Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 7525–7529, August 1995
Biochemistry

A family of Ran binding proteins that includes nucleoporins

COLIN DINGWALL*, STEFANIE KANDELS-LEWIS, AND BERTRAND SÉRAPHIN

Gene Expression Programme, European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany

Communicated by Christine Guthrie, University of California, San Francisco, CA, April 28, 1995

ABSTRACT **Ran, a small nuclear GTP binding protein, is essential for the translocation of nuclear proteins through the nuclear pore complex. We show that several proteins, including the *Saccharomyces cerevisiae* Nup2p and *Caenorhabditis elegans* F59A2.1 nucleoporins, contain domains similar to the previously characterized murine Ran binding protein (RBP, termed RBP1). To test the significance of this similarity, we have used the corresponding domains of Nup2p and a putative *S. cerevisiae* RBP in Ran binding assays and the yeast two-hybrid system. Both proteins bind *S. cerevisiae* Ran, but only the putative *S. cerevisiae* RBP binds human Ran. Two-hybrid analysis revealed Ran–Ran interactions and that yeast and human Rans can interact. These data identify Nup2p as a target for Ran in the nuclear pore complex, suggesting a direct role for it in nuclear–cytoplasmic transport. We discuss the possibility that proteins harboring Ran binding domains link the Ran GTPase cycle to specific functions in the nucleus.**

Nuclear protein import proceeds in two major steps, an energy-independent docking at the nuclear envelope followed by an energy-dependent translocation step (1, 2), both of which are mediated by soluble protein factors (3–8).

An essential factor for the translocation step is Ran, a small nuclear Ras-related GTP binding protein. It is the only known nuclear G protein and, like other G proteins, it undergoes cycles of GTP binding, hydrolysis, and GDP release (9–11). In addition, the GTP-bound form of Ran is stabilized by and binds tightly to a Ran binding protein (RBP) termed RBP1 (12, 13).

We have identified a family of putative RBPs in a range of organisms on the basis of their sequence similarity to the murine RBP1. All of these proteins share a discrete Ran binding domain (RBD), the smaller proteins consisting essentially of a single RBD with short extensions at their N and/or C termini. Our searches identified three RBD-containing proteins from yeast, one of which is the putative yeast homologue of the mammalian RBP1 protein. Interestingly, a RBD was found at the C terminus of the *Saccharomyces cerevisiae* nucleoporin Nup2p (14). Consistent with a role for the RBD at the nuclear pore, two RBDs were also found in a putative nucleoporin (F59A2.1) from *Caenorhabditis elegans*.

By using biochemical methods and the "two-hybrid" system (15), we show that both mammalian Ran and a *S. cerevisiae* Ran homologue, GSP1 (16), bind to the putative RBP in *S. cerevisiae*. We also show in the two-hybrid system that *S. cerevisiae* Ran, but not mammalian Ran, binds to the RBD of Nup2p. We discuss the possibility that proteins with RBDs are specifically associated with different nuclear functions and may act as effector molecules linking the Ran GTPase cycle to these functions.

MATERIALS AND METHODS

Biocomputing. Searches were made by using the FASTA and TFASTA programs of the Genetics Computer Group (Madison, WI) package (17). Profiles were built with PROFILEMAKE and used with the Genetics Computer Group package PROFILESEARCH program (18) and TPROFILESEARCH program (P. Rice, European Molecular Biology Laboratory). Multiple sequence alignments were produced with CLUSTAL W (19). Pairwise identity was calculated using the Genetic Computer Group DISTANCE program.

Cloning, Expression in *Escherichia coli*, and Protein Purification. Clones of mammalian Ran and the Q69L mutant in pET11d have been described (10). The gene encoding *S. cerevisiae* Ran (*GSP1*) was amplified from yeast genomic DNA and cloned into pET11d. Protein was expressed and purified as described (10).

The yeast RBP1 protein and the C-terminal domain of Nup2p (amino acids 556–720) were amplified from yeast genomic DNA. The sequence of 10 clones of the yeast *RBP1* gene revealed a sequencing error in the data base entry (accession no. X65925, EMBL data base release 33); this was corrected (EMBL data base release 40). Glutathione *S*-transferase (GST) fusion proteins were expressed and purified by published procedures (20).

