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The GTPase Ran: regulation of cell life and potential roles in cell transformation

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TABLE OF CONTENTS

1. ABSTRACT

The GTPase Ran plays a crucial role in nucleo–cytoplasmic transport of tumor suppressors, proto-oncogenes, signaling molecules and transcription factors. It also plays direct roles in mitosis, through which it regulates faithful chromosome segregation and hence the generation of genetically stable cells. Ran operates through a group of effector proteins. In this review we summarize growing evidence suggesting that deregulated activity of Ran or its effectors can contribute to pathways of cell transformation and facilitate tumor progression.

2. INTRODUCTION: THE DIVERSE ROLES OF RAN

The small GTPase Ran is the central element of a signalling network with relevant roles in cell life and duplication. The human gene coding for Ran was originally identified for sharing sequence homology with the GTP-binding domain of Ras and was found to encode a mRNA transcript whose abundance was high in teratocarcinoma cells (hence called teratocarcinoma clone 4, TC4), but was down-regulated by exposure to a differentiating agent, retinoic acid (1). That first discovery should have immediately alerted cellular biologists to the involvement of this GTPase in cell differentiation and transformation. In further studies, Ran proved a highly versatile GTPase acting in a variety of processes in budding and fission yeast, the nematode *C. elegans, D. melanogaster,* zebrafish, amphibians, mammalian species and plants. In particular, yeast mutants in the homologous genes encoding the GTPase Ran, or its regulators, exhibited defects in nuclear protein import or in RNA export; these studies pinpointed a requirement for Ran in nucleo-cytoplasmic transport and shifted the focus on the use of informative systems to characterize this process. For some time, it was unclear whether Ran acted primarily in transport, indirectly affecting cell proliferation, or whether it had direct roles in intracellular transport, cell cycle and cell proliferation (2-3). This issue remained unresolved for some time (4) and the potential involvment of Ran in cell transformation was temporarily neglected.

In 1999, a new burst of studies (5-9) showed that Ran has roles in assembly of the mitotic apparatus in systems that contained no nuclear envelope (NE) and in which, therefore, transport was suppressed. The studies that followed (reviewed in 10-12) have clarified that Ran plays direct roles in multiple processes:

- Transport of RNAs and proteins across the NE,
- Mitotic spindle organization and function,
- Nuclear and NE reconstitution in cells that exit mitosis and re-enter interphase.

All three processes affect the proliferating and differentiating ability of cells. Some Ran-dependent processes have been extensively reviewed elsewhere and we will refer to those comprehensive reviews wherever possible. In this review, we will try to relate those established mechanisms to newly emerging evidence that deregulation of Ran activity affects fundamental processes through which the cell fate is established, and may therefore contribute to cell transformation and tumor progression. This will be linked to a retrospective assessment of the literature, which growingly indicates that expression of Ran and its regulators is indeed altered in several cancer types.

3. THE BASIC MECHANISM OF RAN GTPASE SIGNALLING

3.1. The Ran network 'core' and its topological organization in cells

The mode of action of Ran is similar to that of other GTPases and depends on the guanine nucleotide that binds to it. The central concept is that RanGTP selectively interacts with a group of effectors, which in turn regulate downstream target factors: thus, nucleotide turnover on Ran is key to its biological activity.

Three factors regulate nucleotide turnover on Ran and thus influence downstream processes:

- RCC1 is the guanine nucleotide exchange factor (GEF) on Ran and generates RanGTP. Given that RCC1 is a chromatin-binding protein, RanGTP is enriched at and around chromatin;

- RanGAP1 (GTP-hydrolysis activating protein 1) hydrolyses GTP on Ran; RanGAP1 has a largely cytoplasmic distribution, with a significant enrichment around the NE;

- RanBP1 (Ran-binding protein 1) is a cytoplasmic protein that binds RanGTP and renders it accessible to RanGAP1, thus stimulating RanGDP production in the cytoplasm.

The importance of the guanine nucleotide-bound state of Ran has been experimentally defined in studies of nucleo-cytoplasmic transport. Given that in interphase cells RCC1 generates RanGTP in the nucleus, GTP-bound Ran binds its effectors therein and dissociates from them in the cytoplasm, where the hydrolysis factors for Ran reside. Among RanGTP effectors is importin beta, the vector of nuclear protein import. For some proteins, vectorial transport is accomplished by importin beta alone; more frequently, importin beta binds a member of the importin alpha family that acts as an 'adaptor' molecule between importin beta and protein cargoes that carry a nuclear localization signal (NLS). Importin beta is a high affinity effector for RanGTP: upon entry in the nucleus in complexes with its cargoes, it is bound by RanGTP; this causes the dissociation of import complexes. As a result, NLS proteins are released in a free form in the nucleus.

RanGTP has the opposite effect on nuclear export. Proteins that must be transported to the cytoplasm, carrying short leucine-rich sequences that are critical for their targeting out of the nucleus and termed nuclear export signals (NESs), as well as RNAs of all classes, assemble with specific export vectors (exportins) in the nucleus prior to translocation to the cytoplasm. Exportins are also RanGTP effectors and share similarity with importin beta in the Ran-interacting domain. RanGTP binds exportins in the nucleus: this binding facilitates the formation of trimeric export complexes (RanGTP/exportin/export cargo) and is essential for their translocation to the cytoplasm. Thus, by modulating the assembly and disassembly of transport complexes, Ran determines the subcellular localization, and, ultimately, the biological activity, of RNAs and proteins (Figure 1).

3.2. Ran acts through a conserved mechanism in interphase and in mitosis

The same mechanism of RanGTP-dependent release of free NLS-containing proteins, and of relocalization of NES-containing cargoes, continues to operate after NE breakdown – although nucleo-cytoplasmic transport is temporarily suppressed - and regulates the organization and function of the mitotic apparatus. Studies with *Xenopus* oocyte-derived extracts, a system of election to study mitotic spindle assembly, have clearly shown that RanGTP still binds importin beta at mitotic onset; by doing so, it releases a set of mitotic factors containing NLSs in a free, biologically productive form. The first identified mitotic factors under Ran control were found to induce the formation of microtubule (MT) asters and were collectively termed 'spindle assembly factors' (SAFs). Subsequently RanGTP was also shown to regulate the dynamic activity of MTs in the mitotic spindle by activating a variety of MT-associated proteins (MAPs) and some factors with motor activity (reviewed in 13).

Ran also acts in mitosis via CRM1, the export receptor for NES proteins, with important functional consequences; Ran and CRM1 operate at mitotic kinetochores (KTs), which represent the chromosomal 'platform' at which the mitotic spindle checkpoint monitors chromosome attachment to MTs (reviewed in 14). The KT-associated Ran/CRM1 complex regulates the residency of checkpoint factors on KTs, and hence the schedule of the spindle checkpoint activation and release (reviewed in refs. 15-16; see below).

In summary, Ran regulates cell proliferation and differentiation by both determining the proper localization of RNA and proteins in the nucleus or in the cytoplasm during interphase, and by regulating the organization of the mitotic apparatus, responsible for the transmission of their genetic identity to daughter cells during cell division.

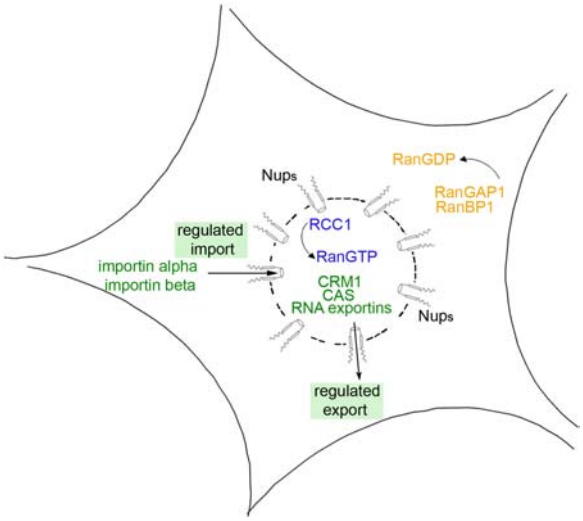

Figure 1. The subcellular distribution of Ran network components in interphase cells. RCC1, the exchange factor for Ran, localizes in the nucleus and therein generates RanGTP (blue); the hydrolysis factor RanGAP1 generates RanGDP in the cytoplasm; RanBP1 also localizes in the cytoplasm and stimulates RanGAP1 activity (orange). RanGTP regulates the activity of import and export receptors (green) which mediate directional transport through nuclear pore complexes. Nups, nucleoporins.

4. TRANSPORT ACROSS THE NE, CELL PROLIFERATION AND TRANSFORMATION

Ran function is required for the subcellular localization of proteins that regulate cell cycle and apoptosis, and acts therefore at a crucial level of control. Ran also regulates pathways of RNA export, which will not be discussed here. It must be recalled, however, that at least one such pathway can impact on cell transformation and cancer progression: the micro RNA export pathway, the efficiency of which is directly regulated by RanGTP. Micro RNAs (miRNAs) are a special class of small non-coding RNAs that regulate gene expression at the post-transcriptional level. They are assuming growing importance in oncogenesis, as it is emerging that distinctive miRNA profiles characterise particular cell types and are altered in cancer cells (17-18). MiRNAs are processed from larger transcripts through the sequential activity of nuclear and cytoplasmic endoribonucleases to yield RNA intermediates (pre-miRNAs), that are exported to the cytoplasm for additional processing; eventually, mature 22-nt long miRNAs are generated, which are biologically effective in post-transcriptional regulation. Nuclear export of pre-miRNAs is therefore a crucial step in the production of functional miRNAs (19). An importin beta-related transport receptor, exportin 5 (exp5), acts as the specific export vector for pre-miRNAs (20-22). The unique role of exp5 in miRNA nuclear export is indicated by depletion experiments that resulted in reduced miRNA production.

