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REVIEWS

Complementary functions for the Ran gradient during division

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ABSTRACT

The Ran pathway has a well-described function in nucleocytoplasmic transport, where active Ran dissociates importin/karyopherin-bound cargo containing a nuclear localization signal (NLS) in the nucleus. As cells enter mitosis, the nuclear envelope breaks down and a gradient of active Ran forms where levels are highest near chromatin. This gradient plays a crucial role in regulating mitotic spindle assembly, where active Ran binds to and releases importins from NLS-containing spindle assembly factors. An emerging theme is that the Ran gradient also regulates the actomyosin cortex for processes including polar body extrusion during meiosis, and cytokinesis. For these events, active Ran could play an inhibitory role, where importin-binding may help promote or stabilize a conformation or interaction that favours the recruitment and function of cortical regulators. For either spindle assembly or cortical polarity, the gradient of active Ran determines the extent of importin-binding, the effects of which could vary for different proteins.

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In addition to its well-described function in nucleocytoplasmic transport, the Ran gradient regulates mitotic spindle assembly and cortical actomyosin-dependent events. These cortical processes include cellularization, polar body extrusion and cytokinesis. The mechanisms by which the gradient facilitates spindle assembly are well-described. However, importin-α and/or -β also can localize to the cortex and regulate the function of cortical proteins [Kiyomitsu & Cheeseman 2013, 1,2]. In this review we describe how during division, the Ran gradient plays complementary roles to spatially and temporally regulate spindle assembly and cortical regulation.

Nucleocytoplasmic transport is the best-known function for the small GTPase Ran and importins [3]. The role of karyopherins in nucleocytoplasmic transport has been reviewed extensively [e.g. 4–10]. If a message needs to be relayed to the nucleus, or if proteins or RNA need to be nuclear-localized, the nuclear envelope poses a logistical challenge [4,10,11]. The nuclear pore, a large multicomplex structure that spans the double membrane of the nucleus, serves as a selective gateway to allow for communication between the cytoplasm and the nucleus. Karyopherins, which includes the family of importins, are able to traverse the nuclear pore to bring proteins into or out of the nucleus [6]. Broadly speaking, the process of nucleocytoplasmic shuttling involves the interplay between importins, Ran and exportins [10]. Importins bind to proteins through their nuclear localization signal (NLS) and transport them into the nucleus. Active Ran triggers their dissociation causing NLS-proteins to remain in the nucleus while importins return to the cytoplasm [4–10]. In addition, some proteins bind to exportins and Ran-GTP for transport out of the nucleus.

This review highlights recent data describing the roles of Ran and importins beyond their transport functions. Many proteins that regulate mitotic spindle assembly and cytokinesis have NLS sequences that may regulate their activity via importin-binding (Table 1). Collectively, studies support a model where the Ran/importin gradient is an elegantly balanced system with dual control of processes close to and away from chromatin – a biological example of the principle of yin and yang. We aim to highlight emerging evidence supporting that the functions of Ran-GTP at the two ends of the gradient are interrelated and complementary functions of one system. Although much remains to be explored, we postulate that the Ran gradient acts as a sliding scale. Our current knowledge supports Ran-GTP as a spatial and temporal cue that influences a variety of processes along the length of its gradient across the cell.

Ran-mediated regulation of the mitotic spindle

The regulation of mitotic spindle assembly is one of the prevalent non-transport functions of importins. The Heald group showed that a gradient of active Ran

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Table 1. Ran-GTP regulation of proteins through importins.

Protein	Protein Function	Interacting Importin	Reference
RCC1	RanGEF	Importin α3 Importin β1	[101,102]
HURP		Importin β1	[33]
Kid		Importin α1 Importin β1	[103,104]
NuMA	Spindle Assembly Factor	Importin α1 Importin β1	[30–32]
TPX2		Importin α1 Importin β1	[27–29]
XCTK2		Importin α1 Importin β1	[105]
Cdc7	Serine/threonine kinase	Importin α2 Importin β1	[39,106]
PTHrP	Various functions	Importin α1 Importin β1	[40,107]
Snail	Transcription factor	Importin α1 Importin α3 Importin α5 Importin β1	[41]
TRF1	Regulator of telomere length	Importin α1 Importin β1	[42]
Ect2	RhoGEF	Importin α* Importin β1	[83,92]
Anillin	Scaffold for the contractile ring	Importin α Importin β1 Importin β2	[1,58,80]
Cyk-4/ MgcRacGAP	Forms central spindle	Importin α1 Importin β1	[88]
MKLP1	Forms central spindle	Unknown	[87,90,91]
GAL4	Transcription factor	Importin α1 Importin β1	[43,108]
N-WASP	Activator of Arp2/3	Importin α1 Importin α5	[51]
GCK-III	Subgroup of Ste20-like serine/ threonine kinases	Unknown	[84]