Two-Hybrid Analysis. We used vectors and yeast strains described by Brent and co-workers (21). Ran, RBP1, and the C terminus of Nup2p (amino acids 556–720) were amplified from *S. cerevisiae* DNA and cloned into pEG202 to produce a lexA DNA binding domain fusion and into pJG4.5 to produce an activation domain fusion. The human Ran and mutant (Q69L) Ran were amplified from the pET11d vectors (10) and cloned into pEG202 and pJG4.5. The control plasmid harboring the Bicoid fusion has been described elsewhere (22). β-Galactosidase assays were as described (23). For each experiment duplicate assays were performed.

Nitrocellulose Blot Assay. To detect RBPs, we used the nitrocellulose blot assay (12, 13) with the following modifications. After electrophoretic transfer, proteins were denatured in 6 M guanidinium chloride, which was diluted in six sequential 1:2 dilution steps. Filters were then probed with 10 nM Ran loaded with [γ-^{32}P]GTP to 150–500 Ci/mol (1 Ci = 37 GBq).

GST Fusion Protein Binding Assay. Nucleotides {GTP, GDP, guanosine 5′[γ-thio]triphosphate (GTP[γS]), or guanosine 5′-[β-thio]diphosphate (GDP[βS])} were bound to mammalian or *S. cerevisiae* Ran as described (10). The GST binding assay was essentially as described for Ras (24). Nucleotide-bound Ran was incubated with GST, GST–RBP, or GST–Nup2p, and complexes were isolated by using glutathione-agarose. Bound proteins were analyzed by SDS/PAGE, electrophoretically transferred, and probed with Ran-specific polyclonal antisera.

Binding of ^{35}S-labeled Ran produced in reticulocyte lysates was assayed by using 10 μl of *in vitro* translation reaction mixture.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RBP, Ran binding protein; RBD, Ran binding domain; GST, glutathionine *S*-transferase; GDP[βS], guanosine 5′-[β-thio]diphosphate; GTP[γS], guanosine 5′-[γ-thio]triphosphate.

*Present address: Department of Pharmacological Sciences, State University of New York, Stony Brook, NY 11794-8651.

RESULTS

Identification of a Family of RBDs. The sequence of the murine RBP1 (12) was used in data base searches to identify related proteins (Fig. 1*A*). Pairwise and multiple sequence alignment of these related protein sequences allowed us to define a domain of ≈170 residues that we refer to as the RBD. Ten residues are conserved in all the RBDs (Fig. 1*B*). These are mostly hydrophilic or charged residues that are, therefore, likely to be on the surface of the domain and may form binding sites. These conserved amino acids are surrounded by residues that are less well conserved but are generally hydrophobic and probably form the core of the domain. The distinct domain organization of the larger proteins also suggests that the RBD is independently folded, and in general it is likely that the RBD is an independent folding unit as the mammalian and yeast RBP1 proteins consist almost exclusively of an RBD.

The best match was found with a human protein that is the homologue of the murine RBP1 protein (95% identity). The RBP found in yeast is encoded by open reading frame near the *NTH1* (neutral trehalase) gene (25). To our knowledge, the presence of an open reading frame at this location has not been reported. The mammalian and yeast RBP1 proteins are composed almost exclusively of one copy of the RBD flanked only by short N- and/or C-terminal extensions (Fig. 1*A*), suggesting that they are indeed homologous proteins. This is further supported by the high level of sequence identity among them (55–95%).

Two other yeast proteins have a region showing significant similarity to mammalian RBP, namely Nup2p, a previously characterized *S. cerevisiae* nucleoporin (14), and SC5610.10, a putative protein identified during the course of the yeast genome sequencing project (Fig. 1). The 720-amino acid yeast nucleoporin Nup2p is organized into distinct domains (14). The central domain (amino acids 181–555) contains 15 FSFG repeats characteristic of a group of nucleoporins (26). The RBD of Nup2p identified here corresponds almost exactly to the domain present downstream of the FSFG repeats at the extreme C terminus of the protein (amino acids 560–720; Fig. 1*A*).

