1. This RanGTP locally stimulates the release of spindle assembly factors (SAF) from the inhibitory binding of importin α/β to promote spindle assembly towards chromosomes. (d) RanGTP inhibits DNA reduplication in the same cell cycle. During S phase, high cyclin-dependent kinase (Cdk) activity phosphorylates MCM helicase. This promotes the interaction of MCM with both RanGTP and Crm1 in the nucleus, thereby preventing MCM from participating in DNA reduplication.

The function of Ran was thought to be solely devoted to nucleocytoplasmic transport until the discovery of its role in regulating multiple microtubule (MT)-based processes [3,4] and spindle assembly [5–9] in mitosis. Surprisingly, for the regulation of spindle assembly RanGTP uses a similar mechanism to nuclear import [10–12] (Figure 1c). The versatility of Ran functions was further extended when it was shown that this small GTPase also regulates DNA replication [13] and nuclear-envelope assembly [14–16]. Interestingly, it appears that Ran does not 'reinvent the wheel' in regulating DNA replication. Instead, Ran uses a mechanism similar to nuclear export to sequester the MCM helicase, thereby preventing DNA reduplication during the same cell cycle [13] (Figure 1d). New studies show that Ran has yet another role in regulating spindle checkpoint during mitosis [17]. Here, we discuss these new studies, and how Ran might act as a molecular switch to silence spindle-checkpoint signals, thereby allowing the transition from metaphase to anaphase.

Spindle checkpoint: a dynamic signaling process

To ensure accurate chromosome segregation, eukaryotic cells have evolved an elegant sensing mechanism to detect correct attachment of every chromosome onto the mitotic spindle. Operating at specialized chromatin structures called 'kinetochores', this sensory system sends a stop signal to prevent metaphase–anaphase transition in the presence of a single inappropriately attached or unattached chromosome. Although the detailed mechanism

