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Mitosis-Specific Regulation of Nuclear Transport by the Spindle Assembly Checkpoint Protein Mad1p

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SUMMARY

Nuclear pore complexes (NPCs) and kinetochores perform distinct tasks, yet their shared ability to bind several proteins suggests their functions are intertwined. Among these shared proteins is Mad1p, a component of the yeast spindle assembly checkpoint (SAC). Here we describe a role for Mad1p in regulating nuclear import that employs its ability to sense a disruption of kinetochore-microtubule interactions during mitosis. We show that kinetochore-microtubule detachment arrests nuclear import mediated by the transport factor Kap121p through a mechanism that requires Mad1p cycling between unattached, metaphase kinetochores and binding sites at the NPC. This signaling pathway requires the Aurora B-like kinase Ipl1p, and the resulting transport changes inhibit the nuclear import of Glc7p, a phosphatase that acts as an Ipl1p antagonist. We propose that a distinct branch of the SAC exists in which Mad1p senses unattached kinetochores and, by altering NPC transport activity, regulates the nuclear environment of the spindle.

INTRODUCTION

Most molecules enter and leave the nucleus through portals in the nuclear envelope (NE) that are formed by massive macromolecular structures termed nuclear pore complexes (NPCs). Because NPCs are impermeable to most macromolecules, transport is facilitated by a group of proteins, termed nuclear transport factors (NTFs), that traverse the central channel of the pores by interacting with NPC proteins (termed nucleoporins or Nups) containing repetitive phenylalanine-glycine (FG) repeats. These interactions are sufficient to partition NTFs into the NPC channel but are of low enough affinity to allow movement of NTFs between the many FG-containing binding sites they encounter as they escort their attached cargos through the NPC (reviewed in Wente and Rout, 2010).

It is thought that during most constitutive nuclear transport events, NTFs primarily interact with FG repeats (reviewed in Wente and Rout, 2010). However, other NTF binding sites lacking FG repeats have been identified in several Nups. These sites are thought to be of higher affinity and specific for individual NTFs (Fontoura et al., 2000; Lusk et al., 2002; Pyhtila and Rexach, 2003). In *Saccharomyces cerevisiae*, a site present in Nup53p binds specifically to the NTF Kap121p and functions to regulate nuclear protein import mediated by Kap121p (Makhnevych et al., 2003). In asynchronous cells, the Kap121p binding domain of Nup53p is masked by its interaction with its neighbor Nup170p. However, when cells are arrested in mitosis, NPC structural rearrangements cause the release of Nup170p from Nup53p, exposing the Kap121p binding site on Nup53p. *S. cerevisiae* cells undergo a "closed" mitosis (i.e., their NEs remain intact), and a consequence of these changing interactions is the inhibition of Kap121p-mediated protein import. Binding to Nup53p slows the movement of Kap121p and its cargo through the NPC while leaving import mediated by other NTFs unaffected.

The identification of the Kap121p transport inhibitory pathway (KTIP) is one of several observations that have established alternative functions for Nups during mitosis. Other important examples have arisen from studies in metazoan cells wherein a subset of Nups binds to kinetochores (KTs) following NE breakdown in mitosis (reviewed in Wozniak et al., 2010). Among these are Nup358 (RanBP2) and members of the Nup107-160 subcomplex, which, intriguingly, contains members that share a domain architecture with the KT-associated RZZ (Rod:Zwilch:Zw10) complex, suggesting a shared ancestry (Civril et al., 2010). The role of Nups during mitosis appears complex; studies suggest they function in centrosome positioning, normal spindle morphology, and KT function (reviewed in Wozniak et al., 2010). Several lines of evidence point to a role for these proteins in the proper attachment of spindle microtubules (MTs) to KTs (Joseph et al., 2004; Platani et al., 2009; Zuccolo et al., 2007). Molecular details that are beginning to emerge include the observations that the Nup107-160 complex plays a role in the centromeric localization of the Aurora B kinase-containing chromosome passenger complex and in the facilitation of KT-MT interactions through its association with the γ-tubulin ring complex (Platani et al., 2009: Mishra et al., 2010).

A functional relationship between NPCs and KTs has also been inferred as a result of their shared interactions with two components of the spindle assembly checkpoint (SAC), Mad1 and Mad2, in both yeast and metazoan cells (Campbell et al., 2001; Iouk et al., 2002). These proteins are bound to NPCs during interphase but are recruited to KTs lacking spindle MT attachments during mitosis (reviewed in Chan et al., 2005). At unattached KTs, Mad1 and Mad2 function to propagate a metaphase