*Ect2 contains a classic NLS, and though direct importin-α interaction was not demonstrated, heterodimer interaction was inferred through importin-β binding.

forms in the vicinity of chromatin, which controls the release of importin-bound spindle assembly factors [SAFs; 12–14]. In interphase cells, RCC1, the RanGEF (guanine nucleotide exchange factor), is enriched in the nucleus, and RanGAP (GTPase-activating protein), is in the cytoplasm [10,15]. Their differential localization creates a gradient of active Ran that is high in the nucleus and low in the cytoplasm. This gradient persists after nuclear envelope breakdown, as RCC1 remains associated with chromatin [13, 16, Figure 1(a)]. Importin-α binds to the classical NLS of SAFs and serves as an adaptor for importin-β via its autoinhibitory IBB (importin-β binding) domain [10,11,15,17–19]. Importin-β-binding causes a conformational change that displaces the IBB and relieves autoinhibition to permit cargo-binding [18–20]. The working model is that binding of the α/β heterodimer impedes SAF function by hindering binding to proteins required for their function in bipolar spindle assembly. When Ran-GTP binds to the importin-SAF complex in the vicinity of chromatin, the SAF is released to carry out its function [7,10,21]. As a result, cells have a gradient of SAF-bound importins that is inverse, although not necessarily proportional to the active Ran gradient (Figure 1(a,b)).

The Ran-GTP gradient was demonstrated in several model systems by the Heald lab [12,13,16]. They generated a fluorescence resonance energy transfer (FRET) probe termed Rango (Ran-regulated importin-β cargo) that indirectly shows Ran-GTP levels. Strikingly, they showed that a gradient of active Ran persists after nuclear envelope breakdown in mitotic *Xenopus laevis* egg extracts and in HeLa cells [13,16]. In both systems, the Ran-GTP gradient is steep with high concentrations near chromatin, and lower concentrations over the length of the spindle, followed by a sharp decrease at spindle poles [13,16]. Importantly, the steepness of the gradient is not the same in every cell, and at least partly depends on ploidy, with chromosomal gain driving a steeper gradient [22]. It will be interesting to determine how the regulation of RCC1 or RanGAP gene expression compares between different cell types, which could indicate different threshold requirements for the Ran-regulation of spindle assembly. For example, this pathway could be more dominant in aneuploid cancer cells to help them avoid mitotic catastrophe.

Several reviews have highlighted how the Ran gradient regulates the function of SAFs [e.g. 23,14]. Spindle assembly requires the coordinated function of MAPs (microtubule associated proteins) required for microtubule nucleation, stability, bundling and/or motors to generate force [23,24]. As cells enter mitosis, centrosomes mature, nucleate microtubules and separate. The length and kinetics of microtubules must be controlled to ensure the formation of stable microtubule attachments at kinetochores, which is necessary for proper chromosome alignment and subsequent separation as cells exit mitosis [25,26]. Factors such as TPX2, NuMA and HURP, which regulate microtubule nucleation, bundling and stability, are all negatively regulated by importin-binding [14,23]. TPX2 is directly inhibited by importin-α of the heterodimer [27–29], while NuMA is sterically hindered by importin-β of the heterodimer [30–32], and HURP is directly inhibited by importin-β-binding [33, Figure 2]. Thus, different SAFs are regulated differently by importin-α, importin-β, or the heterodimer. Since each could have unique contact sites when bound to cargo, their effect on intra- or intermolecular interactions could be different [7,15,30,34]. This fits with the concept that not all SAFs have the same spatial or temporal functional requirements [35].

Different SAFs function in different locations of the cell for mitotic spindle assembly. The mitotic spindle

