PERSPECTIVES: CELL CYCLE

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 proteins, importin 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 G_1, S, and G_2 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.

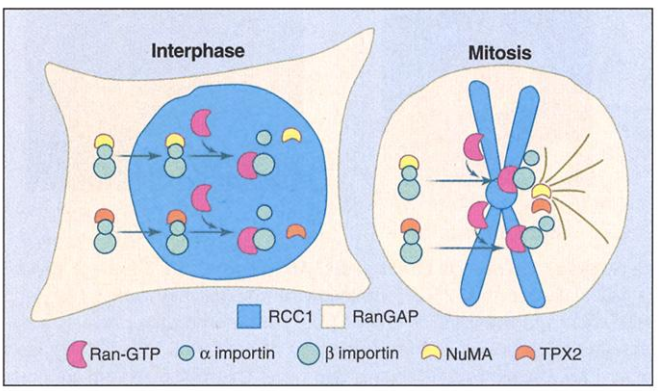
Throughout interphase, the nucleotide-bound state of Ran regulates the binding of importins and exportins to their cargo. During nuclear import, binding of Ran-GTP to importin α and β causes them to release their cargoes within the nucleus. During nuclear export, Ran-GTP is required for the efficient 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 to 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



Doing double duty. The localized production of Ran-GTP by the chromosome-bound GTP exchange factor RCC1 regulates nuclear transport and spindle assembly. (**Left**) During interphase, NuMA and TPX2 are transported into the nucleus by importin α and β. In the nucleus, Ran-GTP produced by RCC1 binds to the importins, causing them to discharge their cargoes including NuMA and TPX2. (**Right**) During cell division (mitosis or meiosis), chromatin-bound RCC1 produces a gradient of Ran-GTP that is greatest closest to the chromosomes. As is the case during interphase, Ran-GTP associates with the importins, inducing them to release NuMA and TPX2, which are then free to stimulate microtubule stabilization and spindle assembly. However, this happens only next to the chromosomes where there is sufficient Ran-GTP to induce the importins to release NuMA and TPX2.

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the targets of importin blocking activity. Both promote microtubule assembly and are found in complexes with importin α and β (*7–9*). TPX2 (*9*) anchors a microtubule-dependent motor (Xklp2) to the spindle poles (*10*); NuMA (*8*), when bound to the microtubule motor dynein, cross-links microtubules into the spindle poles during mitosis (*11*). A connection between NuMA and importin β explains the curious finding that mutations in mammalian RCC1 (the RanGEF) result in mitotic defects that can be overcome by increasing the expression of NuMA (*12*). Inhibiting the importin β block of NuMA activity restores formation of the spindle poles. Together, these findings strongly support a direct link between NuMA and Ran/RCC1 in mammalian cells.

Both NuMA and TPX2 are found in the interphase nucleus, presumably localized there by Ran and importin α and β. Their nuclear localization prevents them from interacting with microtubules in the cytoplasm until after breakdown of the nuclear membrane at the beginning of mitosis or meiosis (see the figure). In the absence of a nuclear membrane, chromatin-bound RCC1 and cytoplasmic RanGAP1 presumably produce a natural gradient of Ran-GTP that is most concentrated at the chromosomes and least concentrated at the cell periphery. Consequently, TPX2, NuMA, and probably other factors regulated by Ran-GTP are preferentially activated in the vicinity of chromatin. Ran mutants that cannot convert GTP to GDP increase net microtubule assembly by increasing the frequency at which disassembling microtubules are converted back into the assembly phase (*13*, *14*). There is also a Ran-GTP–dependent increase in the initiation of microtubule formation by centrosomes (*14*), although the factors involved in this additional Ran-dependent activity are not known.

In the absence of a nuclear membrane, commandeering the nuclear import machinery would prevent assembly of spindles except where there is an appropriate source of RCC1, that is, in or near the chromosomes. Ran stimulates microtubule polymerization and the nuclear import of proteins through similar mechanisms that depend on its ability to abrogate inhibitory interactions between proteins. The association of Ran with the importins and with known microtubule-associated proteins suggests that the nuclear transport machinery directly regulates microtubule organization. The identification of downstream effectors of Ran has provided valuable insights into the intricacies of spindle assembly.

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NOTA BENE: EVOLUTION

Wolbachia and Wasp Evolution

One of the strangest partnerships in nature is the pairing of the symbiotic bacterium *Wolbachia* with a remarkable range of insect hosts. *Wolbachia* live in the cytoplasm of insect cells and apparently do no harm. These endosymbionts do, however, have a startling effect on the reproduction of their insect hosts, which has led biologists to speculate that *Wolbachia* may contribute to reproductive isolation and the creation of new insect species (speciation). Bordenstein *et al.* (*1*) now provide evidence that this indeed may be the case.

When male insects infected with *Wolbachia* mate with uninfected females, no offspring are produced (because the cytoplasm of infected sperm is incompatible with the cytoplasm of uninfected eggs). Yet viable offspring result from all other mating combinations (uninfected males and infected females, infected males and infected females, and uninfected males and uninfected females). This arrangement ensures that *Wolbachia* (which are passed to offspring only through females) spread rapidly through the host species because uninfected females that mate with infected males cannot produce offspring. But is this partial reproductive isolation sufficient to drive the emergence of new insect species? Bordenstein and colleagues speculated that if a host insect population was infected with different *Wolbachia* strains that were incompatible (so that individuals infected with one strain could not produce offspring with individuals infected with the other), then this double reproductive barrier might be sufficient to drive speciation.

They set out to test their hypothesis in two closely related species of parasitic wasp, *Nasonia giraulti* (which inhabits eastern North America) and *Nasonia longicornis* (which inhabits western North America). In both species, individuals were infected with different *Wolbachia* strains and reproductive incompatibility was bidirectional: Matings between *N. longicornis* males and *N. giraulti* females, and between *N. giraulti* males and *N. longicornis* females produced few or no hybrid offspring. But when both species were treated with antibiotics to cure their *Wolbachia* infection, interspecies matings produced normal numbers of hybrid offspring. Working with uninfected wasps, the authors then tested several other reproductive barriers (unrelated to *Wolbachia* infection), such as reduced fertility and hybrid breakdown, that are known to precede the formation of two separate species. Interspecies matings did not reduce the number of eggs laid or the number of viable hybrid offspring produced, and sperm of one species was capable of fertilizing eggs of the other. There was also no evidence of hybrid breakdown because hybrid male offspring mating with female offspring of either species did not show abnormal courtship behavior or reduced fertility.

The authors conclude that microbial-induced reproductive isolation is already apparent between *N. longicornis* and *N. giraulti*, whereas genetically driven reproductive barriers have not yet been formed. Bordenstein *et al.* are careful not to endorse *Wolbachia* as the means of *Nasonia* speciation (geographical isolation is arguably a far more important factor in this case), but their work offers a tantalizing glimpse into how an apparently harmless endosymbiont could alter the course of evolution.

—ORLA SMITH

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