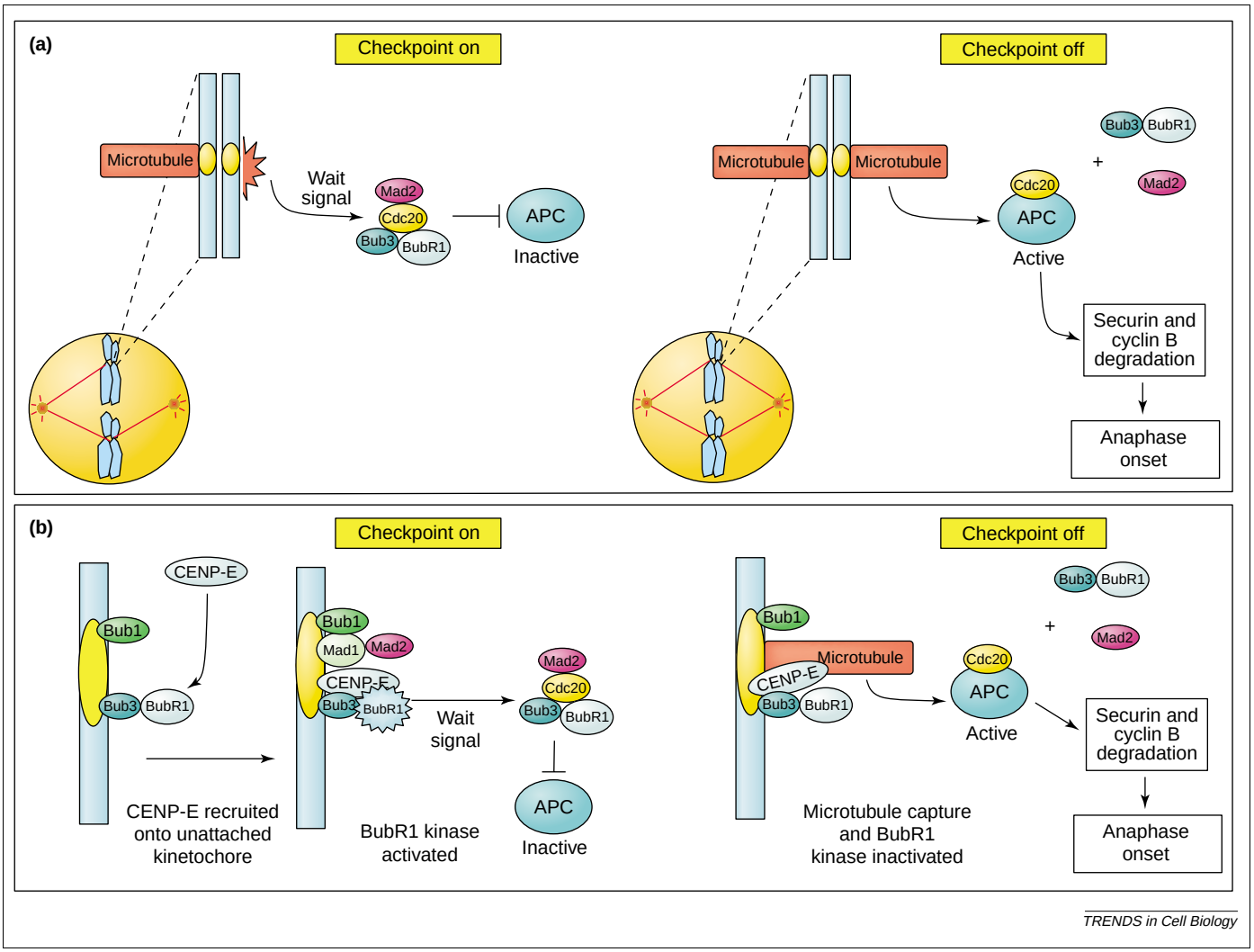

Figure 2. Activation and silencing of spindle checkpoint during mitosis. **(a)** An unattached kinetochore sends out a 'wait' signal to halt cell-cycle progression at the metaphase–anaphase transition. This wait signal is generated by dynamic association of the spindle-checkpoint components with the unattached kinetochore, which leads to the inhibition of Cdc20/FZY by the checkpoint components. As a result, anaphase-promoting complex (APC) is inactivated and the cells are arrested at metaphase. Upon proper attachment of microtubules to all kinetochores, checkpoint components dissociate from Cdc20/FZY, allowing the activation of APC by Cdc20/FZY. Ubiquitination of securin and cyclin B by APC leads to their destruction, thereby triggering chromosome segregation and anaphase onset. **(b)**. CENP-E (kinetochore-associated kinesin)-dependent BubR1 kinase is required for turning on and off of the spindle-checkpoint signal. BubR1 is required for recruiting CENP-E to the unattached kinetochore. Direct association of CENP-E and BubR1 activates BubR1 kinase activity, which is essential for mitotic checkpoint signaling. The spindle checkpoint is silenced by CENP-E dependent capturing of microtubule, which alters or disrupts the interaction of CENP-E with BubR1 and inactivates BubR1 kinase.

remains to be clarified, it appears that the dynamic association of checkpoint proteins, such as Mad1, Mad2, Bub1 and BubR1 with even a single unattached kinetochore activates these proteins [18]. Some of the activated checkpoint proteins then bind to and inhibit Cdc20/FZY, an activator of the anaphase-promoting complex (APC). Once MTs capture all kinetochores in a bipolar orientation, the MT occupancy and/or tension on kinetochores prevent further binding and activation of the checkpoint proteins by kinetochores. Consequently, Cdc20/FZY binds to and activates APC. Chromosome segregation and anaphase ensue when the active APC targets proteins such as securin and cyclin B for degradation through polyubiquitination (Figure 2a).