Finally, F59A2.1, a protein identified during the sequencing of the *C. elegans* genome, displayed two regions similar to mammalian RBP1 (Fig. 1*A*). This *C. elegans* protein was suggested to be a nucleoporin as it contains several XFXFG repeats (26) and a putative $CX_2CX_{10}CX_2C$ zinc finger found in other nucleoporins (27).

In addition we found several expressed sequence tags, including some from plants, encoding partial proteins with similarity to mammalian RBPs (data not shown).

To test the significance of the similarity between these proteins, we assayed the ability of their respective RBDs to interact with *S. cerevisiae* and mammalian Ran.

The Yeast RBP1 Protein Binds Yeast and Human Rans *in Vitro*. The identified sequence of a *S. cerevisiae* RBP1 homologue encodes a protein of 201 amino acids and, therefore, has a molecular weight consistent with it being the major protein

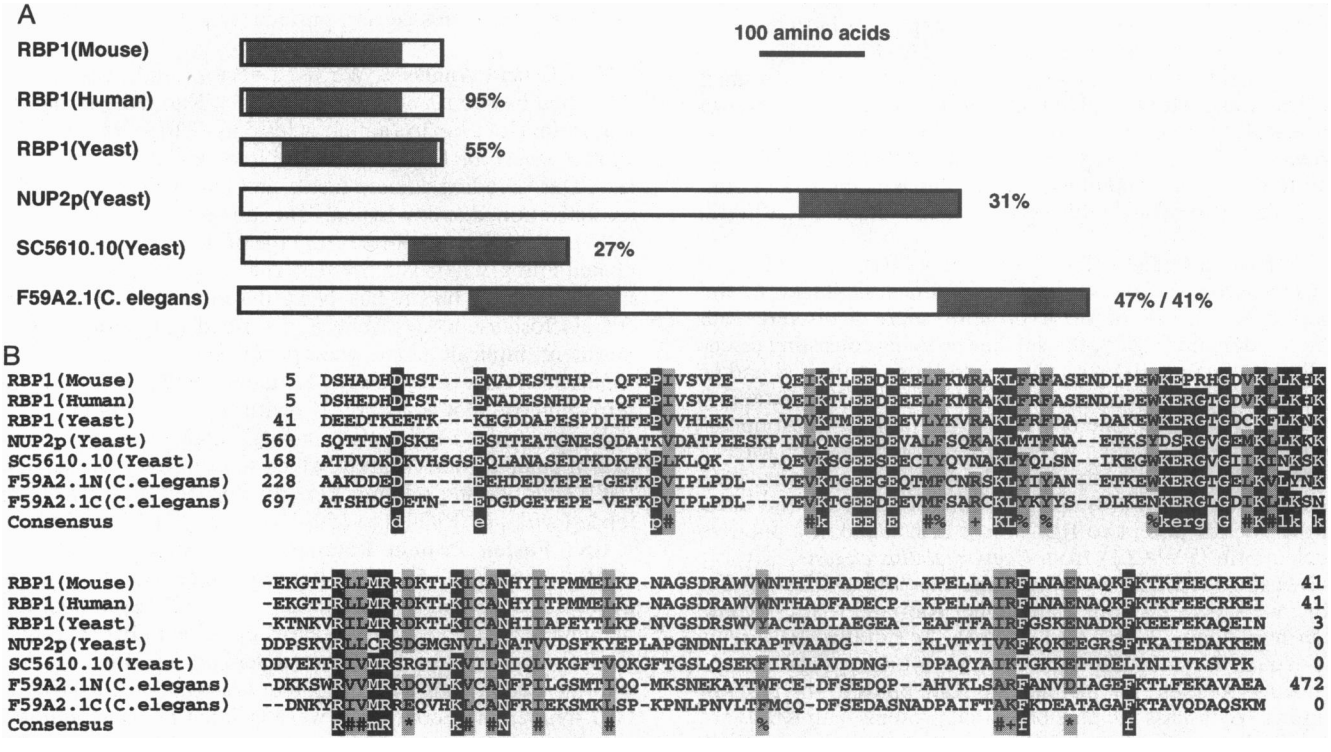

FIG. 1. Domain organization of RBPs and sequence alignment of RBDs. (*A*) RBPs are open boxes and RBDs are shaded boxes. Sequence names (and GenBank accession nos.) are as follows: RBP1(mouse), murine RBP1 (accession no. P34023); RBP1(human), human RBP1 (accession no. P34022); RBP1(yeast), RBP1 from yeast (accession no. X65925); NUP2(yeast), Nup2p from yeast (accession no. P32499); SC5610.10(yeast), yeast protein encoded in open reading frame 10 in λ clone 5610 (accession no. Z38060); F59A2.1(*C. elegans*), protein F59A2.1 from *C. elegans* (accession nos. Z34801), F59A2.1C(*C. elegans*) and F59A2.1N(*C. elegans*) represent the C- and N-terminal RBD, respectively. The percent identity between a given RBD and the murine RBP1–RBD is indicated after each protein. (*B*) Alignment of the RBDs. The sequences of the RBDs from the various proteins were aligned with the CLUSTAL W program, with dashes indicating gaps. The number of residues preceding and following each RBD is shown