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Review

Small GTPase Ran and Ran-binding proteins

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Abstract

Like many other small GTPases, Ran functions in eukaryotic cells as a molecular switch that cycles between GTP- and GDP-bound forms. Through the proper modulation of the GTP/GDP cycle, Ran functions with a number of Ran-binding proteins to control a broad array of fundamental cellular functions, including nucleocytoplasmic transport, mitotic spindle assembly, and nuclear envelope and nuclear pore complex formation. Recent studies have revealed that 'Ran and Ran binding proteins' are involved in a variety of functions involving cell fate determination, including cell death, cell proliferation, cell differentiation, and malignant transformation. In this review, we discuss recent progress on the functional link between the Ran system and tumorigenesis, which give clues to the molecular understanding of cancer biology.

Keywords: cancer; cell fate determination; importin β; nucleocytoplasmic transport; Ran.

Introduction

As will be discussed in this review, Ran and Ran-binding proteins are expected to be deeply involved in multiple aspects of biological functions not only on cellular levels but also on physiological levels. Ran-binding proteins mediate not only the RanGTPase cycling switch (e.g., RCC1, RanGAP, RanBP1, and RanBP2), but also other fundamental activities, such as nucleocytoplasmic transport (e.g., importin β superfamilies). The small GTPase Ran was originally identified in a human teratocarcinoma line as a factor encoding a Ras-like sequence (1). Ran is a highly abundant and strongly conserved GTPase encoding a ~25 kDa protein primarily located in the nucleus (2). On the one hand, as revealed by a substantial body of work, Ran has been found to have widespread functions since its initial discovery. Like other small GTPases, Ran functions as a molecular switch by binding to either GTP or GDP. However, Ran possesses only weak GTPase activity, and several well-known 'Ran-binding proteins' aid in the regulation of the GTPase cycle. Among such partner molecules, RCC1 was originally identified as a regulator of mitosis in tsBN2, a temperature-sensitive hamster cell line (3); RCC1 mediates the conversion of RanGDP to RanGTP in the nucleus and is mainly associated with chromatin (4) through its interactions with histones H2A and H2B (5). On the other hand, the GTP hydrolysis of Ran is stimulated by the Ran GTPase-activating protein (RanGAP) (6), in conjunction with Ran-binding protein 1 (RanBP1) and/or the large nucleoporin Ran-binding protein 2 (RanBP2, also known as Nup358). RanBP1 was originally named HTF9A in the course of a study searching for a bidirectional promoter at the HTF9 locus, the function of which remains unknown (7), but was re-named after the discovery of its RanGTP-binding activity (8). Meanwhile, RanBP2 was identified as a large-sized component of the nuclear pore complex (NPC), encoding a ~358 kDa protein that binds to Ran (9, 10). In addition, as shown in Table 1, at least 21 importin β superfamily members in humans and 14 members in *Saccharomyces cerevisiae* bind to RanGTP. All of the importin β superfamily members contain a helical HEAT (=huntingtin, elongation factor 3, the PR65/A subunit of protein phosphatase 2A, and the lipid kinase Tor) repeats and a Ran-binding domain. Members of the importin β superfamily bind preferentially to RanGTP via their N-terminal region. The details for the importin β superfamily are discussed at length in other reviews (11, 12).

Ran and Ran-binding proteins play critical roles in a broad array of cellular activities, including nucleocytoplasmic transport, mitotic spindle assembly, and nuclear envelope (NE) and NPC reformation. In this review article, we provide an overview regarding the involvement of the Ran system, as exerted by Ran and Ran-binding proteins, in the three fundamental cellular functions mentioned above, focusing on representative factors among the Ran-binding proteins. Furthermore, we introduce recent progress on the newly discovered functions of the Ran system at the cellular level and, where possible, at the organism level. Moreover, we discuss how unsolved problems regarding Ran and Ran-binding proteins will be investigated in the future.

Table 1 Nomenclature of importin β superfamily members in vertebrate systems and their homologs in yeast.

Vertebrate importin β superfamilies	Yeast homologs
Importin β1	Kap95p
Importin β2/transportin	Kap104p
Importin 4/RanBP4	Kap123p
Importin 5/RanBP5	Kap121p
Importin 7/RanBP7	Kap119p/Nmd5p
Importin 8/RanBP8	Kap108p/Sxm1p
Importin 9	Kap114p
Importin 11	Kap120p
Importin 13	–
–	Kap122p/Pdr6p
Transportin SR1, SR2, 3	Kap111p/Mtr2
Exportin 1/CRM1	CRM1p
Exportin 2/CAS(CSE1L)	Cse1p
Exportin 4	–
Exportin 5	Kap142p
Exportin 6	–
Exportin 7/RanBP16	–
Exportin-t	Los1p
RanBP17	–
Snurportin	–

Ran and Ran-binding proteins in nucleocytoplasmic transport

In interphase, Ran and Ran-binding proteins regulate nucleocytoplasmic transport through NPCs, giant protein structures ~125 MDa in size and embedded in the NE. Although small molecules, such as ions and small proteins (smaller than ~40 kDa) are able to diffuse passively through the NPCs, most macromolecules larger than 40–50 kDa are selectively transported through the NPCs by an active process mainly controlled by Ran and Ran-binding proteins. Ran and Ran-binding proteins control the assembly and disassembly of nucleocytoplasmic transport complexes, which are generally composed of cargo proteins with a family of Ran-binding proteins (collectively called 'importins' and 'exportins') that recognize cargo signals [nuclear localization signals (NLSs) or nuclear export signals (NESs)] for localization in the nucleus or the cytoplasm.

