Ran control of mitosis in human cells: gradients and local signals

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Abstract
Roles of the GTPase Ran in cell life and division rely on a largely conserved mechanism, i.e. Ran’s ability to interact with transport vectors. Modes of control of downstream factors, however, are diversified at particular times of the cell cycle. Specificity and fine-tuning emerge most clearly during mitosis. In the present article, we focus on the distinction between global mitotic control by the chromosomal Ran gradient and specific spatial and temporal control operated by localized Ran network members at sites of the mitotic apparatus in human cells.

Introduction
It is exactly 20 years since the GTPase Ran was discovered: in 1990, Mark Rush’s laboratory reported the cloning of four novel genes from human teratocarcinoma cells, identified for their homology with the GTP-binding domain of Ras [1]. One (TC4, teratocarcinoma clone 4), was down-regulated in teratocarcinoma cells induced to differentiate and seemed particularly interesting. TC4 encoded a nuclear protein, representing the only nuclear member of the Ras GTPase superfamily and was renamed Ran (for Ras-related nuclear) [2]. Ran is conserved from yeast to humans. It cycles between GTP- and GDP-bound states under the control of the nuclear guanine-nucleotide-exchange factor RCC1 (regulator of chromosome condensation 1), which generates RanGTP [3], and the cytoplasmic factors RanGAP1 (RanGTPase-activating protein 1) and RanBP1 (Ran-binding protein 1), which co-operate to hydrolyse RanGTP [4,5]. The compartmentalization of Ran regulators on opposite sides of the NE (nuclear envelope) yields RanGTP accumulation in the nucleus, which is essential for Ran’s main function in interphase, i.e. control of nucleocytoplasmic transport.

The first decade after Ran discovery focused on roles in transport, generating in-depth biochemical, molecular and structural knowledge [6]. Ran operates via effectors of the importin β superfamily. Importin β interacts with cargoes directly or through an importin α ‘adaptor’ for proteins carrying NLSs (nuclear localization sequences). RanGTP binds importin β with high affinity and dissociates it from cargoes, releasing the latter in the nucleus; this terminates nuclear import.

A turning point came in 1999, when RanGTP was shown to regulate spindle assembly in XEE (Xenopus egg extract) (see [7–9] for reviews). This role is exerted via either CRM1 (chromosome region maintenance 1) or importin β, the nuclear export and import vectors for protein cargoes respectively; thus cells reuse the same components for nucleocytoplasmic transport and for building up the mitotic spindle. The knowledge accumulated in the XEE system was extended somewhat automatically to all mitotic cells. Overgeneralization, however, leaves gaps to be filled by the end of the second decade. In the present paper, we discuss Ran modes of mitotic control in mammalian cells, focusing on functions mediated by importin β.

RanGTP long-range signals in the XEE system
In XEEs, RanGTP operates in substantial continuity with mechanisms regulating nuclear import, binding importin β and releasing import cargo-like SAFs (spindle-activating factors). RCC1 binds mitotic chromosomes (although with modifications compared with interphase) [10–12] and generates RanGTP therein. RanGTP, diffusing from chromosomes until it ‘hits’ an effector or a hydrolysing factor, generates a concentration ‘gradient’ around chromosomes [13–15], within which active SAFs are released. RanGTP-dependent SAF release around chromosomes is key to chromatin-driven spindle assembly in XEEs. Indeed, RanGTP-coated beads alone can substitute for chromatin in that system and provide a ‘release’ signal that activates MT (microtubule) aster formation.

Chromosome-originated RanGTP, interacting with importin β, can generate a long-range gradient in large cells (e.g. oocytes, Figure 1A), depending on where RanGTP–importin β complexes (assumed to diffuse freely) dissociate in the cytoplasm. MT nucleation and MT stabilization take place at different distances from chromosomes. The gradient model predicts that individual SAFs, depending on their position within the gradient reach, respond to

Key words: importin β, mitosis, Ran GTPase, spindle microtubule.
Abbreviations used: AKAP450, A-kinase-anchoring protein 450; HURP, hepatoma up-regulated protein; KT, kinetochore; MT, microtubule; MTOC, MT-organizing centre; MUG, mitosis with unreplicated genomes; NE, nuclear envelope; NLS, nuclear localization sequence; RanBP1, Ran-binding protein 1; RanGAP1, RanGTPase-activating protein 1; RCC1, regulator of chromosome condensation 1; SAF, spindle-activating factor; SIM2, small ubiquitin-related modifier; TC4, targeting protein for Xklp2; XEE, Xenopus egg extract.