Exp5 binds pre-miRNAs in a RanGTP-dependent manner: therefore, the assembly of RanGTP/Exp5/miRNA complexes is key to the biogenesis of functional miRNAs. Conditions under wich RanGTP production is altered, either caused by cellular stress or due to altered expression of Ran or of its regulators (see below), are therefore likely to affect the efficiency of pre-miRNA export, with potentially deleterious impact on the emergence of cellular phenotypes that can facilitate cell transformation.

Ran-dependent intracellular transport of miRNAs and proteins is so intimately intertwined with control of cell proliferation and its checkpoints that some Ran network members and effectors were first identified as regulators of cell cycle and proliferation, while their role in transport was independently recognized only at a later time. Below, we will briefly recall the most significant instances in which such a dual role in transport and in cell proliferation was identified in independent studies of the same factor.

4.1. RCC1

The regulator of chromosome condensation, RCC1, was identified as a checkpoint gene well before its role as the guanine exchange factor for Ran was recognized: RCC1 was originally identified as a temperature sensitive allele in a mutant baby hamster kidney cell line, called tsBN2, in which cells arrested in S phase by DNA replication inhibitors condensed their chromosomes (hence, the RCC1 acronyme for the wild-type allele) and entered mitosis prematurely when shifted to the restrictive temperature (23). That discovery indicated that the restrictive temperature suppressed a function important to delay mitotic onset until DNA replication was complete. Uncoupling of these two events leads to segregation of incompletely replicated genomes and hence to mutant or unviable cells. Precocious progression into mitosis in tsBN2 cells was accompanied by premature condensation of incompletely replicated chromosomes and activation of the cyclin B1/cdc2 kinase complex, the mitosis-promoting factor (MPF). Further studies clarified that wild-type RCC1 is required for regulated subcellular localization of two protein phosphatases, Cdc25B and Cdc25C, which regulate MPF activation (see 24 for review). Mitotic onset is preceded by variations in the frequency of import and export of these two regulators; this shuttling activity may be part of the intracellular communication that transduces information on the advancement of the cell cycle and culminates with their stable localization in the nucleus just before NE breakdown (reviewed in 25). In tsBN2 cells in which RCC1 is inactive, Cdc25B and Cdc25C are prematurely stabilized in nuclei, causing the unscheduled activation of MPF in the presence of unreplicated DNA. Wild-type RCC1, therefore, couples completion of DNA replication with MPF activation. RCC1 was independently reisolated as the factor responsible for GTP nucleotide exchange on Ran (26). At first the relation between the two processes was not obvious, but it was eventually rationalized by taking into account the role of RanGTP in control of nucleo-cytoplasmic transport: RCC1 inactivation causes a decrease in nuclear RanGTP levels and thus impairs nuclear export of the Cdc25B and Cdc25C phosphatases at a time at which the conditions for mitotic entry have not yet set.

In addition to regulating the S- / M-phases dependence, roles of RCC1 in the mammalian cell cycle

have been extensively reviewed (24, 27). TsBN2 cells have represented a very useful and informative system to pinpoint cell cycle alterations associated with the loss of GEF activity on Ran, with interesting implications for understanding particular mechanisms that may operate during cell transformation. For example, the notion that RCC1 acts to link the onset of mitosis to completion of DNA replication may hint to a requirement for RCC1 in linking mitotic onset to repair of DNA damage, given that similar regulators are involved. Furthermore, caffeine, which disrupts ATR (ataxia telangecatasia related)-dependent checkpoint regulation, is also reported to influence the formation of complexes between RCC1 and Ran, and hence the GEF activity of RCC1 on Ran (28). Finally, it is interesting to note that the loss of RCC1 function prevents the recycling of importin alpha-dependent transport in a very similar manner to that occurring under conditions of cellular stess (29). Since the loss of RCC1 is lethal, no comparable mutation to the tsBN2-specific RCC1 alelle exists in cancer cells, but it is interesting that RCC1 function is targeted under particular conditions in checkpoint-defective cells.

4.2. CRM1

The *Crm1* (Chromosomal region maintenance) gene was identified in a cold-sensitive mutant *S.pombe* strain that showed irregularly deformed nuclear domains containing fibrous or rod-like condensed segments (30). That phenotype implicated Crm1 in maintaining the higher order chromosome structure. *Crm1* is an essential gene in both budding and fission yeast (30-31). Because *Crm1* mutants showed deregulated transcription of some genes (30), Crm1 was hypothesized to act as a chromatin protein with a general role in transcriptional control (31). A *crm1* mutant was independently identified in a screening for *S.pombe* genes conferring resistance to leptomycin B (LMB), a *Streptomyces* antibiotic with cytostatic effects on both mammalian and yeast cells, indicating that the crm1 protein was the molecular target of LMB (32).

Human CRM1 was independently identified as an interactor of the nucleoporin CAN/Nup214. CAN was known to be involved in acute leukemias as part of an oncogenic fusion protein: (i) in myeloid leukemia, a recurrent translocation fuses *CAN* to a gene called *DEK*; (ii) in a case of acute undifferentiated leukemia, *CAN* is fused to a gene termed *SET* (33-34). The search for interactors of these chimaeric proteins yielded the isolation of the human homologue of yeast CRM1 (35). CRM1 was indeed found to be part of a complex containing CAN and another nuclear pore component, Nup88 (36). Localization and protein interaction studies indicated that CRM1 interacts with NPCs and moves between the nucleus and the nuclear pore complex (NPC) cytoplasmic face, similar to some transport factors. Indeed, three papers almost simultaneously identified CRM1 as the export receptor for proteins containing leucine-rich NES sequences (37-39). LMB inhibits the interaction of CRM1 with NES-containing proteins and thus blocks their export (37, 39).

CRM1 shares a domain of homology with importin beta and with a group of proteins of similar size, hence the CRIME acronym for that domain (CRM1, Importin beta, etcetera). These domains interact with GTP-bound Ran. Indeed, CRM1 binds to both NES sequences and to RanGTP in a cooperative way. RanGTP stabilizes the CRM1/NES protein complexes formed in the nucleus, thereby activating export. The role of CRM1 as the export vector for proteins that have a cytoplasmic function accounts for the complexity of the mutant phenotypes and for the different ways in which it was identified. Furthermore, the dependence of the export process on RanGTP binding accounts for scenarios in which altered RanGTP production can hinder aspects of cell proliferation (e.g. cell cycle transitions, cell survival and differentiation) that require specific factors to be targeted to the cytoplasm.

4.3. CAS

CAS is the human homologue of the yeast gene *CSE1* (Chromosome segregation), which was originally isolated in a genetic screening for mutations affecting chromosome segregation (40). The screening made use of a chromosome with a partially functional centromere, so that mutations with mild phenotypes could be identified and mutants in centromere-binding proteins could be selected for their synthetic effect. *Cse1* mutations had relatively small effects on segregation of chromosome with wild-type centromeres, but increased mis-segregation of chromosomes carrying defective centromeres. *Cse1* mutant are more sensitive than wild-type strains to nocodazole (NOC), a MT-depolymerizing drug, and in some cases exhibit abnormal spindle structures. These data implicated CSE1 in cell division (40). A link with nuclear transport emerged from the finding that the *Cse1* phenotype is suppressed by SRP1, the yeast importin alpha homologue (41).

The human homologue of the yeast *CSE1* gene was cloned from an antisense fragment of human cDNA that rendered MCF7 breast cancer cells resistant to cell death induced by bacterial toxins and tumor necrosis factor; the gene was therefore named CAS (cellular apoptosis susceptibility gene) (42). In fact, a more complex picture subsequently emerged, because CAS regulates cell proliferation and certain apoptotic pathways, but does not interfere, for example, with cell death induced by staurosporine, ciclohexymide or etoposide (43).

The human CAS protein localizes to interphase MTs and to the mitotic spindle (44), suggesting a role in cell division, like its yeast homologue. The *CAS* gene maps on the long arm of chromosome 20, in a region frequently amplified in cancer cell lines and specifically correlated with aggressive breast cancer. In addition, *CAS* gene is highly expressed in proliferating and tumor cells (42) and is specifically amplified in BT-474 breast cancer cells; *CAS* extra-copies are also present in breast and colon cancer cell lines and in leukemia cells (45). The *CAS* gene has been proposed to work as a "switch" in determining whether a cell should proliferate or undergo apoptosis and, in that respect, it has been assimilated to genes such as MYC, p53 and BCL2, which regulate both apoptosis and cell proliferation and also function as oncogenes when mutated.

A link to transport was only established in 1997. CAS/CSE1 was found to interact with NPCs and to share

the CRIME domain with importin beta and CRM1 (36, 46). CAS was then characterized as the mediator of importin alpha recycling (47): it was shown to interact with NPCs, to cross the NE and to bind importin alpha in the presence of RanGTP. The CAS/importin alpha/RanGTP complex is exported out of the nucleus and dissociates in the cytoplasm, following RanGTP hydrolysis, thus rendering importin alpha available to reinitiate a novel import cycle.

4.4. The interplay between Ran control of nucleo-cytoplasmic transport and cellular states

Ran function determines the subcellular localization of proteins that then regulate cell proliferation and differentiation. Conversely, conditions that influence the cell cycle and checkpoints can regulate Ran-dependent nuclear transport. Cell cycle mechanisms can 'sense' transport rates and regulate them in a phase-specific manner (48).

Stress-inducing agents can also influence Ran-dependent control of transport. Cellular stress induced by UV irradiation, oxidative stress or heat shock specifically suppresses nuclear export of importin alpha (29): that prevents the recycling of import factors, and, over time, depresses nuclear import of proteins. Stress decreases intracellular ATP levels and alters the balance of GTP / GDP nucleotides, with a net decrease of nuclear RanGTP levels (49). Similar effects are caused by agents that induce ATP depletion, which perturb the subcellular localization of importin alpha and significantly decrease RanGTP levels (50). Thus, the intracellular distribution of Ran and the overall process of nuclear import are sensitive to stress conditions.