There are two types of classical NLSs: (i) the monopartite NLS, consisting of a single cluster of basic amino acids, as exemplified by the NLS (PKKKRKV) in simian virus 40 (SV40) large tumor (T) antigen (13, 14); and (ii) the bipartite NLS containing two clusters of basic amino acids with spacer residues, such as those in nucleoplasmin (KRPAAIKKAGQAKKKK) (15) and Cap-binding protein 80 (CBP 80) (RRRHSCENDGGQPHKRRK) (16). Meanwhile, the NES is generally composed of hydrophobic amino acids, such as leucine, as found in HIV Rev (LQLPPLERLTL) (17, 18).

For nuclear import (Figure 1), the classical NLS-containing cargo protein is initially recognized by an adaptor molecule called importin α (also known as karyopherin α or PTAC 58) (16, 19, 20). These two proteins subsequently form a ternary complex with importin β1 (also called karyopherin β1 or PTAC 97) (21–24), which possesses Ran-binding activity. The nuclear-imported ternary complex is dissociated by the action of nuclear RanGTP via binding to importin β1, and the cargo proteins are consequently released in the nucleus. Nuclear importin α is recycled out of the nucleus as a ternary complex with RanGTP and a specific exporter for importin α, CAS (also called CSE1L in humans) (25–28). CAS was originally identified as a factor that renders breast cancer cells resistant to apoptotic stimuli and has been shown to bind to RanGTP (29). After

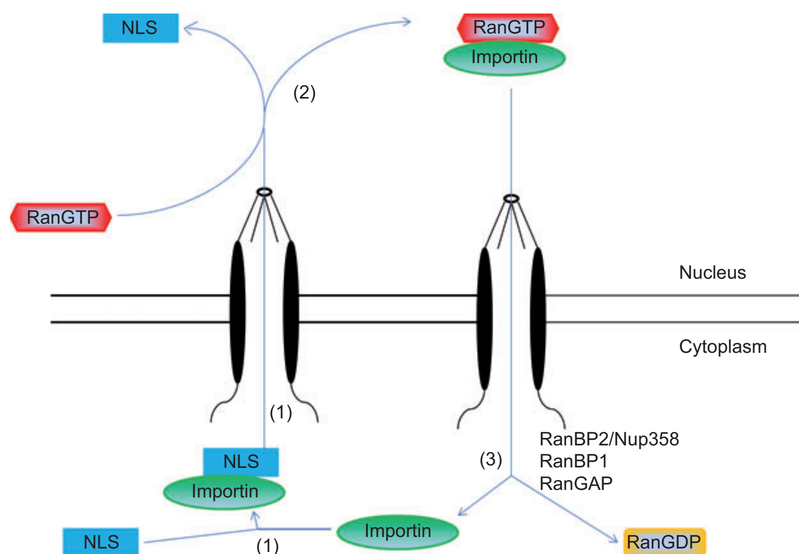

Figure 1 Ran and Ran-binding proteins in the nuclear import cycle.
(1) Nuclear import of NLS-containing proteins mediated by importins α and β (collectively referred to as 'importins') as a ternary complex. (2) Dissociation of the ternary complex by nuclear RanGTP. (3) Dissociation and hydrolysis of nuclear-exported RanGTP-importin complex by the action of RanGAP in conjunction with RanBP1 and/or RanBP2.

translocation through the NPCs, the RanGTP-importin β1 export complex and RanGTP-CAS-importin α export complex are dissociated by cytoplasmic RanGAP with the aid of RanBP1 and/or RanBP2. In this process, GTP bound to Ran is hydrolyzed into GDP, and RanGDP is imported into the nucleus by the RanGDP-binding nuclear transport factor 2, NTF2 (also called p10) (30).

Cytoplasmically injected wheat germ agglutinin (WGA) inhibits active nuclear import but not passive diffusion (31). Furthermore, during the course of screening for inhibitors that block the interaction between RanGTP and importin β1, Soderholm et al. (32) very recently discovered a small molecule named 'importazole' (2,4-diaminoquinazoline). These investigators showed that this compound blocked importin β1-mediated nuclear import both in *Xenopus* egg extracts and cultured cells but did not affect transportin (also called importin β2)-mediated nuclear import. Importazole is expected to serve as a good research tool to study RanGTP/importin β1-involved cellular functions.

