



Lincoln's Inn Fields

Cell Regulation

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Chromosome abnormalities, such as aneuploidy and polyploidy, are most common features among a wide variety of cancer cells. Although whether chromosome instability is a cause or a consequence of tumorigenesis remains under scrutiny, exploring the molecular mechanisms underlying accurate chromosome segregation is one of the most important research fields in basic tumour biology. Mitotic spindle microtubules have been specifically evolved to perform this critical job. Not surprisingly some widely used cancer drugs target microtubules per se (e.g. Taxol) and many new drugs that inhibit other factors that play regulatory roles in microtubule dynamics and chromosome segregation are currently under the way of clinical trials. Our laboratory has been working on the mechanisms of how bipolar spindles are formed using fission yeast as a model system. During 2008, we have made two main achievements as outlined below.

Spatio-temporal regulation of Kinesin 8 Klp5 and Klp6

Kinesins were originally identified as motor proteins that use energy gained from ATP hydrolysis to power processive movement along microtubules. Kinesins consist of a large protein family and recently, it has become clear that several kinesin subfamilies (namely kinesin-8, -13 and -14) possess microtubule depolymerising activities and are, therefore, important regulators of microtubule dynamics. Fission yeast Klp5 and Klp6 belong to the kinesin-8 family. Klp5 and Klp6 are not essential for cell division, but deletion mutants exhibit hyper-stable microtubules with defects in chromosome congression and segregation. Most kinesins function as homodimers. Klp5 and Klp6 are, however, unique members of the kinesin-8 family, as they are the only members reported thus far that form a heterodimer, which is essential for Klp5/6 function.

We have investigated the importance of Klp5/6 dimerisation. We show that Klp5 and Klp6 are mutually dependent on each other for nuclear mitotic localisation. During interphase, these two molecules are exported to the cytoplasm. In sharp contrast, during mitosis, Klp5 and Klp6 remain in the nucleus, which requires the existence of each counterpart (Figure 1). Canonical nuclear localisation signal (NLS) is identified in the non-kinesin C-terminal regions. Intriguingly individual NLS mutants (NLSmut) exhibit loss-of-function phenotypes, suggesting that Klp5 and Klp6 enter the nucleus separately. Indeed whilst neither Klp5-NLSmut nor Klp6-NLSmut enters the nucleus, wild type Klp6 or Klp5 respectively does so with different kinetics. Remarkably chimera strains containing only the N-terminal Klp5 kinesin domains cannot disassemble interphase microtubules during mitosis, leading to the coexistence of cytoplasmic microtubules and nuclear spindles with massive chromosome mis-segregation. In this strain a marked reduction of microtubule dynamism, including microtubule catastrophe/rescue frequency, shrinkage rate and dynamicity is evident. These results prompt us to propose that Klp5 and Klp6 play a vital role in promoting microtubule

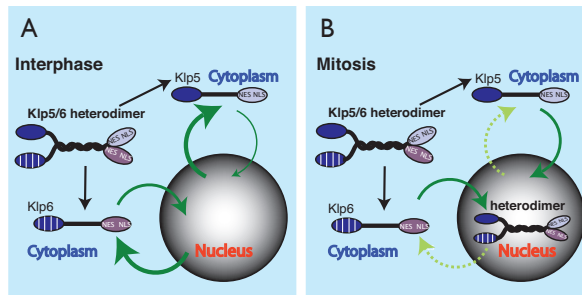


Figure 1. Cell-cycle dependent regulation of Klp5/Klp6 nuclear transport. Klp5 and Klp6 shuttle between the cytoplasm and the nucleus. Nuclear import occurs as monomers (or homodimers) independently of their partner. During interphase (A), Klp5 or Klp6 enters the nucleus in an nuclear import signal (NLS) dependent manner, but is rapidly exported via an LMB-sensitive, therefore Crm1/exportin-1, pathway. During mitosis (B), on the other hand, Klp5 and Klp6 are retained in the nucleus, possibly by forming a heterodimer. This could be achieved due to inhibitory masking of individual nuclear export signals (NESs) or enhanced Klp5's NLS activity or both. It is also possible that Klp5/6 heterodimers are anchored to the nuclear spindles, leading to prevention of nuclear export.

dynamics, which is essential for the spatiotemporal control of microtubule morphogenesis (Unsworth *et al.*, 2008, *Mol. Biol. Cell* 2008,19:5104-15).

