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MITOSIS

Microtubules protect spindle assembly factors

The spindle assembly factors HURP (hepatoma up-regulated protein), NuSAP (nucleolar and spindle-associated protein) and TPX2 (targeting protein for XKLP2) associate with microtubules to recruit additional regulators of spindle formation, and they are degraded by APC/C (anaphase-promoting complex, also known as the cyclosome)-mediated ubiquitylation when they have fulfilled their mitotic roles. These factors are activated by their release from importin-β but, as this also sensitizes them to APC/C-meditated degradation, how do they execute their function before they are degraded? Rape and colleagues now show that HURP is protected from degradation by microtubules.

The authors assessed the stability of HURP, NuSAP and TPX2 in cell extracts in the presence or absence of taxol, a chemical that induces soluble tubulin in extracts to form microtubules. Spindle assembly factors were degraded by APC/C^{CDC20} or APC/C^{CDH1} in the absence of microtubules (APC/C requires the cofactor CDC20 or CDH1 (CDC20 homologue 1) to degrade substrates). By contrast, microtubules inhibited the APC/C^{CDC20} or APC/C^{CDH1}-mediated ubiquitylation of HURP, NuSAP and TPX2. Thus, microtubules protect spindle assembly factors from APC/C-mediated degradation *in vitro*.

The authors focused on HURP and identified two motifs — microtubule-binding domain 1 (MBD1) and MBD2 — that are important for HURP–microtubule binding. They found that substituting the positively charged residues in the HURP MBD1 with Ala ($HURP^{MBD1*}$) decreased the ability of this binding domain to bundle, but not bind, microtubules. However, the HURP–microtubule interaction was completely abolished when these residues were substituted in both MBD1 and MBD2 ($HURP^{MBD1/2*}$). *In vivo* analysis suggested that MBD1 is the dominant MBD in HURP–microtubule interactions during interphase.

So, are these MBDs important for the stabilization of HURP by microtubules? *In vitro* assays revealed that microtubules could stabilize, and prevent the APC/C-mediated ubiquitylation of, HURP and $HURP^{MBD2*}$, but not $HURP^{MBD1*}$ or $HURP^{MBD1/2*}$. Thus, MBD1 is essential for the microtubule-induced stabilization of HURP. It also seems to be sufficient to impart this protection, as fusing the MBD1 to other APC/C substrates targeted them to microtubules and protected them from APC/C-mediated ubiquitylation.

Do microtubules protect HURP from degradation during mitosis? Wild-type HURP was completely degraded once all sister chromatids had been divided into the two daughter cells. By contrast, $HURP^{MBD1*}$ and $HURP^{MBD1/2*}$ were completely degraded shortly after sister chromatid separation began, but could be stabilized by inhibiting the proteasome or APC/C function. Thus, microtubules seemed to stabilize HURP during mitosis. Interestingly, the degradation of HURP later in mitosis is as important as its stabilization early on, as the expression of HURP that could not be targeted by APC/C caused mitotic defects.

Finally, the authors asked how APC/C and importin-β interplay to regulate spindle formation and function. Spindle formation was highly compromised when APC/C and importin-β function were inhibited together but not when they were inhibited individually. Thus, APC/C and importin-β cooperate to control the stability and activity of spindle assembly factors and ensure spindle structure and function.

In short, this study reveals that while HURP, and presumably other spindle assembly factors, act at microtubules to regulate spindle formation, the microtubules that form the spindle are protecting them from degradation.

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ORIGINAL RESEARCH PAPER Song, L., Craney, A. & Rape, M. Microtubule-dependent regulation of mitotic protein degradation. *Mol. Cell* **53** 179–192 (2014)