On the one hand, for nuclear export (Figure 2), NES-containing cargos form a complex with RanGTP and exportin 1 (also called CRM1) (33–37). In addition, Ran-binding protein 3 (RanBP3), which exhibits a weak binding affinity for RanGTP compared with RanBP1 and RanBP2, functions as a co-factor for RanGTP/exportin 1-mediated nuclear export by increasing the affinity of exportin 1 with both RanGTP and the cargos (38–40). Leptomycin B (LMB), originally identified as a potent antifungal antibiotic, inhibits the nuclear export process by binding to exportin 1 to block the interaction between exportin 1 and the NES-containing cargos (41). The discovery of LMB has promoted the study of the nuclear export machinery for multiple proteins (42).

On the other hand, several examples of nucleocytoplasmic transport occurring independently of the RanGTPase cycle have been found, including the nuclear import of β-catenin (43), importin β1 (44), U1A (45, 46), U2B (46), and importin α (47). Yokoya et al. (43) found that the nuclear accumulation of β-catenin was not inhibited even when a dominant-negative Ran mutant defective in GTP hydrolysis activity [Ran (G19V)] was injected into the cytoplasm. Furthermore, this study demonstrated that the nuclear import of β-catenin was not affected in a tsBN2 cell line possessing a point mutation in the *RCC1* gene, indicating that the nuclear import of β-catenin does not depend on the RanGTPase cycle (43). Furthermore, Miyamoto et al. (47) found that importin α has an ability to enter the nucleus in a Ran/importin β1-independent manner. These findings imply the presence of an unexpected diversity in the nucleocytoplasmic transport machinery.

Ran and Ran-binding proteins in mitotic spindle assembly

During mitosis, chromosome segregation must occur in a highly ordered manner to prevent aneuploidy, which can lead to unlimited cell proliferation or cell death. By separating into two identical chromosome sets, a mother cell divides into two daughter cells, with the mitotic apparatus controlling this process modulated by microtubules gathered around the chromosomes. Assembled mitotic spindles consist of microtubules

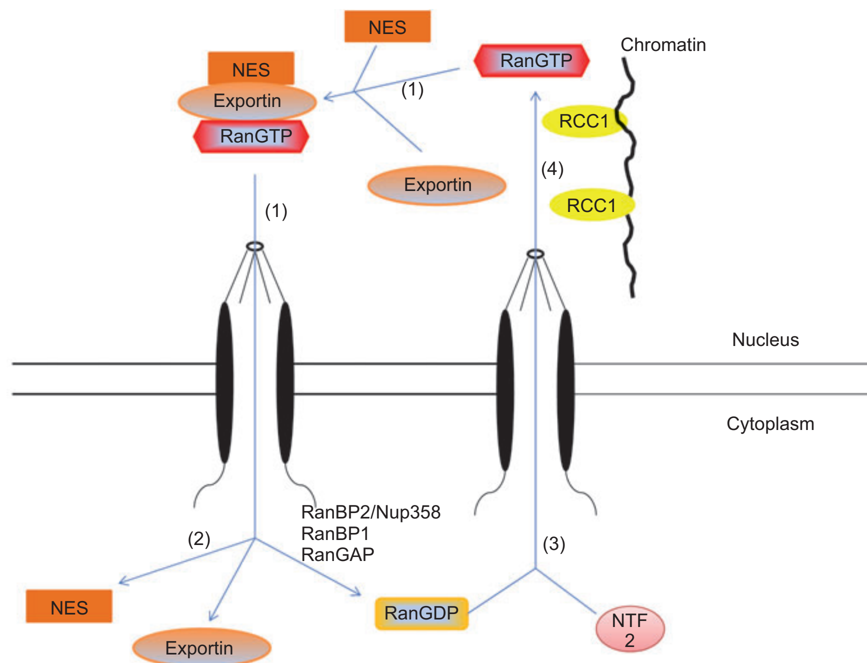

Figure 2 Ran and Ran-binding proteins in the nuclear export cycle.
(1) Nuclear export of NES-containing proteins mediated by exportin and RanGTP as a ternary complex. (2) Dissociation of the ternary complex and hydrolysis of RanGTP triggered by the action of RanGAP in conjunction with RanBP1 and/or RanBP2. (3) Nuclear import of RanGDP mediated by NTF2. (4) Conversion of RanGDP into RanGTP by the action of chromatin-associated RCC1.

that extend from each of the two poles to the chromosomes at the middle of spindles. Numerous studies have demonstrated the involvement of Ran and Ran-binding proteins in the regulation of mitotic spindle assembly (Figure 3). The first evidence was acquired in 1999 in a reconstitution system using M-phase-arrested *Xenopus* egg extracts (48–52) and demonstrated critical roles for Ran in the assembly of microtubule asters and mitotic spindles. These studies showed that in M-phase-arrested extracts, a RanGTP-mimicking mutant [Ran (Q69L)] or excess RCC1 induced microtubule assembly, whereas a mutant with decreased affinity for both GTP/GDP [Ran (T24N)] failed to induce microtubule assembly, suggesting a model in which RCC1-mediated production of RanGTP around the chromosomes stabilizes microtubules. In agreement with this model, a RanGTP gradient was observed around mitotic chromosomes, as visualized through fluorescence resonance energy transfer-based biosensors of Ran in *Xenopus* egg extracts (53) and mitotic somatic cells (54).

