Cell

A Network of Nuclear Envelope Membrane Proteins Linking Centromeres to Microtubules

Megan C. King,[1,*] Theodore G. Drivas,[1] and Günter Blobel[1]
[1]Laboratory of Cell Biology, The Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
*Correspondence: mking@rockefeller.edu
DOI 10.1016/j.cell.2008.06.022

SUMMARY

In the fission yeast *S. pombe*, nuclei are actively positioned at the cell center by microtubules. Here, we show that cytoplasmic microtubules are mechanically coupled to the nuclear heterochromatin through proteins embedded in the nuclear envelope. This includes an integral outer nuclear membrane protein of the KASH family (Kms2) and two integral inner nuclear membrane proteins, the SUN-domain protein Sad1 and the previously uncharacterized protein Ima1. Ima1 specifically binds to heterochromatic regions and promotes the tethering of centromeric DNA to the SUN-KASH complex. In the absence of Ima1, or in cells harboring mutations in the centromeric Ndc80 complex, inefficient coupling of centromeric heterochromatin to Sad1 leads to striking defects in the ability of the nucleus to tolerate microtubule-dependent forces, leading to changes in nuclear shape, loss of spindle pole body components from the nuclear envelope, and partial dissociation of SUN-KASH complexes. This work highlights a framework for communication between cytoplasmic microtubules and chromatin.

INTRODUCTION

To alter nuclear position, the cytoskeleton must exert sufficient force on the nucleus to drive its movement, while, simultaneously, the nucleus must buffer these forces so that its entire mass is efficiently propelled through the cytoplasm without affecting nuclear integrity. How the cytoskeleton and nuclear envelope (NE) interface to achieve this balance is poorly understood. The first molecular details of the proteins that form this interface have come from the identification and characterization of the conserved SUN and KASH domain-containing proteins (Figure 1A). In *metazoa*, integral outer nuclear membrane (ONM) proteins possessing KASH domains link the NE to all major classes of cytoskeletal elements (Starr and Fischer, 2005; Wilhelmsen et al., 2006) and tether the primary microtubule-organizing center (MTOC), the centrosome, to the NE (Malone et al., 2003). In turn, KASH domain proteins bind (in the NE lumen) to integral inner nuclear membrane (INM) proteins containing the conserved SUN domain (Tzur et al., 2006). Thus, integral membrane proteins of the NE link the cytoplasmic cytoskeleton to the nuclear interior. Disruption of SUN-KASH interactions leads to expansion of the NE lumen, illustrating the importance of this complex in maintaining nuclear structure (Crisp et al., 2006). However, given the large forces exerted on SUN-KASH complexes (potentially up to 100 *p*N during mammalian cell migration [Szabo et al., 2004]), it is likely that additional macromolecules reinforce these NE-cytoskeletal connections. Candidates include additional NE membrane proteins, soluble proteins that reside in the NE lumen, and structural elements within the nucleus. In *metazoa*, the nuclear lamina, a meshwork of the intermediate filament lamins, provides one important structural scaffold to which SUN domain proteins are coupled (Crisp et al., 2006; Gruenbaum et al., 2005; Haque et al., 2006).

We have chosen to further study the molecular linkages between the cytoskeleton and the NE in the fission yeast, *Schizosaccharomyces pombe*. Several aspects of *S. pombe* physiology are conserved with that of metazoans and are notably absent in the budding yeast, *S. cerevisiae*. First, the primary MTOC in *S. pombe* (termed the spindle pole body, or SPB), is tethered to the ONM during interphase (Figure 1B) (Ding et al., 1997). Second, *S. pombe* contain clear homologs of both SUN and KASH domain proteins. Tethering of the SPB to the ONM likely involves one or both *S. pombe* KASH domain proteins, named Kms1 and Kms2 (Miki et al., 2004; Niwa et al., 2000; Shimanuki et al., 1997), although the contribution of these proteins to the SPB-NE interface during interphase has not been examined in detail. Kms1 and Kms2 interact with the *S. pombe* SUN domain protein, Sad1 (Miki et al., 2004), thus providing a means of coupling the SPB to the nuclear interior. Sad1 is required for SPB duplication at the onset of mitosis (Hagan and Yanagida, 1995) and oscillates along the NE in a microtubule-dependent fashion, suggesting that it is coupled to the SPB (Tran et al., 2001). Importantly, although Sad1 colocalizes with SPB components at the level of the light microscope, Sad1 is an integral INM protein. Therefore, Sad1 defines a specific region of the NE to which the SPB is attached (Figure 1B). We call this discrete region of the NE the MTOC attachment site, or MAS. Because the MAS spans both the INM and ONM, its components include inner MAS proteins (Imas) and outer MAS proteins (Omas). In addition to the SPB, a second type of interface between the NE and microtubules (MTs) exists in *S. pombe*, called the interphase MTOC

