Ran at kinetochores

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Abstract

The Ran GTPase controls many cellular functions, including nucleocytoplasmic trafficking, spindle assembly, nuclear assembly and cell-cycle progression. Considerable evidence suggests that diffusible Ran-GTP near mitotic chromatin facilitates the release of critical factors from nuclear transport receptors, thereby promoting organization of mitotic spindles with respect to chromosomes. In addition to this role of soluble Ran-GTP, Ran has two important but less understood roles at mitotic kinetochores. Namely, it is essential for regulation of the spindle assembly checkpoint and for assembly of microtubule fibres that attach kinetochores to spindle poles. Here, I will briefly summarize evidence for these kinetochore-associated functions and mention some of the issues that remain to be addressed regarding them.

Introduction

The Ran GTPase is required for many cellular functions, including nucleocytoplasmic trafficking, spindle assembly, nuclear assembly and cell-cycle control [1]. The exchange factor for Ran, RCC1 (regulator of chromosome condensation 1), is chromatin-associated throughout the cell cycle. The GAP (GTPase-activating protein) for Ran, RanGAP1, is cytoplasmic during interphase. This distribution of Ran’s regulators leads to a high concentration of Ran-GTP within nuclei and a low concentration of Ran-GTP in interphase cytosol [2]. Ran’s primary effectors are Ran-GTP-binding proteins that act as nuclear transport receptors [2]. Ran-GTP regulates association between these proteins and their transport cargoes. Import receptors bind to their cargo in the cytoplasm. After translocation into the nucleus, they bind Ran-GTP and release their cargo. In contrast, export receptors form ternary complexes within the nucleus that contain both cargo molecules and Ran-GTP. They dissociate from their cargo within the cytoplasm after RanGAP1-mediated GTP hydrolysis.

During mitosis in metazoan cells, a significant fraction of RanGAP1 becomes associated with kinetochores [3]. Nevertheless, chromatin-bound RCC1 promotes increased concentrations of Ran-GTP near mitotic chromosomes [4,5]. The heterodimeric Importin-α/β import receptor binds and inhibits factors required for spindle assembly [6]. The locally high concentration of diffusible Ran-GTP near mitotic chromatin may release Importin-α/β from these targets, promoting organization of mitotic spindles with respect to chromosomes. In addition to this role of freely soluble Ran-GTP, Ran has two important roles at mitotic kinetochores. Namely, it is essential for regulation of the SAC (spindle assembly checkpoint) [7] and for assembly of microtubule fibres that attach kinetochores to spindle poles [k-fibres (kinetochore fibres)] [8]. Here, I will briefly summarize evidence for these kinetochore-associated functions, and mention some of the issues that remain to be addressed regarding them.

Targeting of Ran pathway components to kinetochores

RanGAP1-mediated GTP hydrolysis by Ran is facilitated by a family of Ran-GTP-binding proteins. These proteins dissociate Ran-GTP from transport receptors, thereby allowing RanGAP1 binding and catalysis [2]. The largest member of this family is the mammalian RanBP2 (Ran-binding protein 2; also called Nup358), a 358 kDa nucleoporin that possesses four Ran-GTP-binding domains and a series of other structural motifs [9,10]. RanGAP1 becomes modified through conjugation to SUMO-1 (small ubiquitin-related modifier-1) [11,12]. RanGAP1–SUMO-1 binds stably to RanBP2 throughout the cell cycle in a complex that also includes the SUMO-1 ligase, Ubc9 [13,14]. This complex is targeted to spindles and particularly to the kinetochore in a microtubule-dependent manner in mitotic mammalian cells [3].

Kinetochore targeting of the RanBP2–RanGAP1 complex requires a nuclear export receptor, Crm1, which is found on kinetochores throughout mitosis [8]. Like all export receptors, Crm1 binds to cargo in ternary complexes that contain Ran-GTP [2]. Inhibition of Crm1 ternary complex formation using LMB (leptomycin B), a highly specific chemical inhibitor, blocks kinetochore recruitment of RanBP2–RanGAP1 [8]. The essential role of ternary complex assembly can be independently verified using tsBN2 cells, a hamster cell line with a temperature-sensitive allele of the RCC1 gene. Upon shift to the restrictive temperature, tsBN2 cells protolyse RCC1, so that Ran accumulates in its GDP-bound form [15], thereby preventing Crm1 ternary complex

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Abbreviations used: APC/C, anaphase-promoting complex/cyclosome; CENP-E, centromere protein E; GAP, GTPase-activating protein; k-fibre, kinetochore fibre; kMT, kinetochore microtubule; LMB, leptomycin B; NPC, nuclear pore complex; RanBP2, Ran-binding protein 2; RCC1, regulator of chromosome condensation 1; SAC, spindle assembly checkpoint; SUMO-1, small ubiquitin-related modifier 1.