Subsequently, two RanGTP-dependent factors in mitotic spindle assembly (SAFs), TPX2 and NuMA (nuclear mitotic apparatus protein), were identified (55–57). TPX2, a microtubule-associated protein, targets a motor protein, Xklp2, to microtubule minus ends (58) and activates Aurora A kinase (59), which is involved in centrosome functions (60, 61). TPX2 is inactivated by associating with importin α via its NLS domain, whereas the RanGTP-mediated dissociation of the complex allows TPX2 to be activated and to mediate microtubule assembly and spindle pole formation (55).

However, Wiese et al. (56) and Nachury et al. (57) have shown that the mitotic roles of RanGTP are largely mediated through importin β1 and that importin β1 inhibits mitotic spindle assembly by sequestering at least NuMA, an important factor for microtubule organization in the spindle. In particular, importin β1 binds to NUMA via an NLS domain in the NUMA C-terminus; this binding is also dissociated by the action of RanGTP.

A number of factors that are targets of the RanGTP-importin β1 pathway have been identified, including HURP (Hepatoma up-regulated protein) (62, 63), RAE1 (64), NuSAP (65), kid (61, 66), Cdk11 (67), and XCTK2 (68). Among them, HURP forms a large complex with TPX2, XMAP215, Aurora A kinase, and Eg5 in *Xenopus* egg extracts (62). HURP is a target protein of importin β1 and is involved in the stabilization of K-fibers and the capture of microtubules by kinetochores. Importin β1 inhibits HURP to nucleate and cross-link the microtubules (61, 62). Thus, RanGTP-dependent dissociation of SAFs from importins appears to provide a general molecular mechanism for microtubule nucleation along the mitotic chromosomes.

Furthermore, APC/C (anaphase-promoting complex) was recently shown to promote proper ubiquitination and degradation of four factors, Bard1, Hmmr, HURP, and NuSAP, required for RanGTP-dependent mitotic spindle assembly (69). Song and Rape (69) demonstrated that among these factors, importin β1 inhibited the ubiquitination of HURP and NuSAP by APC/C in a dose-dependent manner and that importin β1 mutants showing poor binding efficiency for HURP and NuSAP failed to inhibit this ubiquitination, indicating that the importin β1-RanGTP pathway controls the ubiquitination and degradation of specific SAFs. Collectively, the APC/C and importin β1-RanGTP pathways may undergo tight regulation for controlling the amount of SAFs to ensure

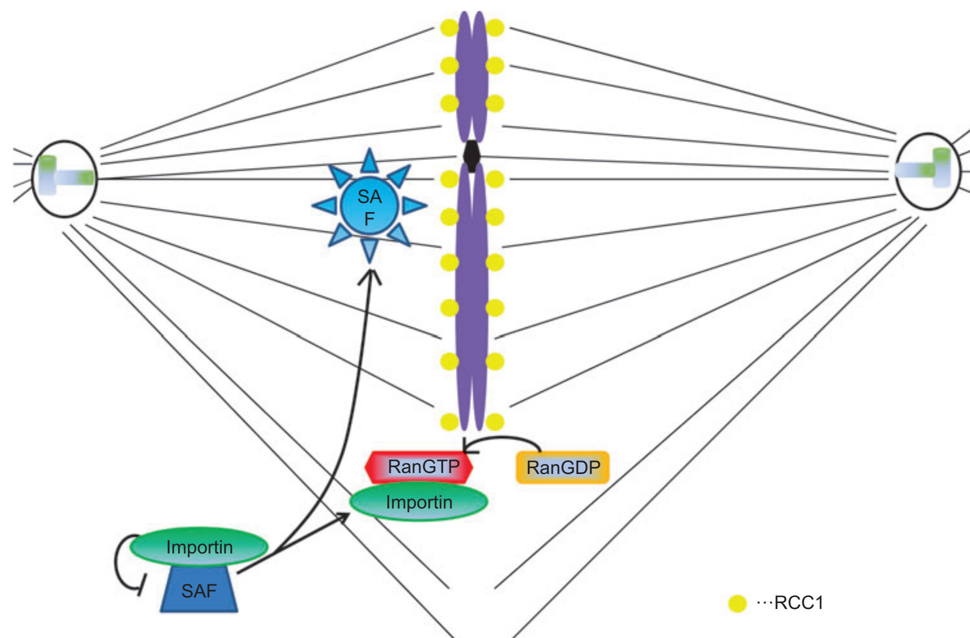

Figure 3 Ran and Ran-binding proteins in mitotic spindle assembly.
In mitosis, SAFs, such as TPX2, NuMa, and HURP, are inactivated through their association with importins; this inactivation is released by the action of RanGTP, which was converted from RanGDP by chromatin-associated RCC1. The activated SAFs subsequently exert their activity in regulating mitotic spindle microtubules.

the orderly assembly of the mitotic spindle. The importin β1-RanGTP pathway is also possibly involved in other basic cellular machineries in mitotic spindle assembly.