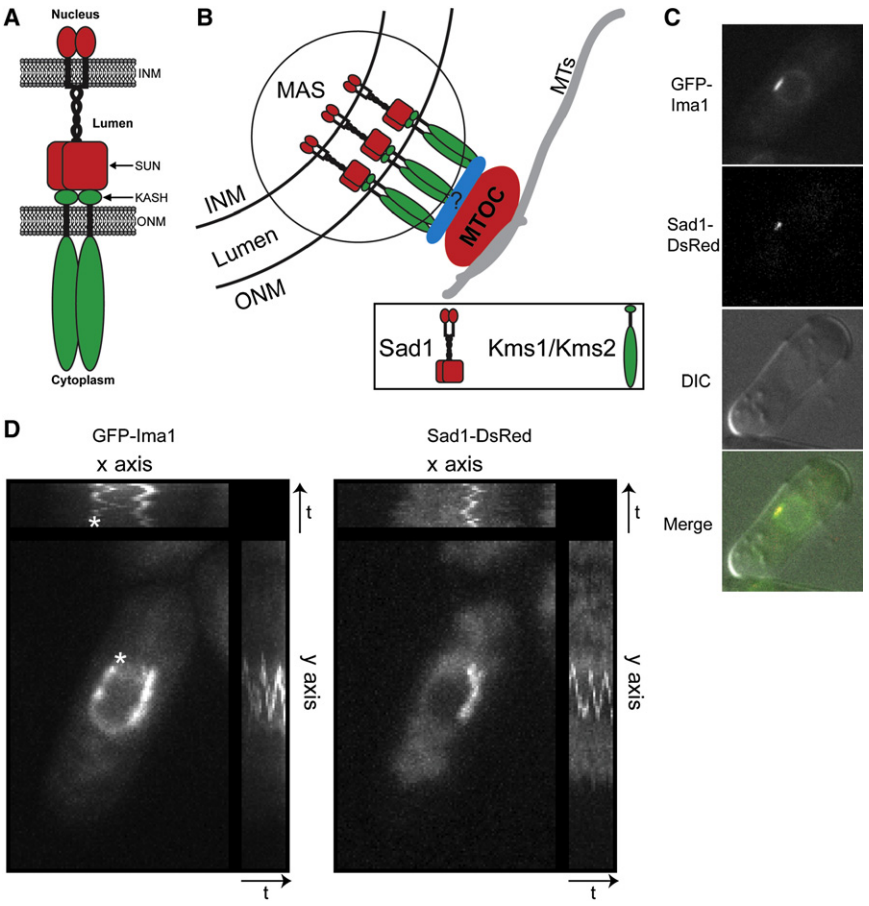

Figure 1. Ima1 Is a Conserved, Integral Inner Nuclear Membrane Protein that Is Enriched at the Site of MTOC Attachment

(A) Cartoon of SUN-KASH interactions at the nuclear envelope (NE). The SUN domain protein (red) is integrated into the inner nuclear membrane (INM). The protein has an N-terminal nucleoplasmic domain followed by a single transmembrane segment, a lumenal coiled-coil region, and the conserved SUN domain. The KASH domain protein (green) is integrated into the outer nuclear membrane (ONM). The variable N-terminal cytoplasmic domain interacts with cytoskeletal elements, and the C terminus contains the KASH domain, which is composed of the transmembrane segment (black) and a small lumenal tail (labeled KASH). Both proteins are shown as homodimers.