The status of cell proliferation and differentiation also affect importin alpha family members. Human cells contain one importin beta vector and six importin alpha isoforms that interact with NLS subtypes with subtle sequence specificity (51). In transformed cell lines in which proliferation was inhibited by serum starvation, the expression of some nuclear transport factors was markedly decreased; serum readdition restored high levels of expression (51). Specific differentiation pathways also regulate differentially importin alpha members. For example, rat pancreatic AR42J cells can be stimulated to differentiate towards a neuroendocrine or an acinar phenotype by specific inducers; these conditions up-regulate the levels of importin alpha3 and alpha4. In contrast, importin alpha1 and 4 are down-regulated in leukemia HL60 cells induced to differentiate. These results indicate that proliferation and differentiation pathways affect the abundance of specific importin alpha isoforms, and hence the efficiency of transport of particular protein cargo types (51). These lines of evidence indicate that transport pathways are linked to the cell cycle and cell proliferation machinery. Recent data also suggest that transport pathways may be deregulated in some cancer types, associated with altered expression of transport receptors or of componenets of the NPCs (52).

4.5. Control of the subcellular localization of tumor suppressor and oncogenes

Most tumor suppressor proteins such as APC, p53, VonHippel-Lindau (VHL) and others, as well as proto-oncogenes such as BRCA1 and c-Abl, contain NLS and NES signals that regulate their shuttling between the nucleus and cytoplasm (Table 1). In particular conditions, their transport can be specifically modulated: for example, stress or DNA damage may induce post-translational modifications, and/or interactions with protein partners, that can stabilize them in a specific compartment, e.g. the nucleus. While it would not be possible to list all factors relevant to cell proliferation that are subjected to nucleo-cytoplasmic transport (e,g, cyclins, mitogen-associated kinases etc.), some paradigmatic examples are summarized in Table 1. Thus, even in cells in which tumor suppressor genes have a wild-type status, their protein products can be rendered biologically inactive due to failed subcellular compartmentalization.

In discussing the impact of nucleo-cytoplasmic transport on cancer onset and progression, p53 is a paradigmatic example worth examining in some depth. Genotoxic damage induces p53 tetramerization, nuclear import and stabilization in the nucleus; therein, p53 activates transcription of genes acting either in damage repair or in the induction of apoptosis. In order to accumulate functional p53 in the nucleus, therefore, pathways of nuclear export must be down-regulated. p53 export to the cytoplasm is associated to rapid p53 degradation and is LMB-sensitive: this implicates CRM1 and RAN as upstream regulators of p53 turn-over in normal cells. Human HMD2 (mdm2 in murine cells) is a p53-interacting RING finger protein, with E3 ligase activity, that ubiquitinates p53 and promotes its degradation. Interestingly, the *HDM2/mdm2* genes are transcriptional targets of p53 activation (reviewed in refs. 53-54) and are therefore part of a regulatory loop in which p53 can regulate its own degradation. In the presence of DNA damage or other oncogenic stimuli, HDM2/mdm2 proteins are negatively regulated by p14/ARF-INK4a (54), a cyclin kinase inhibitor that interacts with them and prevents their binding to p53; by doing so, p14 blocks the ubiquitination and degradation of p53, thus contributing to the nuclear accumulation of p53. When damage is repaired/removed, and the cell cycle is resumed, p14 is eliminated and HDM2/mdm2 can again regulate p53 export and target it to degradation.

A clear circumstantial link exists between p53 binding by HDM2/mdm2 and its nuclear export, ubiquitination and proteasome-dependent degradation, but the precise mechanism of p53 export has been a matter of debate. p53 contains a NES located within its tetramerization domain, which underlies its cytoplasmic localization in cells non-exposed to DNA damage (55). HDM2/mdm2 also harbors a NES that is responsible for its cytoplasmic targeting. Overexpression of HDM2/mdm2 causes p53 export from the nucleus in a manner that is dependent on binding of HDM2/mdm2 to p53, and requires the p53 NES, and the ubiquitin ligase activity of HDM2/mdm2 (56-57). CRM1 overexpression increases nuclear export of p53, although it has been noted that

Table 1. Nucleo-cytoplasmic transport of cell proliferation regulatory factors mediated by Ran and its effectors

Factor	Function	Localization / function relation	Regulation of transport	Ref
p53	Tumor suppressor	Active in nucleus, degraded in the cytoplasm	See text for details	55-60
BRCA1	Tumor suppressor in breast epithelia; Response to DNA damage	Active in nucleus	NLS-dependent nuclear import; two NESs direct CRM1-dependent export	157,158
c-Abl	Oncogenic kinase	Shuttling protein; In the cytoplasm: regulates cell proliferation and survival; In the nucleus: regulates apoptosis	Regulated nuclear import in response to DNA damage	159
RB	Tumor suppressor; Co-transcriptional regulator: suppresses transcription of proliferation genes	Active in the nucleus	NLS-dependent import; high affinity for importin alpha; LMB- and phosphorylation-sensitive export	160 161
Von Hippel-Lindau (VHL)	Tumor suppressor	Largley cytoplasmic, but antitumor properties are associated with nuclear localization	Shuttling due to NES and NLS signals	162,163
APC	Tumor suppressor; Controls proliferation of epithelial cells by regulating the activity of catenin molecules	Shuttling protein; Density-dependent relocalization	NLSs direct nuclear import and NESs direct export; Phosphorylation around NLS is critical for regulated shuttling	164,165 166
p73	DNA repair, apoptosis	Active in nucleus	Transport via NLS and NES; stability is NLS- and NES-dependent	167
p27Kip1	Inhibitor of cyclin-dependent kinases	Shuttling: active in the nucleus in growth-arrested cells; cytoplasmic in cycling cells, associated with 14-3-3- binding	CRM1 association is up-regulated on cell cycle entry and directs nuclear export; NLS-dependent import mediated by importin alpha 3/5 and inhibited by phosphorylation	168 169,170
p21Cip	Inhibitor of cyclin-dependent kinases	Shuttling, active as suppressor of proliferation in the nucleus	NLS direct nuclear import; Export is inhibited by phosphorylation	171 172
Stat1/2	Transcription factor	Cytoplasmic before activation; Cytokine stimulation induces phosphorylation, dimerization and nuclear accumulation	LMB-sensitive export; export block interferes with response to Interferon-gamma	173
Stat 3	Transcription factor	Cytoplasmic before activation; Cytokine stimulation induces phosphorylation, dimerization and nuclear accumulation	Nuclear import mediated by importin alpha, beta and Ran	174
E2F4	Transcription factor	Nuclear in G0 and quiescent cells	CRM1-dependent LMB-sensitive export upon cell cycle entry	175
E2F5	Transcription factor	Shuttling: nuclear in non-dividing differentiated cells	NLS-dependent import and NES-dependent LMB-sensitive export	176
Survivin	Part of the passenger protein complex; regulator of mitosis and apoptosis	Nucleo-cytoplasmic shuttling in interphase, relocalizes to kinetochores in mitosis and midbody at cytokinesis; export-defective mutants do not protect against apoptosis	Non canonical NES but CRM1-dependent LMB-sensitive export from nucleus; CRM1-dependent recruitment at kinetochores	177,178
NF-kB	Heterodimeric transcription factor	Shuttling: cytoplasmic when inactive; active in the nucleus	CRM1-dependent cytoplasmic localization of inactive dimers; NLS-dependent import mediated by importin alpha 3/4	179 180

export, per se, may not necessarily result in p53 degradation (58). Interestingly, however, the cell response to some types of DNA damage actively relies on reprogramming of p53 export cycles, by triggering phosphorylation and inhibition of a previously unrecognized NES in p53 outside the tetramerization domain (59). In addition, a mutant form of p53 found in certain cancers retains an intact NES that specifies its cytoplasmic retention; this has been correlated to higher susceptibility to become ubiquitinated compared to wild-type p53; the introduction of mutations that prevent ubiquitination restore the nuclear localization of p53 (60): these findings provide direct evidence for the implication of the nucleo-cytoplasmic machinery in the tumor suppressor function of p53. Togheter these data illustrate the importance of subcellular transport for the stability and function of a fundamental tumor suppressor. The status of the Ran network, as a global regulator of subcellular transport, and particulalry the formation of CRM1/NES export subtsrates under RanGTP control, does therefore provide one level of control in these processes. These discoveries have lead to envisage the possibility to target nucleo-cytoplasmic transport as a strategy for therapeutic intervention in cancer, based on the following rationale:

- Intracellular localization is essential for the activity of many signaling molecules associated with cell proliferation and checkpoints;
- The recruitment of signaling proteins in the nucleus is an essential step in activating transcription of specific genes in response to an extracellular signal;
- Tumor suppressors and inducers of apoptosis are subjected to Ran-dependent nucleo-cytoplasmic transport;
- Dysfunction of the mechanisms of nucleo-cytoplasmic transport can result in a number of pathological situations and diseases, including cancer.

The discovery that LMB inhibits cell cycle progression, and the identification of the target of LMB as

CRM1, seemed at first very promising to that scope (61). However, inhibition of the overall export activity by LMB may cause non-specific effects, because CRM1-interacting NESs are present in many proteins, and different CRM1-interacting NESs show different "strengths" as export substrates; for example, a strong NES is present in RanBP1, which regulates the overall Ran GTPase turnover, whereas p53 and p53-regulated proteins p21 and HDM2 harbor comparatively weaker NESs (62). LMB however has proven a powerful tool to identify export signals and the protein domains in which they reside, which can significantly help the design of selective drugs. That line of reasoning has stimulated, among others, the idea that inhibiting the p53-HDM2 interaction might be a useful approach to favour the nuclear retention of p53, and hence its apoptosis-inducing activity in tumor cells (63-64). A range of selective compounds have been designed with the scope of stabilizing wild-type p53 in nuclei (65).

5. RANBPM, A CHAPERON PROTEIN WITH ROLES IN CELL SIGNALING, ADHESION AND MIGRATION

Ran-binding domains are not only present in direct regulators of nucleotide turn-over on Ran and transport vectors. RanBPM is another Ran partner and can specifically interact with RanGTP, though not being a canonical member of the Ran network "core". RanBPM acts in multiple cell signaling pathways, with direct implications in cell proliferation and motility, as briefly recapitulated below. It is presently unclear how RanGTP regulates RanBPM, and/or conveys it to perform particular functions in specific cellular contexts and in response to specific stimuli. By analogy with other Ran effectors, it may be envisaged that RanGTP binds RanBPM and modulates its association with or dissociation from interacting partners, thus modulating their function.