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formation. Notably, temperature shift of tsBN2 cells is accompanied by complete release of the RanBP2–RanGAP1 complex from kinetochores [8]. Crm1 itself requires neither ternary complex assembly nor microtubules for kinetochore association [8]. The component(s) at kinetochores that is directly involved in Crm1 binding and recruitment is currently unknown. However, it is attractive to speculate that other nucleoporins that are constitutively associated with kinetochores throughout mitosis [16] may have a role in Crm1 recruitment (see below).

Interestingly, there is considerable variability between different species and cell types regarding the fraction of Crm1 and RanBP2–RanGAP1 recruited to mitotic kinetochores [17], with a commensurate variability in the sensitivity of different mitotic cells to disruption by LMB. There are at least two potential sources of such variability: it is possible that cells emphasize particular Ran effector pathways to different extents during mitosis. For instance, Importin-α/β appears to be a dominant Ran-GTP effector for spindle assembly in Xenopus egg extracts [18–20], while many of the spindle assembly defects in temperature-shifted tsBN2 cells may arise from Crm1-dependent pathways [8] (see below). Alternatively, functions associated with the RanBP2–RanGAP1 complex may be important in all cases, but the extent to which they must be concentrated on to kinetochores may vary between cell types.

Ran and the SAC

To assure the fidelity of chromosome segregation in eukaryotic cells, the onset of mitosis is tightly controlled through the SAC [21]. This regulatory pathway monitors the attachment of microtubules to kinetochores, and prevents activation of the APC/C (anaphase-promoting complex/cyclosome) until all chromosomes are correctly attached and aligned on to the metaphase plate. The APC/C is an ubiquitin ligase that is responsible for the destruction of a number of key mitotic regulatory proteins [22]. Components of the SAC include: Mad1, Mad2, Mps1, Bub1, Bub3, BubR1 and CENP-E (centromere protein E) [21]. In Xenopus egg extracts, elevated levels of RCC1 or Ran-GTP abrogate SAC-mediated mitotic arrest, allow APC/C activation and disrupt the kinetochore localization of SAC components [7]. Along with other manipulations of Ran-GTP levels in egg extracts, these experiments suggest that the SAC is directly responsive to the overall concentration of Ran-GTP [7].

tsBN2 cells that have been shifted to the restrictive temperature retain the ability to arrest in mitosis in response to treatment with the microtubule-depolymerizing agent nocodazole, despite the absence of RCC1 [8]. However, shifted tsBN2 cells progress more quickly through the metaphase–anaphase transition upon release from nocodazole than unshifted control cultures [17]. At the same time, temperature-shifted tsBN2 cells form defective k-fibres (see below), which typically cause extreme mis-segregation of chromosomes [8]. Taken together, these results indicate that although neither RCC1 nor Ran-GTP are required for SAC activation in response to fully unattached kinetochores in tsBN2 cells, they are required to prevent anaphase in response to inappropriate attachments or misassembled k-fibres.

LMB-treated HeLa or U2OS cells can delay anaphase onset in response to mis-assembled k-fibres [8] (see below). This observation indicates that neither RanBP2–RanGAP1 recruitment to kinetochores nor Crm1 activity itself is indispensable for the SAC. Data from Xenopus egg extracts also argue that the Importin-α/β import receptor is unlikely to act as a Ran-GTP effector in the SAC [7]. The identification of the SAC effector is thus a major issue for future investigation.

