A new cap for kinetochore fibre minus ends

Sabine Petry and Ronald D. Vale

In mitotic spindles, each sister chromatid is directly attached to a spindle pole through microtubule bundles known as kinetochore fibres. Microspherule protein 1 (MCRS1) is now shown to support spindle assembly by localizing to the minus ends of kinetochore fibres and protecting them from depolymerization.

The mitotic spindle, a dynamic ensemble of microtubules, microtubule-associated proteins and motor proteins, aligns sister chromatids in the middle of the cell before segregating them to opposite poles[1]. Mitotic spindle assembly is initiated by microtubule polymerization at chromosomes through a RanGTP gradient, and at centrosomes when present[1]. An important component of this machinery is the kinetochore fibres (K-fibres), stable microtubule bundles that connect the kinetochore on each sister chromatid with one of the two spindle poles. The aligned sister chromatids separate at the onset of anaphase and K-fibres decrease in length while maintaining their attachment to the kinetochore, thereby moving each sister chromatid to opposite poles. Considerable work has been devoted to understanding how microtubule plus ends attach to kinetochores and how their dynamics are regulated[2]. However, much less is known about the minus ends of K-fibres, the dynamics of which are likely to be subject to complex regulation. On page 1406 of this issue, Meunier and Vernos now show that MCRS1 plays an important role in mitotic spindle formation by localizing to K-fibre minus ends to regulate their stability[3].

MCRS1 is known to be involved in the activation of ribosomal RNA transcription[4], the regulation of RNA polymerase-II-dependent transcription[5] and the inhibition of telomerase activity[6] (Fig. 1a). Based on its reported localization to nucleoli[4] and centrosomes[7], Meunier and Vernos postulated that MCRS1 could be a RanGTP-regulated spindle-assembly factor. Investigation of MCRS1 localization led to the striking observation that during mitosis MCRS1 is localized to the minus-end region of K-fibres, but not to other spindle microtubules. MCRS1 depletion by RNA interference (RNAi) resulted in a striking mitotic phenotype: spindle assembly was significantly delayed and cells remained in mitosis for more than 8 hours, in contrast to control cells, which completed cell division in 1 hour. During this time, MCRS1-depleted spindles cycled through phases of collapse and reassembly while displaying an activated spindle checkpoint, a safety mechanism that prevents chromosome mis-segregation and aberrant mitotic progression.

To investigate the mechanism by which MCRS1 contributes to spindle assembly, the authors treated mitotic cells with the microtubule-depolymerizing drug nocodazole and studied microtubule regrowth into astral microtubule arrays, termed asters, following drug washout. The results showed a marked defect in microtubule assembly around chromatin but not centrosomes, a phenotype that resembles the loss of function of TPX2, a factor that promotes microtubule growth around chromosomes[8]. Consistent with this phenotype, MCRS1 was localized within the centre of chromosomal microtubule asters shortly after nocodazole washout, but was absent from microtubule asters at centrosomes. In contrast, microtubule nucleating and stabilizing proteins, such as γ-tubulin and TPX2, localize to both centrosomal and chromosomal asters in similar experiments[8]. These data suggest that MCRS1 is specifically involved in chromosomal microtubule assembly.

MCRS1 could promote the growth of chromosomal asters either by nucleating new microtubules or by stabilizing pre-existing ones. Several MCRS1-depletion phenotypes support the latter mechanism and indicate that MCRS1 specifically stabilizes microtubule minus ends. The authors observed that in the absence of MCRS1, K-fibres could still form in monopolar spindles (where they could be most clearly visualized), but were 40% shorter than K-fibres of control cells. In addition, when exposed to cold treatment, which depolymerizes microtubules, the shorter K-fibres of MCRS1-depleted cells depolymerized more rapidly than those of wild-type cells, suggesting that they were less stable. When assessing poleward flux, a hallmark of mitotic and meiotic spindles resulting from microtubule minus-end depolymerization at the poles coupled with plus-end polymerization[9], the authors discovered that in the absence of MCRS1, tubulin subunits in spindle microtubules fluxed more rapidly towards the spindle poles. Moreover, MCRS1-depleted cells had shorter spindles and increased distances between kinetochore pairs, indicating an increase in pulling forces on K-fibres[10]. Taking into account prior work on kinetochore microtubules[11], the faster poleward flux, a shorter spindle and increased kinetochore distance all point to faster minus-end depolymerization in the absence of MCRS1. These results, combined with the localization of MCRS1 at K-fibres, imply that MCRS1 is protecting K-fibre minus ends against microtubule depolymerization.

