J. Biochem. **120**, 207-214 (1996)

RCC1 in the Ran Pathway

Takashi Seki, Naoyuki Hayashi, and Takeharu Nishimoto[1]

Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Maidashi, Higashi-ku, Fukuoka 812

Received for publication, May 2, 1996

RCC1 is a chromosomal protein that functions as a GEF of the nuclear G protein Ran, which GTPase activity is enhanced by RNA1 located in the cytoplasm. RCC1 has no preference for GTP or GDP-bound Ran, so that GTP-Ran formation *in vivo* is regulated by relative concentrations of GTP/GDP and regulatory proteins interacting with RCC1, Ran, and RNA1. Proteins possessing the special Ran-binding motif have been found to be conserved in species ranging from yeasts to mammalians. The finding of RanBP2/NUP358 clearly indicates the involvement of the Ran pathway in the nuclear pore transport function, in agreement with the finding that both *rcc1*$^-$ and *rna1*$^-$ show defects in this process. However, loss of RCC1 induces premature initiation of mitosis, resulting in G1 arrest with the micronuclei possessing mitotic condensed chromosomes. How both the cell cycle and nucleocytoplasmic transport are regulated by the RCC1-Ran pathway is a major question.

Key words: Ran, RanBP1, RanBP2, RCC1, RNA1.

Chromatin is decondensed in the G1 phase and begins to be condensed from the S phase onwards. Such a dynamic structure of chromatin is regulated by cdk/cyclin complexes and their regulators. Condensed chromatin is neither replicated nor transcribed. Therefore, how the structure of chromatin is coupled with cell cycle events is a major issue in cell cycle regulation. Since loss of RCC1 (*R*egulator of *C*hromatin *C*ondensation) induces either G1 arrest or premature initiation of mitosis (*1*), RCC1 is thought to be involved in cell cycle regulation. However, yeast *rcc1*$^-$ has been found to be defective in diverse functions (*2-4*).

RCC1 is a guanine nucleotide-exchanging factor (GEF) acting on the nuclear Ras-like G protein Ran (*5*). Ras functions as a molecular switch cycling between GTP-bound "on" and GDP-bound "off" states. By analogy, Ran may function as a biological switch, and RCC1 may carry out its biological functions through Ran, which is essential for nuclear pore transport function (*6*). Thus, the question arises whether diverse phenotypes of *rcc1*$^-$ are a consequence of a defect in the nuclear pore transport function.

Human RCC1 and its transcripts

Two human *RCC1* cDNAs have been isolated that differ in the 5′ untranslated regions transcribed from different promoters but share an open reading frame (ORF) of 1,425 bp that encodes a protein of 44,847 kDa possessing seven internal repeats of 60 amino acid residues (Fig. 1) (*7*). Another *RCC1* cDNA (*RCC1-1*) has been amplified by PCR which has an extra 93 bp of nucleotide inserted into the valine[25] codon of RCC1 outside the RCC1 repeat (*8*), indicating that it is an alternative splicing product of primary *RCC1* transcript. Both *RCC1* and *RCC1-1* cDNAs complement the tsBN2 mutation with similar efficiency. The same alternative splicing product as *RCC1-1* mRNA is also found in hamster BHK21 cells and *Xenopus*, indicating that the alternative splicing of *RCC1* mRNA has some biological meaning yet unknown. Thus, human cells express four types of RCC1 mRNAs transcribed from two promoters and spliced alternatively. The gene encoding RCC1 has been mapped on human chromosome 1 (*9*).

RCC1 homologues

RCC1 homologues are identified in *Drosophila*, and both budding and fission yeasts, in addition to hamster and *Xenopus*.

***Drosophila* RCC1 homologue BJ1:** Twenty-nine of 41 independent hybridoma clones producing antibodies against proteins extracted from *Drosophila* embryonic nuclei, recognized an antigen designated as BJ1 (*10*). Its cDNA of 2.2 kb, corresponding to the size of its mRNA, encodes a polypeptide of 59 kDa, but its electrophoretic mobility indicates a molecular mass of 68 kDa.

BJ1 possesses two internal amino acid repeats: an RCC1 repeat from amino acid 46 to 417 (45% identical to human RCC1 repeat), and a BJ1 repeat from amino acid 418 to 520. The BJ1 repeat consists of three repeats of about 35 amino acid residues that are highly homologous to each other, in contrast to the case of RCC1 repeat. The C-terminus of BJ1 possesses a cluster of glutamic acids followed by a lysine-rich sequence (EK-stretch), and to some extent it is similar to nucleoplasmin of *Xenopus* and histone H1.

Budding yeast RCC1 homologue (SRM1, PRP20, and MTR1): Budding yeast RCC1 homologue has been cloned as genes complementing mutants isolated from diverse view points of cellular functions, namely, mating pathway (*srm1*) (*2*), mRNA splicing (*prp20*) (*3*), and mRNA export (*mtr1*) (*4*).

The *srm1-1* (*s*uppressor of *r*eceptor *m*utation) allele is a suppressor restoring mating capacity to the receptorless mutants *Δste2* and *Δste3* (*2*), but it does not suppress the deletion mutants *Δste4* and *Δste5*. At the restrictive temperature, *FUS1* is constitutively expressed and the