RanBPM was first isolated as a 55 kDa protein in a two-hybrid screen using Ran as a bait (66). Antibodies produced to that first isolated clone revealed centrosomal localization, as well as co-sedimention with the purified centrosomal fraction, hence the original name of RanBPM (Ran-binding protein in the microtubule-organizing center, later renamed Ran-binding protein 9, RanBP9). RanBPM was proposed to regulate MT nucleation; consistent with this, anti-RanBPM antibodies inhibited the formation of MT asters. These results provided a first hint that Ran may regulate centrosome functions through RanBPM (66). Later experiments indicated that the first isolated RanBPM clone was in fact a truncated version, alerting to the possible caveat that the reported involvment in MT nucleation may in fact have reflected a 'dominant negative' effect. The full-size RanBPM was found to encode a 90 kDa protein (67) that is part of a large multimeric complex together with Muskelin, a mediator of cell spreading, and with four more proteins, all of which contain LisH/CTLH motifs, present in proteins involved in MT dynamics, cell migration and chromosome segregation (68). A role of RanBPM in MT nucleation and organization of the MT cytoskeleton was further strengthened by its association with MT- interacting factors, such as, for example hSMP-1, a human sperm membrane protein expressed during male germ cell differentiation that modulates MT assembly (69). Furthermore, many RanBPM-interacting proteins are expressed in neuronal cells, which are particularly rich in MTs: among those, the fragile X mental retardation protein (**FMRP**) (70) is implicated in RNA transport and translation in neurons; the tyrosine kinases **p75NTR** (71) and **TrkA** (72) mediate signal transduction in neural cells; **Plexin-A1** is a component of a receptor complex in axonal outgrowth (73).

RanBPM has a widespread ability of to interact with receptors that regulate cell adhesion and migration. Some of these factors have direct roles in cell proliferation and altered signaling is associated with cell transformation.

- The cell adhesion molecule L1-CAM is a RanBPM partner; furthermore, RanBPM overexpression in primary neurons inhibits L1-mediated neurite outgrowth and branching, while, in COS cells, it reduces L1-triggered Erk1/2 activation: thus, RanBPM may act as an adaptor protein in L1-dependent signaling in cell adhesion, migration and proliferation, possibly via the Erk/MAPK pathway (74). Interestingly, LI-CAM is also expressed in non-neuronal cancer cells and can induce metastasis when overexpressed (75).

- **MET**, the receptor protein-tyrosine kinase for hepatocyte growth factor (HGF), controls the growth, morphogenesis and motility of hepatic cells. MET is overexpressed or mutated in several human cancers. RanBPM interacts with MET and HGF strengthens the interaction. RanBPM stimulates the HGF-MET signaling pathway, the formation of active RasGTP, Erk phosphorylation and activation of serum-responsive promoters. It has been suggested, therefore, that high levels of RanBPM may cause constitutive activation of Ras pathway via MET (76).

- **MIRK** (Minibrain-related kinase/Dyrk1B) is a kinase active in muscle development and expressed in various carcinomas (77). Mirk up-regulation inhibits cell migration, whereas HGF (which stabilizes the interaction of RanBPM and Met), or high RanBPM levels, attenuate the inhibition. Furthermore, RanBPM inhibits the kinase activity of Mirk. These findings suggest that RanBPM and Mirk regulate cell migration in an antagonistic manner.

- **Integrin family members** regulate cell adhesion, motility, proliferation, differentiation and survival. RanBPM interacts with the beta(2) integrin LFA-1 and with the cytoplasmic domain of beta(1) and co-localizes with them at the cell membrane (78). RanBPM also cooperates with LFA-1-mediated adhesion in AP-1-dependent transcriptional activation, and can therefore be viewed as an adaptor protein modulating integrin-dependent signaling.

- Similarly, RanBPM interacts with members of the nuclear receptor superfamily, including thyroid hormone receptor (**TR**) (79) and androgen receptor (**AR**) (80), and can increase transcriptional activation by these receptors when overexpressed. In synthesis, therefore,

RanBPM can cooperate with membrane-bound signalling molecules to transduce signals culminating with transcriptional activation of specific genes.

- Finally, RanBPM can establih specific interactions with partners in cancer cells. For example, **Axl** is a transmembrane receptor tyrosine kinase, the ligand of which is the growth/survival factor Gas6, that interacts constitutively with RanBPM in several cancer cell lines (81). **Psoriasin** (82) is also a RanBPM interactor that is highly expressed in pre-invasive breast cancer and often down-regulated with breast cancer progression. A study of their levels of expression showed high RanBPM mRNA levels in tumor-derived compared to normal breast epithelial cells (82), suggesting therefore that the interaction of RanBPM with psoriasin influences both epithelial and stromal cells, thus contributing to breast tumor progression. RanBPM may also influence cell transformation through its ability to interact with proliferation regulatory proteins p73 (83), and homeodomain-interacting protein kinase-2 (HIPK2) (84), though the biological consequences of these interactions have yet to be fully clarified.

As recalled above, RanBPM is not a direct component of the Ran system and does not influence the overall netrwork activity. However, its Ran-binding domain makes it susceptible to modulation by RanGTP in its interactions with signaling partners. Therefore, intracellular RanGTP levels can influence RanBPM1-dependent functions in cell proliferation, motility, migration and adhesion.

6. RAN IN CONTROL OF CELL DIVISION AND GENETIC STABILITY

Mitotic spindle alterations can lead to unbalanced chromosome segregation, and hence to the generation of aneuploid cells; if not eliminated by cell death, these celsl can give rise to a genetically unstable clone and initiate tumorigenesis. Indeed, aneuploidy is a distinctive hallmark of cancer (85-86). In addition, levels of expression of genes that regulate the mitotic apparatus are important in the response of cells to drugs that target MTs (87). Dysfunction of Ran or its regulators can influence both of these aspects:

a) they can contribute to the onset of genomic instability through faulty cell division, by causing errors in the assembly or function of the mitotic spindle, and

b) they can influence the response to MT-targeting drugs that are employed in chemotherapeutic treatment of cancer.

As recalled above, many Ran functions are exerted by releasing factors that are otherwise complexed to importin beta (reviewed by 11-12), either directly or via importin alpha members. The dynamic interactions between (i) Ran, (ii) the regulators of its nucleotide-bound state, (iii) importin vectors and (iv) NLS-containing importin partners, are critically important in these processes. Ran also operates via CRM1 and regulates its interaction with NES-containing proteins. It is now well-established that, at NE breakdown, these same actors assume new functions in regulating mitosis. The most important source for mechanistic information is the Xenopus system, supported by genetic evidence from yeast, in which Ran members genetically interact with some mitotic regulators, indicating cooperation in mitotic control pathways. Growing studies are currently aiming to clarify the role of Ran in spindle formation, chromosome alignment and mitotic checkpoint in normal and transformed human cells in vivo.

RCC1 remains bound to chromatin throughout mitosis; therefore, the bulk of RanGTP is generated at and around mitotic chromosomes and dilutes away from them, a distribution that is commonly referred to as the RanGTP 'gradient'. It is however emerging that Ran regulates mitosis through more subtle signals, that are not only concentrated around chromosomes, but reach critical sites of the mitotic apparatus, i.e. MTs, centrosomes, KTs, and regulates local processes with astonishing spatial precision therein. These processes have been largely reviewed elsewhere (12, 13, 15-16, 88). Here we will briefly recapitulate Ran-dependent functions that can be a source of genetic instability when deregulated.

6.1. Ran in centrosome duplication in interphase

Centrosomes are the major MT-organizing centres in somatic cells and organize the spindle poles at mitosis. They were the earliest cellular organelles to be recognized, over one century ago, as being distinctively altered, either in structure or in number, in cancer cells (89-92). Supernumerary or structurally abnormal centrosomes give rise to multipolar spindles, which are not detected by the spindle checkpoint (93); no 'corrective mechanism' therefore operates to arrest progression of multipolar mitoses: thus, chromosomes can aberrantly segregate at more than two poles, giving rise to genetically unstable cells. A fraction of Ran localizes at centrosomes throughout the cell cycle, tethered via AKAP450, a large coiled-coil protein (94) The Ran/AKAP450 complex contributes to anchoring MTs to centrosomes and organizing the structure of MT asters in interphase (94).

Ran is also implicated in control of centrosome duplication, probably by recruiting specific regulators to centrosomes. Tight regulation of centrosome duplication once per cell cycle is necessary to ensure that bipolar spindles form (89-92). A role of Ran in centrosome duplication emerged from the observation that Ran is targeted by viral oncoproteins: Papillomavirus E7, Adenovirus E1A and SV40 large T antigen (95). These oncoproteins inactivate cell cycle regulators, stimulate S phase and induce centrosome amplification, ultimately causing segregation abnormalities in mitosis and aneuploid daughter cells. The ability of E1A to deregulate centrosome duplication depends on its physical interaction with Ran and on the presence of functional RCC1 (95), suggesting that viral oncoproteins use a Ran-dependent mechanism to induce centrosome amplification.

A role in centrosome duplication has also been demonstrated for CRM1, a fraction of which localizes at

centrosomes (96). CRM1 interacts with the HBx protein from the Hepatitis B virus (HBV), which plays a role in liver carcinogenesis. HBx contains a NES and its binding to CRM1 sequesters the latter in the cytoplasm. Interestingly, wild-type but not NES-defective HBx induces supernumerary centrosomes. Similar to HBx, LMB also blocks the centrosomal localization of CRM1 and induces supernumerary centrosomes (96). These data suggest that the centrosomal CRM1 fraction contributes to restrict centrosome duplication to once per cell cycle (Figure 2). CRM1 regulates the centrosomal localization of nucleophosmin (NPM), a factor thought to bind to newly duplicated centrosomes to prevent their reduplication within the same cell cycle. NPM is often mutated in human cancers, consistent with its role in genomic stability (97). NPM contains a functional NES that is required for its association with centrosomes, and LMB disrupts NPM centrosomal localization; this implicates the formation of a local complex containing Crm1 and RanGTP that targets NPM at centrosomes and regulates their duplication. The data suggest a mechanism whereby CRM1 sequestration by HBx, or inhibition by LMB, would similarly render CRM1 unavailable for the centrosomal recruitment of NPM, hence giving rise to supernumerary centrosomes that can organize multipolar spindles in mitosis (98).