(B) Diagram of the microtubule-organizing center (MTOC) attachment site (MAS) at the NE of *S. pombe* (circled). SUN domain protein Sad1 (red) and KASH domain proteins Kms1 and/or Kms2 (green) interact within the lumen of the NE to link the MTOC to the NE either directly or through an as yet unidentified adaptor protein(s) (blue). MTs, microtubules.

(C) GFP-Ima1 localizes to the NE and MAS. Fluorescent micrographs, DIC, and merged images are shown of a representative single cell of strain MKSP58 expressing GFP-Ima1 and Sad1-DsRed.

(D) GFP-Ima1 comigrates with Sad1-DsRed as the SPB oscillates along the NE. A composite image of time-lapse frames taken every 15 s for 5 min of one MKSP58 cell (see above) expressing GFP-Ima1 and Sad1-DsRed. The asterisk indicates a second focus of GFP-Ima1 that oscillates along the NE. The time axes are indicated by the arrow and "t."

(iMTOC; Sawin and Tran, 2006). Although poorly characterized, iMTOCs play important roles in regulating the interphase MT architecture and also contain the MAS protein Sad1 (Tran et al., 2001).

Here, we describe the function of a previously uncharacterized integral membrane protein (Ima1) that resides at the inner face of the MAS and supports the buffering of MT forces at the NE. Surprisingly, Ima1 achieves this role by linking the MAS to heterochromatic regions within the nucleus. By examining cells lacking Ima1, we uncover a general role for the centromeric heterochromatin in buffering MT-dependent forces on the NE. This provides a framework for functional cooperation and communication between the cytoplasmic cytoskeleton and chromatin throughout the cell cycle.

RESULTS

Ima1 Is a Conserved Inner Nuclear Membrane Protein

A proteomic approach in mammalian cells first suggested that Ima1 is a NE membrane protein (NET5; Schirmer et al., 2003). A single gene homologous to *IMA1* is present in all *metazoa* and the fission yeast *S. pombe* (systematic name SPCC737.03c) but is absent in the budding yeast *S. cerevisiae*. Across species, an N-terminal cysteine-rich region is the most highly conserved domain of Ima1 (Figure S1 available online).

Consistent with the expectation that Ima1 resides at the NE, a GFP fusion of Ima1 localizes specifically to the nuclear rim in *S. pombe* (Figure 1C). Using immunoelectron microscopy and antibodies directed against the GFP tag, we found that the majority of gold particles associated with the NE are found along the INM (90%, n = 40; Figure S1), suggesting that Ima1 resides at the inner face of the NE. The presence of the GFP antigen within the nucleus combined with glycosylation analysis (Figure S2) suggests that *S. pombe* Ima1 adopts the topology indicated in Figure S2. In all species, the C-terminal hydrophilic domain contains a nuclear localization signal, which likely promotes trafficking of Ima1 to the INM (Lusk et al., 2007).

At the nuclear rim, GFP-Ima1 is enriched in distinct regions of the NE (Figure 1C). Using time-lapse imaging of live cells, we found that GFP-Ima1-enriched regions of the NE are dynamic and oscillate along the NE largely parallel to the long axis of the cell (Movie S1). On average, Ima1 foci undergo one full oscillation (returning to the same location at the NE) in 189 ± 50 s (n = 25), with the average oscillation being 1.7 ± 0.7 μm in size. Such oscillations are reminiscent of the movement of the SPB as it is pushed by polarized MT bundles (Hagan et al., 1990; Tran et al., 2001), suggesting that GFP-Ima1 may enrich in the MAS. Consistent with this, such GFP-Ima1-rich regions frequently colocalize with the MAS protein, Sad1 (Figure 1C). To better illustrate the amplitude and path of GFP-Ima1 oscillations and investigate their association with the MAS, we created a single, composite image of GFP-Ima1 and Sad1-DsRed localization over 5 min by overlaying an entire time-lapse series