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Figure 1 | The chromosomal/cytoplasmic ratio in oocytes and somatic cells

The extension of the chromosomal and cytoplasmic space is shown in a mouse oocyte (A) and in a human metaphase cell (B). Different enlargements are used to obtain comparable perimeter sizes (delimited by white circles in the leftmost panel). Chromosome-originated RanGTP signals propagate in a gradient in oocytes and require specific local adjustment in mammalian cells. DAPI, 4',6-diamidino-2-phenylindole.

A RanGTP landmark in mammalian cells

Most mammalian somatic cell types have a small cytoplasm (Figure 1B); although RanGTP is still generated around chromosomes, RanGTP-rich and RanGTP-depleted regions are less well separated than in oocytes [16,17]. Furthermore, centrosomes are dominant MTOCs (MT-organizing centres) instead of chromatin, imposing some topological reorganization of the Ran network.

Does this mean that the RanGTP gradient is ineffective in mammalian cells? Evidence from various sources indicate a global ‘directional’ role of RanGTP-rich chromosomes. In XEEs supplemented with sperm nuclei carrying centrosomes, the RanGTP gradient is required even when chromatin-directed MT nucleation is suppressed. Under these conditions, somehow abnormal spindles assembled, yet they connected properly to sperm chromatin; disruption of the gradient instead prevented MT connection to chromatin [14]. These findings are consistent with modelling data indicating that chromosomal RanGTP facilitates ‘search-and-capture’, a phase that would extend over several hours if based on purely stochastic MT movements [18]. Moreover, modelling the gradient in mammalian cells suggests that the chromosomal RanGTP concentration increases from prometaphase to metaphase [17]; the increased RanGTP signal as chromosomes progressively align would facilitate further MT capture.

Human MUG (mitosis with unreplicated genomes) cells, where chromatin is peripherally located and KTs (kinetochores) are visible in isolation, provide perhaps the most compelling information on RanGTP function in directing MT growth; in the MUG system, spindle MTs assemble from KTs, i.e. outside the areas of highest RanGTP concentration, yet MT growth orients directionally towards RanGTP-rich chromatin [19]. These data suggest that, in mammalian cells, the chromosomal RanGTP gradient provides a landmark guiding MT growth, regardless of their nucleation source.

Ran localization/function in mammalian mitotic cells

After NE breakdown, the distribution and mutual affinity between Ran network members determine where and when they interact, disassemble and release factors. Conformation-specific antibodies against the Ran C-terminal region, which extends outwards when Ran binds GTP [20], can be used to depict free or importin-bound RanGTP in intact cells (but not RanGTP fractions interacting with Ran-binding-domain-containing proteins, which engage the same region recognized by the antibody) (Figure 2). Intense signals are seen at asters, spindle poles and MTs until anaphase; this suggests that much RanGTP diffusing from chromatin, but a fraction that associates with MTs, is easily solubilized in the absence of NE. At later stages, the signal is transiently enriched at the midbody and eventually returns nuclear.
Ran is recruited to centrosomes by AKAP450 (A-kinase-anchoring protein 450), a coiled-coil protein acting as a scaffold for various centrosomal components. When AKAP450 was displaced from centrosomes by expressing a dominant-negative mutant retaining the centromere-binding domain, Ran was also displaced: under those conditions, the MT nucleation capacity of centrosomes was impaired, indicating a requirement for centrosomal Ran [22].

The centrosomal RanGTP/RanBP1 balance also regulates centrosome cohesion during spindle pole formation. RanBP1 overexpression, which decreases the availability of free RanGTP, generates multipolar spindles with split centrioles that remain connected to MT arrays [23]. Some viral oncoproteins target centrosomal Ran and/or RanBP1 and induce multipolar spindles and chromosome missegregation [24,25], underscoring the implication of centrosomal RanGTP/RanBP1, when deregulated, as a source of genetic abnormalities in mammalian cells.