6.2. Ran control of mitotic centrosome function and spindle pole formation

Spindle pole organization requires a balance between factors that confer structural support to the poles, and factors that counteract excessive rigidity in these stuctures, so as to allow sister centrioles to move as MTs become tethered and organize in polar arrays. Multipolar spindles form not only as a result of deregulated centrosomes duplication, but also when centrosomes fragment or when the two sister centrioles that constitute a centrosome separate. Like Ran, RanBP1 localizes at centrosomes throughout the cell cycle (99). RanBP1 overexpression, though not altering centrosome duplication in interphase, causes sister centrioles to split in mitosis, giving rise to spindles with fragmented poles (99, Figure 2). The fact that RanBP1 overexpression induces a mitotic phenotype, though being a constitutive centrosomal resident throughout the cell cycle, suggests that it operates via factor(s) that reach the asters/spindle poles after NE breakdown. RanBP1 excess causes an unbalance between Ran and RanBP1 at spindle poles; the resulting phenotypes suggests that either a factor facilitating centriole separation becomes abnormally activate, or, on the contrary, a factor conferring stuctural cohesion is inhibited. It is of note that the inactivation of another spindle pole-located Ran-interacting protein, harboring a RCC1-homologous domain and called Nercc1, also causes multipolar spindles with fragmented poles (100-101).

A similar phenotype is also generated by the Tax protein encoded by the human T-cell leukemia virus type-1 (HTLV-1) (102). Tax localizes at centrosomes/spindle poles in a Ran- and RanBP1-dependent manner and induces centrosome fragmentation in mitosis. Tax harbors a Ran-interacting domain that specifies its localization to centrosomes. However, Ran binding per se is not sufficient to target Tax to centrosomes or to induce spindle pole abnormalities. In the search for other responsible factors, RanBP1 was found to interact with Tax; furthermore, eliminating RanBP1 by RNAi rescued Tax-dependent spindle pole abnormalities. These data suggest a model in which Tax is targeted to centrosomes of HTLV-1 leukaemic cells after NE breakdown; a Tax/Ran/RanBP1 complex forms at centrosomes, which disrupts the cohesion of sister centrioles, causing them to split apart and giving rise to spindles with abnormal poles.

Importin beta and alpha also localize at spindle poles in mitosis (103). Importin beta overexpression induces multipolar spindles and centrosome fragmentation, chromosome misalignement and mitotic delay (103-104). After NE breakdown, importin beta is transported to poles along MT in complexes with pole-organizing factors and with dynein (103), a minus-end directed motor protein involved in spindle pole organization (105). TPX2, a RanGTP-dependent SAF, also reaches poles at least in part via dynein (106). In vitro, TPX2 is functionally inhibited in complexes with importin alpha and beta, but can still bind MTs (107-108). Furthermore, TPX2 inactivation by RNAi causes spindle pole fragmentation (109-110) and abolishes importina beta localization at spindle poles (103). These data suggest a model of mutual regulation between importin beta and TPX2, in which complexes containing importin beta, alpha and their TPX2 cargo are transported along MTs to the poles via dynein; importin binding maintains TPX2 inactive in these complexes. At poles, centrosomal RanGTP binds importin beta and thus removes its inhibition on TPX2. The model accounts for the observation that importin beta excess inhibits the release of active TPX2 at poles, resulting in spindle pole fragmentation similar to that caused by TPX2 inactivation. Thus, the balance between Ran network components and Ran-regulated proteins at spindle poles is essential for the establishment and maintenance of the spindle bipolarity.

6.3. The role of Ran in microtubule function

In addition to the formation of spindle poles, Ran regulates the dynamic activity of MTs, which is essential during the “search-and capture” phase in which mitotic MTs are projected from centrosomes in the cytoplasm until they make contacts with single KTs.

A complete inventory of spindle-associated proteins has been recently compiled after tandem mass spectrometry analysis of purified mitotic spindles (111). Several factors known to be RanGTP-sensitive for their activity were identified (TPX2, TOG1p, Aurora-A, NPM, NuMA NuSAP, Rae1/Gle1, HURP), as expected. In addition, spindle preparations also contained Ran partners and effectors, including Ran itself, RanBP2, RanGAP1, the miRNA vector exportin 5, transport receptors for ribosomal and splicing factors (importin 4, 7 and 8; transportin 1 and 2; RanBP5), and many transport receptors for proteins: exportin 1/CRM1, CAS/CSE1, importin alpha 1, 2 and 7 and importin beta. Several nucleoporins did also co-purify with spindles. For some components, i.e. RanBP1 (112), importin alpha and beta (103), RanGAP1 and RanBP2 (113-114), this proteomic survey is backed-up by in-depth studies of their distribution and function in mitotic spindles.

A role of RanBP1 in control of MT function has been recently demonstrated (112). Earlier findings that RanBP1 protein abundance is highest from early G2 to anaphase suggested that modulation of Ran-dependent interactions in mitosis requires increased RanBP1 concentrations compared to earlier cell cycle phases (115). RanBP1 inactivation by RNAi causes prolonged prometaphase delay, often followed by apoptosis (112, 116). Cells that remain viable have hyperstable MTs that are resistant to both NOC- and to cold-induced MT depolymerization. These hyperstable MTs show an abnormally spread distribution of the MT-stabilizing factor HURP over their entire length (112); normally, HURP is restricted at the plus-end region of MTs, proximal to KTs, but a similar spread pattern is induced by a GTP-locked Ran mutant (117). HURP is a direct binding partner of importin beta and dissociates from it in the presence of RanGTP. These results suggest that RanBP1 activity is required to modulate HURP release from importin beta and to regulate its spatial localization on MTs. Associated with unrestricted HURP distribution and MT hyperstability, RanBP1-depleted cells display lagging chromosomes in anaphase, suggestive of a type of attachment defined merotelic, in which one same KT is attached to MTs emanating from opposite poles (Figure 2). Merotelically attached chromosomes are not detected by the spindle checkpoint, because they are attached to the spindle, but they cannot segregate and are therefore a cause of aneuploidy (reviewed in 118). Thus, RanBP1 regulates chromosome segregation by modulating the interactions of particular MAPs with importin beta and RanGTP, thus ensuring their correct spatial distribution and activity (112).

The Ran effector CAS/CSE1 also localizes to the spindle MTs in mitosis (44). Interestingly, CAS is phosphorylated by MEK1 (119). Taxol is a MT-directed drug that suppresses the dynamic activity of MTs, arresting mitotic progression and activating apoptosis. Inhibition of MEK1-mediated phosphorylation increases paclitaxel (Taxol)-induced apoptosis in breast, ovarian, and lung tumor cell lines. It has been suggested therefore that altering the activity/phosphorylation status of CAS via MEK1 inhibition may present a potential strategy in experimental cancer therapy (120).

Several more MAPs regulate MT functions under Ran control, including Rae1/Gle1, TPX2, RHAMM, TOG1p, NuMA, NuSAP, HURP; their interactions with MTs under Ran control have been reviewed elsewhere (13). Ran network dysfunction can cause deregulated activity of these MAPs, as briefly recalled above for TPX2 and HURP; this can explain at least some pathways through which Ran affects the organization and dynamic functions of the spindle, and thus faithful chromosome transmission to genetically balanced daughter cells.

6.4. The role of Ran at kinetochores

Ran has two important roles at mitotic KTs: it contributes to the spindle checkpoint function and it regulates the formation of MTs that will establish stable interactions with KTs and pull chromosomes towards opposite poles. The spindle checkpoint monitors that all chromosomes are attached to the spindle MTs via their KTs and is accompanied by high activity of cyclin B and securin, a regulator of proteins called cohesins that tether sister chromatids together until all chromosomes are attached (14). When all chromosomes have attached, the spindle checkpoint is inactivated, concomitantly with timely degradation of these proteins, sister chromatids separate and begin to segregate (121-122). The ability of cells to activate the checkpoint is of extreme importance to prevent that mitotic cells harboring unattached chromosomes will proceed to incomplete segregation. Cyclin B, securin and other mitotic proteins are targeted for degradation after ubiquitination by an E3 ligase called APC/C. A specialized network of KT-associated proteins, including Mad1, Mad2, Mps1, Bub1, Bub3, BubR1 and CENP-E, can “sense” the local tension generated by MT attachment, or the lack thereof, and regulates the activity of the APC/C in consequence; in particular, Mad2 is indispensable in this signaling (14, 123). In the presence of an incompletely assembled spindle or faulty MT attachments to KTs, Mad2 remains tethered to KTs and causes a delay in APC/C activation, thus preventing the ubiquitination and degradation of cyclin B and securin.

The spindle checkpoint function is extremely relevant to cancer treatment (87, 124). NOC, which prevents Mt assembly and attachment to KTs, induces sustained activity of the checkpoint and blocks mitotic progression. Vinblastine has a similar mechanism of action and is employed in chemotherapeutic treatments of cancer to arrest mitosis; after prolonged arrest, mitotic cells eventually undergo apoptosis. Taxol does not affect MT assembly but blocks their dynamics; this also causes mitotic arrest and apoptosis; a taxol derivative, paclitaxel, is also widely employed in cancer chemotherapeutic treatment.

