EDITORIALS: CELL CYCLE FEATURES

How the kinetochore switches off the spindle assembly checkpoint

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The eukaryotic kinetochore senses whether or not it is attached to spindle microtubules. If unattached, it activates the signaling cascade of the Spindle Assembly Checkpoint (SAC) and delays cell division. The biochemistry of SAC signaling is well-understood. However, the molecular mechanism that couples SAC activation and inactivation to the attachment state of the kinetochore has been an enduring question in cell biology. Three recent studies address this question, but propose 2 different mechanisms, one mechanical in nature and the other based on biochemical competition.^{1–3} Here we highlight key results that led us to the ‘mechanical switch’ model, and propose that the SAC is silenced by a hybrid mechanism that uses biochemical competition as well as a mechanical switch.

An unattached kinetochore initiates SAC signaling by enabling the phosphorylation of the kinetochore protein Spc105/KNL-1 by Mps1 kinase. This allows the kinetochore to recruit SAC proteins and generate the ‘wait-anaphase’ signal. After the kinetochore forms end-on microtubule attachment, it sheds SAC proteins and stops signaling. Two types of mechanisms can explain the attachment-induced removal of SAC proteins from the kinetochore. Microtubule attachment may interfere with the binding of one or more SAC proteins either by creating steric hindrance or by competing for a common binding site in the kinetochore. Alternatively, it may disrupt protein interactions mechanically, by changing protein organization in the kinetochore.⁴

To understand the mechanism of SAC silencing, we investigated the relatively simple budding yeast kinetochore that has a well-defined protein organization.⁵ We found that the yeast kinetochore encodes a microtubule-operated mechanical switch to control the SAC. In a mechanical switch, the closing of 2 terminals allows electricity to flow, while opening them stops this flow. In a similar manner, the Calponin-Homology domains of the Ndc80 complex and the phosphodomain of Spc105 operate as 2 protein terminals in the yeast kinetochore. They come together when the kinetochore is unattached, allowing Mps1 bound to the Calponin-Homology domains to phosphorylate Spc105 and activate the SAC (Fig. 1, top). Because these domains contain microtubule-binding sites, end-on microtubule attachment separates them by ~30 nm (Fig. 1, bottom). Now, Mps1 can no longer phosphorylate Spc105, and the SAC is inactivated. Thus, the nanoscale protein organization of the

end-on kinetochore-microtubule attachment serves as the mechanical signal for SAC silencing.

The signaling role for the kinetochore architecture is based on 4 key results.¹ First, we found that microtubule attachment to the kinetochore must disrupt the interaction between Mps1 and Spc105 to silence the SAC; if this interaction is forced to occur, SAC protein recruitment occurs even if the kinetochore is attached. Second, attachment does not disrupt the Mps1-Spc105 interaction simply by removing Mps1 from the kinetochore. A residual pool of Mps1 persists at the kinetochore even after the formation of end-on microtubule attachment. Third, where Mps1 binds in the yeast kinetochore is critical to the ability of the kinetochore to inactivate the SAC after it forms end-on

Figure 1. Top: An unattached kinetochore activates the SAC by allowing Mps1 to phosphorylate Spc105/KNL-1 (artistic rendering). Bottom: Two-step model of SAC silencing. Step 1: microtubule attachment interferes with the binding of Mps1 to the Calponin-Homology domains of the Ndc80 complex. Step 2: Calponin Homology domains and the phosphodomain of Spc105 separate, and prevent the residual Mps1 from phosphorylating Spc105.

attachment. If Mps1 is ectopically localized near Spc105, in the inner kinetochore, it phosphorylates Spc105 even after end-on kinetochore-microtubule attachments form. Consequently, SAC signaling becomes constitutive, insensitive to the attachment state of the kinetochore. In contrast, localization of Mps1 in the outer kinetochore allows attachment-sensitive SAC operation. Finally, we showed that Mps1 localizes in the outer kinetochore even after the kinetochore is attached, likely by binding to the Calponin-Homology domains of the Ndc80 complex. Physical separation between these domains and Spc105 in the attached kinetochore prevents the residual Mps1 from phosphorylating Spc105.

Our work confers an essential signaling role to the stereotypical architecture of the kinetochore-microtubule attachment that is observed not only in budding yeast, but also in humans. However, recent studies of the human kinetochore report that Mps1 binds to the Calponin-Homology domains of the Ndc80 complex, and that the microtubule outcompetes Mps1 in binding to these domains to silence the SAC.^{2,3} However, this mechanism alone is unlikely to be sufficient for SAC silencing. Even in human kinetochores, a residual pool of Mps1 persists after the formation of stable attachments.² This residual Mps1 can activate the SAC, if Mad1 is forced to localize to the kinetochore.⁶ Moreover, the distance between CH-domains of Ndc80 and the phosphodomain of Spc105/KNL-1 changes with microtubule attachment in accordance with the mechanical switch model.⁷ Therefore, we propose a 2-step mechanism for SAC silencing (Fig. 1). First, biochemical competition diminishes the binding of Mps1 to the Calponin-Homology domains. Then, the formation of a mature end-on attachment separates the Calponin-Homology domains from KNL-1 to facilitate SAC silencing.

Even with the new insights, key questions remain regarding the mechanism of SAC silencing, especially in the human kinetochore. This complex kinetochore forms dynamic attachments with many microtubules, gaining and losing them continuously. Therefore, it probably contains a subset of Ndc80 molecules that are unattached even in metaphase. Do these molecules bind Mps1? What is the location of the residual Mps1 within stably attached human kinetochores? Does the organization of this kinetochore play a role in preventing the residual Mps1 from activating the SAC? Future work will answer these and other questions, and determine whether and how the protein architecture of the vertebrate kinetochore influences attachment-sensitive operation of the SAC.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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