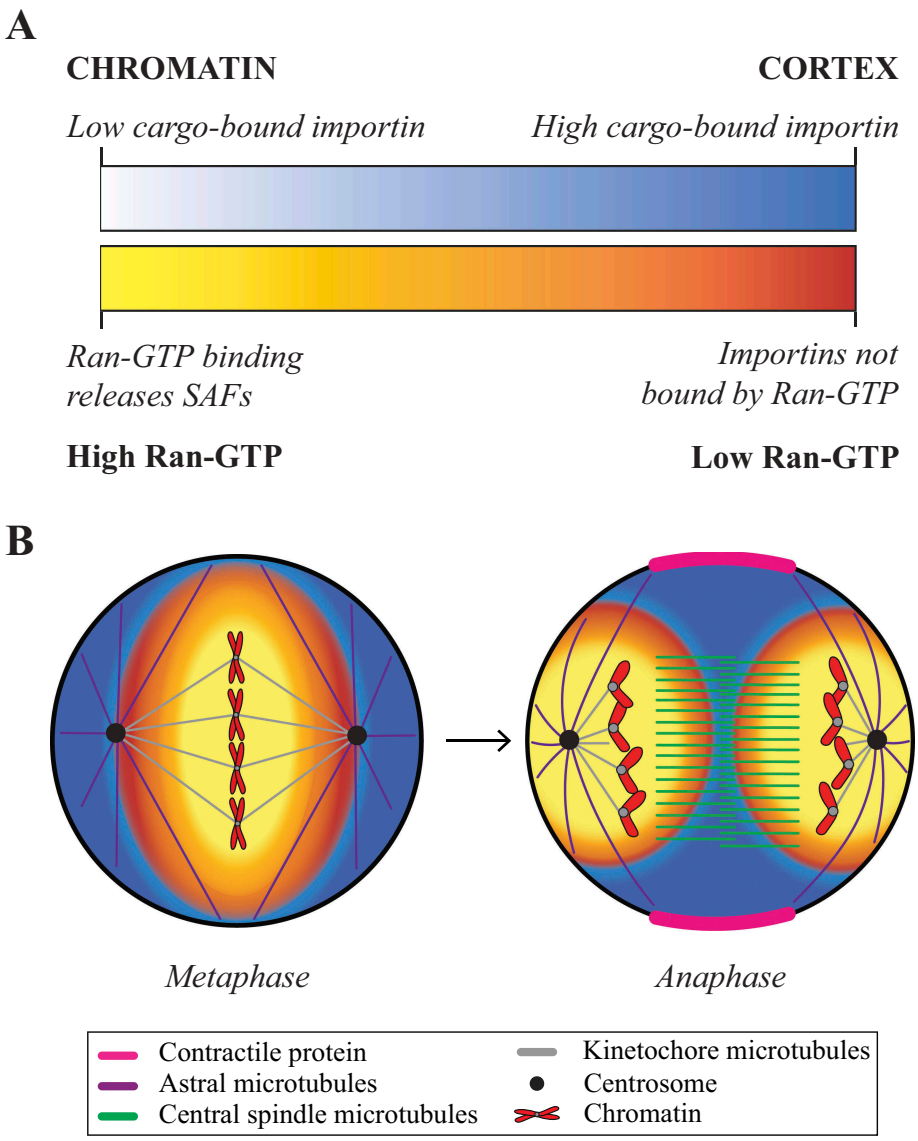

Figure 1. The Ran gradient regulates different stages of mitosis. (a) Following nuclear envelope breakdown, active Ran (Ran-GTP) levels remain as a gradient that decreases from chromatin towards the cortex (orange gradient – dark orange is low) [13,16]. RCC1, the RanGEF that generates active Ran, remains associated with chromatin, while RanGAP generates inactive Ran (Ran-GDP) and is cytosolic [12]. There is an inverse gradient of importins bound to NLS-containing proteins, which is highest near the cortex (blue gradient – dark blue is high) [4]. (b) Cartoon schematics show a cell in metaphase (left) and anaphase (right) with the relative locations of active Ran (orange gradient) and importin-bound proteins (blue gradient) [13,16]. The legend indicates the components of the cell with chromatin (red), centrosome (black), central spindle microtubules (green), astral microtubules (purple), kinetochore microtubules (grey) and contractile proteins (pink). During metaphase, the spindle is controlled by the high levels of Ran-GTP around chromatin, while in anaphase, importin-binding facilitates the cortical localization of proteins such as anillin to control polarity [1,12].

occupies a large proportion of the cell, with the spindle poles positioned away from chromatin where Ran-GTP levels are highest [13]. Some SAFs are required close to chromatin for chromosome alignment, such as HURP and the chromokinesin Kid, while others function at the poles and/or over a larger distance, such as XCTK2, TPX2 and NuMA for minus end stability and/or microtubule nucleation [12,35,36]. The spatial and temporal control of SAFs could be achieved through their different binding affinities for importin-α, -β, or the heterodimer, or steepness of the Ran-GTP gradient. Our understanding of the spatial requirements for the Ran/importin gradients could benefit from computational models of spindle assembly [37,38]. Further, visualizing these gradients in different cell types and cell cycle stages would help verify and improve these models, and predict where they function.