Recent studies have shed light on how the capture and/or tension on kinetochores of MT stop the checkpoint signal. CENP-E, a kinetochore-associated kinesin, has been implicated as a sensor that mediates the capture of MT at the kinetochore and transduces the signal of capture and/or tension to components of the checkpoint machinery. Interestingly, CENP-E has now been shown to bind to and activate BubR1, a checkpoint kinase [19]. Because the capture of MTs by kinetochore is facilitated by CENP-E and because capture silences the kinase activity of BubR1, these new findings lead to an elegant model of how CENP-E might mediate both the activation and silencing of checkpoint through BubR1 [19] (Figure 2b).

Excess RCC1 stops the checkpoint signal by releasing checkpoint proteins from kinetochores in *Xenopus* egg extracts

Because RanGTP is required for spindle assembly, Arnaoutov and Dasso set out to determine whether this GTPase also regulates mitotic progression. Under normal cell cycle conditions, the *Xenopus* egg extract can oscillate between interphase and mitosis. However, in the presence

of a high concentration of sperm nuclei and the MT depolymerization reagent nocodazole, the spindle checkpoint is activated and blocks cyclin B ubiquitination and degradation. Remarkably, Arnaoutov and Dasso found that the addition of excess RCC1 to such an egg extract overrides the checkpoint, resulting in the ubiquitination and degradation of cyclin B. Further analyses have shown that excess RCC1 promotes the association of APC with its activator Cdc20/FZY in egg extracts, which leads to polyubiquitination of both cyclin B and securin by $APC^{FZY/Cdc20}$ [17].

How might excess RCC1 activate APC? Examining the localization of checkpoint proteins revealed that excess RCC1 released Bub1, Bub3, Mad2 and CENP-E from kinetochores in the presence of excess sperm nuclei and nocodazole. To exclude the possibility that excess RCC1 might only be able to release checkpoint proteins in the presence of nocodazole, Arnaoutov and Dasso performed experiments in the absence of nocodazole. Previous studies have shown that, although the checkpoint is not activated, spindles assembled in CSF egg extracts have Bub1 retained on the kinetochores assembled from unreplicated DNA. Interestingly, excess RCC1 also released Bub1 from the kinetochores in the absence of nocodazole. These new studies suggest that the elevated RCC1 level releases checkpoint proteins from kinetochores downstream of MT occupancy and/or tension. Dislodging checkpoint proteins from kinetochores would prevent them from inhibiting Cdc20/FZY, thereby activating APC.

RanGAP1 and RanBP1 counteract the effect of RCC1 in regulating spindle checkpoint

Because RCC1 catalyzes RanGTP production, checkpoint override in the presence of excess RCC1 might be the result of an elevated RanGTP level in the egg extracts. Indeed, when the levels of RanGAP1 and RanBP1 are elevated in the egg extracts, RCC1-mediated checkpoint override is largely reversed [17]. Therefore, RanGTP hydrolysis induced by excess RanGAP1 and RanBP1 can overcome the effect of excess RCC1 [17]. This strongly suggests that high RanGTP levels silence the mitotic checkpoint. Consistent with this idea, Arnaoutov and Dasso further demonstrated that depletion of RanGAP1, RanBP1 and RanBP2 from the egg extracts also blocked the binding of checkpoint proteins to kinetochores.

One would predict that checkpoint override caused by excess RCC1 should be fully recapitulated by excess RanGTP. Indeed, adding excess RanGTP also overrides checkpoint in *Xenopus* egg extracts (M. Dasso, pers. commun.). Arnaoutov and Dasso favor the idea that proper chromosome alignment at the metaphase plate might trigger an increased binding of RCC1 to chromosomes and an increase in local RanGTP concentration, thereby ejecting the checkpoint proteins from the kinetochores. The challenge is to determine whether and how RCC1 binding to mitotic chromosomes is regulated by chromosome alignment.

It is noteworthy that a steep RanGTP gradient is maintained on the mitotic chromosomes [20] by RCC1, which is also a highly mobile enzyme that couples chromosome binding to RanGTP production [21]. If proper chromosome alignment indeed causes increased RCC1 binding to chromosomes, the residence time of RCC1 on the properly aligned metaphase chromosomes might be different from the RCC1 on prometaphase chromosomes. Because a RCC1–GFP fusion protein is fully functional *in vivo* [21], it should be possible to study RCC1 behavior in living cells under checkpoint conditions.

