"HURP on" we're off to the kinetochore!

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RanGTP has a central role in spindle assembly, but the Ran-regulated factors required to initiate spindle bipolarity and stabilize MT growth toward the chromosomes remain unknown. However, three recent papers (Koffa et al., 2006; Sillje et al., 2006; Wong and Fang, 2006) have identified a single factor, HURP, that may encompass both of these properties.

The assembly of a bipolar spindle is essential for the faithful segregation of a cell's genetic material. Assembly involves the reorganization of many cellular components to form arguably the most complex machine in the metazoan cell. Microtubules (MTs) are the essential infrastructure of this machine. In mitosis, MTs predominantly grow from the microtubule-organizing center, the centrosome, toward the chromosomes and attach to the kinetochore or chromosome arms. MTs then move the chromosomes to the metaphase plate in a process known as congression. In addition, MTs can be nucleated around chromosomes, in a process believed to be Ran dependent (Gadde and Heald, 2004).

RanGTP has a profound influence over the formation of the spindle, affecting MT nucleation, stabilization, and organization (Hetzer et al., 2002). A RanGTP gradient centered around chromosomes prevents the binding of nuclear transport receptors to a subset of spindle assembly factors (SAFs) that are nuclear in interphase (Li and Zheng, 2004; Caudron et al., 2005; Kalab et al., 2006). Despite the identification of many Ran-regulated SAFs, some key questions remain: how does Ran stimulate spindle bipolarity? What is the Ran-dependent microtubule stabilizing factor involved in directing microtubules toward chromosomes? Three recent studies may now have identified a factor that sheds light on both of these questions (Koffa et al., 2006; Sillje et al., 2006; Wong and Fang, 2006).

Stabilization of K-fibers to and their targeting to kinetochores

A new factor, hepatoma up-regulated protein (HURP), was identified as having a role in chromosome congression (Koffa et al., 2006; Sillje et al., 2006; Wong and Fang, 2006). HURP had previously been identified as an Aurora A substrate up-regulated in hepatomas (Yu et al., 2005). In each of the current studies, HURP was identified using a different approach. The Mattaj laboratory biochemically fractionated MAPs from *Xenopus* egg extracts to identify factors required for spindle assembly. The Nigg laboratory used a proteomics approach to purify and identify spindle components, while the Fang laboratory mined microarray data to identify proteins whose expression was induced during G2 or G2/M of the cell cycle and that were also coregulated with known mitotic proteins. All three labs identified HURP as a MAP that could bundle MTs in vitro and which localized predominantly to the portion of K-fibers (bundles of spindle MTs that attach to kinetochores in metazoans) closest to the chromatin. Interestingly, Sillje and coworkers found that HURP did not localize to astral MTs in HeLa cells (Sillje et al., 2006), suggesting that HURP's role is specific to K-fibers in somatic cells. Consistent with this observation, the loss of HURP resulted in misaligned chromosomes at the metaphase plate, suggesting a role for HURP in chromosome congression. This misalignment stemmed from a failure, in many cases, of K-fibers to attach to kinetochores (Fig. 1). Further experiments demonstrated a role for HURP in increasing K-fiber stability, suggesting that either the bundling of MTs to form a K-fiber stabilizes them or that HURP has additional roles in stabilizing MTs. However, HURP's role in congression may not be restricted to stabilizing and bundling K-fibers. Using an MT regrowth assay, Wong and Fang found that HURP is required for de novo MT production from chromosomes in a manner similar to TPX2 (Tulu et al., 2006).

Interestingly, despite chromosome misalignment in HURP-depleted cells, the cells entered into anaphase after a prolonged period in prometaphase. Wong and Fang examined this event more closely and found that the spindle checkpoint was activated despite progression into anaphase. However, checkpoint activation was not just due to a lack of MT attachment to kinetochores, but also a lack of tension between amphitelically attached sister kinetochores. The reduced tension resulted from reduced MT stability within the K-fibers (Fig. 1). However, Wong and Fang showed that HeLa cells, used by all groups, can override the spindle checkpoint when MTs are partially destabilized through a variety of means. Therefore, the spindle checkpoint override may not be a direct consequence of HURP activity, but may rather be due to a general destabilization of MTs, in HeLa cells at least. How the checkpoint is over-ridden remains unclear, but once deciphered will provide considerable insight into how the checkpoint operates.