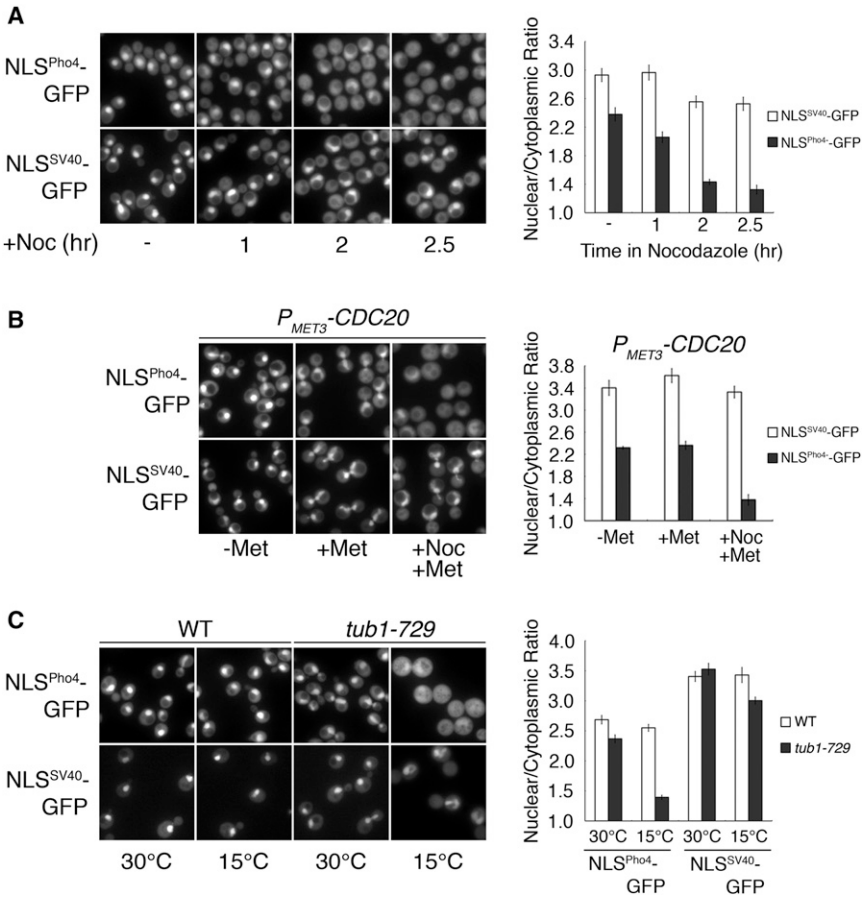

Figure 1. MT Destabilization during Metaphase Activates the KTIP

Localization of transport reporters imported by Kap121p (NLS^{Pho4}-GFP) or Kap60p-Kap95p (NLS^{SV40}-GFP) was assessed via epifluorescence microscopy under the indicated conditions (left panels). Bar graphs represent a quantification of the reporter signals in cells visualized via microscopy and are given as nuclear/cytoplasmic fluorescence intensity ratios (right panels; $n \geq 50$ cells). Error bars express standard error.

(A) WT cells expressing *NLS^{Pho4}-GFP* or *NLS^{SV40}-GFP* were grown to mid-log phase (−), treated with nocodazole, and analyzed at the indicated times (+Noc).

(B) *P_{MET3}-CDC20 NLS^{Pho4}-GFP* and *P_{MET3}-CDC20 NLS^{SV40}-GFP* cells were grown in media lacking methionine (−Met). Methionine was added to the media alone (+Met) or with nocodazole (+Met +Noc), and the cultures were incubated for 2.5 hr.

(C) WT (DF5) and *tub1-729* containing strains expressing *NLS^{Pho4}-GFP* or *NLS^{SV40}-GFP* were incubated at a permissive (30°C) or nonpermissive (15°C) temperature for *tub1-729* growth.

arrest signal through activation of the soluble mitotic checkpoint complex (MCC), which inhibits the anaphase-promoting complex/cyclosome (APC/C) (reviewed in Musacchio and Salmon, 2007). Here we show that the ability of yeast Mad1p to sense KT-MT detachment is also used to regulate nuclear transport during SAC arrest. We present data for a model whereby Mad1p, in response to the activity of the yeast Aurora B-like kinase Ipl1p, triggers the KTIP. Furthermore, we show that the KTIP contributes to establishing a nuclear environment that supports Ipl1p function by reducing nuclear levels of the Glc7p phosphatase.

RESULTS

Destabilization of Microtubules during Metaphase Alters Nuclear Transport

Treatment of yeast cultures with MT-destabilizing drugs (e.g., nocodazole) disrupts the mitotic spindle and its attachment to KTs, leading to activation of the SAC and metaphase arrest. In arrested cells, structural rearrangements occur within NPCs that inhibit translocation of Kap121p through the NPC and import of its cargos (Makhnevych et al., 2003). These events are readily monitored by a loss in the nuclear accumulation of a Kap121p-specific import reporter, such as NLS^{Pho4}-GFP (Figure 1A). Using this assay, we investigated the mechanism underlying the nocodazole-induced activation of the KTIP. We tested whether metaphase arrest, independent of depolymerization of the mitotic spindle, was sufficient for activating the KTIP. For these experiments, Cdc20p was depleted to prevent APC/C activation, which in turn induces metaphase arrest in the presence of an intact spindle (Uhlmann et al., 2000; data not shown). Under these conditions, import of NLS^{Pho4}-GFP was robust, suggesting that metaphase arrest was not sufficient for activating the KTIP (Figure 1B). However, if the Cdc20p-depleted cells were treated with nocodazole, nuclear accumulation of NLS^{Pho4}-GFP was lost, consistent with the inhibition of Kap121p-mediated import. In contrast, import mediated by other NTFs (Kap60p-Kap95p) was not affected (Figure 1B, NLS^{SV40}-GFP). These results were consistent with the conclusion that MT depolymerization activates the KTIP. In support of this idea, we also observed that cells harboring a cold-sensitive α-tubulin mutation (*tub1-729*), which exhibit MT defects at the nonpermissive temperature (15°C) and arrest in metaphase (Schatz et al., 1988), also induce the KTIP (Figure 1C). Moreover, inhibition of Kap121p transport appears restricted to periods of mitosis prior to chromosome segregation. Cells arrested in G1 phase, S phase, or late anaphase and then treated with nocodazole exhibit no inhibition of NLS^{Pho4}-GFP import (Figure S1 available online). Thus, the signal inducing the KTIP arises as a consequence of MT destabilization during metaphase.

Loss of MT-KT Attachment Activates the KTIP

Studies characterizing the *tub1-729* mutant reported that the mutant protein destabilized KT-MT interactions, leading to KT detachment (Abruzzi et al., 2002). Therefore, we investigated