We have previously shown that fission yeast homologues of TACC (Transforming Acidic Coiled Coil protein) and TOG (Tumour Overexpressing Gene), Alp7 and Alp14 respectively, form a complex, which shuttles between the cytoplasm and the nucleus (Sato and Toda, 2007, *Nature*, 44, 334-337), reminiscent of Klp5/6 localisation patterns. Importantly Alp7/14 and Klp5/6 regulate microtubule dynamics in an antagonistic but coordinated fashion as microtubule stabilising and depolymerising factors, respectively, throughout the cell cycle. This fact raises an interesting possibility that common regulatory mechanisms, involving the Ran-GTPase signaling pathway (a central player for nuclear transport and spindle microtubule assembly), exist to ensure proper spatiotemporal control of microtubule morphogenesis. Further elucidation of such molecular mechanisms would lead to a better understanding of microtubule-dependent cell morphogenesis, bipolar mitotic spindle formation and coordinated chromosome segregation.

Mitotic role of the Dam1/DASH complex

The kinetochore, a specialised proteinaceous structure on the centromeric DNA, must attach to spindle microtubules in a bipolar manner to ensure high fidelity sister chromatid segregation. Budding yeast Dam1 (or DASH) complex is an outer kinetochore complex that consists of 10 essential proteins, Ask1, Duo1, Dam1, Dad1-4, Hsk3, Spc34 and Spc19. This complex is postulated to play an important role in facilitating the attachment of the spindle microtubules to the kinetochore in metaphase, but how it regulates microtubule

dynamics remains unknown. Unlike budding yeast, the fission yeast complex is non-essential, however it promotes bipolar microtubule attachment in conjunction with microtubule-depolymerising kinesin-8 Klp5 and Klp6 (see above). We screened for *dam1* temperature sensitive mutants in a *kfp5* null background and identified the *dam1-A8* mutant. *dam1-A8kfp5* double mutant cells display massive chromosome missegregation with lagging chromosomes and monopolar attachment of sister chromatids to one SPB (Spindle Pole Body, fungi equivalent of the animal centrosome). Unexpectedly contrary to a *dam1*-deletion mutant that is hypersensitive to microtubule-destabilising drugs, *dam1-A8* is resistant and furthermore the temperature sensitivity of *dam1-A8kfp5* is rescued by addition of these drugs. This indicates that the hyper-stabilised rigidity of kinetochore-spindle mal-attachments is the primary cause of lethality (Figure 2). Our result shows that fine-tuning of Dam1 activity is essential for chromosome bi-orientation. (Griffiths *et al.*, 2008, *Biochem. Biophys. Res. Commun.* 368, 670–676).

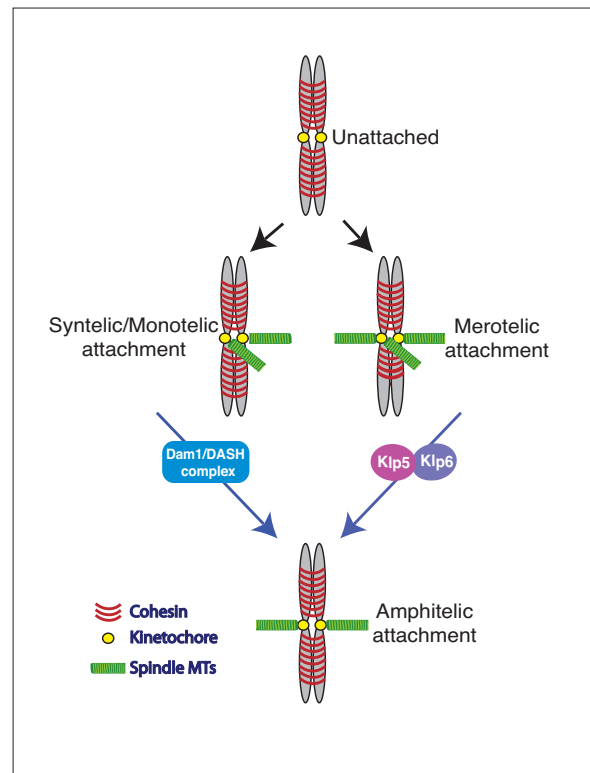


Figure 2. Mitotic roles of the Dam1 complex. Resolution of a merotelic kinetochore (attachment to both spindle poles by one or more microtubules) may occur in a stochastic manner, in which microtubule depolymerising Klp5 and Klp6 are required for the processivity of this reaction. Monotelic or syntelic kinetochore (attachment of both kinetochores to the same pole or only one kinetochore to one pole, respectively) would be resolved in a Dam1 complex dependent manner, leading to proper end-on amphitelic configurations.

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