How might RanGTP silence the checkpoint?

Although Ran regulates diverse cellular functions, where the mechanism is known, Ran appears to achieve regulation through nuclear transport receptors (Figure 1). Arnaoutov and Dasso have shown that the transport receptor importin β, which regulates nuclear import during interphase, mitotic-spindle assembly and postmitotic nuclear assembly [16], is not required for regulating the spindle checkpoint. The authors suggest that RanGTP might regulate the spindle checkpoint through certain other transport receptors and/or nuclear pore components [17].

Interestingly, some nuclear-pore components are found at the kinetochore during mitosis. Because both Mad1 and Mad2 are found at nuclear pores during interphase [22] and at kinetochores during mitosis, the kinetochore-localized nuclear-pore proteins could tether Mad1 and Mad2 at the kinetochore [17]. If RanGTP prevents the tethering, then a transient increase in RanGTP concentration on mitotic chromosomes at the metaphase–anaphase transition would release checkpoint proteins from kinetochores. It is not yet known whether or how transport receptors might regulate checkpoint proteins. However, it is tempting to speculate that certain transport receptor(s) might interact with checkpoint proteins in mitosis in a manner analogous to the interaction between transport receptors and their import or export cargos during interphase. For example, a high RanGTP concentration on condensed chromosomes might facilitate the binding of certain export receptor(s) to some checkpoint proteins. This binding might sequester the checkpoint proteins away from kinetochores, leading to checkpoint silencing.

Concluding remarks

Considering the large number of transport receptors discovered thus far, it is perhaps not surprising that RanGTPase is involved in regulating a diverse range of cellular activities. Knowledge gained from studying Ran-regulated nuclear transport, spindle assembly and DNA replication should help to elucidate the mechanism of RanGTP-induced override of the spindle checkpoint. A bigger challenge is to understand what regulates the increase of RanGTP production during the metaphase–anaphase transition. Arnaoutov and Dasso suggest that proper attachment of all kinetochores to the metaphase spindle might lead to increased binding of RCC1 to mitotic chromosomes [17]. Investigating how proper alignment of chromosome is translated into increased RanGTP concentration on mitotic chromosomes offers exciting challenges and opportunities for future research.

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Septate and paranodal junctions: kissing cousins

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A series of recent publications significantly advances our knowledge about the evolution and composition of septate junctions in arthropod and chordate species. These papers indicate that insect septate junctions share several structural and functional components with paranodal junctions, which join myelinating glial cells to axons in the vertebrate nervous system, and that both probably evolved from a common ancestral precursor.

Two recently published articles provide new insights into the structure and function of septate junctions in insects that are evolutionarily related to paranodal junctions in vertebrates [1,2]. The studies identify novel proteins that are important for proper barrier function of the septate junctions in *Drosophila melanogaster* and assess the implication of mutations in genes encoding these proteins. These findings are not only of great importance for a better understanding of the function and structure of epithelial septate junctions in invertebrates but will also lead to a better understanding of the neuron–glial interactions in the nervous system of the vertebrates.

Sealing junctions play an important role in the function of epithelia and neurons [3,4]. The seals preserve ionic differences across the junction, allowing for vectorial ion transport and rapid neuroconduction. In the epithelia of *Drosophila*, this sealing function is performed by septate junctions that lie below the adherens junction on the lateral side of these cells. This is different from mammalian epithelia, which have tight junctions that are localized apically in relation to adherens junctions. The reasons for this evolutionary difference are not entirely clear, but the location of the mammalian tight junction might provide enhanced regulation of cell polarity and improved ion selectivity.

Evolutionary relationship between septate and paranodal junctions

In invertebrate species, septate junctions also provide the functional equivalent of the vertebrate blood–brain

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