How might MCRS1 protect the minus ends of K-fibres? The kinesin-13 MCAK is a microtubule depolymerase that is important for the control of microtubule stability, the rate of poleward flux, and overall spindle length[12]. Patronin, a microtubule minus-end capping protein, is known to block the depolymerizing action of kinesin-13 depolymerases[13], and Meunier and Vernos suggest a similar mechanism for the function of MCRS1 at K-fibres. The authors demonstrated that as was previously shown for Patronin, double RNAi depletion of kinesin-13 and MCRS1 could rescue at least a subset of the MCRS1 defects *in vivo*. Furthermore, kinesin-13 reduced centrosome-nucleated microtubules in *Xenopus* egg-extract experiments, but was unable to do so in the presence of MCRS1. In addition, although MCRS1 and kinesin-13 did not interact *in vitro*, purified MCRS1 could displace kinesin-13 from microtubules in a microtubule-pelleting assay. Based on these observations the authors proposed that MCRS1 helps to stabilize K-fibres by suppressing the activity of kinesin-13 at microtubule minus ends (Fig. 1b).

Finally, Meunier and Vernos investigated whether MCRS1 is regulated by RanGTP, which was the question that prompted the study in the first place. A well-studied system for examining the effects of RanGTP is *Xenopus* egg extract arrested in meiosis II (ref. 14). Addition of a constitutively active Ran mutant induces chromatin-mediated generation of microtubules by releasing spindle assembly factors, such as TPX2, from

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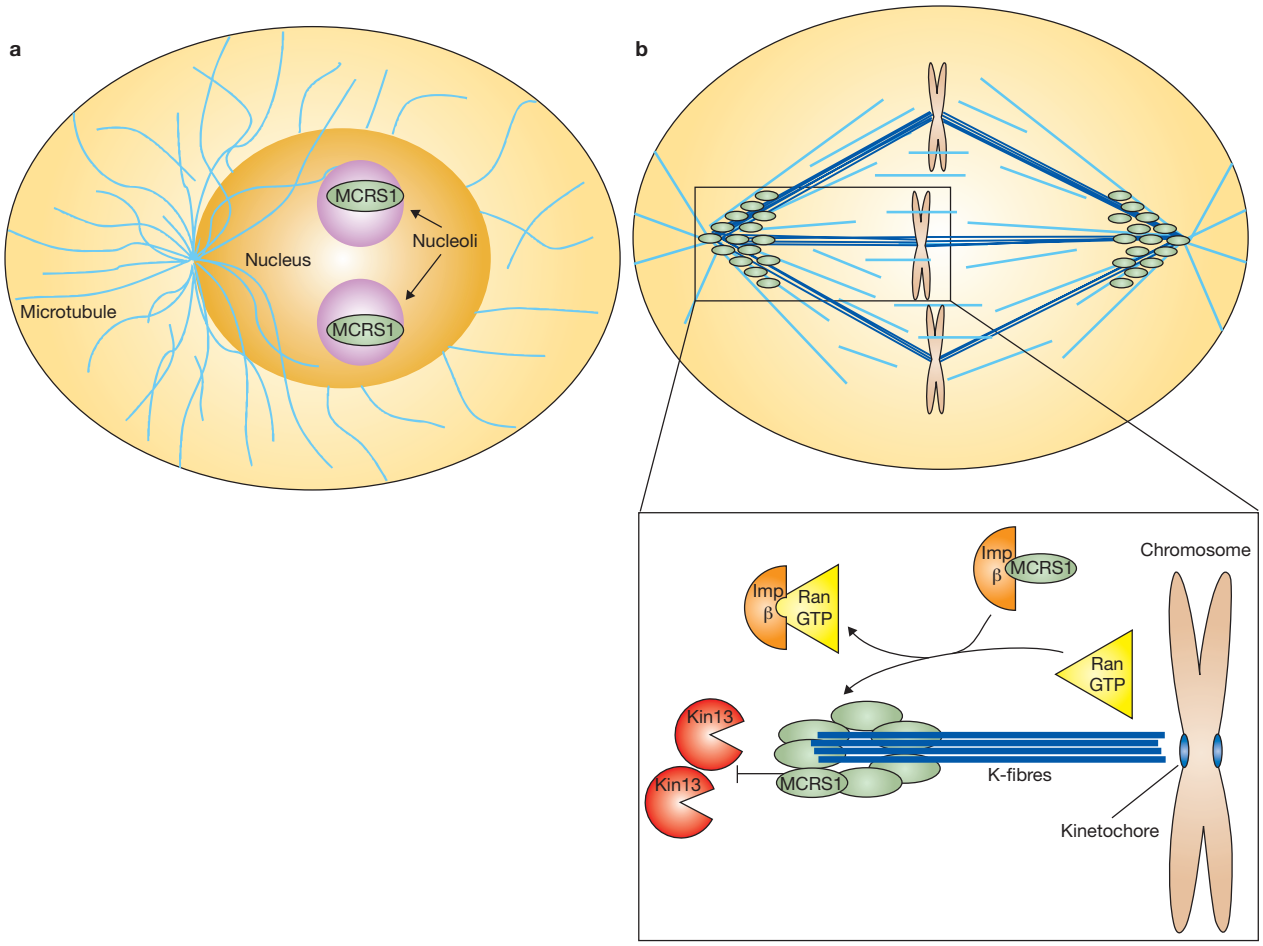