[1] To whom correspondence should be addressed.

```
Human          39 TLGQGDV-GQLGLGE-NVM-ERK-KP----ALVSIPEDVV-----------------QAEAGGMHTVCLSKSGQVY--
Hamster        39 TLGQGDV-GQLGLGE-SVL-ERK-KP----ALVPLLODVV-----------------QAEAGGMHTVCLNQSGQVY--
Xenopus        44 TLGQGDV-GQLGLGE-DIM-ERK-KP----ALVTLTEDIV-----------------QAAAGGMHTVCLGASGSIY--
Drosophila     46 VCGNGDV-GQLGLGE-DIL-ERK-RL----SPVAGIPDAM-----------------DISAGGMHNLVLTKSDGIY--
S.cerevisiae   46 CWGTGSM-CELGLGP-LAK-EVK-RPRLNPFIPRDEAKII-----------------SFAVGGMHTLALDEESNVW--
S.pombe        65 VFGSGSM-NELGMGE-EEM-DVVYRPRLNPILSTDKVGVV-----------------DLAVGGMHSAALLHDGRVY--

                  SFGCNDE-GALGRDT-SVEGSEM-VP----GKVELQEKVV-----------------QVSAGDSHTAALTDDGRVFLW
                  SFGCNDE-GALGRDT-SVEGSEM-VP----VQVQLDMPVV-----------------QVSAGDSHTAALTEDGRVFLW
                  TFGCNDE-GALGRDT-SEEGSEM-QP----GKVELAEKVV-----------------QVSAGDSHTAALTEDGRVFVF
                  SFGCNDE-GALGRDT-SEDGSES-KP----DLIDLPGKAL-----------------CISAGDSHSACLLEDGRVFAW
                  SWGCNDV-GALGRDT-SNAKEQL-KD----MDAD*GHKVV-----------------QLAATDNMSCALFSNGEVYAW
                  TWGVNDD-YALGRLT-KDQKDEN-GD----KVDND*-RVT-----------------KVICSDNLTAAITDNGCCFTW

                  G-SFRDNNGVIGLLE-P-MKKSM-VP----VQVQLDVPVV-----------------KVASGNDHLVMLTADGDLY--
                  G-SFRDNNGVIGLLE-P-MKKSM-VP----VQVQLDMPVV-----------------KVASGNDHLVMLTTDGDLY--
                  G-SFRDNNGVIGLLE-P-MKKSM-VP----VQVQINTPVI-----------------KIASGNDHLVLLTVDGDLY--
                  G-SFRDSHGNMGLTI-DGNKRT---P----IDLMEGTVCC-----------------SIASGADHLVILTTAGKVF--
                  G-TFRCNEGILGFYQ-DKIKIQK-TPWK--VPTFSKYNIV-----------------QLAPGKDHILFLDEEGMVF--
                  G-TFRCSDGVLGYSDSQKRTAEPTQM-R--LP-----EIC-----------------QLATGTDHIIALTTTGKVY--

                  TLGCG-EQGQLGRVP-ELFANRG-------GRQGLERLLVPKCVMLKSRGSRGHVRFQDAFCGAYFTFAISHEGHVY--
                  TLGCG-EQGQLGRVP-ERFINRG-------GRQGLERLLVPKCVLLKSRGSRGRVRFQDAFCGAYLTFAISREGHVY--
                  TSGCG-EQGQLGRVP-ERFINRG-------GRKGLERLLVPQCIHLKAKGS-GRVHFQDVFCGAYFTFAVSQEGHYY--
                  TVGCA-EQGQLGRLS-ERSISGE-------GRRGKRDLLRPTQLIITRAK----PFEAIWATNYCTFMFESQTQVIW-
                  AWGNG-QQNQLGR-------------------KVMERFRL-KTLDPRPFGLR-HVKYIAS--GENHCFAITKDNKLV--
                  TWGNG-QQFQLGR-------------------RMLERRRL-QGLTPQPLALK-NIISVGA--GSYHSFAIDNKGRVY--

                  GFGLS-NYHQLGT-F-GTE-SCFIPQNLT-SFKNSTKSWV-----------------GFSGGQHHTVCMDSEGKAY--
                  GFGLS-NYHQLGT-P-GTA-SCFIPQNLT-SFKNSTKSWV-----------------GFSGGQHHTICMDSEGKAY--
                  GFGLS-NYHQLGT-K-NTQ-ACYAPQNLT-SFKNSTKSWI-----------------GFSGGQHHTVCVDSEGKAY--
                  ATGLN-NFKQLAH-E-TKG-KEF---AL-TPIKTELKDIR-----------------HIAGGQHHTVILTTDLKCS--
                  SWGLN-QFGQCGV-S-EDVEDGALVTKPKRLALPDNVVIR-----------------SIAAGEHHSLILSQDGDIY--
                  AWGLN-ITRQCGI-EVEDEEEGAVITKPTLVDALEGYNVK-----------------SITGGEHHTLALLEDGRVL--

                  SRGRA-EYGRLGL-G-EGAEEKS-IP----TLI-SRLPAVS-----------------SVACGASVGYAVTKDGRVF--
                  SLGRA-EYGRLGL-G-EGAEEKS-IP----TLI-SRLPVVS-----------------SVACGASVGYAVSKDGRVF--
                  SLGRA-EYGRLGL-G-ENAEEQS-EP----TPI-PDLPKIN-----------------SVASGASVSYAVSTDGCVF--
                  VVGRP-EYGRLGL-G-DVKDVVE-KP----TIV-KKITEKIV----------------SVGCGEVCSYAVTIDGKLY--
                  SCGRL-DMFEVGI-P*KARAVP--LP----TKL-NNVPKFK-----------------SVAAGSHHSVAVAQNGIAY--
                  AWGRD-DRHQLGI-P*NNYYLS--TP----TII-PGLTNVI-----------------QVVCGTHHNLAVTSDGKVY--

                  AWGMGTNY-QLGT-GQDEDAWS--PVEMM-GKQ-LENRVVL-----------------SVSSGGQHTVLLVKDKEQS 421
                  AWGMGTNY-QLGT-GQDEDAWS--PVEMT-GKQ-LENRVVL-----------------TVSSGGQHTVLLVKDKEQS 421
                  AWGMGTNL-QLGT-GEEEDVWS--PEQMT-GKH-LEDREVL-----------------SVSSGGQHTVLLVRKR--S 424
                  SWGSGVNN-QLGV-GDGDDELE--PIVVV-SKN-TQDKHML-----------------LASGGGQHAIFLVKADKCD- 421
                  SWGFGETY-AVGL-G*EDDTEV--PTRIK-NTA-TQDHNII-----------------IVGCGGQFSV*GVKLSDED- 472
                  SWGSAENY-EVGQ-G*GEDVAV--PTLVR-SKA-IKEVAIR-----------------VAGAGGQFSI*GIPNASEE- 477

                  * insertion of several a.a.
```

Fig. 1. **Alignments of RCC1 repeat.**

haploid *srm1-1* arrests in G1, while no cell cycle-specific arrest occurs in the homozygous *srm1-1* a/α diploid. In contrast to other *rcc1*$^-$, there is no mRNA accumulation in *srm1-1* (*4*).

The *prp20* (*p*re-*R*NA *p*rocessing) allele was isolated using an accumulation of pre-mRNA as a selection-marker and shows alterations in mRNA steady-state levels, defective mRNA splicing and changes in transcription initiation or termination (*3*).

Three different recessive mutants alleles of the *MTR1* (mRNA transport) gene, *mtr1-1, 1-2* and *1-3* have been isolated (*4*) by a method similar to that used for *prp20*. At the restrictive temperature, in these mutants, mRNA accumulates even in the absence of p34^{cdc28} kinase activation and protein synthesis, both of which are essential for PCC (premature chromatin condensation) induction of tsBN2 cells (*1*), and the maturation of rRNA and tRNA is inhibited, but nuclear import of NLS (E1a) fused BSA continues at least for 6 h.

RCC1 homologue in budding yeast is essential for viability. Its mRNA is about 1.6 kb and encodes a protein of 52 kDa with seven internal RCC1 repeats that is 30% identical to human RCC1.

Fission yeast RCC1 homologue pim1-D1: To eluci-

date the mechanism of coupling between the completion of DNA replication and the initiation of mitosis, *pim1* (*p*remature *i*nitiation of *m*itosis) has been isolated from fission yeast, which enters mitosis in the presence of hydroxyurea (HU), an inhibitor of DNA replication, and which turns out to be defect in a human RCC1 homologue (*11*). The same gene has been isolated as a gene required for decondensation of mitotic chromosomes and designated *dcd* (*d*efect in *c*hromatin *d*econdensation) (*12*). Thus, *pim1* is renamed as *pim1-d1*^{ts}. The *pim1*$^{+}$ encodes a polypeptide of 58 kDa with seven internal RCC1 repeats. Pim1 is 30% identical to both human RCC1 and budding yeast SRM1 (Fig. 1).