The formation of importin-SAF complexes also could vary depending on intra-/intermolecular inhibition or post-translational modifications. A recent study

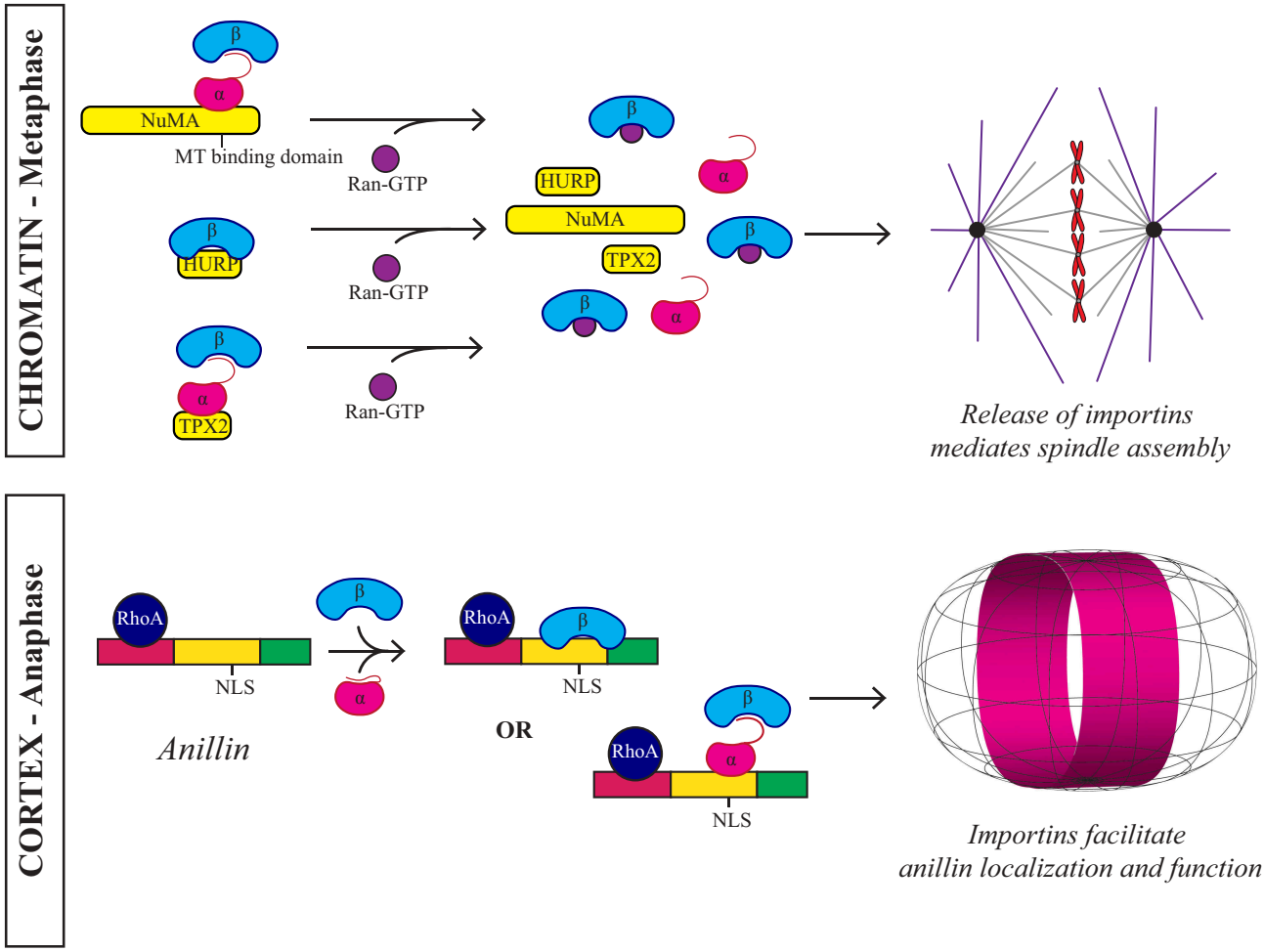

Figure 2. Ran regulates proteins required for spindle assembly and cortical polarity. During metaphase, the Ran gradient regulates spindle assembly in the vicinity of chromatin. Among other proteins, importin-binding inhibits the function of TPX2, NuMA and HURP (all in light yellow), although the mechanism by which this occurs differs for each protein [14,23]. The microtubule-bundling activity of HURP is directly inhibited by importin-β (blue) binding [33], whereas the α/β heterodimer inhibits TPX2 and NuMA. In the heterodimer, importin-β sterically hinders the microtubule binding site of NuMA [30–32]. Importin-α (pink) directly inhibits TPX2, but requires importin-β for TPX2-binding [27–29]. The release of SAFs from importins by Ran-GTP (purple) permits them to carry out their function in spindle assembly [12]. The spatial location of cargo release from importins would depend on various factors including binding affinity to importins (-α, -β or the heterodimer) and post-translational modifications. Thus, where the importin and Ran gradients are functionally relevant, as well as the gradient steepness and length-scale of these gradients could be unique to each NLS-containing cargo. During anaphase, importin-binding regulates cortical proteins [1]. In particular, anillin is a conserved protein that crosslinks components of the contractile ring for cytokinesis. The C-terminus of anillin contains a RhoA-GTP Binding Domain (RBD; red), a C2 domain (yellow), and a Pleckstrin homology domain (PH; green) [95]. RhoA-GTP (dark blue) binds to the RBD, causing a conformational change that relieves autoinhibition of the NLS in the neighbouring C2 domain. This domain also contains binding sites for phospholipids, microtubules and Ect2, the GEF required for RhoA activation. Importin-β-binding facilitates cortical recruitment, by stabilizing a conformation that may favour these other interactions [1]. We propose that other NLS-containing contractile proteins could similarly be regulated by importin-binding.

