A Mad that Wears Two Hats: Mad1’s Control of Nuclear Trafficking

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In a recent issue of Molecular Cell, Cairo et al. (2013) report that the spindle checkpoint protein Mad1 shuttles between unattached kinetochores and nuclear pores. Mobile Mad1 inhibits the import receptor Kap121p, thus indirectly changing spindle dynamics. This adds nuclear transport control to the mitotic roles of Mad1.

Nuclear pore complexes (NPCs) perforate the nuclear envelope (NE) of eukaryotic cells, providing a selective barrier for the trafficking of macromolecules between the nucleus and the cytoplasm (Wente and Rout, 2010). NPCs consist of around 30 proteins, called nucleoporins or Nups. A family of transport factors called karyopherins binds to protein cargoes and mediates their movement through NPCs. Karyopherins move through the central NPC channel by virtue of their capacity to make many low-affinity interactions with Nups that possess domains with phenylalanine-glycine-repeat motifs (FG-Nups). This translocation mechanism explains much about bulk trafficking through NPCs; however, less is understood about how transport is fine-tuned in response to physiological conditions. A new report from Cairo et al. (2013), published in Molecular Cell, provides interesting insights into how Mad1, a spindle checkpoint protein, becomes repurposed for modulation of nuclear trafficking in response to faulty spindle assembly, as well as how this mechanism is utilized for correcting spindle defects.

The spindle assembly checkpoint (SAC) is a regulatory pathway that prevents anaphase onset until the kinetochores of all mitotic chromosomes become properly attached to spindle microtubules (Lara-Gonzalez et al., 2012). The Mad1 and Mad2 proteins are key SAC components. When active, the SAC sequesters the Cdc20 protein in a complex called the mitotic checkpoint complex (MCC). Incorporation into the MCC prevents Cdc20 from fulfilling its role as an activator of the anaphase-promoting complex/cyclosome (APC/C), a ubiquitin ligase that mediates the degradation of important mitotic substrates, including Cyclin B and Securin.

Interestingly, many SAC components associate with NPCs during interphase, including Mad1 and Mad2 (Campbell et al., 2001; Wozniak et al., 2010). In yeast, Mad1 directs a structural reorganization of the NPC after SAC activation to reveal a karyopherin binding site on the nucleoporin Nup53 (Makhnevych et al., 2003). This binding site is distinguished both by the fact that it is specific to an individual karyopherin, Kap121p, and by the fact that it is much higher affinity than binding sites within FG-Nup domains used in NPC transit. Nup53 binding causes Kap121p transport complexes to become arrested; this mechanism is termed the Kap121p-transport inhibitory pathway (KTIP).

Cairo et al. (2013) examined the role of Mad1 in the KTIP and the mitotic consequences of this pathway. They monitored Kap121p activity through the localization of a synthetic Kap121p import substrate (NLSgrpl4-GFP). They observed that KTIP activation is directly sensitive to the presence of unattached kinetochores per se, rather than simply mitotic delay. Moreover, Mad1 was essential for KTIP activity, whereas Mad2 was dispensable. This finding is remarkable because Mad1 and Mad2 act in tandem within the SAC (Lara-Gonzalez et al., 2012); the absence of a requirement for Mad2 indicates that the KTIP does not require the Mad1-Mad2 complex or MCC production. Consistent with this idea, Mps1 kinase overproduction results in ectopic SAC activation in the absence of unattached kinetochores and Mad1/Mad2-dependent APC/C inhibition, but not KTIP induction.

Notably, recruitment of Mad1 to kinetochores is not sufficient in itself for KTIP activity, because Mps1 overexpression is accompanied by the accumulation of an immobile population of Mad1 at bi-oriented kinetochores. Rather, KTIP signaling requires active shuttling of Mad1 between unattached kinetochores and NPCs: KTIP activity was lost in mad1 mutants lacking either the capacity to bind Nup53 or kinetochores. KTIP was also lost after the inhibition of Mad1 movement by the inactivation of another karyopherin, Xpo1p (Scott et al., 2009). These data suggest a model in which Mad1 acquires an enhanced ability to induce the KTIP through a transient association with unattached kinetochores and in which it acts through direct association to the NPC.

In addition to this activation mechanism, the authors investigated the target and physiological role of the KTIP. They examined the import of Glc7p, a protein phosphatase and Ipl1 antagonist. The Ipl1 kinase is the yeast ortholog of Aurora B; it promotes correct spindle assembly by destabilizing fragile microtubule-kinetochore attachments (Lampson and Cheeseman, 2011). They found that Glc7p acts as expected for a KTIP substrate: it accumulated within nuclei in a Kap121-dependent manner but was redistributed to the cytoplasm in a Mad1-dependent manner upon microtubule depolymerization with the drug nocodazole. Interestingly, although ipl1-321 cells retain the capacity to undergo SAC arrest and to target Mad1 to detached kinetochores, they lack the capacity for KTIP activation, suggesting that the Ipl1p kinase is required in this pathway. Together, these data suggest that Ipl1
triggers KTIP activation, which in turn enhances Ipl1 effectiveness by decreasing the nuclear concentration of its antagonist, Glc7. This model does not exclude the possibility that trafficking of other Kap121 cargo molecules may play important roles during checkpoint arrest.

These findings provide an elegant example of how nuclear trafficking can be specifically adjusted in response to physiological stimuli. Of course, important questions remain regarding this pathway. In particular, Mad1 is constitutively associated to NPCs throughout the cell cycle, but it only invokes the KTIP under conditions where it is cycling on and off of unattached kinetochores in an Xpo1-dependent manner. The features that Mad1 acquires during its visit to the kinetochore remain mysterious, as does the cycling process itself. As Cairo et al. (2013) note, it is attractive to speculate that phosphorylation of Mad1 or one of its binding partners by Ipl1 could contribute to Mad1’s activity in the KTIP. At the same time, it remains to be understood how this kinetochore-enhanced population of Mad1 promotes NPC reorganization, whereas the constitutively bound Mad1 population does not.

Yeast undergoes a closed mitosis, with an intact NE, whereas metazoans disassemble their NE during each cell division. At face value, this fact argues against the operation of a KTIP-like mechanism during the metazoan metaphase, because nuclear compartmental identity is lost during this interval. Nevertheless, it is imaginable that nucleoporins and karyopherins could form stable, Mad1-dependent complexes in response to mitotic signals and that these complexes could sequester or inactivate key mitotic factors (Figure 1).

Figure 1. Mad1 Remodels Nucleoporin Complexes during SAC Arrest
(A) In yeast, Mad1 associates with unattached kinetochores (KT), promoting Mad1’s distinct roles in SAC signaling and in Ipl1p-dependent KTIP activation. Mad1 cycles from unattached kinetochores to the NPC, where it interacts with Nup53 and causes reconfiguration of nucleoporin interactions to arrest Kap121 transport of Glc7 and possibly other cargo proteins.

(B) During vertebrate mitosis, NPCs disassemble into complexes containing multiple nucleoporins. As a speculative model, it is possible that kinetochore-activated Mad1 (Mad1*) could cause the rearrangement of those complexes and the unmasking of high-affinity Karyopherin binding sites (Nup*). The formation of stable karyopherin-cargo complexes in association with these sites could alter the localization or function of karyopherin-bound cargo proteins (Cargo*).

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