In accordance with the conservation of the RCC1 repeat, budding yeast *rcc1*$^{-}$ *prp20-1* and *srm1-1* can be complemented by human RCC1 and *vice versa*.

RCC1 is located on the chromatin as a protein-protein complex

RCC1 is released from isolated HeLa nuclei by treatment with 0.3 M NaCl or digestion with 100 μg/ml of DNase I (*13*), or by treatment with 25 mM chloroquine, a DNA intercalation drug (*14*). Almost all RCC1 is released from hamster nucleus after sequential treatment with DNase I (100 μg/ml) and 2 M NaCl, indicating that it is not located on the nuclear matrix.

To elute BJ1 from *Drosophila* Kc cell nuclei by treatment with 100 mM NaCl, it is essential to employ ethidium bromide (7.5 mM) (*10*), indicating that BJ1 binds to DNA, either directly or indirectly. On sucrose gradient analysis of chromosomes, BJ1 is cosedimented with nucleosomes, from which it is released as a complex of 7S at 450 mM NaCl and of 15S at 250 mM NaCl. Therefore, BJ1 is bound to chromatin as a protein-protein complex that is released at increased ionic strength and falls apart at an even higher salt concentration.

SRM1 extracted from asynchronously growing budding yeast can be retained on double-stranded (ds) DNA-cellulose, but *Escherichia coli*-produced SRM1 can not (*15*). In *in vitro* reconstitution assays, SRM1 binds to dsDNA-cellulose through a multiprotein complex composed of six to seven proteins, with a collective molecular mass greater than 150 kDa, of which three are suggested to bind GTP. Interestingly, such a SRM1-complex losses its DNA-binding ability during DNA replication, indicating that the SRM1-complex is somehow modified in the cell cycle.

In contrast to SRM1, *E. coli*-produced human RCC1 efficiently binds to ^{32}P-labeled pUC18 DNA digested with *Eco*RI (*16*). Such DNA-binding ability is, however, not essential for the function of RCC1, since human *RCC1* mutants without DNA-binding abilities complement tsBN2 mutation with an efficiency similar to wild-type *RCC1* (*16*). In *ts*$^{+}$ transformants of tsBN2 cells complemented by Δ8-29 *RCC1*, in which DNA binding domain is deleted, most Δ8-29 RCC1 protein is localized in the cytoplasm, indicating that the DNA-binding domain functions as a nuclear location signal. Indeed, the determined DNA-binding domain of human RCC1, which is localized in the N-terminus outside the RCC1 repeat, contains a sequence similar to a bipartite nuclear location signal of nucleoplasmin. Interestingly, in these transformants, a significant amount of Δ8-29 RCC1 protein is cosedimented with chromosomal DNA by sucrose gradient analysis. Since Δ8-29 RCC1 protein itself has no DNA-binding activity, this finding indicates that RCC1 is tethered to chromosomal DNA by some chromosomal proteins.

In mitosis, RCC1 is dispersed into the cytoplasm, but some RCC1 still binds to mitotic chromosomes, because RCC1 is purified from HeLa cells using antikinetochore autoimmune sera that specifically stain mitotic chromosomes (*14*). A small protein tightly bound to RCC1 has been purified along with RCC1 from HeLa cells. Its amino acid residues is similar but not identical to the reported amino acid sequence of TC4, a member of the Ras family (*17*), and it has been designated Ran (*Ras*-like *n*uclear G protein) (*5*). The RCC1·Ran complex is stable in 3 M guanidine hydrochloride in the absence of nucleotide and Mg^{2+} (*14*).

Ran GTPase

In consistent with the finding that RCC1 has a GEF activity acting on Ran, *Spi1* (suppressor of *pim1*$^{-}$) that is the fission yeast Ran homologue has been isolated as a multicopy suppressor of *pim1*$^{-}$ (*11*). Similarly two Ran homologues, *GSP1* and *GSP2* (GTP-binding suppressor of *PRP20*), have been isolated from budding yeast as a multicopy suppressor of *prp20* (*18*). Independently, budding yeast Ran homologues *CNR1* and *CNR2* (*C*onserved *N*uclear *R*as homologue) have been isolated by PCR amplification using degenerate primers (*4*). *GSP1/CNR1* and *GSP2/CNR2* encode proteins of 219 and 220 amino acids (97% identical amino acid residues), respectively and are functionally exchangeable. Both are over 88% identical at the amino acid level to two other GTPases, Ran and Spi1. Levels of *GSP1/CNR1* transcript are about 10-fold those of *GSP2/CNR2*. *GSP1/CNR1* is essential for survival, while GSP2/CNR2 whose expression is carbon-source dependent, is not (*18*).

Human Ran is a 25-kDa protein abundant in the nucleus. The soluble monomeric form of Ran accounts for about 0.36% of total cellular proteins of HeLa cells, or 10^{7} copies per cell, which is 25-fold molar excess over the RCC1·Ran complex form (*5*). The Ran·GDP·Mg^{2+} complex has been crystallized and compared with Ras, revealing that while the G-domain is conserved, the region involved in GDP and Mg^{2+} coordination is varied and that the N terminus (MAAQGEP) and the acidic tail (DEDDDL) are flexible in the crystal structure (*19*). It is suggested that a big conformational change occurs upon GTP binding. Analogously to Ras, T24N mutant results in a dominant negative inhibitor tightly binding to RCC1 (*20*), and Q69L mutant blocks Ran guanine nucleotide activating protein (RanGAP) (*21*).

Unlike other Ras family proteins, Ran lacks the C-terminal lipid modification motif but possesses an unusually acidic tail (-DEDDDL) (*17*), which stabilizes GDP binding to Ran and is required for stable interaction with RanBP1 (*22*).

RanGAP

Human RanGAP, which converts GTP-Ran to GDP-Ran, has been purified from HeLa cells as a homodimer (150 kDa) of a 65-kDa protein. Its cDNA is 2.9 kb and turns out to be a human homologue of budding yeast RNA1 (*23*), consistent with the finding that a ts mutant, *rna1-1*, exhibits defects in mRNA splicing and nuclear export similar to *prp20-1* (*24*).

Murine *RanGAP* has been cloned as a gene required for

postimplantation development, designated *fug1* (*F*ailure to *u*ndergo *g*astrulation), which was mapped on chromosome 15 (*25*). *fug1* encodes a protein of 589 amino acids, of which the first 400 are 88% identical to human RanGAP and 38% identical to budding yeast RNA1. A long acidic domain and 11 leucine-rich motifs, thought to mediate high affinity protein-protein interactions, are well conserved. During screening of Ca^{2+}-binding proteins, fission yeast *rna1*$^+$ has been isolated (*26*). It shares 42% identical amino acids with budding yeast RNA1 and complements the *rna1-1*, indicating that fission yeast *rna1*$^+$ is a functional homologue of budding yeast RNA1. Like budding yeast RNA1, it is essential for growth.