Figure 1 MCRS1 has multiple functions in interphase and mitosis. (**a**) MRCS1 localizes to nucleoli in interphase, where it is involved in the regulation of transcription, including the activation of ribosomal RNA transcription. It has also been shown to inhibit telomerase activity (among other activities; not depicted here). (**b**) At the beginning of prometaphase, RanGTP in the vicinity of chromatin induces the release of spindle-assembly factors from importin-β. Subsequently, MCRS1 binds to K-fibre minus ends and protects them from depolymerization by kinesin-13 in the metaphase spindle.

importin-β (ref. 15). Similar to TPX2, Meunier and Vernos showed that MCRS1 was associated with importin-β and could be released from this complex by RanGTP in *Xenopus* egg extracts and *in vitro*, indicating that it is a RanGTP-regulated spindle-assembly factor (Fig. 1b).

In identifying MCRS1 as a K-fibre regulator, this work brings a previously unrecognized protein to the spotlight in the mitosis field. This protein seems to share a similar, but not identical, mitotic phenotype to Patronin. Both molecules regulate spindle length by controlling the poleward flux of microtubules, a phenomenon that involves the depolymerization of microtubule minus ends by kinesin-13. Simultaneous depletion of kinesin-13 rescues the RNAi depletion phenotypes of both Patronin[13] and MCRS1, as demonstrated in the present study. However, the two proteins are not redundant in function, as depletion of either one alone generates a marked phenotype. Although these phenotypes have not yet been rigorously examined side-by-side in the same cell type, this raises questions as to why the cell has two minus-end protectors and what may be the distinct roles of Patronin and MCRS1. Furthermore, the exact mechanism of MCRS1 function at K-fibre minus ends remains to be determined. Patronin has been shown to bind very specifically to microtubule minus ends to block kinesin-13-induced depolymerization. Thus far, MCRS1 seems to bind along the length of microtubules *in vitro*, raising questions as to how it stabilizes microtubule ends against the action of kinesin-13 and how it concentrates towards the poleward ends of K-fibres. Moreover, the present work does not demonstrate protection against kinesin-13-induced depolymerization with purified MCRS1, and thus whether MCRS1 alone is sufficient for this effect remains unknown. Additionally, the ability of MRCS1-coated beads to promote microtubule assembly in *Xenopus* egg extracts suggests that it can recruit microtubule growth promoters. However, whether this biochemical activity reflects a second role of MRCS1 in spindle formation, in addition to its better-characterized effects on stabilizing microtubule minus ends against kinesin-13, is unclear from the present work.

Although much remains to be understood about the regulation and transitions of MRCS1 between its very different functions in mitosis and interphase, the work of Meunier and Vernos adds to our knowledge of mitotic spindle assembly and provides a basis for interesting future studies.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Pulling together and pulling apart: collective cargo movement in eukaryotic cells

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To establish and maintain their internal organization, living cells must move molecules to their correct locations. Long-range intracellular movements are often driven by motor molecules moving along microtubules, similarly to trucks driving along a highway. Recent work demonstrates that some randomly dispersed cargos can generate actin filaments that form a connected network whose contraction drives collective cargo movement.