before and after each sequence, respectively. Conserved residues in at least six of the seven sequences are in white on a black background. Positions with conserved physiocochemical properties are shaded. The consensus line indicates conserved residues with the following notation: uppercase type, residue conserved in the seven sequences; lowercase type, residue conserved in six out of seven sequences; *, acidic residue (Asp or Glu) in at least six of the seven sequences; +, basic residue (Lys or Arg) in at least six of the seven sequences; #, small hydrophobic residue (Ala, Ile, Leu, Met, or Val) in at least six of the seven sequences; %, aromatic residue (Phe, Tyr, or Trp) in at least six of the seven sequences.

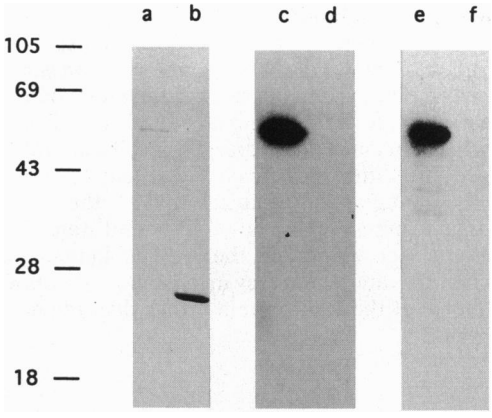

FIG. 2. Detection of Ran–GTP binding to *S. cerevisiae* RBP by gel overlay with Ran–[γ-^{32}P]GTP. Lanes: a and b, Coomassie blue-stained SDS/polyacrylamide gel; c and d, autoradiogram of renatured blots probed with 10 nM *S. cerevisiae* Ran–[γ-^{32}P]GTP; e and f, autoradiogram of renatured blots probed with 10 nM mammalian Ran–[γ-^{32}P]GTP; a, c, and e, 0.2 μg of GST–RBP fusion; b, d, and f, 2.0 μg of GST.

present in yeast extracts that binds Ran *in vitro* (ref. 13 and our unpublished data). The protein was expressed in *E. coli* as a GST fusion protein and purified. The mammalian and yeast Ran proteins were expressed in *E. coli* as untagged proteins and purified to homogeneity from bacterial cell lysates by using sequential steps of ammonium sulfate precipitation, gel filtration chromatography, and two ion-exchange chromatography steps (10).

The ability of the pure RBP1–GST fusion protein to bind directly to Ran was determined in a nitrocellulose blot assay (12, 13) by using mammalian or yeast Ran bound to [γ-^{32}P]GTP as the probe. We found that the yeast RBP1–GST fusion binds specifically to both yeast Ran and mammalian Ran (Fig. 2).