RNA1 is located in the cytoplasm, possibly enriched in the nuclear periphery of fission yeast (*26*). In HeLa cells, however, a significant amount of RanGAP activity is associated with the nuclear fraction, which can be extracted by increasing the NaCl concentration to 400 mM, but neither by 25 mM chloroquine nor by digestion of DNA with DNase I at 20 μg/ml (*23*).

Interaction of Ran with RCC1 and RNA1

Since Ran lacks the lipid modification motif, the reaction between Ran and its related proteins is considered to proceed as if it were an enzyme reaction in homogenous solution. Thus, the interaction of Ran with its related proteins can be analyzed using the Mechaelis-Menten equation (*27*), where RCC1, for example, is the enzyme, and Ran·nucleotide is the substrate, as shown in Fig. 2.

Ran has low intrinsic GTPase and GDP dissociation rate constants that are of similar magnitude, 1.5×10^{-5} and 1.8×10^{-5} s^{-1}, respectively. Both reactions are stimulated 10^{-5}-fold by RNA1 and RCC1. In contrast to $p21^{ras}$, RCC1 has almost no preference for the nucleotide state of Ran. The association of GTP with Ran·RCC1 complex is much slower than that of GDP, and Ran has higher affinity for GDP than for GTP. Based on the intracellular concentration of GTP (327 μM), GDP (91 μM), and Ran (6 μM which is 25-fold molar excess over RCC1), and on the kinetics of RCC1-stimulated nucleotide exchange, Klebe *et al.* (*27*) calculated that 38% GTP-bound Ran can be formed, and they suggested that this relative amount of GTP-Ran is formed regardless of the absolute Ran concentration. Therefore, in addition to the regulation of Ran by proteins such as RCC1 and Ran-binding proteins, the intracellular concentration of GDP and GTP influences the biological activity mediated by the Ran pathway.

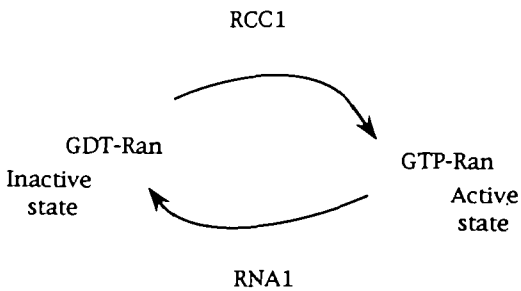
Ran binding proteins

So far, several proteins possessing the Ran-binding motif (RanBD) which specifically recognize GTP-Ran have been identified. In addition, there are Ran-binding proteins possessing no clear Ran-binding motif, like RCC1 and RNA1. Together, these proteins may function for tuning the Ran pathway.

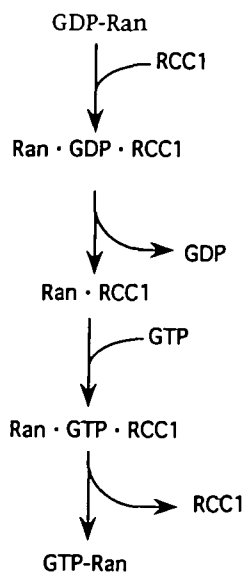
RanBP1: mouse RanBP1 has been identified as a protein of 27 kDa bound to GTP-, but not GDP-Ran, using an overlay assay (*21*). Human RanBP1 preferentially inhibits GTP release from Ran, favoring an increment of GTP-Ran (*28*), but it also coactivates RanGAP RNA1, since overexpression of RanBP1 suppresses the *rna1-1* (Noguchi, unpublished data).

Budding yeast RanBP1 homologue, YRB1 has been identified by screening a gene whose overexpression affects chromosome stability (*29*) and it has been purified based on its affinity to GTP-GSP1 using GST-fused G21V (GSP1 mutant blocked in GTP form) (*30*). *YRB1* has also been identified in a search for mutations suppressing the mating deficiency of *fus1* mutants, which are defective in cell fusion (Trueheart and Thorner, in preparation). YRB1 is localized in the cytoplasm and is suggested to exist at the nuclear periphery, since the isolated nuclei, but not the cytosol, of a ts mutant *yrb1* which is defective in both nuclear protein import and RNA export, has defects *in vitro* nuclear protein import assay (*30*). Together, these reports indicate that YRB1, which is essential for viability, is involved in both cell cycle regulation and nuclear pore transport function.

RanBP2/nup358: By a two-hybrid method using human Ran as a bait or by screening a HeLa cell expression library with GTP-Ran overlay method, two groups independently isolated a series of overlapping cDNA clones encoding a protein designated either RanBP2 or NUP358



A: basic RanGTPase cycle



B: RCC1-catalyzed guanine nucleotide exchange reaction

Fig. 2. **Reaction of RCC1, Ran, and RNA1.** The model of RCC1-catalyzed guanine nucleotide exchange reaction is taken from Klebe *et al.* (*27*).

(*31, 32*). Henceforth, we shall refer to it as RanBP2.

RanBP2 is a protein of 358 kDa encoded by about 10 kb of mRNA. As Fig. 3 shows, it contains the N-terminal leucine-rich region of 700 amino acid residues, four Ran-binding domains (RanBDs) that are highly homologous to RanBP1 and flanked by nucleoporin-characteristic XFXFG, eight zinc finger motifs that are the same as those of NUP153 (*33*), and the C-terminal domain, which is 67% identical to human cyclophilin A. Consistent with the presence of the nucleoporin-specific FXFG, RanBP2 is localized on the nuclear pore, particularly on the top of cytoplasmic nuclear filaments (*31, 34*). Therefore, the zinc finger domains of RanBP2, which bind DNA in a zinc-dependent manner as reported for NUP153 (*33*), should bind factors other than DNA.

Is RCC1 bifunctional?

RCC1 is located on the chromatin, and loss of RCC1 function disperses Ran into the cytoplasm, indicating that RCC1 functions as a center of the Ran pathway. On the chromatin, RCC1 may sense a conformational change of chromatin which occurs during the progression of the cell cycle, and inform it to the cytoplasm through Ran. This is an intriguing idea from the point of view of cell cycle regulation and suggests the presence of two functional domains in RCC1: one required for taking upstream signals and one for transferring them to the downstream events.

The mutual complementation of *rcc1*$^-$ between yeast and mammalian homologues indicates that the putative RCC1 repeat which is conserved through evolution is responsible for RCC1 function. Indeed, the *rcc1*$^-$ isolated so far are mapped in the RCC1 repeat region (*4*). Interestingly, not all of those *rcc1*$^-$ mutants are suppressed by overexpression of Ran or by a newly isolated suppressor. The *rcc1*$^-$ mutants *mtr1-1, prp20-1*, and *mtr1-3*, which are mapped near the C-terminus, can be suppressed by overexpression of GSP1, but those mapped in the N-terminus, *srm1-1* and *mtr1-2*, can not (*4*). These results together suggest that the RCC1 repeated domain possesses two functional domains.