Actin filaments and microtubules are two building blocks of the cytoskeleton, a network of filamentous polymers that gives cells their shape and organizes their contents. Many important cellular cargos move through the cytoplasm along microtubules that in several animal cells form a polarized radial array (Fig. 1a). This array informs cargo molecules scattered throughout the cytoplasm of the direction 'out' towards the plasma membrane and 'in' towards the nucleus and the Golgi apparatus. Cargo can reach its destination by binding to the appropriate motor molecule. On page 1431 of this issue, however, Melina Schuh demonstrates a completely different strategy by which membrane-bound vesicles, scattered throughout the cytoplasm of mouse oocytes, can move distances of many microns out towards the cell periphery[1]. In this case, instead of relying on pre-formed microtubules, the vesicles generate their own transport tracks by polymerizing actin filaments from their surfaces. These filaments interact with neighbouring vesicles and the cell cortex to form a disordered mesh (Fig. 1b). Actin-based molecular motors on the vesicles then cause the entire network to contract. By itself, this process should cause the vesicles to coalesce in towards the centre of the cell, perhaps into a single large clump. The collective outwards movement is directed by the interaction of some cargo-generated actin filaments with other actin filaments and motor proteins anchored in the cortex at the periphery of the cell. These motors generate a uniform, outwards-directed force that

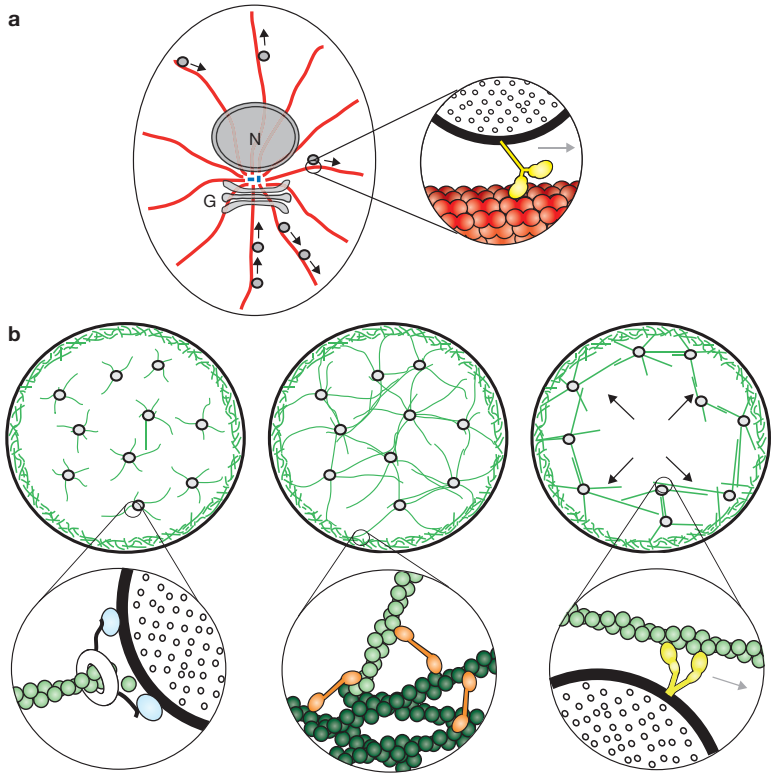

Figure 1 Movement of intracellular cargo. (**a**) Schematic view of a microtubule network (red) in a typical animal cell. Microtubules are often arranged in a radial array around a central structure, called the centrosome (blue), which is closely associated with the nucleus (N) and the Golgi apparatus (G). Intracellular cargos (small circles) move towards the cell centre or the periphery by recruiting molecular motors (yellow) that carry them in the appropriate direction along the polarized microtubules (expanded region). (**b**) Schuh's model of collective cargo movements driven by network contraction. Left: Cargo-containing vesicles generate actin filaments (green) using nucleation factors Spire and Formin2. The expanded region shows the barbed end of an actin filament (green) tethered to a vesicle by the FH2 domain of Formin2 (white torus). Potential interactions between Spire and the end of the filament are not shown. The factors anchoring Formin2 to the membrane (light blue) are unclear but might include the Spire FYVE domain or a Rab-family protein. Centre: Cargo-generated filaments interact to form a connected network or gel. In the expanded region, filaments (light green) that reach the actin-rich cell cortex (dark green) are anchored by interactions with crosslinking proteins (orange) and molecular motors (not shown). Right: Motor molecules associated with cargo vesicles (yellow, expanded region) move along filaments in the network, causing it to contract. Strong interactions with the cortex drive contraction outwards, carrying trapped cargo vesicles towards the cell surface.

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