We also used a solution binding assay to confirm this result, which also allows us to test the nucleotide requirement for binding (24). As there is an activity in the reticulocyte lysate that stimulates GTP hydrolysis by Ran (data not shown), nonhydrolyzable nucleotide analogues were also used to stabilize Ran in the nucleotide-bound states. Yeast Ran was loaded with GTP, GTP[γS], GDP, or GDP[βS] and incubated with the GST fusion protein bound to glutathione-agarose. Bound proteins were analyzed by SDS/PAGE, transferred to nitrocellulose, and probed for Ran by using a rabbit polyclonal antiserum. Ran loaded with GTP[γS] binds specifically to the GST–RBP fusion but not to GST (Fig. 3*A*). No binding was detected with Ran–GTP, Ran–GDP, or Ran–GDP[βS]. The same binding assay was performed by using ^{35}S-labeled Ran produced *in vitro* by translation of *in vitro*-transcribed RNA. Identical results were obtained (Fig. 3*B*). In parallel experiments, the binding of mammalian Ran to the GST–RBP1 fusion protein was studied with identical results (data not shown).

By using the nitrocellulose blot assay, we observed the binding of mammalian and yeast Ran–GTP to RBP1 (Fig. 2). However, this was not detected by using the solution binding assays (Fig. 3). This inconsistency is probably due to the solution binding assay being less sensitive than the nitrocellulose blot assay and due to the reticulocyte lysate stimulating GTP hydrolysis by Ran leading to the rapid hydrolysis of bound GTP.

By using the same range of assays, we were unable to demonstrate a direct interaction between the RBD of Nup2p and yeast or mammalian Ran (data not shown). This could be due to this interaction being of a lower affinity than the RBP–Ran interaction, which has an estimated dissociation constant of 10^{-10} M (13). Alternatively, additional factors may stabilize the interaction between Ran and the RBD of Nup2p *in vivo*, which are absent from the *in vitro* assays. We therefore assayed the ability of the RBD of Nup2p and the yeast RBP1 to bind Ran *in vivo* by using the two-hybrid system in yeast (15, 21).

The Ran Binding Domain of Nup2p Binds Ran *in Vivo*. By using plasmid vectors and yeast strains that have been described (21), we made fusions between the DNA binding domain of lexA and yeast Ran, human Ran, or the Q69L human Ran mutant. In the latter construct, the Q69L mutation blocks the GTPase activity of Ran and leads to the accumulation of GTP-bound Ran (28). As controls, we used fusions between the lexA DNA binding domain and Rab3a, a small GTP binding protein involved in exocytosis (29) or *Drosophila* bicoid (22). The ability of the lexA fusions to enter the nucleus and bind DNA was demonstrated by the repression of transcription from a reporter plasmid in which the *lexA* DNA recognition sequence was located between the GAL_{UAS} and the *lacZ* gene (data not shown). Activation domain fusions were made among the B42 transcription activation domain, a nuclear localization signal, a hemagglutinin epitope tag, and the yeast RBP1, the RBD of Nup2p, or yeast Ran. As controls we used fusions harboring either a randomly chosen human cDNA or an SH3 domain. These plasmids were introduced into the yeast strain EGY48 and quantitative β-galactosidase assays were performed. The results of these experiments are presented in Fig. 4.

No transcriptional activation was observed when we used any of the lexA fusions in combination with a random human cDNA fused to the B42 activation domain (Fig. 4, bars p–t). Yeast Ran and Bicoid were also tested in combination with an SH3 domain fusion and no activation was observed (Fig. 4, bars u and v). This demonstrates that the various lexA fusions are unable to activate transcription on their own or in combination