To identify a functional domain of RCC1 for GEF activity on Ran, a series of mutants in which charged amino acid residues of the human RCC1 repeat are changed to alanine was prepared and their GEF activities on Ran were analyzed using the Mechaelis-Menten equation. Alanine mutants located on the N-terminus of the RCC1-repeat have an increased K_m, but a normal k_{cat} (19 s^{-1}) for the GEF reaction on GDP-Ran. On the contrary, alanine mutants of the C-terminal histidine residues have a normal K_m (2.2×10^{-6} M) but a reduced k_{cat} for the GEF reaction on GDP-Ran (*35*). These kinetics data indicate that the repeated domain has at least two domains that are functionally different from each other.

Nuclear pore transport function of the Ran pathway

Two cytosolic fractions (A and B) from *Xenopus* oocytes have been identified as being required for protein import into the nucleus of digitonin-permeabilized cells. Ran was identified as one of the two components required for full fraction-B activity (*6*).

Proteins imported to the nucleus have nuclear localization sequences (NLSs) that are recognized by NLS receptor/Impotin p60/karyopherin α, and the complex formation of NLS-protein and karyopherin α is further enhanced and tightened by binding with p97/Imortin p90/karyopherin β. Fraction A consists of karyopherin α and β. The triple complex of NLS-proteins, karyopherin α and β is transferred onto NPC in an ATP-independ manner. In digitonin-permeabilized cells, GTPγS accumulates on the cytoplasmic periphery of the nucleus, where RanBP2 is the sole Ran-binding protein, so that the NLS-complex is suggested to first bind to RanBP2 on the top of the cytoplasmic filament of NPC (*34*). With the aid of GTP-Ran and p10, both of which comprise fraction B, NLS-fused proteins are then translocated into the nucleoplasm, depending on the binding of ATP and the hydrolysis of GTP bound to Ran. RanGAP is, therefore, another factor required for nuclear import of proteins. In fact, cytosols extracted from the *rna1-1* are defective in nuclear protein import and, by the addition of wild-type RNA1 proteins into this extract, protein import activity is restored in a dose-dependent manner (*24*).

The finding that the nuclei, but not the cytosol prepared from a ts mutant *yrb1* that was pre-incubated at the nonpermissive temperature, are defective in nuclear protein import in the presence of wild-type cytosol suggests that YRB1 functions for protein import on the budding yeast nuclear membrane (*30*).

Similar to nuclear protein import, GTP hydrolysis on Ran is needed for poly(A)$^+$ RNAs export, since a Ran mutant locked in the GTP form inhibited both nuclear protein import and mRNA export in budding yeast (*36*), and *rna1-1* causes nuclear accumulation of mRNA (*24*) as well as defect in protein import. Each class of RNA is suggested to have its own carrier protein, which has the nuclear export signal (NES) and shuttles between the nucleus and cytoplasm. Thus, RNA export can be considered as cytoplasmic transport of RNA carrier proteins. In this context, it is notable that NUP153, which has the same zinc finger motif as RanBP2, is located on the nucleoplasmic side of NPC (*33*). The presence of proteins containing the same zinc finger motif on both nuclear and cytoplasmic

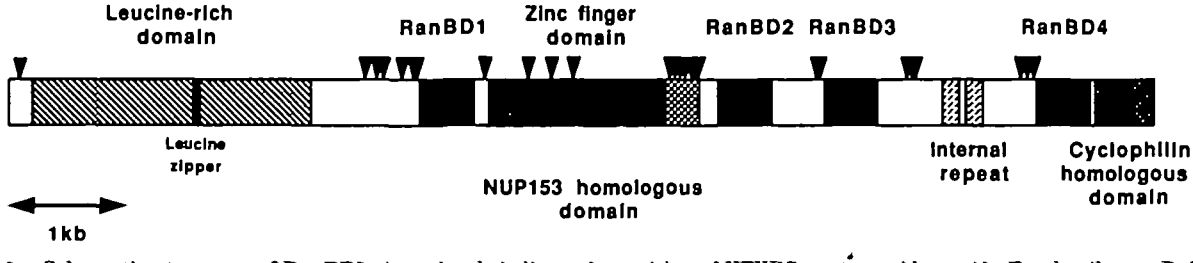


Fig. 3. **Schematic structure of RanBP2.** Arrowheads indicate the position of XFXFG pentapeptide motifs. For details, see Ref. *32*.

sides of NPC may indicate that this domain recognizes the same kind of proteins shuttling between the nucleus and the cytoplasm, while there is no evidence to indicate the involvement of NUP153 in RNA export.

In *rcc1*$^-$ other than *srm1-1*, the most prominent phenotype is a defect in mRNA export rather than nuclear protein import. In fact, the deletion of *MTR1* blocks mRNA export. This occurs immediately after a shift-up of temperature in the absence of protein synthesis and p34^{cdc28} kinase activation (*4*). Using tsBN2 cells, Cheng *et al.* (*37*) reported that RCC1 is required for nucleocytoplasmic transport of precursors of small nuclear RNAs (snRNAs), intranuclear transport of U3 snRNA, and processing of ribosomal RNAs, but not for export of transfer RNAs; and they suggested that RCC1 functions in a step that is a prerequisite for the export of pre-snRNAs and poly(A)$^+$ RNAs, that is, transport to—rather through—the NPCs. In accordance with this suggestion, it has been reported that *prp20-1* causes a loss of cap-like nucleolus staining and abolishment of uniform nucleoplasmic structure, but the nuclear envelope and the nuclear pores remain structurally intact (*3*). Indeed, NPC of tsBN2 cells functions for nuclear import of proteins fused with a large number of NLS peptides even after 6 h of incubation at 39.5°C, a restrictive temperature (*4, 38*). Interestingly, the *srp1-31* a ts mutant of budding yeast karyopherin α homologue, shows a severe defect in nuclear protein import, but does not affect mRNA export (*39*), indicating that a block of nuclear protein import does not inevitably cause mRNA accumulation. Thus, mRNA accumulation may be induced by loss of RCC1 function, independently from the defect of nuclear protein import.