showed that a fraction of importin-α is palmitoylated and associated with the plasma membrane [2]. Hyper-palmitoylation caused a decrease in spindle and nuclear size, suggesting that sequestering importin-α at the membrane reduces the cytosolic pool regulating SAF function and nuclear import [2]. However, hyper-palmitoylation did not prevent a bipolar spindle from forming, suggesting that many SAFs remained functional likely because they are regulated directly by importin-β. This also raises the question as to whether importin-β binds to palmitoylated -α. This study highlights the unique localization and/or functions of importin-α, and it would be interesting to understand the different threshold requirements for the function of importin-α or -β as monomers vs. the heterodimer.

An increasing number of studies is requiring us to re-evaluate the conventional view on how the Ran/importin system regulates NLS-containing proteins.

For example, several studies showed that the importin-β-mediated nuclear localization of proteins such as Cdc7 [39], PTHrP [40], Snail [41] and TRF1 [42] is inhibited by importin-α. Another study showed that importin-α acts as a coactivator of the transcriptional activator GAL4 when it is bound to DNA [43]. Thus, importins can play negative or positive roles in different contexts, and the binding of importin-α and/or -β does not have to impede the function of a target protein, but rather could facilitate conformational changes that are favourable for binding to other partners and/or function. As discussed in the following sections, the location of a particular protein along the Ran gradient could also correlate with whether importins positively vs. negatively regulate protein function.

Ran-mediated regulation of the cortex

Ran in meiosis

The Ran-GTP gradient also regulates polar body formation in mouse oocytes. During meiosis, polar bodies extrude complements of DNA to reduce ploidy [reviewed in 44, 45]. The small, acentrosomal meiotic spindle forms near the cortex and positions the chromosomes for segregation into the polar body (Figure 3(a)). Prior to extrusion of the polar body, the cortex is polarized by the formation of an F-actin cap [44,45]. Dumont et al. [46] used the previously mentioned FRET probe Rango to show that a Ran-GTP gradient forms around meiotic DNA in the mouse oocyte. In a separate study, Deng et al. [47] showed that Ran-GTP is required to establish cortical polarity and induce the formation of the cortical F-actin cap. By injecting beads coated with bacterial or yeast plasmid DNA into MII oocytes, they found that the cap could still form in response to any type of DNA. Interestingly, the elicited response was both DNA-dosage and -distance dependent. Both the amount of input DNA and the distance of DNA to the cortex correlated with the magnitude of response; one DNA bead induced a smaller cortical cap than three beads, and three DNA beads could elicit a response within 10 μm of the cortex, less so at 20 μm, and not at 30 μm. Thus, the authors hypothesized that the Ran-GTP gradient helps cells sense chromatin position by serving as a molecular ruler.

Additional studies revealed that there could be crosstalk between Ran and Cdc42, although they did not explore the mechanism by which this occurs [48]. Active Cdc42 recruits N-WASP to regulate Arp2/3 for the nucleation of actin filaments that form the cortical cap [49]. N-WASP has an NLS and it would be interesting to determine if it can be directly regulated by importin-binding [50,51]. Burdyniuk et al. [52] proposed a unique role for Ran-GTP in regulating F-actin for chromosome alignment in starfish oocytes. They found that an Arp2/3-nucleated F-actin network forms around chromosomes during meiosis in a Ran-GTP-dependent manner to collect chromosomes scattered over a large distance [52]. Since these F-actin patches prevent microtubule-kinetochore attachments, they must disassemble before attachments can be made, which would help prevent aneuploidy.

In addition to regulating chromosome alignment, having a cue associated with meiotic chromatin that regulates the cortex would ensure that actin and myosin assembly for polar body formation occurs only when chromatin is at an ideal distance to the cortex to prevent aneuploidy. Deng et al. [47] also reported that injection of constitutively active Ran^{Q69L} inhibited cap formation rather than inducing larger or multiple caps, which is similar to observations from studies on the role of Ran in cytokinesis [1]. Further studies using ooctyes that vary in size, and from different species, will expand our knowledge of the molecular mechanisms of the Ran pathway in meiosis.