Ran and Ran-binding proteins in reformation of the NE and NPC

At the end of mitosis, the mitotic spindle is disassembled, followed by the reformation of the NE and NPCs, enabling nucleocytoplasmic transport to restart at interphase. Using cell-free *Xenopus* egg extracts, the Ran system has been shown to be required for NE reformation (70, 71) and post-mitotic NPC assembly (72, 73) (Figure 4). Zhang et al. (74) demonstrated that bead-immobilized Ran was able to induce the formation of NE-like structures. Intriguingly, the NE-like structures that formed around the beads contained NPCs. Nucleocytoplasmic transport was also shown to occur. Moreover, Hetzer et al. (71) showed that both Ran and RCC1 were required for NE reformation at a very early stage in this process. In particular, before NE reformation, RanGDP associates with chromatin, followed by the recruitment of RCC1, which leads to the production of RanGTP around the chromatin surface (74). The Ran system is also required for the NE integrity of *Caenorhabditis elegans* (75, 76) and *Drosophila melanogaster* (77), indicating the universal role of the Ran system in NE formation in eukaryotes.

The molecular mechanism of post-mitotic NPC assembly by the Ran system remains to be elucidated, but importin β1 appears to function as an important player in this process (72, 78–85). In a *Xenopus* nuclear reconstitution system, excess importin β1 was shown to block nuclear membrane fusion and NPC assembly, whereas RanGTP was observed to stimulate these events. The inhibitory effects of importin β1 on nuclear membrane fusion was reversed by RanGTP, whereas the inhibitory effect on post-mitotic NPC assembly was unaffected by RanGTP in a cell-free *Xenopus* egg extract system (86), suggesting the existence of an additional factor(s) involved in post-mitotic NPC assembly.

Indeed, subsequent studies using a HeLa cell line and a cell-free *Xenopus* nuclear reconstitution system demonstrated that the Nup107-Nup160 complex was critical for post-mitotic NPC assembly (78–80). Boehmer et al. (78) and Waltzer et al. (79) found that when Nup107 or Nup133, components of the Nup107-Nup160 complex, was depleted by small interfering RNA (siRNA) in HeLa cells, the number of multiple nucleoporins and the density of NPCs in the NE decreased. Furthermore, Waltzer et al. (79) demonstrated that the immunodepletion of Nup107-160 components in *Xenopus* egg extracts induced NE formation without NPCs and that the phenomena were reversible only when the Nup107-160 complex was re-added before NE formation. Harel et al. (80) also found that immunodepletion of Nup85 or Nup133 resulted in nuclei showing severely defective nuclear import activity and lacking functional nucleoporins. These findings indicate that the Nup107-160 complex plays a pivotal role in NPC assembly. Collectively, these results show that importin β1 likely functions as a negative regulator of post-mitotic NPC assembly by sequestering several nucleoporins (the Nup107-160 complex) from the chromatin surface. However, this negative effect of importin β1 is overcome by RanGTP generated around the chromatin by chromatin-bound RCC1, which allows the recruitment of the Nup107-160 complex.

In proliferating cells, the number of NPC components should double during interphase before mitosis. Using a cell-free NPC insertion assay with *Xenopus* egg extracts, D'Angelo et al. (81) found that the components of the NPC were recruited from both sides of the NE and that this process also required a properly functioning RanGTPase cycle and the Nup107-160 complex, implying role(s) for 'cytoplasmic' RanGTP in this process. Furthermore, the NPC has been found to assemble *de novo*, not through splitting of pre-existing pores.