A number of kinetochore-associated components of the SAC, including Mad1, Mad2, and Mps1, localize to budding yeast and vertebrate NPCs (nuclear pore complexes) during interphase [16,23–25]. Interestingly, release of Mad2 from NPCs is coupled to spindle checkpoint activation in yeast [24]. Moreover, mutations that disrupt Ran-GTP concentration within the nucleus of both yeast and vertebrate cells also cause displacement of Mad2 from NPC [26]. Given that numerous vertebrate NPC components localize to kinetochores during mitosis (see below), it is possible to speculate that Ran’s capacity to control the spindle checkpoint might function through the interactions of spindle checkpoint proteins with these NPC components. On the other hand, disruption of Mad1 and Mad2 recruitment to NPC does not cause gross dysfunction of the checkpoint in budding yeast [27], arguing against this hypothesis.

Finally, RCC1’s chromatin-binding behaviour is tightly regulated during mitosis in both Xenopus egg extracts [7] and tissue culture cells [28], such that it appears to bind more stably as cells undergo the metaphase–anaphase transition. This change may be associated with a commensurate increase in Ran-GTP production [29] that could help to silence the checkpoint as cells enter anaphase or to keep it from becoming re-activated by the absence of tension across kinetochores after cohesion is dissolved upon sister chromatid separation [30].

Ran and k-fibre formation

Several lines of evidence suggest that the Ran pathway has an important role in k-fibre assembly in metazoan cells. Metazoan k-fibres include both microtubules that are attached to kinetochores at their plus-ends (kMTs (kinetochore microtubules)) and microtubules that are not [31]. kMTs form coherent bundles throughout their length without entanglement to other fibres, and k-fibres have cross-bridges between their microtubules that may contribute towards their integrity [31,32]. Mature k-fibres form through multiple pathways: plus-ends of dynamic astral microtubules attach to kinetochores through capture mechanisms [33], and kinetochores actively nucleate microtubules that can become oriented into bipolar spindle structures through interaction with astral microtubules [34]. During prometaphase, lateral contacts between kinetochores and microtubules are also observed [35,36], and these contacts have recently been
shown to play an important role in congression of monotelic chromosomes on to the metaphase plate [37]. Such lateral contacts are less prevalent over time, and the preponderance of microtubule–kinetochore attachments in mature k-fibres occurs through microtubule plus-ends.

RNA interference-mediated depletion of RanBP2 in HeLa cells causes dissociation of RanGAP1 from kinetochores and mitotic arrest [38,39]. RanBP2-depleted cells show defects in spindle assembly, including failure to align chromosomes on to the metaphase plate and failure to form cold-resistant k-fibres. Mitotic arrest in RanBP2-depleted cells requires components of the SAC (such as Mad2), although SAC proteins are not stably associated with the kinetochores as would typically be observed during checkpoint arrest. Nevertheless, kinetochores of RanBP2-depleted cells recruit checkpoint proteins after treatment with microtubule-depolymerizing agents, such as nocodazole, strongly suggesting that their kinetochores are competent to bind checkpoint proteins. Together, these findings would be consistent with the idea that cells make transient or unstable microtubule attachments in the absence of RanBP2 that are sufficient to release some checkpoint proteins but that are insufficient for k-fibre stability or for full silencing of the SAC.

This notion is further supported by analysis of LMB-treated mitotic cells HeLa or U2OS cells, which show a prolonged metaphase, followed by substantial chromosome missegregation [8]. As with RanBP2-depleted cells, LMB-treated cells lack cold-stable k-fibres. Interestingly, further analysis of k-fibres in these cells showed striking defects, particularly the absence of discrete k-fibre attachments at kinetochores [8]. Rather, chromosomes in LMB-treated cells show pole-to-pole microtubule bundles that span their centromeres (Figure 1), indicative of atypical and/or highly merotelic microtubule attachment. At the same time, the discrete nature of k-fibres is lost, and LMB-treated cells frequently show structures that appear to arise from fusion of k-fibres, with centromeres of multiple chromosomes apparently coalesced into agglomerated structures [8]. Temperature-shifted tsBN2 cells show similar k-fibre defects [8], consistent with the notion that Ccm1 ternary complex formation plays an important role in k-fibre integrity.