RanGAP1 regulates RanGTP localization in centrosome- and KT-driven nucleation

Centrosomal MT nucleation, for which centrosomal RanGTP is required, can be abolished under certain circumstances and KTs take over as MTOCs. RanGTP is also required for KT-directed nucleation [26]. This is associated with RanGTP accumulation at KTs just before nucleation onset [27], which enables KT recruitment of γ-tuRC (γ-tubulin ring complex) components [28] and TPX2 (targeting protein for Xklp2) activation [26]. Some mechanism must therefore determine RanGTP localization.

In human cells, a SUMO (small ubiquitin-related modifier)-conjugated RanGAP1 fraction localizes to mitotic KTs [29,30] in a manner requiring MTs and RanBP2, a Ran-binding protein with SUMO-ligase activity. SUMO-conjugated RanGAP1 can hydrolyse RanGTP at KTs (reviewed in [21]). When centrosomal nucleation is abolished, RanGAP1 localization at KTs is prevented and RanGTP accumulates therein [27]. The reversible localization of RanGAP1 therefore provides a rapid switch between MT nucleation pathways; RanGAP1 loss from KTs can trigger KT-directed MT nucleation, whereas SUMO-dependent targeting at KTs can negatively regulate it when centrosomes are functional. RanBP2, which localizes at spindle poles, MTs and KTs [29], may act as ‘sensor’ of centrosomally nucleated MTs and regulate in response RanGAP1 localization at KTs, enabling or preventing RanGTP accumulation therein. The fact that in XEEs, where KTs are the only MTOCs, RanBP2/RanGAP1 are never recruited to KTs [21], despite the conservation of all components, is consistent with this view.

Importin/TPX2 interplay at mitotic MTs and poles

Fractions of RanGTP [31,32], RanBP1 [31,33] and RanGAP1 [29,30] co-localize at spindle MTs, implying that RanGTP localization

The localization of Ran network components and effectors is crucial to their function in mitotic cells, as illustrated in the examples below. Ran also has roles in KT/MT attachments (reviewed in [21]), which we do not discuss in the present paper for reasons of space.

Ran and centrosomal MT nucleation

Centrosomes contain Ran, at least in part GTP-bound [22], as well as RanBP1 [23]. Possibly, Ran associates with centrosomes as RanGTP and the centrosomal RanBP1 fraction modulates its interactions during MT nucleation and pole organization. The identification of centrosomal RanGTP represented the first significant difference in the spatial arrangement, and functional diversification, of the Ran network in mammalian cells compared with XEEs.
can be hydrolysed therein. Importin α/β also associate with MTs after NE breakdown and become part of complexes with dynein and TPX2 [34], the first identified RanGTP mitotic target in XEEs. Indeed, TPX2 activation by RanGTP near chromosomes is required to ensure its MT-nucleation activating function in XEEs (reviewed in [35]). Spindles can instead form in human cells without TPX2, but their poles fragment [36–38]. A similar phenotype is caused by importin β overexpression, which is rescued by simultaneously co-expressing TPX2 or generic NLSs that ‘buffer’ importin β excess [34]; this indicates that importin β excess causes spindle pole disruption via TPX2 inhibition. The formation of MT-interacting complexes containing dynein, TPX2 and its own importin inhibitor suggests, however, that some negative regulation of TPX2 along MTs is required as the spindle assembles. TPX2 can bind MTs directly and has MT cross-linking activity [39]. Dynein-dependent polewards transport of TPX2 in importin-containing complexes would ensure TPX2 accumulation and release at poles by local RanGTP, avoiding excess TPX2 activity along MTs [34]. TPX2 actually moves along MTs in an Eg5-, dynein- and flux-dependent manner, and motor-dependent polewards movement is necessary for incorporating KT–MTs in the spindle, while down-regulating MT nucleation near chromosomes [40]. Thus local pools of active TPX2 are required at different sites (e.g. chromosomes, MTs, poles) in somatic cells to organize MTs into a bipolar spindle. Tight control of the local TPX2 distribution is therefore essential, requiring increased specificity of Ran- and importin-dependent spatial regulation in mitotic cells.