Moreover, ELYS (also called MEL-28 in *C. elegans*) has recently been revealed to be required for the RanGTP-dependent targeting of the Nup107-160 complex to the chromatin during post-mitotic NPC assembly (82–85, 87) but not interphase NPC assembly (88). ELYS binds to AT-rich chromatin regions via an AT-hook DNA-binding motif at its C-terminus, with this recruitment of ELYS to the chromatin requiring RanGTP (83, 85). Very recently, POM121 has been

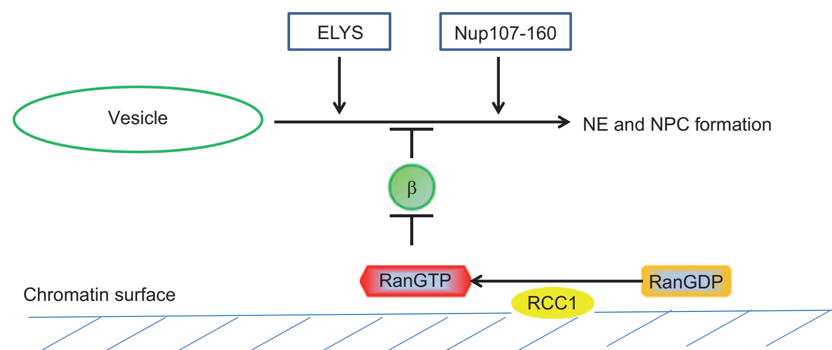

Figure 4 Ran and Ran binding proteins in NE and NPC formation.
NPC is assembled during late telophase as an early event in NE reformation. Localized RanGTP, produced by chromatin-bound RCC1, recruits nucleoporins, including Nup107-160 complex, which has been sequestered by importin β1. The RCC1-generated RanGTP dissociates the nucleoporins from importin β1, thereby liberating the nucleoporins to bind to chromatin.

revealed to be important for the early stage of interphase NPC assembly (88–92). POM121 interacts with importin α/β and a subset of nucleoporins via its NLS domains, with the activity of the NLS important for interphase NPC assembly (89, 91). Indeed, the introduction of NLS mutants in POM121 caused defective nuclear transport and abnormal cytoplasmic membrane stacks in a U2OS cell line (89). In addition, in HeLa and U2OS cell lines, the NLS of POM121 has been shown to target the inner nuclear membrane in a RanGTP-dependent manner (91). Further analysis is anticipated to reveal more detailed mechanisms of NPC assembly (and disassembly) both in the post-mitotic stage and in interphase.

Novel aspects of Ran and Ran-binding proteins

Through investigations in knockout and transgenic mice, Ran and Ran-binding proteins have been revealed to be involved in a variety of physiological phenomena. For example, mice heterozygous for a Ran mutation did not show germline transmission (93), whereas Ran-overexpressing transgenic mice showed abnormalities in T-cell responses, including compromised lymphokine secretion and proliferation after T-cell receptor activation, as well as defects in the nuclear accumulation of AP-1 transcription factors, such as c-Jun and c-Fos (94). This indicates that Ran is involved in the nuclear retention of particular transcription factors in T cells. Furthermore, Dishinger et al. (95) demonstrated that ciliary targeting of KIF17, a kinesin 2 motor, was regulated by importin β2 (transportin) in a RanGTP-dependent manner and that KIF17 interacted with importin β2 via its 'ciliary localization signal' in its tail domain, suggesting that Ran regulates cell compartmentalization by modulating the transport of cytoplasmic proteins into either the nucleus or cilia.

Similarly, Yudin et al. (96) found that in sensory neurons, RanBP1 and RanGAP controlled the formation of a retrograde injury-signaling complex. That is, the injury-dependent translated RanBP1 stimulated the dissociation of RanGTP from importin β1 locally through the hydrolysis of RanGTP by RanGAP, followed by the formation of a new complex of cargo protein and importin α/β1. This cargo complex was transported from axons to cell bodies via the direct interaction of importin α with dynein, a microtubule motor protein, as revealed by Hanz et al. (97). Thus, retrograde injury signals are delivered along the microtubules toward the neuronal cell body in a locally translated RanBP1-dependent manner.

On the one hand, RanBP1-knockout mice have recently been established and were unexpectedly viable (98), in contrast to the known knockout mice for other RanGTPase cycle-related molecules, such as Ran, RanGAP, RanBP2, and importin β1 (93, 99–102). Viable RanBP1-knockout mice showed growth retardation after birth and male-specific infertility due to a block in spermatogenesis, presumably resulting from both the downregulation of RanBP2 and the lack of RanBP1 during spermatogenesis. Intriguingly, mouse embryonic fibroblasts (MEFs) from *RanBP1*$^{-/-}$ embryos showed a normal growth rate as compared to those from the wild-type littermates. However, *RanBP1*$^{-/-}$ MEFs exhibited severely reduced cell viability upon RanBP2-siRNA treatment, whereas MEFs from wild-type littermates showed a lesser extent of damage upon the same treatment, indicating that the functions of RanBP1 can be complemented in normal tissues and cells, probably by RanBP2 (98).