Cell cycle regulation by RCC1 and the Ran pathway

Upon temperature shift up, hamster tsBN2 cells mutated in the *RCC1* do not enter the S phase after release from G1 blocked either with serum starvation or isoleucine deprivation. But they prematurely enter mitosis after release from the G1/S boundary blocked with hydroxyurea (2.5 mM), resulting in premature chromatin condensation (PCC) (*1*), as shown in Fig. 4. Cells showing PCC normally traverse mitosis and then the micronuclei possessing intact nuclear membranes and nuclear pores, as shown by electron microscopy, are reformed; but the decondensation of chromosomes does not occur, so that cells are arrested prior to entering the next cell cycle at 39.5°C, a restrictive temperature (*1*), similarly to *pim1-d1*^{ts} (*12*). These phenomena occur even in the presence of HU, strongly indicating that loss of RCC1 uncouples DNA replication from mitosis.

cdc25C, a tyrosine phosphatase of p34^{cdc2}, is localized in the cytoplasm. To activate MPF, therefore, cdc25C must enter the nucleus. Indeed, in tsBN2 cells, cdc25C enters the nucleus prior to MPF activation (*40*). In the presence of cycloheximide an inhibitor of protein synthesis, MPF cannot be activated. Under these conditions, cdc25C does not enter the nucleus, while RCC1 is lost due to the mutation. Regarding the absence of nuclear import of cdc25C in the presence of cycloheximide, two possibilities can be considered. First, an initial activation of pre-MPF, which occurred in the nucleus by loss of RCC1, was inhibited by cycloheximide, leading to abolition of the nuclear import of cdc25C, that may depend on an initial activation of pre-MPF. Second, the accumulation of GDP-Ran induced by loss of RCC1 may inhibit the nuclear import of proteins including cdc25C. In tsBN2 cells arrested at the G1/S boundary at 33.5°C a permissive temperature, cdc25C already accumulated in the cytoplasm (*40*). Thus, cdc25C must enter the nucleus prior to mitosis, although it is unknown how the nuclear entrance of cdc25C is triggered. Since nuclear protein import is not profoundly affected in tsBN2 cells by loss of RCC1 function (*38*), the first idea seems to be the more plausible.

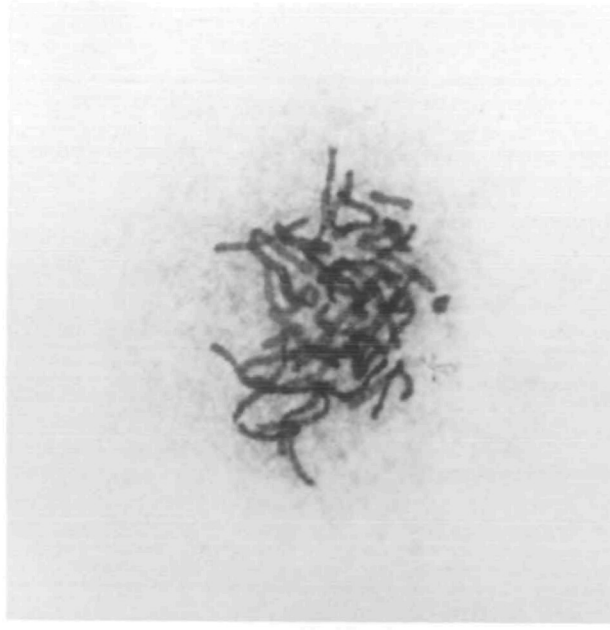


Fig. 4. A typical G2 PCC induced by loss of RCC1 function. tsBN2 cells were released from HU block and incubated at 39.5°C (*50*).

It has been suggested that p34^{cdc2} can be activated directly by GTP-Ran (*20*). However, this suggestion is not consistent with the observation in tsBN2 cells that loss of RCC1 leads to accumulation of GDP-Ran rather than GTP-Ran, or with the finding in mammalian cells that Ran mutant (G19V, Q69L double mutant) blocked in the GTP-form inhibits the cell cycle progression at both the G1/S and G2/M boundaries (*41*). We injected GTP- and GDP-Ran into tsBN2 cells that had been preincubated for 90 min at 39.5°C to sequester RCC1 function. Under these conditions, GTP-Ran, but not GDP-Ran, inhibited PCC induction (*42*). Furthermore, GTPγS-Ran also inhibited PCC induction by nucleoplasmic injection. These observations together indicate that GTP-Ran by itself functions to inhibit premature activation of MPF in the S phase.

The depletion of RCC1 from *Xenopus* egg extract inhibits the replication of added sperm DNA and the nuclear assembly, while a small nucleus is formed (*43*). The same effect is observed on addition of *E. coli*-produced T24N mutant of Ran, which tightly binds to RCC1 and inhibits its GEF activity. On addition of an excess of wild-type GTP-Ran, both nuclear assembly and chromosomal DNA replication are rescued in RCC1-depleted *Xenopus* extract (*43*). Thus, GTP-Ran is an active form of Ran for interphase functions in *Xenopus* extracts as well as hamster cells.

Suppressor of *rcc1*$^-$

Isolation of either suppressors or synthetic lethal mutants of target genes is a main avenue to genetically identify factors interacting with the target protein. As a suppressor of *pim1*, in addition to *spi1*, another suppressor of *pim1* has been isolated as a cold-sensitive mutant of *esp1/ppe1* (*44*) which encodes a protein 71% identical to budding yeast SIT4, a type 2A-related protein phosphatase. Due to the mutation, PIM1 disappears and can not make a complex with SPI1 at 36°C, a restrictive temperature. Such a phenotype does not recover as a result of a ts mutation of *esp1/ppe1*, while a double mutant, *esp1-pim1*, grows at 36°C, indicating that ESP1 act downstream of SPI1/Ran. *esp1*$^+$/*ppe1*$^+$ is not essential, but its deletion makes fission yeast cold-sensitive for growth. Deletion of *esp1*$^+$/*ppe1*$^+$ can be suppressed by overexpression of three classes of fission yeast genes, *ppa1*$^+$ and *ppa2*$^+$ encoding type 2A-like phosphatase, *dis3*$^+$, and *pck1*$^+$ encoding a protein kinase C-like kinase, and by overexpression of budding yeast *SIT4* (*45*). The double mutants, *ppe1-ppa2* and *ppe1-pck1* are lethal. Recently, we found that Ran binds directly to DIS3 (Noguchi *et al.*, in press). *dis3-54* shows failure in chromosome disjunction and is synthetic lethal in combination with *dis1*^{cs} or *dis2*^{cs} a catalytic subunit of type 1 phosphatase, both of which show defects similar to *dis3-54* (*46*). These findings together suggest that the Ran pathway is involved in the phosphatase network which regulates the cell cycle progression.

GTR1 encodes a putative GTPase (*47*) and its cold-sensitive mutation suppresses both the N-terminal and the C-terminal budding yeast *rcc1*$^-$, in contrast to other suppressors of *rcc1*$^-$, which cannot simultaneously suppress budding yeast *rcc1*$^-$ mapped at both ends of the RCC1 repeat. The same mutation of *gtr1* also suppressed *rna1-1*, indicating that the isolated *gtr1* mutant functions as a suppressor for defects in the Ran pathway (Nakashima *et al.*, in press).

NuMA is a protein involved in the maintenance of nuclear structure and the assembly of the mitotic spindle. Overexpression of this protein partly suppresses the terminal phenotype of tsBN2 cells (*48*). This finding may indicate the involvement of the Ran pathway in the maintenance of nuclear structure, as suggested by Cheng *et al.* (*37*) and Aebi *et al.* (*3*). In this connection, it is notable that overexpression of yeast RCC1 homologue suppresses class 2 α-tubulin mutant (*49*).