Other nuclear pore proteins during mitosis

The RanBP2–RanGAP1 complex is not the only component of the interphase NPC that associates with the kinetochore during mitosis: the Nup107–Nup160 complex, which includes nine nucleoporins [40], localizes at mitotic kinetochores in mammalian cells [41]. In contrast with the RanBP2–RanGAP1 complex, however, Nup107–Nup160 remains associated with the kinetochore throughout mitosis [41–43]. Interestingly, this complex shows enhanced accumulation on unattached kinetochores in nocodazole-treated cells [44], in a manner reminiscent of some dynamic outer kinetochore proteins, such as BubR1, Mad2, dynein and CENP-E [45,46].

Deletion of the Nup160 subunit of this complex causes mitotic spindle defects in fission or budding yeast [47,48], and the Nup107–Nup160 complex is required for spindle assembly in Xenopus egg extracts [44]. Detailed analysis of spindle formation in egg extracts indicates that initial
Scheme 1 | Pathways of Ran action
Schemes for Ran-GTP function in SAC (left) and k-fibre assembly (right) are shown. See text for discussion.

microtubule nucleation around spermatid centrosomes occurs normally in meiotic Xenopus extracts depleted of the Nup107–Nup160 complex. However, later stages of spindle formation failed, leading to spindles with dramatically decreased microtubule densities, or residual structures around spermatid chromosomes that were entirely lacking of associated microtubules. Xenopus egg extracts form microtubule asters in response to elevated levels of Ran-GTP, and subsequently re-arrange these structures into bipolar ‘microspindles’ [49]; neither of these responses to Ran-GTP are impaired by depletion of the Nup107–Nup160 complex [44]. These findings do not exclude the possibility that the Nup107–Nup160 complex may be an upstream regulator of Ran-GTP, but argue against the possibility that it works downstream as a Ran effector in spindle formation. Finally, the Nup107–Nup160 complex is not required for activation of the SAC in egg extracts, nor for SAC silencing by increased levels of Ran-GTP [44].

Summary
It is informative to consider the differences and similarities of Ran’s roles in k-fibre assembly versus SAC regulation. For k-fibre assembly, Crm1, RanGAP1 and RanBP2 appear to function within an autoregulatory negative feedback loop (Scheme 1, right) [8]: RCC1 generates Ran-GTP, which in turn allows Crm1 ternary complex assembly at kinetochores and RanBP2–RanGAP1 complex recruitment. The activity of RanGAP1, however, promotes Ran-GTP hydrolysis, dissolution of Crm1 ternary complexes and its own release from kinetochores. So what is the goal of this futile cycle? It is possible that kinetochore-bound Crm1–RanBP2–RanGAP1 improves k-fibre assembly through local suppression of Ran-GTP levels in order to reduce kinetochore-driven microtubule assembly through Importin-α/β or other effectors. While kinetochore-nucleated microtubules might function during early stages of chromosome attachment to spindles [50], RanBP2–RanGAP1 could thus decrease their utilization as attachments. Alternatively, some components associated with the RanBP2–RanGAP1 complex might act to regulate k-fibre formation. Indeed, RanBP2 itself has multiple domains that function enzymatically or through binding of other proteins [9,10,51–54]. It is further interesting to note that changes of RCC1 behaviour during anaphase could substantially alter the generation of Ran-GTP [7,28,29] and thus the persistence of the RanBP2–RanGAP1 complex at kinetochores. Major questions posed by this model include the nature of the activity being recruited and its mechanism of action in k-fibre formation.

In contrast, SAC regulation by Ran appears to be a more straightforward switch in Xenopus egg extracts, where high levels of Ran-GTP can allow SAC bypass (Scheme 1, left). The effectors of this pathway again remain unknown, and current evidence argues against several of the most obvious candidates (Importin-α/β [7], Crm1 [8] and the Nup107–Nup160 complex [44]). One interesting question regarding the SAC concerns the fact that it appears to be significantly weakened in temperature-shifted tsBN2 cells [17], despite their low overall concentrations of Ran-GTP. This point cannot currently be resolved, but it is possible to speculate that the RanBP2–RanGAP1 complex could again be involved and that its displacement from kinetochores (and possible inactivation) could compensate for the decrease in Ran-GTP levels. In any case, the most direct interpretation of the switch model that arises from the Xenopus results would suggest that changes of RCC1 dynamics at anaphase could be pivotal in silencing the SAC. In some ways this idea is attractive, since it would unite the dynamic behaviour of the k-fibre and the SAC under a single regulatory pathway.

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References