Importins in cellularization

Another cortical process that was shown to be regulated by importins is cellularization in *Drosophila* (Figure 3(b)). After 9 mitotic divisions, the nuclei of the syncytial embryo migrate to the periphery and subsequently become separated by membranes via a process of cellularization, which begins during the 14th division [e.g. 53, 54, 55]. This process gives rise to a layer of polarized epithelial cells connected via adherens junctions, and occurs due to the trafficking of vesicles for directed membrane growth [56]. The end-stages of cellularization have some similarity to cytokinesis. Anillin, a scaffold protein that binds to actin, myosin and septins and has well-described roles in cytokinesis, is also required for cellularization, although its role in this process is not well-understood [57]. Silverman-Gavrila et al. [58] showed that importins could regulate anillin's localization during cellularization. They found that over-expression of importin-α decreases anillin's cortical localization, and showed that importin-α/β could outcompete the septin Peanut for anillin-binding [58]. However, since the nuclei are enclosed during cellularization and Ran-GTP would be sequestered, it is not clear how importin-binding regulates anillin localization. Based on our studies of anillin in cytokinesis (see below), one hypothesis is that cytosolic importins promote anillin's recruitment to the cortex by modulating its conformation for septin and/or lipid-binding, but its enrichment to precise locations is governed by binding to active RhoA.

Other studies showed that importins can regulate proteins independently of Ran for mitotic Golgi