Elevated levels of RCC1 or RanGTP, however, abrogate the spindle checkpoint-dependent mitotic arrest in the presence of NOC, disrupt the KT localization of spindle checkpoint components and allow APC/C activation. In particular, Bub1 and Bub3 localization to KTs are directly responsive to RanGTP levels and can be modulated by manipulating the levels of RCC1 and RanGAP1 (125). In tsBN2 cells lacking RCC1 function, NOC does still activate the checkpoint, but cells progress more quickly through the metaphase-to-anaphase transition after NOC release and form defective k-fibres that cause mis-segregation of chromosomes (126). Thus, RCC1 and RanGTP are required to prevent anaphase in the presence of MT misattachment or misassembly. LMB also induces a lack of stable k-fibres, consistent with a role of the Crm1/RanGTP complex in proper k-fibre assembly. During mitosis, RanGAP1 is targeted to KTs in a SUMO-modified form, together with RanBP2/Nup358, a Ran-binding protein with SUMO-ligase activity (113). CRM1 and RanGTP are required for RanGAP1/RanBP2 recruitment to KTs and for efficient chromosome segregation (126). The RanGAP1/RanBP2 complex plays a role in stabilizing MTs attachments to KTs:

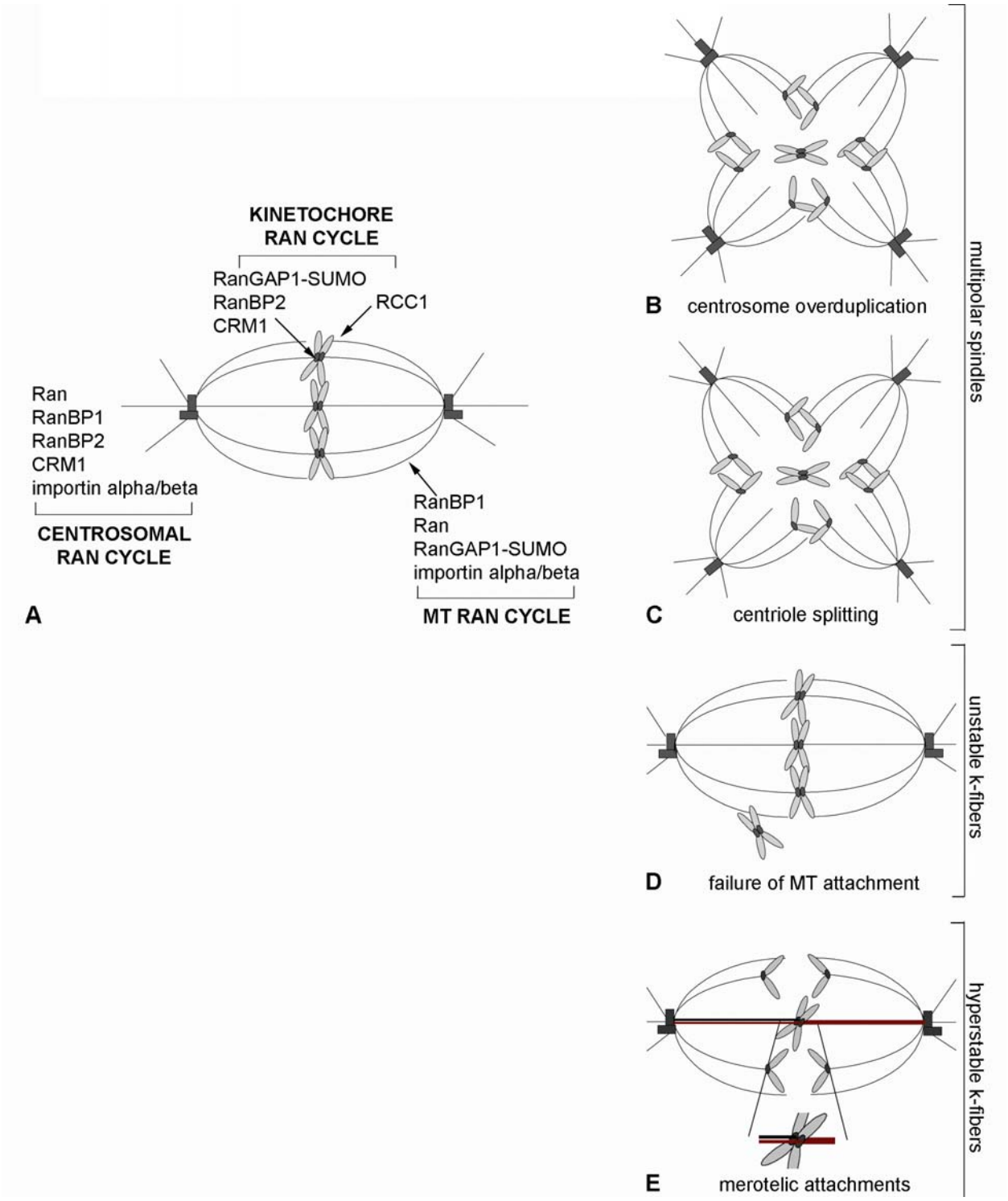

Figure 2. The Ran network can affect chromosome segregation in multiple ways. A. A schematic representation of Ran network components arranged in local networks at specific structures of the mitotic apparatus. Deregulated activity or dysfunction of single components in these networks can induce different mitotic defects, represented on the right: unbalanced activity of the centrosome-associated Ran network can cause centrosomal amplification (panel B), fragmentation or splitting (panel C); Ran dysfunction at the level of MTs and kinetochores can cause either unstable MT/kinetochore attachments (panel D), or, on the contrary, hyperstable merotelic attachments (represented as a thick red line in panel E) (see text for details). MT, microtubules.

RanBP2 depletion caused the dissociation of RanGAP1 from KTs, failure to assemble stable k-fibers, defects in spindle assembly and chromosome mis-alignment (114, 127). Crm1/RanGTP, RanGAP1 and RanBP2 have been proposed to work in an auto-regulatory feedback loop, in which RCC1 would regulate the recruitment of RanGAP1/RanBP2 at KTs, essentially by generating RanGTP that is then available for association with CRM1. RanGAP1/RanBP2 in turn promote RanGTP hydrolysis and catalyze their own release from KTs: at this point, high RanGTP concentrations at KTs would facilitate the release of spindle checkpoint factors, thus causing the checkpoint inactivation in normal cells or its override in the presence of NOC (reviewed in 15-16). In summary, inhibiting CRM1 or RCC1 function yields segregation errors associated with a lack of cold-resistant K-fibers; RanBP1 depletion also yields segregation errors, but with an opposite phenotype, that is not due to lack of attachment but, on the contrary, to excessively stable MT attachments (Figure 2). Therefore, levels of Ran regulators are critical for proper attachment of chromosomes to MTs and hence faithful segregation.

6.5. Emerging roles of nucleoporins in cell division

A body of evidence implicate Ran in control of proteins that relocalize dynamically from interphase NPCs to mitotic KTs. NPCs are the macromolecular assemblies that 'fenestrate' the NE and permit transport of molecules across it. They are highly complex structures, formed of multiple copies of several different nucleoporins that assemble in specific sub-complexes forming the basic constituents of NPCs.

- Some KT-associated factors that act in the spindle checkpoint, including Mad1, Mad2, Mps1 and others are "stored" at the NE after mitosis. In particular, human MAD1 localizes to NPCs in interphase, an its overexpression can drive formation of structures called anulate lamellae, that represent storage compartments for NPC proteins during NE disassembly (128), suggesting a possible role of MAD1 in the recruitment of NPC proteins (129).

- Conversely, some NPC constitutive components localize to KTs during mitosis (see below).

These observations have triggered interest for the dynamic localization of these proteins and the role of Ran in these regulated movements. Emerging data suggest that dysfunction of NPC-associated proteins that are normally regulated by Ran and importin beta influence the spindle checkpoint function and the outcome of cell division.

In interphase, RanGTP regulates the dissociation of some nucleoporins (Nup107, Nup153 and Nup358) from importin beta, their targeting to chromatin and their assembly in NPC subcomplexes (130). By analogy, at NEB Ran may regulate in a reverse manner nucleoporin dissociation from NPCs and recruitment at KTs.

A functional link between Ran and NPC association of spindle checkpoint components was elicited in *S. cerevisiae*. Interestingly, in *S.cerevisiae,* in which the NE does not break down during mitosis*,* Mad1 and Mad2 reside at NPCs during the cell cycle, associated to a specific NPC subcomplex (131). Mad2 localization to NPC is linked to proper nuclear compartmentalization of the yeast Ran homologue. Mutations that disrupt the interaction of Ran with its import factor NTF2 *(ntf2-2),* or caused the loss of function of RCC1 (*prp20-1*) or RanGAP1 (*rna1-1*) displaced Mad2 from NPCs, indicating that disruption of the Ran cycle profoundly alters Mad2 localization to NPCs (132). Ran remained at the nuclear periphery in all mutants, but failed to fill the nuclear compartment. Experiments with *tsBN2* cells indicate a conserved requirement for RCC1 function in Mad2 localization in mammalian cells*.* Interestingly, defective mutants for Ran import in nuclei (*ntf2-2)* or for RCC1-dependent RanGTP formation (*prp20-1)* were resistant to the MT-destabilizing drug benomyl, while double mutants that simultaneously lacked mad2 function (i.e. ntf2-2/mad2 delta and prp20-1/mad2 delta) showed restored sensitivity. Thus, the loss of nuclear RanGTP links the release of Mad2 from NPCs and spindle checkpoint function. These data, together with the demonstrated role of high RanGTP levels in disrupting the KT localization of Mad2 and in spindle checkpoint override in mammalian cells (125), support the conclusion that the nucleotide-bound state of Ran influences both Mad2 mitotic localization and activity.