On the other hand, although the homozygous null mutation of RanBP2 (*RanBP2*$^{-/-}$) resulted in embryonic lethality, *RanBP2*$^{+/-}$ mice were viable but showed defects in glucose metabolism in the central nervous system (100). Furthermore, mice expressing low amounts of RanBP2 were found to be viable but highly susceptible to tumor formation. Indeed, MEFs with low amounts of RanBP2 exhibited defects in the SUMOylation of topoisomerase IIα and showed severe aneuploidy due to the inability of topoisomerase IIα to accumulate at the inner centromeres (101). *C. elegans* and *D. melanogaster* possess direct homologs of *RanBP2* as an essential gene but not of *RanBP1*, while *S. cerevisiae* possesses a *RanBP1* homolog as an essential gene but not of *RanBP2* (103). When these observations are taken into consideration, the functions of RanBP1 may possibly be compensated, at least partially, by RanBP2 in normal mammalian cells (98). Moreover, RanBP2/Nup358 function has been shown to be a central determinant of NPC remodelling during the muscle differentiation of C2C12 cells (104). By using atomic force microscopy, Asally et al. (104) found a correlation between the number of RanBP2/Nup358 molecules and the NPC structure and demonstrated that siRNA-mediated depletion of RanBP2/Nup358 inhibited the myotube formation of myoblasts, suggesting the critical role(s) of RanBP2/Nup358 in myogenesis.

RanBP3 has been shown to function as one of the target molecules of signal integration between the Ras/ERK/Rsk and the PI3K/Akt signaling pathways. Yoon et al. (105) found that Rsk- and Akt-mediated phosphorylation of RanBP3 contributed to the modulation of a Ran gradient between the nucleus and the cytoplasm and thus nucleocytoplasmic transport efficiency, suggesting that Ras/ERK/Rsk and PI3K/Akt signaling affect nucleocytoplasmic transport efficiency. Furthermore, Smad2 and Smad3, critical signal transducers in the transforming growth factor (TGF)-β signaling pathway, were found to be exported out of the nucleus by RanBP3 (106). Dai et al. (106) found that RanBP3 directly bound to Smad2/3, which were dephosphorylated by nuclear Smad phosphatases, and mediated the nuclear export of Smad2/3 in a Ran-dependent manner. Thus, by enhancing the nuclear export of Smad2/3, RanBP3 is involved in termination of TGF-β signaling.

Human CAS (hCAS) has been shown to associate with a subset of p53 target genes, including PIG3, p53AIP1, and p53R2 (107). Tanaka et al. (107) found that hCAS bound to chromatin and was involved in the selective activation of genes through modulation of the trimethylation of histone H3 lysine 27 and that siRNA-mediated depletion of hCAS resulted in the downregulation of camptothecin-dependent PIG3 expression.

Exportin 4, which was originally recognized as the most distant member of the importin β superfamily and which is conserved only in higher eukaryotes (108), was identified as a

tumor suppressor factor in liver cancer (109). By establishing a focused shRNA library against murine orthologs of genes deleted in hepatocellular carcinoma and screening shRNAs that promote tumorigenesis of hepatocellular carcinoma, Zender et al. (109) identified *exportin 4* as 1 of 13 possible tumor suppressor genes for liver carcinogenesis. Exportin 4 suppressed mouse hepatocellular carcinoma proliferation by exporting eukaryotic initiation factor 5A2 (eIF5A2) out of the nucleus, the overexpression of which is tightly associated with human tumors (109). In addition, exportin 5, the specific exporter for precursor microRNAs (pre-microRNAs) (110–112), was found to contribute to tumorigenesis through the nuclear accumulation of pre-microRNAs, when it carried a mutation that rendered it functionally defective (113).

Schulze et al. (114) found that recombinant N-terminal domain of Ran-binding protein 10 (RanBP10) had unexpected RanGEF activity (114). Furthermore, this study showed that RanBP10 was associated with microtubules in megakaryocytes. Indeed, siRNA-mediated abrogation of RanBP10 resulted in a defect in microtubule organization, suggesting that temporally and spatially restricted cytoplasmic RanGTP may affect the organization of specialized microtubules required for proper thrombopoiesis.

Various cell stresses, including oxidative stress, are well-known to provoke RanGTPase cycle perturbation, thereby resulting in a nuclear retention of importin α (115–120). In examining the nuclear roles of importin α, Yasuda et al. (120) very recently demonstrated that nuclear KPNA2 (a subtype of importin α, also called Rch1, importin α2, in humans) upregulates the expression of the mRNA encoding serine/threonine kinase 35 (STK35) in response to oxidative stress. This study has also reported that KPNA2 bound to the promoter region of STK35 and that the constitutive expression of STK35 induced caspase-independent cell death. These observations indicate that nuclear-localized KPNA2 influences gene expression and directly contributes to cell fate determination, including non-apoptotic cell death.