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Abbreviations used in this paper: HURP, hepatoma up-regulated protein; MT, microtubule.

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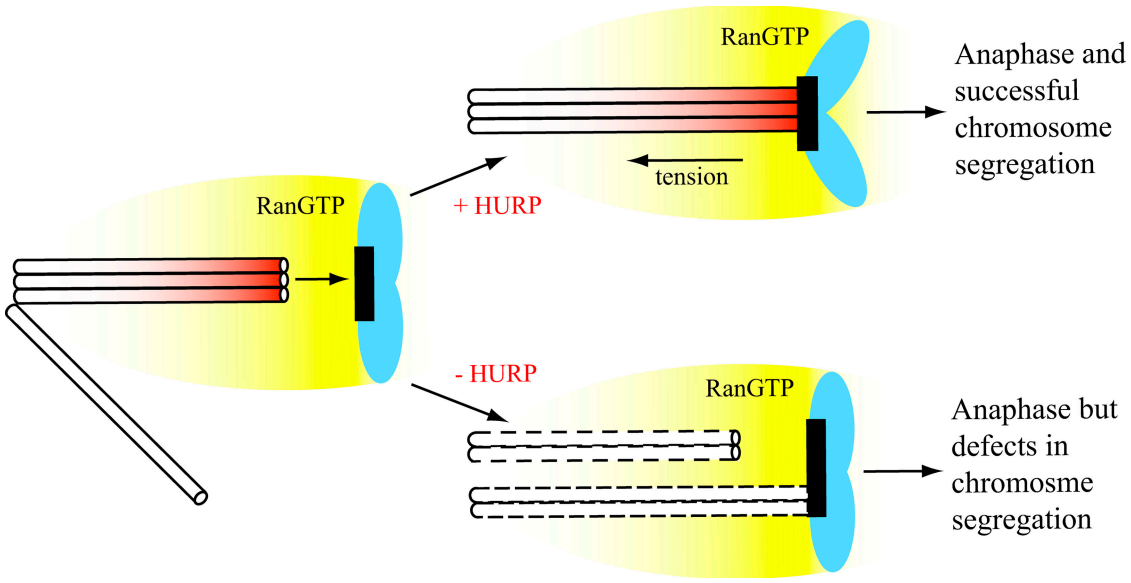

Figure 1. **HURP (red) is involved in stabilizing and targeting K-fibers to chromosomes (blue).** Depletion of HURP leads to unstable K-fibers, which either fail to attach to kinetochores (black) or fail to generate tension between sister kinetochores; yet these cells eventually enter anaphase. Yellow depicts the gradient of RanGTP emanating from the chromosomes.

Recent modeling and experimental studies suggest a role for the Ran gradient in directing MT growth toward chromosomes (Wollman et al., 2005; Silverman-Gavrila and Wilde, 2006). HURP localizes to and stabilizes K-fiber ends closest to chromosomes, suggesting that it could be the long sought after Ran-dependent MT stabilizing factor (Fig. 1). HURP localization appears to be particularly sensitive to RanGTP concentrations. Upon overexpression of an allele of Ran locked in the GTP bound form, RanQ69L, which should elevate RanGTP levels in the cell, HURP relocalizes to regions of the spindle closest to the poles. Assuming then that HURP is only active at the highest concentrations of RanGTP within the cell, it would be active close to the chromosomes, thereby facilitating the final run-in of MTs to the kinetochore.

Stimulating bipolar spindle assembly

Bipolar spindle assembly requires the balance of plus- and minus-end–directed motor activities. Previous studies using *Xenopus* egg extracts have shown a correlation with RanGTP-dependent changes in the dynamics of the mitotic kinesin Eg5, on astral microtubules and the initiation of bipolar spindle assembly (Wilde et al., 2001). These data suggested that RanGTP