Conclusion

In view of their high nuclear abundance, there is no doubt that Ran and RCC1 play important roles in the nucleus. Thus far, their involvement in nuclear pore function has become clear. The accumulation of mRNA may reflect another nuclear function of the Ran pathway. It is still obscure how the defect of nuclear function is related to the cell cycle regulation. But the Ran pathway will provide a clue to how the nuclear structure is related to, and is regulated during the progression of cell cycle.

The authors thank the colleagues in Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, for providing unpublished data and Hiroaki Seino for preparing Fig. 1.

REFERENCES

1. Nishitani, H., Ohtsubo, M., Yamashita, K., Iida, H., Pines, J., Yasuda, H., Shibata, Y., Hunter, T., and Nishimoto, T. (1991) Loss of RCC1, a nuclear DNA-binding protein, uncouples the completion of DNA replication from the activation of cdc2 protein kinase and mitosis. *EMBO J.* **10**, 1555-1564
2. Clark, K.L. and Sprague, G.F., Jr. (1989) Yeast pheromone response pathway: Characterization of a suppressor that restores mating to receptorless mutants. *Mol. Cell. Biol.* **9**, 2682-2694
3. Aebi, M., Clark, M.W., Vijayraghavan, U., and Abelson, J. (1990) A yeast mutant, *PRP20*, altered in mRNA metabolism and maintenance of the nuclear structure, is defective in a gene homologous to the human gene *RCC1* which is involved in the control of chromosome condensation. *Mol. Gen. Genet.* **224**, 72-80
4. Kadowaki, T., Goldfarb, D., Spitz, L.M., Tartakoff, A.M., and Ohno, M. (1993) Regulation of RNA processing and transport by a nuclear guanine nucleotide release protein and members of the Ras superfamily. *EMBO J.* **12**, 2929-2937
5. Bischoff, F.R. and Ponstingl, H. (1991) Catalysis of guanine nucleotide exchange on Ran by the mitotic regulator RCC1. *Nature* **354**, 80-82
6. Moore, M.S. and Blobel, G. (1993) The GTP-binding protein Ran/TC4 is required for protein import into the nucleus. *Nature* **365**, 661-663
7. Ohtsubo, M., Kai, R., Furuno, N., Sekiguchi, T., Sekiguchi, M., Hayashida, H., Kuma, K., Miyata, T., Fukushige, S., Murotsu, T., Matsubara, K., and Nishimoto, T. (1987) Isolation and characterization of the active cDNA of the human cell cycle gene (RCC1) involved in the regulation of onset of chromosome condensation. *Genes Dev.* **1**, 585-593
8. Miyabashira, J., Sekiguchi, T., and Nishimoto, T. (1994) Mammalian cells have two functional RCC1 proteins produced by alternative splicing. *J. Cell Sci.* **107**, 2203-2208
9. Nishimoto, T., Seino, H., Seki, N., and Hori, T. (1994) The Human CHC1 gene encoding RCC1 (regulator of chromosome condensation) (CHC1) is localized to human chromosome 1p36.1. *Gemonics* **23**, 719-721
10. Frasch, M. (1991) The maternally expressed *Drosophila* gene encoding the chromatin-binding protein BJ1 is a homologue of the vertebrate gene regulator of chromosome condensation, RCC1. *EMBO J.* **10** 1225-1236
11. Matsumoto, T. and Beach, D. (1991) Premature initiation of mitosis in yeast lacking RCC1 or an interacting GTPase. *Cell* **66**, 347-360
12. Sazer, S. and Nurse, P. (1994) A fission yeast RCC1-related protein is required for the mitosis to interphase transition. *EMBO J.* **13**, 606-615
13. Ohtsubo, M., Ozaki, H., and Nishimoto, T. (1989) The RCC1 protein, a regulator for the onset of chromosome condensation locates in the nucleus and bind to DNA. *J. Cell Biol.* **109**, 1389-1397
14. Bischoff, F.R., Maier, G., Tilz, G., and Ponstingl, H. (1990) A 47-kDa human nuclear protein recognized by antikinetochore autoimmune sera is homologous with the protein encoded by RCC1, a gene implicated in onset of chromosome condensation. *Proc. Natl. Acad. Sci. USA* **87**, 8617-8621
15. Lee, A., Tam, R., Belhumeur, P., DiPaolo, T., and Clark, M.W. (1993) Prp20, the *Saccharomyces cerevisiae* homolog of the regulator of chromosome condensation, RCC1, interacts with double-stranded DNA through a multi-component complex containing GTP-binding proteins. *J. Cell Sci.* **106**, 287-298
16. Seino, H., Hisamoto, N., Uzawa, S., Sekiguchi, T., and Nishimoto, T. (1992) DNA-binding domain of RCC1 protein is not essential for coupling mitosis with DNA replication. *J. Cell Sci.* **102**, 393-400
17. Drivas, G.T., Shih, A., Coutavas, E., Rush, M.G., and D'Eustachio, P. (1990) Characterization of four novel RAS-related genes expressed in a human teratocarcinoma cell line. *Mol. Cell. Biol.* **10**, 1793-1798

