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Joined at the hip: kinetochores, microtubules, and spindle assembly checkpoint signaling

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Error-free chromosome segregation relies on stable connections between kinetochores and spindle microtubules. The spindle assembly checkpoint (SAC) monitors such connections and relays their absence to the cell cycle machinery to delay cell division. The molecular network at kinetochores that is responsible for microtubule binding is integrated with the core components of the SAC signaling system. Molecular-mechanistic understanding of how the SAC is coupled to the kinetochore–microtubule interface has advanced significantly in recent years. The latest insights not only provide a striking view of the dynamics and regulation of SAC signaling events at the outer kinetochore but also create a framework for understanding how that signaling may be terminated when kinetochores and microtubules connect.

The SAC at a glance

Achieving and maintaining proper interactions between chromosomes and spindle microtubules is the be-all and end-all of faithful chromosome segregation. Proper interactions are obtained by embedding the plus ends of microtubules into the microtubule-attachment sites of chromosomes, known as kinetochores. Such 'end-on' attachments enable chromosome biorientation, a state in which the two sister chromatids of a chromosome are attached to opposing spindle poles allowing them to be pulled to opposite sides during cell division. Improper attachments are corrected by the error-correction machinery, orchestrated by the Aurora B kinase. Aurora B phosphorylates microtubule-binding proteins at the outer kinetochore, triggering changes in the dynamics of the microtubules and weakening the affinity of the kinetochore for microtubules [1]. Thus, during mitosis, kinetochore–microtubule interactions are frequently created and destroyed until finally all chromosomes are bioriented and attachments stabilized. Throughout, the lack of stable attachments needs to be communicated to the cell cycle machinery, which cannot be permitted to initiate chromosome segregation (anaphase)

and cell division. The messenger is the SAC (also known as the mitotic checkpoint) (Figure 1).

The transition to anaphase is triggered by the E3 ubiquitin ligase APC/C, which tags inhibitors of mitotic exit (CYCLIN B) and of sister chromatid disjunction (SECURIN) for proteasomal degradation [2]. The SAC has a one-track mind, inhibiting APC/C as long as incorrectly attached chromosomes persist. It goes about this in the most straightforward way possible: it assembles a direct and diffusible inhibitor of APC/C at kinetochores that are not connected to spindle microtubules. This inhibitor is named the mitotic checkpoint complex (MCC) (Figure 1).

The kinetochore-derived SAC signal is generated at a supercomplex called the KMN network, formed by three different subcomplexes: KNL1-C, MIS12-C, and NDC80-C. This network is the pre-eminent kinetochore interface for contacting microtubules and the main target of the error-correction machinery (Box 1). Thus, the attachment site at kinetochores is intimately connected with the SAC machinery. The connection is a two-way street: many SAC components can alter the microtubule affinity of the KMN network and improve error correction, thereby ensuring that error correction and SAC activities are coordinated in space and time (Box 2).

MCC assembly is orchestrated by the kinase MPS1, which associates with the NDC80-C subcomplex of the KMN network (Figure 2) [3,4]. There, it orchestrates the recruitment of an interlinked network of SAC proteins including the components initially identified in budding yeast genetic screens: BUB1, BUB3, MAD1, MAD2, and Mad3/BUBR1 (human protein nomenclature is used unless otherwise stated). MAD2, BUB3, and BUBR1 constitute the final effector of the pathway (the MCC) and its assembly critically depends on BUB1 and MAD1, along with some additional auxiliary proteins [5,6]. Since the discovery of this pathway more than 20 years ago, the field is progressing rapidly, and several recent excellent reviews have compiled the history, basics, and principles of the SAC [5–9]. The aim of this review is to expand on these with recent insights and a particular focus on the integration of the KMN and SAC networks. This is a narrative in reverse, from effector back to initial and local response, to ensure focus on the events that matter for SAC effector assembly.

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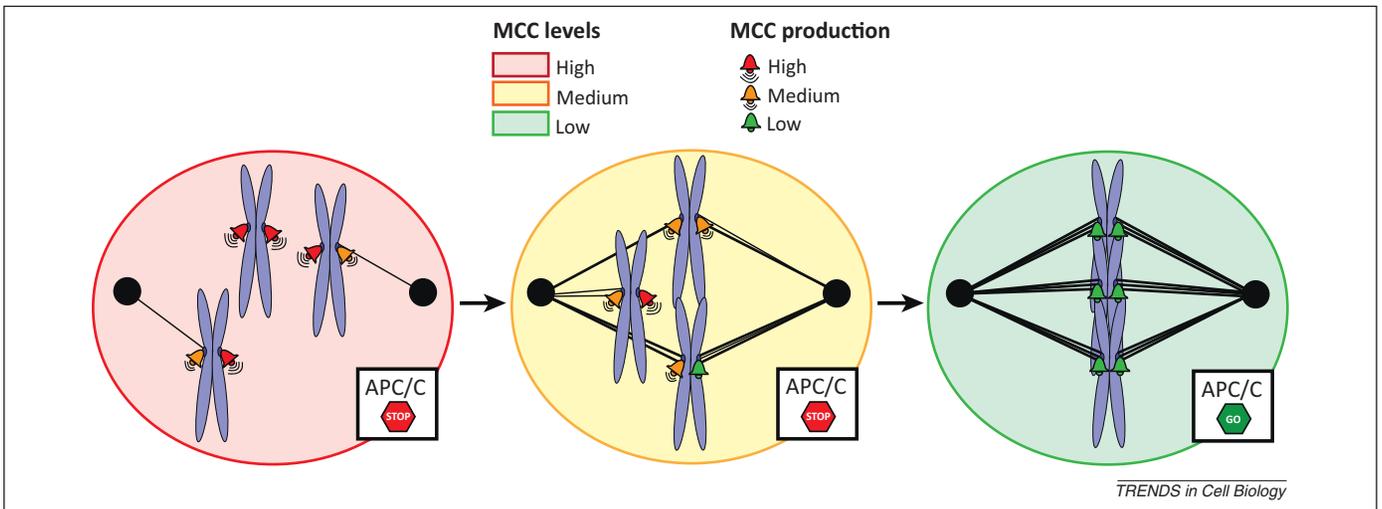
Figure 1. The spindle assembly checkpoint (SAC) response. Unattached kinetochores activate the SAC response (bells), which culminates in assembly of the APC/C-inhibitory mitotic checkpoint complex (MCC). Total MCC levels (indicated by background colors) are determined by the rate of MCC production at kinetochores, which is initially high (red bells) but declines as kinetochores connect with increasing numbers of microtubules (yellow bells), until finally production is halted altogether (green bells). The SAC must nevertheless maintain MCC levels above an undefined threshold to ensure sufficient APC/C inhibition (STOP signals) and prevent anaphase onset and mitotic exit. Only when all kinetochores have met the conditions for correct chromosome segregation is the block on APC/C released (GO signal), allowing cells to proceed to anaphase.