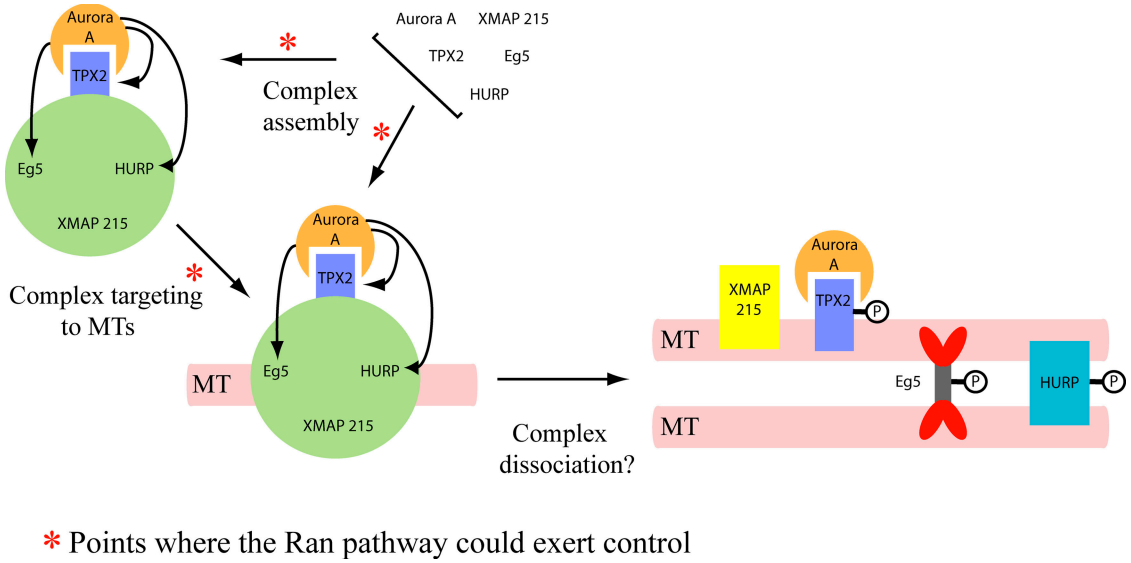

Figure 2. **Model for the potential mode of action of the HURP complex.** The components of the complex assemble in an MT-dependent or -independent manner. Once formed, Aurora A can phosphorylate components of the complex (arrows lead to known substrates of Aurora A). The complex may then function as a single entity or dissociate into smaller units to carry out specific functions in different parts of the spindle.

could stimulate bipolar spindle assembly by regulating the balance of motor protein activity. However, the biochemical connection of Ran to Eg5 remained unclear. Using an in vitro assay, Koffa et al. (2006) fractionated MAPs isolated from *Xenopus* egg extracts and identified a large molecular weight complex that stimulated the reorganization of asters into bipolar spindles. This complex contained two of the usual suspects known to be directly regulated by Ran: Aurora A and TPX2 (Schatz et al., 2003; Trieselmann et al., 2003; Tsai et al., 2003). Intriguingly, the complex also contained Eg5, XMAP215, and HURP, thus providing a biochemical link between Eg5 and Ran that opens up exciting new avenues to further define the mechanism by which RanGTP stimulates bipolar spindle assembly.

How the complex serves to regulate Eg5 remains to be determined. Eg5 activity could be modulated by virtue of its assembly into the complex, either through a conformational change and/or its phosphorylation by Aurora A (Fig. 2). The discovery of this complex suggests the interesting possibility of a relationship between microtubule stability and the balance of motor activity required for bipolar spindle assembly.

Another fascinating problem is how Ran exerts its effect on the HURP complex and how the complex functions (Fig. 2). Ran may regulate targeting of the complex to MTs by regulating the MT binding activity of HURP (Sillje et al., 2006). Indeed, the recruitment of Aurora A and KLP61F (the *Drosophila* Eg5 homologue) to MTs is also dependent on Ran in vivo (Silverman-Gavrila and Wilde, 2006). In addition, assembly of the complex is dependent upon Aurora A activity (Koffa et al., 2006), suggesting that Ran could exert its affect through the characterized activation of Aurora A by TPX2, which is known to depend on MTs (Trieselmann et al., 2003; Tsai et al., 2003). Intriguingly, the spindle localization of HURP complex components does not completely overlap, raising the possibility that the complex is transitory within the cell. This transient interaction may facilitate the Aurora A–dependent phosphorylation of the complex before dispersal.

Our understanding of this exciting complex is in its early stages, but it raises the possibility that several processes in spindle assembly are regulated through one complex. However, we do not know the full extent of the complex yet: are there more components? What are the interactions within the complex? Is the full complex required for each process? Defining answers to these questions will shed more light on the underlying mechanisms behind spindle assembly and reveal in greater detail how Ran regulates mitosis.

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