Nucleoporins can directly influence the spindle checkpoint. One NPC subcomplex, termed the Nup107-160 complex and comprising nine nucleoporins, relocalizes from interphase NPCs to mitotic KTs until late anaphase in yeast, *Xenopus* and human cells (133-135). RNAi-mediated depletion of all Nup107-160 complex components significantly delays mitosis, eventually leading to cell death. Importantly, RNAi to Seh1, one of the nine nucleoporins of the complex, has a minor effect on NPC assembly but specifically prevents the recruitment of Nup107-160 complex to KTs, and yields defects in chromosome congression and delay at the metaphase-anaphase transition; Seh1 depletion increased the frequency of unattached chromosomes that fail to align properly and caused the formation of unstable k-fibers (136). Interestingly, depletion of the Nup107-160 complex from KTs also prevents the localization of Crm1 at KTs: thus, the Nup107-160 complex can be viewed as an upstream regulator of CRM1 targeting at kinetochores, and thus of the kinetochore recruitment of RanGAP1/RanBP2, which is important for the formation of stable k-fibers and faithful chromosome segregation, as summarized above.

Experiments to investigate how nucleoporins and spindle checkpoint factors might interplay and to address the role of Ran in the process, showed an increased accumulation of Nup107-160 complex on unattached KTs in NOC-treated cells. The Nup106-170 complex, however, is not required for checkpoint

activation, because depleted *Xenopus* egg extracts effectively arrested in the presence of NOC, nor does it affect the checkpoint override induced by elevated RanGTP levels, because RCC1 addition caused a comparable release of Bub1 and Mad2 from chromosomes in both depleted and in control extracts in which Nup107-160 was functional (135). The Nup107-160 complex, though dispensable for the initial formation of MT asters, is critical at later steps of spindle formation, because its depletion induces mitotic Figures in which condensed chromatin is surrounded by a decreased MT density (135), consistent with the idea that the Nup107-160 complex regulates efficient formation of spindle MTs and KT interactions.

Finally, the NPC component Nup98 plays an unexpected role in modulating the activity of Rae1, a mRNA export factor that was re-isolated in a search for spindle assembly proteins regulated by importin beta (137). RNAi-dependent inactivation of Rae1 hinders mitotic progression, yielding defective spindles and chromosome mis-segregation. Rae1 shares homology with the spindle checkpoint factor Bub3 (138) and localizes to unattached KTs at mitotic onset (139). Rae1 activity is RanGTP-dependent and is inhibited by direct importin beta binding (137); Nup98 enhances Rae1 interaction with importin beta in a RanGTP-sensitive manner (140). Nup98 also synergizes with Rae1 in regulating chromosome segregation by binding to APC/cdh1 and inhibiting ubiquitination of securin. These data indicate a direct contribution of Nup98 in modulation of the spindle checkpoint and are interesting in the light of the finding that Nup98 is often found as a fusion partner in oncogenic proteins in leukemia cells (141).

In summary, by regulating the localization of crucial regulatory factors at centrosomes, MTs and KTs, the Ran network directly regulates the mitotic apparatus and its checkpoints, and thus balanced chromosome transmission to daughter cells.

7. AN 'ONCOGENOMICS' ANALYSIS OF THE RAN SYSTEM

The last few years have witnessed enormous progress in the identification of gene profiling "signatures" that caracterise particular types of cancer. The genes that are found to be implicated in such distinctive cancer signatures do not necessarily carry structural mutations that directly alter their products, but are often expressed at aberrant levels; this can determine unscheduled occurrence, or altered rates, of the processe(s) in which they act, with detrimantal consequences on cell growth. Cancer is clearly a multi-step process, in which dys-regulation of 'master' genes, though not being per se necessarily causative of cell transformation, can cooperate with other factors and/or failure of regulatory systems to favour tumor growth.

No structural mutations or gene loss of Ran network components have been identified in cancer cells, most likely reflecting the fact that loss and mutation of these genes are lethal, as observed in all model systems in which the Ran network has been examined. There is, however, growing circumstantial evidence to suggest that abnormal Ran network activity can accompany or cause cell transformation. Indeed, some direct targets downstream of Ran in mitotic control are distinctively altered in some cancers: HURP, for example, was originally isolated as a hepatoma up-regulated protein (142). Furthermore, evidence from single gene studies (Table 2) indeed indicate that Ran network components can be expressed at abnormal levels in transformed cells; in certain cases, expression of their coding genes is regulated by transcription factors with a proto-oncogenic potential and is deregulated in cells in which the activity of these factors is abnormal.

- The *RanBP1* gene has been studied in some detail from the point of view of transcriptional control, because it is the only Ran network member to be expressed in a cell cycle-regulated manner in actively proliferating cells, while being expressed at very low levels in non-proliferating cells (143). E2F and RB family members play a major role in cell cycle-regulated *RanBP1* gene transcription (144). Interestingly, *Mad2* (145) and several other genes encoding mitotic products (146) are also under E2F/RB control; these findings may account for a possible origin of mitotic abnormalities in transformed cells in which the E2F/RB pathway is disrupted (147).

- The *RCC1* gene promoter is a direct transcriptional target of *c-myc* and drives aberrant levels of *RCC1* gene transcription in c-*myc* overexpressing cells (148-149). Human tumors in which *c-myc* regulation is disrupted (e.g. lymphomas) may, therefore, express abnormally high RCC1 levels. It is interesting to note that concomitant up-regulation of Ran and RanBP1 has been reported in cells expressing deregulated c-myc activity (150)

- Ran itself is also highly expressed in cancer cell lines, as first found in human teratocarcinoma cells in the initial cloning of the human coding gene (1). Interestingly, both *Ran* (151) and *RanBP1* (143) genes are highly expressed in murine erythroleukemia (MEL) cells and both undergo down-regulation during hexamethylene bisacetamide (HMBA)-induced differentiation; actually, Ran undergoes down-regulation during differentiation, except for a transient window (24-48 hours after HMBA exposure), during which expression is high; this temporary up-regulation of Ran levels may be necessary for cell survival and perhaps reflect a need to 'adjust' the levels of other Ran effectors, such as CAS for example, which affects apoptosis. Ran levels are steadily reduced once the differentiation program is established (151).

- The general idea that Ran is highly expressed in cancer has recently received specific support from a study aiming to identify genes

Table 2. Differential expression of Ran network members and effectors in normal and transformed cells and tissues: single genes studies

Cell or tissue type	Expression	Method of analysis	Ref
Breast	**RanBPM** is highly expressed in breast cancer cell lines compared to normal breast epithelial cells	RT-PCR	82
Ovaria	**Ran** high expression correlates with poor prognosis in serous ovarian tumors	Microarray, Tissue array	181
Cervix	**Ran** is overexpressed in invasive cervival cancer and cervical epithelial neoplasia (CIN)	Microarray, RT-PCR	182
Colon	**Ran** is upregulated in colon tumors compared to normal colon tissue samples	Microarray	183
	Ran is upregulated in colon carcinomas and adenomas compared to normal colon epithelia	Microarray	184
	RanBP7 (import factor for ribosomal proteins) mRNA levels were increased in colorectal tumors	Subtractive hybridization, RT-PCR	185
Esophageal	**RanBP1** is highly expressed in esophageal carcinoma compared with normal epithelium	Gene chip	186
	KPNA2 (importin alpha-2) is overexpressed in esophageal cancer compared to normal organ tissue	Microarray, RT-PCR	187
Lung	**RanBP1** is overexpressed in squamous lung cancer compared to normal lung tissue	Microarray	188
	RanBP1 is differentially expressed in small cell lung, compared to adenocarcinoma of the lung and squamous cell lung cancer	Microarray	189
Nasopharyngeal	**Ran** was significantly overexpressed in many nasopharyngeal carcinomas	Microarray, RT-PCR	190
Melanoma	**KPNA2** (importin alpha-2) shows a significant association with distant metastasis-free survival and overall survival in human primary cutaneous melanomas	Microarray, RT-PCR, Immunohistochemistry	191
Lymphoma	**RCC1** is upregulated in mantle-cell lymphoma cells versus normal tonsillar B lymphocytes	Microarray, Western Blot	192
Myeloma	A high expression of **Ran** is associated with rapid relapse in myeloma patients	Microarray	193
	RanBP1 is differentially expressed between different subgroups of myeloma	Microarray	194
Cell lines from various cancers	**Ran** is overexpressed in cells lines from: Gastric adenocarcinoma, Lung adenocarcinomas, Lung squamous cell carcinoma, head and neck carcinoma, colon adenocarcinoma, pancreatic adenocarcinoma and human chronic myelogenous leukemia, however, it is not induced in normal cells and tissues	Northern Blot	152
Rat fibroblast cell line	**Ran** and **RanBP1** are upregulated by c-Myc in rat fibroblast cells	Microarray	150
HaCaT cell line (keratinocyte cell line)	**RanBP1** is downregulated during differentiation in HaCaT cells	Microarray	195
NTera2 cells (human teratocarcinoma)	**Ran** is overexpressed in the human teratocarcinoma cell line NTera2 and is downregulated during retinoic acid-induced differentiation	Northern Blot	1
Murine erythroleukemia cells (MEL)	**Ran** is downregulated during HMBA-induced differentiation, after a short window of upregulation necessary for cell survival	Northern Blot	151
Various cell lines and tissues	**RanBP1** is downregulated in serum-starved NIH/3T3 fibroblasts and during HMBA-induced differentiation of MEL cells (Friend cell line); it is upregulated in NIH/3T3 fibroblasts induced to proliferate and during liver regeneration	Northern Blot	143

encoding tumor-associated antigens (152). The *Ran* gene was found to encode epitopes recognized by specific HLA-restricted cytotoxic T lymphocytes (CTLs) established from T cells infiltrating into gastric adenocarcinoma. The authors noted that *Ran* expression was high in most cancer-derived cells and tissues, at both the mRNA and protein levels, but not in the surrounding normal cells or tissues. They also generated Ran-derived synthetic peptides and identified two that were capable of inducing tumor-reactive CTLs in peripheral blood from epithelial cancer patients. Because of its increased expression in cancer cells, and involvement in malignant transformation and in increased proliferation of cancer cells, these two Ran-directed peptides have been proposed as candidates for use in specific immunotherapy against some types of epithelial cancers.

It is also of note that a recently developed anticancer compound that targets telomerase in cancer cells caused the concerted down-regulation of both *RanBP1* and *RCC1* genes (153), further strengthening the notion that Ran network components are highly expressed in cells in which the cell cycle machinery is highly active, as in cancer cells, and need be down-regulated when control of proliferation is restored.