Inhibiting anaphase: a matter of MCC dynamics

Assembly of the MCC delays anaphase onset by preventing CDC20 from activating APC/C (Figure 2). Recent evidence suggests that the MCC is also continuously disassembled [10–13], possibly to allow rapid APC/C activation when the last chromosome has achieved proper attachments. The ability to maintain mitotic delay is thus likely to depend on higher MCC assembly rates compared with disassembly rates.

For proper SAC function, stable association between the MCC and APC/C is required [14]. Despite continuing uncertainty regarding the exact roles of each MCC subunit, it is clear that MAD2 and BUBR1 are indispensable for APC/C inhibition. BUBR1 and its orthologs harbor a KEN box near their N termini, a motif normally recognized by APC/C coactivators as a degron [2]. The KEN box of BUBR1, however, competes with other substrates for

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Box 1. The KMN network: Velcro for microtubules

A fully attached human kinetochore is bound by approximately 20 microtubules. In the recently proposed 'lawn' model, hundreds of microtubule-binding protein complexes on a human kinetochore cooperate to maintain attachment [96]. The predominant microtubule-binding complex is known as the KMN network [7,97]. It is assembled from three subcomplexes: KNL1-C (KNL1 and Zwint-1), MIS12-C (NNF1, MIS12, DSN1, and NSL1/MIS14), and NDC80-C (HEC1, NUF2, SPC24, and SPC25) (see Figure 2 in main text). MIS12-C connects the inner kinetochore with KNL1-C and NDC80-C [7,97], which extend to the cytosol to directly contact microtubules. NDC80-C interacts with microtubule filaments through binding the interface between two tubulin subunits [98]. For this, it utilizes two globular CH domains present near the N termini of HEC1 and NUF2.

An important role is reserved for an 80-amino acid N-terminal sequence of HEC1 known as the tail. It is suggested to promote end-on attachments by enhancing NDC80-C oligomerization or by directly contacting the microtubule lattice [97]. In addition, it is the key target of the error-correction machinery. Through multisite phosphorylation of the HEC1 tail, Aurora B is proposed to create electrostatic repulsion between tail and microtubule and hence lower the microtubule-binding affinity of kinetochores (see Figure 2 in main text) [1,7]. In addition, a long coiled-coil region that follows the CH domain of Hec1 is disrupted by a loop that is involved in the recruitment of multiple factors that help in the formation of stable kinetochore–microtubule interactions, including the SKA complex in human cells and the Dam1 complex in yeast [99].

The third component, KNL1-C, also exhibits affinity for microtubules, via a basic patch near the N terminus of KNL1, but the importance of this for the stability of kinetochore–microtubule connections is less well defined [42,89]. KNL1 does play a critical role as a scaffold for the assembly of error-correction and SAC signaling modules (see main text for details). Finally, via a C-terminal coiled-coil region, KNL1 interacts with Zwint-1, thereby recruiting the RZZ complex and Spindly – and thus dynein – to kinetochores [39,43,82].

Box 2. Feedback regulation of error correction by the SAC

The error-correction and SAC machineries are coordinately activated on unattached kinetochores. Unsurprisingly, therefore, the two pathways feed back on each other. As outlined in the main text, Aurora B potentiates MPS1 activation in prophase, ensuring maximal SAC function at the onset of mitosis. Conversely, various SAC proteins modulate error correction by regulating either Aurora B or its targets. BUB1 ensures inner-centromere localization of Aurora B by phosphorylating histone H2A in centromeric nucleosomes to indirectly create a docking site for the Aurora B-associated protein Borealin [100]. BUBR1 harbors a KARD motif that constitutes a docking site for the phosphatase PP2A-B56 (see Figure 2 in main text) [101,102]. BUBR1-associated PP2A-B56 helps to stabilize microtubule attachments by (partially) dephosphorylating Aurora B substrates within the KMN network [98,101]. MPS1 promotes both the activity and the localization of Aurora B [103,104] and impacts on error correction indirectly via the localization of BUB1 and BUBR1 [52]. These examples illustrate the complexity of signaling feedback at kinetochores: MPS1 and BUB1 promote Aurora B actions at kinetochores while simultaneously – by localizing BUBR1/PP2A-B56 – counteracting those actions. It seems likely that such networks have evolved to install features like robustness, switchability, and/or multilevel regulation on the system. Detailed spatiotemporal analysis of signaling events coupled with mathematical modeling may be required to capture the intricacies of this network and predict its behavior under various conditions. Such an approach may help to incorporate added levels of complexity such as the impact of CDK1 and PLK1 on some of these signaling connections.