Some Importin News About Spindle Assembly

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Chromosomes are known to influence the assembly of the spindle—the apparatus of microtubules to which replicated chromosomes become attached—during both mitosis (division of somatic cells) and meiosis (division of germ cells that develop into egg and sperm). During meiosis of mammalian oocytes, for example, a spindle forms next to chromosomes in the absence of centrosomes (organelles that instruct tubulin α and β to polymerize into microtubules). Many different research groups are beginning to elucidate exactly how chromosomes influence spindle assembly. Recent work has shown that microtubule polymerization requires a concentration gradient of Ran—a guanosine triphosphatase (GTPase) that is crucial for the transport of macromolecules into and out of the nucleus (1–6). A recent flurry of papers (7–9) report that the nuclear transport importins, α and β, which also bind to Ran, directly regulate the activities of at least two microtubule organizing components (NuMA and TPX2). These studies therefore suggest that nuclear transport and spindle assembly are somehow intimately intertwined.

Ran, a highly conserved GTP-binding protein of the Ras superfamily, was originally identified as an essential component of the machinery that transports macromolecules into and out of the nucleus. Like other small GTPases, the nucleotide-bound state of Ran is modulated by a series of accessory factors. Conversion of Ran-GTP into Ran-GDP requires the GTPase-activating protein RanGAP1 (and its associated factor, RanBP1); exchange of GDP for GTP is promoted by the guanine nucleotide exchange factor (RanGEF) RCC1. Although Ran is found throughout the cell during G1, S, and G2 phases of the cell cycle (interphase), RanGAP1 and RanBP1 are restricted to the cytoplasm, and RCC1 is confined to the nucleus (see the figure). Because of the compartmentalization of RanGAP1 and RCC1, nuclear Ran is primarily bound to GTP, whereas cytoplasmic Ran is primarily bound to GDP. The different locations of Ran-GTP and Ran-GDP enable Ran to regulate the import and export of molecular cargo through its effects on the importin and exportin nuclear transport receptors.

Throughout interphase, the nucleotide-bound state of Ran regulates the binding of importins and exportins to their cargo. During nuclear import, binding of Ran-GTP to importin α and β causes them to release their cargoes within the nucleus. During nuclear export, Ran-GTP is required for the efficient binding of nuclear export receptors (such as Xpo1) to their cargo. Imported proteins are promptly released as soon as the importins bind to Ran-GTP; meanwhile, other nuclear proteins bind to the exportins ready to be transported out of the nucleus. Conversely, maintenance of Ran-GDP in the cytoplasm (by RanGAP1/RanBP1) causes dissociation of exported proteins from their exportins and allows other proteins that need to move into the nucleus to associate with the importins.

Maintaining the compartmentalization of Ran-GTP and Ran-GDP is essential once the nuclear envelope breaks down, a prelude to cell division (see the figure). Ran-GTP induces microtubule polymerization in extracts of Xenopus eggs during both meiosis and mitosis (1, 3–5). In the absence of the interphase nucleus, RCC1 remains associated with chromatin, whereas RanGAP1 and RanBP1 are spread throughout the cytoplasm. Consequently, a higher concentration of Ran-GTP should exist in the vicinity of the chromosomes, whereas Ran-GDP would be expected to have a distribution throughout the rest of the cell.

Inducing cells to express a mutant Ran that cannot be converted from the GTP to the GDP form causes the spontaneous formation of spindle asters (star-shaped microtubule arrays) in Xenopus egg extracts (1, 3–5). Ran does not, however, colocalize with the microtubules, suggesting that its action is indirect (8). Intriguingly, depletion of Ran-GTP-binding proteins from these egg extracts also induced spontaneous microtubule aster formation (7, 8). Thus, Ran seems to be abrogating the inhibitory effects of other factors on microtubule polymerization.

A series of experiments have now shown that these inhibitory factors may be the importins. Addition of egg extracts of albumin fused to the SV40 large T antigen nuclear localization signal (which binds to importin α and prevents it from binding to its cargo) resulted in spontaneous aster formation (9). Furthermore, addition of importin α or β to the egg extracts could inhibit aster formation even if large amounts of Ran-GTP were also added (8, 9). In fact, a truncated form of importin β that failed to bind to Ran but could still bind to its cargo was slightly more efficient than full-length importin β in preventing aster formation. Indeed, microinjection of truncated importin β into mammalian cells prevented spindle assembly. The blocking of microtubule organization and spindle assembly by importins is due to their binding to (and inhibition of) other cellular factors. The promotion of spindle assembly by Ran-GTP may reflect its ability to induce the release of one or more microtubule-stabilizing proteins by the importins.

Two microtubule-associated proteins, TPX2 and NuMA, have been identified as