18. Belhumeur, P., Lee, A., Tam, R., DiPaolo, T., Fortin, N., and Clark, M.W. (1993) GSP1 and GSP2, genetic suppressors of the prp20-1 mutant in *Saccharomyces cerevisiae*: GTP-binding proteins involved in the maintenance of nuclear organization. *Mol. Cell. Biol.* **13**, 2152-2161
19. Scheffzek, K., Klebe, C., Fritz-Wolf, K., Kabsch, W., and Wittinghofer, A. (1995) Crystal structure of the nuclear Ras-related protein Ran in its GDP-bound form. *Nature* **374**, 378-381
20. Kornbluth, S., Dasso, M., and Newport, J. (1994) Evidence for a dual role for TC4 protein in regulating nuclear structure and cell cycle progression. *J. Cell Biol.* **125**, 705-719
21. Coutavas, E., Ren, M., Oppenhelm, J.D., D'Eustachio, P., and Rush, M.G. (1993) Characterization of proteins that interact with the cell-cycle regulatory protein Ran/TC4. *Nature* **366**, 585-587
22. Richards, S.A., Lounsbury, K.M., and Macara, I.G. (1995) The C terminus of the nuclear Ran/Tc4 GTPase stabilize the GDP-bound state and mediates interactions with RCC1, Ran-GAP and HTF9A/RanBP1. *J. Biol. Chem.* **270**, 14405-14411
23. Bischoff, F.R., Krebber, H., Kempf, T., Hermes, I., and Ponstingl, H. (1995) Human RanGTPase-activating protein RanGAP1 is a homologue of yeast Rna1p involved in mRNA processing and transport. *Proc. Natl. Acad. Sci. USA* **92**, 1749-1753
24. Corbett, A.H., Koepp, D.M., Schlenstedt, G., Lee, M.S., Hopper, A.K., and Silver, P.A. (1995) Rna1p, a Ran/TC4 GTPase activating protein, is required for nuclear import. *J. Cell Biol.* **130**, 1017-1026
25. DeGregori, J., Russ, A., Melchner, H., Rayburn, H., Priyaranjan, P., Jenkins, N.A., Copeland, N.G., and Ruley, H.E. (1994) A murine homolog of the yeast RNA1 gene is required for postimplantation development. *Genes Dev.* **8**, 265-276
26. Melchior, F., Weber, K., and Gerke, V. (1993) A functional homologue of the RNA1 gene product in *Schizosaccharomyces pombe*: Purification, biochemical characterization, and identification of a leucine-rich repeat motiff. *Mol. Biol. Cell* **4**, 569-581
27. Klebe, C., Prinz, H., Wittinghofer, A., and Goody, R.S. (1995) The kinetics mechanism of Ran-nucleotide exchange catalyzed by RCC1. *Biochemistry* **34**, 12543-12552
28. Hayashi, N., Yokoyama, N., Seki, T., Azuma, Y., Ohba, T., and Nishimoto, T. (1995) RanBP1, a Ras-like nuclear G-protein binding to Ran/TC4, inhibits RCC1 via Ran/TC4. *Mol. Gen. Genet.* **247**, 661-669
29. Ouspenski, I.L., Mueller, U.W., Matynia, A., Sazer, S., Elledge, S.J., and Brinkley, B.R. (1995) Ran-binding protein-1 is an essential component of the Ran/RCC1 molecular switch system in budding yeast. *J. Biol. Chem.* **270**, 1975-1978
30. Schlenstedt, G., Wong, D.H., Koepp, D., and Silver, P.A. (1995) Mutants in a yeast Ran binding protein are defective in nuclear transport. *EMBO J.* **14**, 5367-5378
31. Wu, J., Matunis, J., Kraemer, D., Blobel, G., and Coutavas, E. (1995) Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. *J. Biol. Chem.* **270**, 14209-14213
32. Yokoyama, N., Hayashi, N., Seki, T., Pante, N., Ohba, T., Nishii, K., Kuma, K., Hayashida, T., Miyata, T., Aebi, U., Fukui, M., and Nishimoto, T. (1995) A giant nucleopore protein that binds Ran/TC4. *Nature* **376**, 184-188
33. Sukegawa, J. and Blobel, G. (1993) A nuclear pore complex protein that contains zinc finger motifs, binds DNA, and faces the nucleoplasm. *Cell* **72**, 29-38
34. Melchior, F., Guan, T., Yokoyama, N., Nishimoto, T., and Gerace, L. (1995) GTP hydrolysis by Ran occurs at the nuclear pore complex in an early step of nuclear protein import. *J. Cell Biol.* **131**, 571-581
35. Azuma, Y., Seino, H., Seki, T., Uzawa, S., Klebe, C., Ohba, T., Wittinghofer, A., Hayashi, N., and Nishimoto, T. (1996) Conserved histidine residues of RCC1 are essential for nucleotide exchange on Ran. *J. Biochem.* **120**, 82-91
36. Schlenstedt, G., Saavedra, C., Loeb, J.D.J., Cole, C.N., and Silver, P.A. (1995) The GTP-bound form of the yeast Ran/TC4 homologue blocks nuclear protein import and appearance of poly(A)$^+$ RNA in the cytoplasm. *Proc. Natl. Acad. Sci. USA* **92**, 225-229
37. Cheng, Y., Dahlberg, J.E., and Lund, E. (1995) Diverse effects of the guanine nucleotide exchange factor RCC1 on RNA transport. *Science* **267**, 1807-1810
38. Tachibana, T., Imamoto, N., Seino, H., Nishimoto, T., and Yoneda, Y. (1994) Loss of RCC1 leads to suppression of nuclear protein import in living cells. *J. Biol. Chem.* **269**, 24542-24545
39. Loeb, J.D.J., Schlenstedt, G., Pellman, D., Kornitzer, D., Silver, P.A., and Fink, G.R. (1995) The yeast nuclear import receptor is required for mitosis. *Proc. Natl. Acad. Sci. USA* **92**, 7647-7651
40. Seki, T., Yamashita, K., Nishitani, H., Takagi, T., Russell, P., and Nishimoto, T. (1992) Chromosome condensation caused by loss of RCC1 function required the cdc25C protein that is located in the cytoplasm. *Mol. Biol. Cell* **3**, 1373-1388
41. Ren, M., Coutavas, E., D'Eustachio, P.D., and Rush, M.G. (1994) Cell cycle arrest induced by mutant Ran/TC4 proteins. *Mol. Cell. Biol.* **14**, 4216-4224
42. Ohba, T., Seki, T., Azuma, Y., and Nishimoto, T. (1996) Premature chromatin condensation induced by loss of RCC1 is inhibited by GTP- and GTPγS-Ran, but not GDP-Ran. *J. Biol. Chem.* **271**, 14665-14667
43. Dasso, M., Seki, T., Azuma, Y., Ohba, T., and Nishimoto, T. (1994) A mutant form of the Ran/TC4 protein disrupts nuclear function in *Xenopus laevis* egg extracts by inhibiting the RCC1 protein, a regulator of chromosome condensation. *EMBO J.* **13**, 5732-5744
44. Matsumoto, T. and Beach, D. (1993) Interaction of the pim1/spi1 mitotic checkpoint with a protein phosphatase. *Mol. Biol. Cell* **4**, 337-345
45. Shimanuki, M., Kinoshita, N., Ohkura, H., Yoshida, T., Toda, T., and Yanagida, M. (1993) Isolation and characterization of the fission yeast protein phosphatase gene ppe1+ involved in cell shape control and mitosis. *Mol. Biol. Cell* **4**, 303-313
46. Kinoshita, N., Goebl, M., and Yanagida, M. (1991) The fission yeast *dis3*$^+$ gene encodes a 110 kDa essential protein implicated in mitotic control. *Mol. Cell. Biol.* **11**, 5839-5847
47. Bun-Ya, M., Harashima, S., and Oshima, Y. (1992) Putative GTP-binding protein, Gtr1, associated with the function of the Pho84 inorganic phosphate transporter in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12**, 2958-2966
48. Compton, D.A. and Cleveland, D.W. (1993) NuMA is required for the proper completion of mitosis. *J. Cell Biol.* **120**, 947-957
49. Kirkpatrick, D. and Solomon, F. (1994) Overexpression of yeast homologs of the mammalian checkpoint gene RCC1 suppresses the class of α-tubulin mutations that arrest with excess microtubules. *Genetics* **137**, 381-392
50. Nishimoto, T., Ishida, R., Ajiro, K., Yamamoto, S., and Takahashi, T. (1981) The synthesis of protein(s) for chromosome condensation may be regulated by a post-transcriptional mechanism. *J. Cell. Physiol.* **109**, 299-308