Expert opinion

In future studies, understanding the dynamic regulation exerted by Ran and Ran-binding proteins at the organismal level will require interdisciplinary approaches. For example, biological-system methodologies and computer-simulation strategies will be helpful in determining the spatial and temporal regulation of the behaviors and functions of Ran and Ran-binding proteins. Furthermore, more sophisticated imaging technologies, such as single-molecule imaging techniques, super-resolution microscopy, and four-dimensional imaging, will strengthen our insights into the cellular functions of Ran and Ran-binding proteins. Automated high-throughput analysis will also enable us to examine the phenotypes of individual cells in detail. Moreover, the application of techniques used to generate knockout mice will help reveal the physiological roles of Ran and Ran-binding proteins in mammals. Given the past studies showing that null mutations of Ran and Ran-binding proteins result in embryonic lethality, generating gene knockouts in a spatially, temporally limited manner using the Cre-LoxP system should be helpful. In addition, next-generation DNA sequence technologies focusing on genes encoding Ran and Ran-binding proteins in individual patients with disorders such as cancer will shed light on the functional link between previously unknown disorders and Ran and Ran-binding proteins.

Outlook

In the next decade, investigations will reveal in finer detail how the Ran system, as exerted by Ran and Ran-binding proteins, is functionally linked to various types of cell fate determination, including cell death, proliferation, differentiation, immortalization, and malignant transformation. For example, recent studies have indicated that nucleocytoplasmic transport machineries play essential roles in embryonic stem cell differentiation and spermatogenesis (121–124). Furthermore, accumulating clinical evidence has revealed that the dysregulation of Ran and Ran-binding protein function is involved in unlimited cell proliferation, such as cancer [reviewed in (125)]. Ran is overexpressed in multiple human cancers, such as ovarian cancer (126) and renal cell carcinoma (127). Moreover, forced expression of Ran or a particular Ran mutant (F35A) provokes malignant transformation in a rat mammary cell line (R37) (128) or a mouse NIH3T3 cell line (129), respectively. Conversely, siRNA-based screening has identified Ran and its target molecule TPX2 as suppressors of the survival of multiple human tumor cell lines, such as non-small cell lung carcinoma, breast cancer, and colon cancer (130). Furthermore, Ran has been shown to be a partner molecule of survivin, which is a critical regulator of mitosis and overexpressed in cancer cells. The Ran-survivin complex is required for the recruitment of TPX2 to microtubules in cancer cells (131).

In addition to Ran, RanBP1 may be a good candidate of target molecules for cancer therapy in that (i) the abrogation of RanBP1 in several cancer cell lines causes significant cell death (132–134, and M. Nagai, unpublished observations); (ii) RanBP1 is also highly expressed in various types of human cancers, such as breast carcinoma, testis seminoma, brain oligodendroglioma, small cell lung cancer, squamous cell lung cancer, lung adenocarcinoma, bladder carcinoma, colorectal carcinoma, tongue squamous cell carcinoma, melanoma, and leukemia [reviewed in (125)]; and (iii) RanBP1 is not necessarily indispensable for the survival of mice and the survival and proliferation of normal mouse (98) and human fibroblasts (Nagai, unpublished observations), probably owing to the functional compensation by RanBP2.

Spatiotemporal regulation of proteins is one of the most important post-translational controls in the various cellular functions. Therefore, it is easy to speculate that the dysregulation of Ran and Ran-binding protein functions disrupts proper subcellular protein localization, causing abnormal cell fate determination. In fact, several tumor suppressor proteins, such as p53 (135, 136), p27 (kip1) (137–140), and PTEN (141), have been shown to have altered nucleocytoplasmic

localization in several cancers [reviewed in (142)]. Taken together, these findings suggest that the pathogenesis of unsolved, complex symptom-revealing diseases may be explained by a 'defect of proper subcellular localization' of proteins due to the dysregulation of Ran system, even though their encoding DNA sequences are intact.

Furthermore, the cytoplasmic functions of Ran and Ran-binding proteins should also be investigated in a more intensive and comprehensive manner [reviewed in (143)]; this issue will be of importance to understanding these proteins and prompts a number of questions. For instance, are the cytoplasmic functions of Ran and Ran-binding proteins maintained not only in certain specific cell types but also in general cell types? How is the exertion of the Ran system switched between the nucleus and the cytoplasm in a single cell? Are there additional factors that facilitate the cytoplasmic functions of the Ran system?

Collectively, we expect that increasingly greater progress will be made in the field of Ran and Ran-binding proteins, which are apparently important for multiple biological activities.

Highlights

- Ran and Ran-binding proteins regulate nucleocytoplasmic transport machinery, primarily depending on importin and exportin-mediated transfer of cargo proteins.
- Ran and Ran-binding proteins regulate mitotic spindle formation.
- Ran and Ran-binding proteins regulate NE assembly, as well as post-mitotic and interphase NPC assembly.
- Ran and Ran-binding proteins are expected to be tightly involved in cell fate determination, including proliferation, differentiation, immortalization, transformation, and death.
- For understanding the spatial and temporal regulation of the Ran system in detail, multiple techniques from interdisciplinary research fields should be combined.
- The cytoplasmic functions of Ran systems should be an area of research focus.
- There is the intriguing possibility that Ran and Ran-binding proteins may be good candidate targets for cancer therapy.

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Conflict of interest statement

The authors declare that no conflict of interest exists.

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