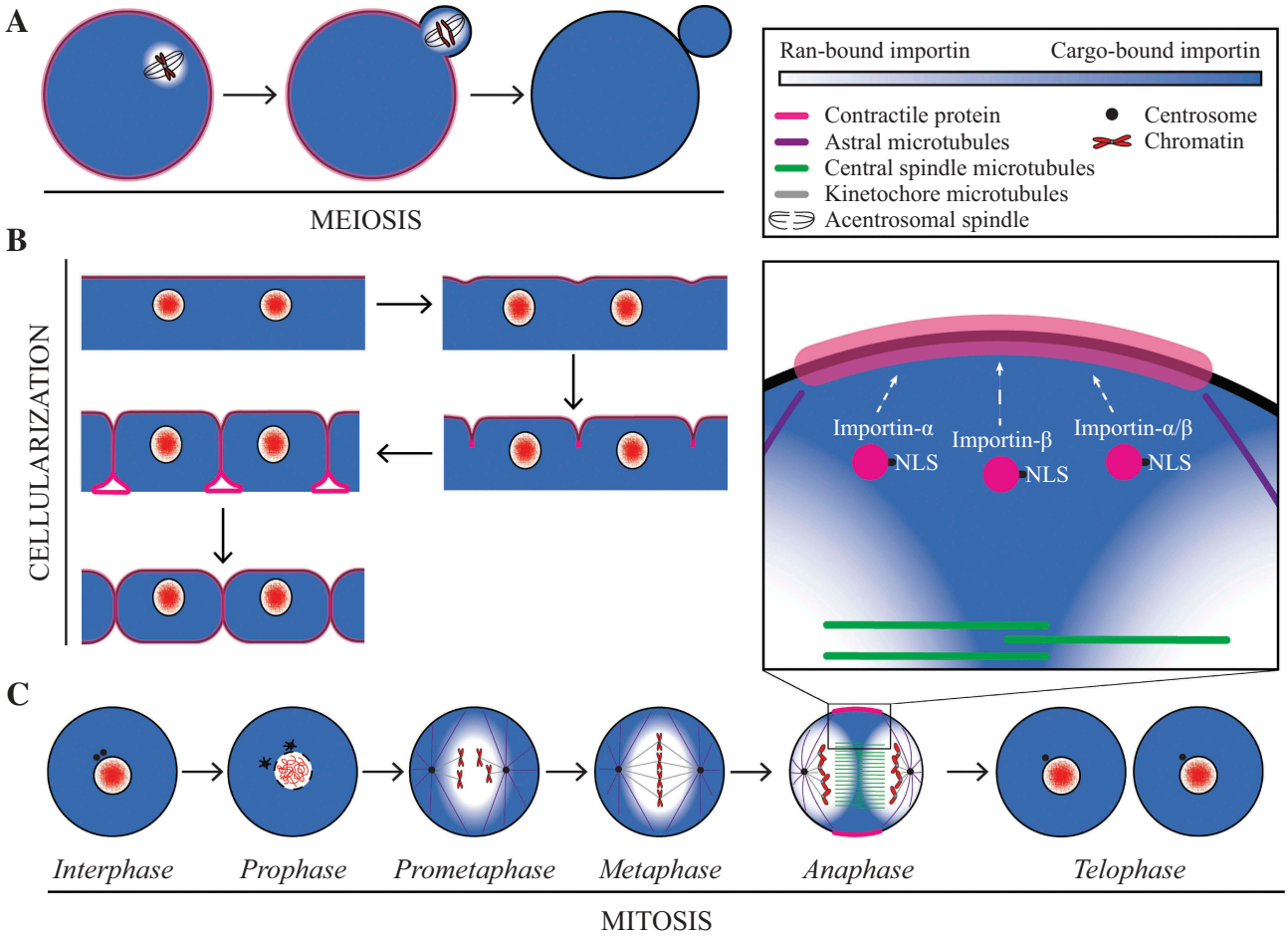

Figure 3. The Ran gradient regulates cortical proteins. (a) Cartoon schematics show an oocyte undergoing polar body extrusion. The legend describes the cell components for the cells in (a–c); contractile proteins (pink), astral microtubules (purple), central spindle microtubules (green), kinetochore microtubules (grey), centrosome (black) and chromatin (red). The Ran gradient is enriched around chromatin, which is positioned close to the cortex and functions as a molecular ruler to direct actin cap formation [47]. (b) During cellularization, Ran-GTP is sequestered in closed nuclei, and importin-binding could increase the localization of proteins at the cortex. The enrichment of proteins at the ingressing membrane would be directed by other factors. (c) In mitotic somatic cells Ran-GTP is generated at chromatin by RCC1 (RanGEF), which is hydrolysed by RanGAP in the cytosol to form a gradient. An inverse gradient of importin-bound proteins forms so that they are high near the cortex. In prometaphase and metaphase, Ran-GTP regulates spindle formation by releasing active spindle assembly factors from importin-binding [12]. In anaphase, importin-binding facilitates anillin's localization to the equatorial cortex for cytokinesis, and we propose that other cortical proteins could similarly be regulated by importin-binding [1]. Ran-GTP is sequestered in the nucleus in telophase as the nuclear envelope reforms (Clarke & Zhang, 2008, 4).

disassembly [59]. Importin-α and other karyopherins may thus have interactions and functions that occur outside of the Ran pathway. This raises the possibility that in the context of cellularization – a closed system in which little to no Ran would be found in the cytoplasm – importins could be functioning at the cortex in a Ran-independent manner to form separate cells.

Ran in cytokinesis

Cytokinesis occurs at the end of mitosis to separate a cell into two daughters (Figure 3(c)). This highly conserved process must occur with high precision to avoid aneuploidy or changes in cell fate [60–62]. Multiple pathways regulate cytokinesis, and can be microtubule-dependent or -independent [60,62]. While these pathways likely function redundantly in symmetrically dividing cells, the preference for one over another may depend on cell fate, architecture, or ploidy. Cytokinesis occurs due to the ingression of a RhoA-dependent contractile ring. The central spindle, which arises between segregating chromatids in anaphase, stimulates the accumulation of active RhoA in the equatorial cortex via the regulation of cortical complexes that activate Ect2, a RhoA guanine nucleotide exchange factor [GEF; e.g. reviewed by 60–62]. Ect2 forms an anaphase-dependent complex with Cyk-4/

MgcRacGAP and MKLP1 at the cortex, which potentiates its activity [61,63–65]. Astral microtubules, which emanate from the centrosomes towards the polar cortex, globally inhibit cortical contractility, leading to the equatorial accumulation of contractile proteins as the spindle elongates [61,62,66]. In addition, MP-GAP globally inhibits RhoA and functions together with astral microtubules to ensure that cortical contractility is dampened outside the equatorial plane [67]. p190RhoGAP also controls contractility in the division plane by modulating RhoA activity [68–71]. It is not clear how astral microtubules regulate cytokinesis, although data supports that these microtubules could sequester anillin, which has a microtubule-binding domain [72,73]. As shown in *C. elegans* embryos, another mechanism could involve the TPXL-1 (TPX2)-mediated polar clearance of contractile proteins by Aurora A kinase [74]. Microtubule-independent pathways also regulate cytokinesis, by signalling through the centrosomes, kinetochores and chromosomes [1,47,58,67,75–79]. In particular, sensing chromatin position could help prevent aneuploidy, especially in asymmetrically dividing cells, and will be discussed below.

In mammalian cells, several studies demonstrated a correlation between chromatin position and cortical contractility during anaphase [1,77,79]. The Ran gradient persists into anaphase (Figures 1(a,b) and 3(c)), and Kiyomitsu & Cheeseman [77] showed that elongation of the cortex occurs in response to spindle positioning. In particular, the site of ingression shifts to recover the equatorial plane when the spindle is displaced towards one of the poles. They proposed that cortical proteins are negatively regulated by Ran-GTP associated with chromatin. In support of this model, they found that cortical proteins polarize in response to chromatin position in BHK (baby hamster kidney epithelial) cells with depolymerized microtubules forced to exit mitosis, which fail to occur upon loss of RCC1 [77]. Overall, this data showed that Ran-GTP inhibits contractility at the cortex, although the mechanism by which it does this was not known [77].