RanBP1 regulates a subset of MT-associated factors

RanBP1 is the only network member to be quantitatively regulated in mammalian cells: it increases in early mitosis and declines in mid-telophase [33]. RanBP1 binds to and is hypothesized to limit the area of RanGTP–importin β complex formation, and hence of SAFs release, but what localizes RanBP1 to that area is not defined. In mammalian mitotic cells, RanBP1 up-regulation suggests that RanGTP–importin β complexes require specific tuning and increased dissociation rates compared with interphase; its co-localization at MTs with Ran and importin α/β suggests that MTs can provide a physical platform where these interactions occur. Indeed, RanBP1 inactivation yields abnormally stable MTs and hampers mitotic progression in human cells [17,31]. Lagging chromosomes appear in anaphase, probably reflecting non-corrected merotelic attachments, accompanied by a widespread distribution of the MT-stabilizing factor HURP (hepatoma up-regulated protein) [31], an importin β direct interactor that normally concentrates at MT-plus ends [43,44]. The distribution of classical NLS-containing SAFs (e.g. TPX2) is instead unaffected. Thus RanBP1 regulates mitotic factors with specificity in space (along MTs), time (with an increased rate from mitotic onset to early telophase) and selectivity for a subset of importin β-dependent targets.

Since the discovery of RAN/TC4 as a highly expressed gene in teratocarcinoma cells, the notion that Ran network members are aberrantly expressed in cancer has extended (reviewed in [45]). E2F/RB (retinoblastoma)-dependent regulation of RanBP1 expression [46] represents perhaps the most direct link between a major cancer-related pathway and a Ran regulator. The finding that RanBP1 abundance is critical for mitotic MT function opens a new issue, raising the possibility that it may influence the efficacy of MT-targeting chemotherapeutic drugs when deregulated in cancer cells. Indeed, RanBP1 silencing increased the sensitivity of transformed cells to taxol [47]; retrospectively, this can account for the observed paclitaxel resistance in RanBP1-overexpressing breast cancers (http://www.ncbi.nlm.nih.gov/geo; see RanBP1 in dataset record GDS630). Therefore, in a newly emerging scenario, RanGTP activity can amplify or antagonize the effect, and hence the therapeutic outcome, of MT-targeting drugs in cancer cells. In line with this view, Ran overexpression attenuates paclitaxel-induced cell death in glioblastoma cells [48].

Spatial and temporal regulatory functions of Ran and importin β

There is increasing evidence that Ran and importin β play subtle roles in spatial and temporal mitotic control, beyond the simple activation versus inhibition of SAFs. For example, the NLS-containing kinesin hKid is recruited to chromosomes in complexes with importin α/β, which inhibit its binding to MTs. RanGTP releases hKid and enables its deposition on chromosomes; given that hKid can only bind MTs after being released, RanGTP-dependent chromosomal localization may be viewed as a necessary step for subsequent MT localization [49]. This suggests that importins and RanGTP do not simply regulate the free versus bound state of hKid, but operate a sophisticated mechanism that orchestrates the balance between chromosome-interacting and MT-interacting populations in stepwise cycles of recruitment/deposition at mitotic sites.

A novel role of importin β has also been identified in protecting some direct interactors [e.g. NUSAP (nucleolar spindle-associated protein), HURP, but not TPX2], from premature APC/C (anaphase-promoting complex/cyclosome)-dependent degradation [50]. That finding may seem paradoxical, as importin β is regarded as an inhibitor of HURP and therefore the reason for preserving HURP from destruction is not obvious. The work, in fact, suggests that importin β is not just a negative regulator of HURP, but modulates its activity in subtle ways. HURP stabilizes MT-plus end contacts with KTs [43,44]; the requirement for active HURP may vary, both in amount and in space, until all attachments are established. Possibly, what was interpreted as an inhibitory function on
HURP when importin β was overexpressed uncovered a more subtle regulation mode, whereby importin β limits HURP release and activation where and when required, while at the same time shielding it from premature degradation.

The emerging theme is that Ran network members convey spatially restricted signals to factors in the mitotic apparatus in mammalian cells. Their relative ratio and dynamic interactions at specific sites are critical to spindle organization and function, whereas the chromosomal RanGTP gradient guides directional MT growth. Thus Ran network components and downstream factors are conserved, but regulatory mechanisms have evolved to adapt to different spatial contexts. Their regulatory specificity is linked to their topological reorganization in those contexts.

Funding

Our work is supported by Fondo Investimenti Ricerca di Base (FIRB) [grant number RBIN0417MT], Progetti di Ricerca di Interesse Nazionale (PRIN) [grant number 200879X9N9-004], Assicurazioni Generali and Fondazione Roma Terzo Settore.

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Received 23 June 2010
doi:10.1042/BST0381709