Oncogenomics reveals the importance of gene profiling studies, both for understanding the generation of the transformed phenotype (the search for "cancer signatures") and for determining the sensitivity of resistance to specific chemotherapeutic treatments, which can help to rationalize the choice of therapies. To gain direct evidence that transformed and tumor cells express deregulated levels of Ran network members, we have examined, in retrospect, a number of microarrays studies from different cancer types. Oncomine (www.oncomine.org) is a compendium that consists of more than 22.0.00 cancer transcriptome profiles, organized so that information about differential gene expression between normal and cancer cells can be easily retrieved.

Table 3. Differential expression ($p<0.001$) of Ran network members (Ran, RanBP1, RCC1) or effectors (importin beta, CRM1) in microarrays profiling of transformed cells/tissues versus their normal counterpart (source: www.oncomine.com

Tumor type	Tissue type	Overexpressed		Underexpressed	
		Gene	Ref	Gene	Ref
Breast	Breast Carcinoma	Ran, RanBP1, RCC1, CRM1, Importin beta	196, 197		
Ovary	Ovarian Clear Cell Adenocarcinoma	RCC1, Importin beta	198	CRM1, Importin beta	198
	Ovarian Endometrioid Adenocarcinoma	Importin beta	198		
	Ovarian Serous Adenocarcinoma			Importin beta	198
	Ovarian Mucinous Adenocarcinoma			CRM1	198
Prostate	Prostate Carcinoma	Ran, RCC1	199-202	Importin beta	203
Testis	Embryonal Carcinoma	CRM1	204		
	Seminoma	RanBP1	205		
	Adult Male Germ Cell Tumor	RCC1	206	RCC1, Importin beta	206
Brain	Oligodendroglioma	RanBP1, RCC1, CRM1, Importin beta	207	Ran	207
	Astrocytoma	RCC1, CRM1, Importin beta	207	Ran	207
	Glioblastoma multiforme	RanBP1, RCC1, CRM1, Importin beta	207	Ran, CRM1	207
	Glioblastoma			Ran, Importin beta	208
Lung	Small Cell Lung Cancer	RanBP1, CRM1, Importin beta	209		
	Squamous Cell Lung Cancer	Ran, RanBP1, RCC1, CRM1	209-211		
	Lung Adenocarcinoma	Ran, RanBP1, RCC1, CRM1, Importin beta	209 211-213		
	Carcinoid	RanBP1, CRM1, Importin beta	209		
Bladder	Superficial Transitional Cell Carcinoma and Invasive Transitional Cell Carcinoma	Ran, RanBP1, RCC1, Importin beta, CRM1	214	RanBP1	214
	Bladder Carcinoma	Ran, RanBP1, RCC1, Importin beta	215	Ran	215
Kidney	Chromophobe Renal Cell Carcinoma	Importin beta	216		
	Renal Clear Cell Carcinoma	Ran, Importin beta	216, 217		
Colon	Colorectal Carcinoma	Ran, RanBP1	218		
	Colon Adenocarcinoma	Ran, RanBP1, Importin beta	219, 220		
Liver	Hepatocellular Carcinoma	CRM1, Importin beta	221		
Pancreas	Pancreatic Ductal Adenocarcinoma			Importin beta	222
Head and Neck	Head and Neck Squamous Cell Carcinoma	RanBP1, CRM1, Importin beta	223		
	Oral Squamous Cell Carcinoma	CRM1	224		
	Tongue Squamous Cell Carcinoma	Ran, RanBP1, RCC1, CRM1	225		
Skin	Melanoma	RanBP1, RCC1, Importin beta	226		
Leukemia	Acute Myeloid Leukemia	Ran, RanBP1, RCC1	227		
	T-Cell Acute Lymphoblastic Leukemia	Ran, RanBP1, RCC1, CRM1, Importin beta	227		
	B-Cell Lymphoblastic Leukemia	Ran, RanBP1, RCC1, CRM1, Importin beta	227		
	Chronic Lymphocytic Leukemia	CRM1	228	Ran, RCC1	228, 229
	Follicular Lymphoma			RCC1	229
Myeloma	Smoldering Multiple Myeloma	Ran, RCC1, Importin beta	230		

Oncomine searching indicated that virtually all tumor types differentially express Ran, RanBP1, RCC1, CRM1 and importin beta compared to their normal tissue counterpart ($P<0.0.01$) (Table 3). Furthermore, it indicates that some Ran members are aberrantly expressed, in a collective manner, in some cancer types, suggesting constitutively altered activity of the entire Ran network. This circumstantial evidence strengthens the notion that regulated Ran signalling is essential for regulated growth and genetic stability of non transformed cells.

8. PERSPECTIVES

In this review we have summarized evidence that indicates that deregulated activity of Ran network components, or their effectors, can affect several regulatory pathways that can in turn contribute to cell transformation through several pathways (Figure 3). It is important to note that Ran can also influence the response of cancer cells to therapy. Chemotherapeutic regimen to treat cancer always include cocktails of DNA-damaging and MT-targeting drugs that are chosen on a largely empirical basis. The activity of Ran network members influences the localization of several factors acting in repair of DNA damage or in apoptosis induction; we have examined the case of p53 to exemplify the importance of Ran control of the localization/function relationship of this fundamental tumor suppressor. Ran network members also act directly in the mitotic apparatus and, therefore, play

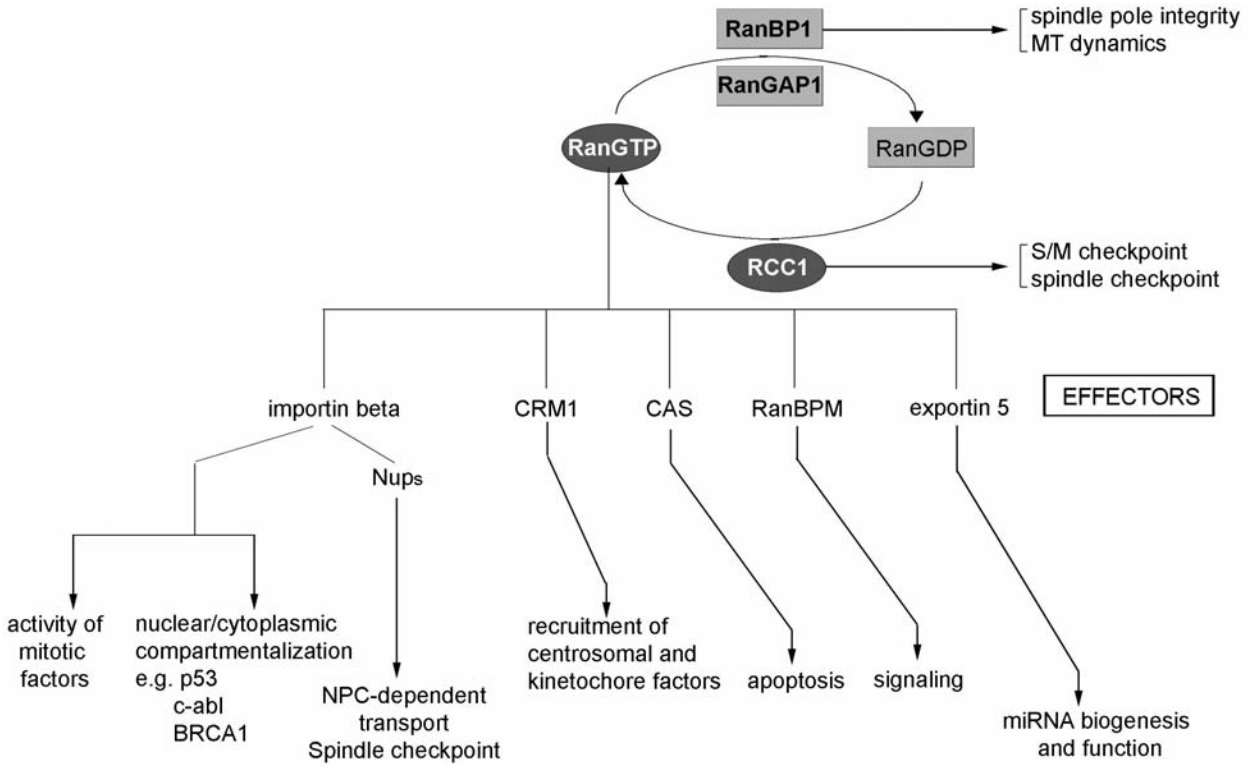

Figure 3. An overview of cellular processes regulated by the Ran GTPase**.** The biochemical mechanism regulating RanGTP/RanGDP turnover is schematically represented in the upper panel. By acting through various effectors, Ran regulates diverse cellular pathways (arrowed) that can impact on cell transformation and tumor progression. MTs, microtubules; NPC, nuclear pore complexes; Nups, nucleoporins.

direct roles in control of chromosome segregation. Early evidence for a cross-talk between Ran network members and MT-targeting drugs was obtained from studies of yeast genetics (154). In mammalian cells, Ran network members influence the response to drugs that target the mitotic apparatus (112, 115-116, 125-126), in some cases with a direct contribution to apoptosis induction (112, 116). It will be important to develop these studies, not only to unravel the multiple mechanisms through which cells can become transformed, but also to help design rational, patient-directed therapies (155-156): in the future, it may become possible to adjust and optimize treatment options for particular cancers according to the level of specific Ran regulators that they express, thus avoiding unnecessary toxicity and increasing the efficacy of the therapeutic outcome.

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Abbreviations: KT: kinetochore; LMB: leptomycin B; MAP: microtubule-associated protein; miRNA: microRNA; MPF: mitosis-promoting factor; MT: microtubule; NE: nuclear envelope; NES: nuclear export signal; NLS: nuclear localization signal; NOC: nocodazole; NPC: nuclear pore complex; SAF: spindle assembly factor.

Key Words: Ran GTPase, RCC1, RanBP1, RanBPM, Transport Factors, Nucleo-Cytoplasmic Transport, Mitotic Apparatus, Aneuploidy, Cancer, Review

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