A more recent study by our group offers insight into the molecular mechanism of how Ran-GTP regulates the cortex for cytokinesis [1]. We found that importin-β binds to a conserved C-terminal NLS in anillin, and point mutations that disrupt importin-binding decrease anillin's cortical affinity and function for cytokinesis (Figure 2). The NLS, which is in the C2 domain, is autoinhibited by the neighbouring RhoA-GTP binding domain (RBD). This led us to propose a model where active RhoA initially induces conformational changes in anillin, that then could be stabilized by importin-binding (Figures 2 and 3(c)). Our model also considers that importins optimally regulate anillin function at an ideal concentration. Similar to the findings from Silverman-Gavrila et al. [58] for cellularization, over-expressing importin-β also decreases anillin's cortical affinity during cytokinesis [1]. We propose that anillin's affinity for importins is lower than that of its other binding partners such as phospholipids, RhoA regulators and septins to permit a 'hand-off' from importins to these other components at the equatorial membrane.

Several cytokinesis regulators have at least one NLS that mediates nuclear localization during interphase, and it would be exciting to explore their regulation by the Ran pathway during mitotic exit [i.e. 80–83]. For example, mammalian GCK-III proteins have an NLS [84], and recent studies showed that GCK-1 (*C. elegans*) may counteract active RhoA by restricting the amount of anillin and myosin in the contractile ring to brake contractility [85,86]. Other key cytokinesis regulators with an NLS include Ect2, Cyk-4, and MKLP1 [83,87,88]. Having an NLS could permit the regulation of cytokinesis proteins in various ways by importin-binding. For example, Ect2 and MKLP1 have phosphorylation sites for cell cycle kinases in/near their NLS's, and phosphorylation could affect importin-binding, causing them to accumulate in the cytosol during prophase and/or prevent their sequestration after nuclear reformation [89–92]. However, another role to consider for importin-binding could be to control their cortical localization and function. Interestingly, human anillin has more than one NLS; the N-terminal NLS mediates nucleocytoplasmic transport through importin-β2-binding, while the highly-conserved C-terminal bipartite classic NLS binds to importin-β for anillin's cortical recruitment and function in cytokinesis [1,80]. This raises an interesting question as to whether the highly conserved, C-terminal NLS initially arose in metazoans to mediate nuclear localization, but then was co-opted into a second function of controlling cortical localization and function, or vice-versa.

In a biological context, there are many advantages to having the Ran pathway regulate cytokinesis proteins. The enrichment of importins available to bind to NLS-containing proteins near the cortex can facilitate the recruitment of cortical regulators prior to central spindle-dependent mechanisms. In cells where chromatin is asymmetrically positioned, this can create an asymmetric distribution of contractile proteins for asymmetric furrow ingression. In cells where ploidy is high, this could delay contractile protein recruitment until chromosomes have already begun segregating

towards their poles, which could tightly couple ingression with chromosome segregation to prevent aneuploidy.

Most of our knowledge of cytokinesis is from studies done using cultured cells, either from *Drosophila* (S2 cells) or mammalian cells (HeLa cells), or in the one-celled *C. elegans* or sea urchin embryo [e.g. 93–95, 64]. It is assumed that the preference for different mechanisms regulating cytokinesis depends on the organism, but this could also be due to differences in cell fate, geometry, ploidy or the number of neighbouring cells. For example, the central spindle is quite small in the early embryo relative to cell size in *C. elegans*, echinoderms and *Xenopus*, and the astral spindle pathway more dominantly regulates cytokinesis in these cells [76,96,97]. A recent study by Davies et al. [98] showed that P_2 and EMS cells rely differently on F-actin-dependent mechanisms, as well as intrinsic vs. extrinsic cues. This highlights the need to explore mechanisms regulating cytokinesis of cells in their native tissue and in developmental contexts. Since few studies have explored the role of the Ran pathway in cytokinesis, we are studying its role in regulating cytokinesis of AB and P_1 cells in early *C. elegans* embryos. The AB cell, which is larger and divides first, is fated to be many tissues of the body, while the P_1 cell is fated to become the germline [99]. It will be interesting to determine if the Ran pathway differently regulates cortical contractility for cytokinesis in these cells.

Concluding remarks

To summarize, the Ran/importin gradient is a beautiful example of the principle of yin and yang where the cortex and spindle are regulated in opposing, but complementary ways by the same system. Cortical regulation and spindle assembly occur at opposite ends of the gradient, which acts as a sliding scale that ties these functions together. However, the gradient likely is not linear and the impact on proteins will vary depending on their binding affinities for importin-α, -β or the heterodimer, post-translational modifications, and accessibility or levels at particular cellular locations. Also, since few cortical targets have been identified, the extent to which the gradient regulates cortical polarity is not clear. The finding that RanBP1 (Ran-binding protein 1) controls cortical neuron polarity via regulating LKB1/Par4 [100] suggests that other Ran pathway components also influence protein function. Thus, there may be many layers of complexity in how the Ran pathway regulates polarization in different cell types.

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