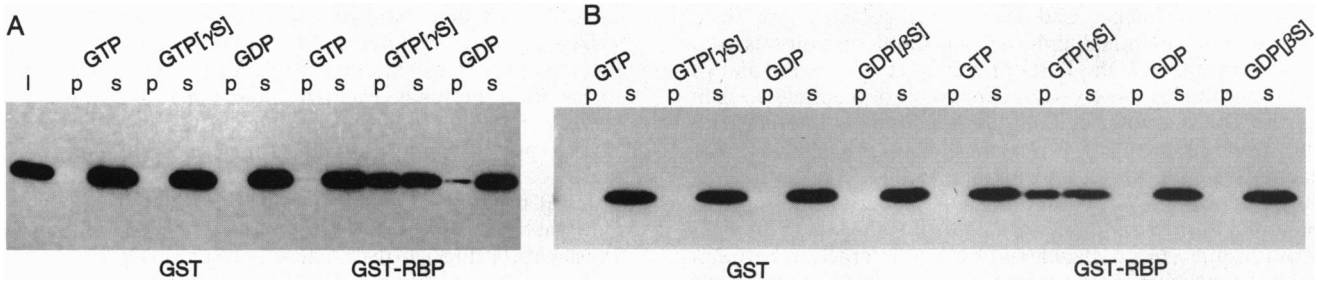

FIG. 3. Binding of *S. cerevisiae* Ran to GST fusion proteins. (*A*) Western blot of proteins bound to GST or GST–RBP. Nucleotides were bound to *S. cerevisiae* Ran and binding assays were carried out. Proteins in the pellet (lanes p) and supernatant (lanes s) fractions were analyzed by SDS/PAGE and transferred to nitrocellulose, and the filter was probed with a Ran-specific rabbit antiserum. (*B*) Different nucleotides were bound to ^{35}S-labeled *S. cerevisiae* Ran protein produced by *in vitro* translation. Binding assays were carried out. Proteins in the pellet (lanes p) and supernatant (lanes s) fractions were detected by autoradiography.

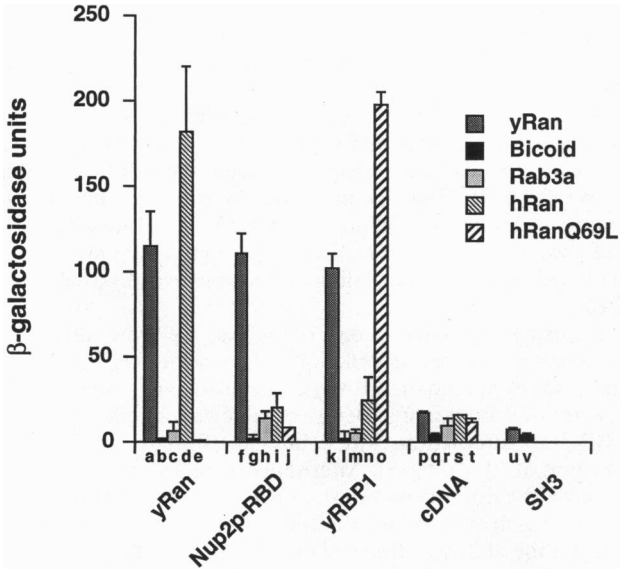

FIG. 4. Results from the two-hybrid assays. The histogram shows the results of the two-hybrid assays with the *y* axis indicating the level expression in β-galactosidase units. Names below the *x* axis indicate the protein fused with the activation domain, and the pattern of the bars indicates the protein fused with the lexA DNA binding domain. yRan, yeast Ran; hRan, human Ran; hRanQ69L, Q69L mutant of hRan; Nup2p–RBD, RBD of Nup2p; RBP1 (yeast), the yeast RBP1 protein; cDNA, a random human cDNA; SH3, phospholipase Cγ subdomain. Two experiments were carried out with each combination. For each experiment, β-galactosidase activity was determined in duplicate. The minimal and maximal levels of β-galactosidase units are indicated.

with activation domain fusions with which they do not specifically interact.

In contrast, the yeast RBP1 protein interacts with both yeast Ran and the Q69L mutant human Ran (Fig. 4, bars k and o). Lower levels of β-galactosidase expression were observed with the wild-type human Ran (Fig. 4, bar n) confirming the results of the *in vitro* interaction study (Fig. 2). An interaction between Ran–GTP and the yeast RBP1 protein is consistent with our biochemical data (see above) and published data showing that RBPs bind to and stabilize Ran in the GTP-bound conformation (12, 13). The difference between the wild-type and Q69L forms of human Ran in this assay may suggest that only low levels of the GTP-bound form of this protein accumulate in yeast. As controls for the specificity of the interaction, we used Rab3a and the *Drosophila* Bicoid fusions and observed no interaction between either of these with the yeast RBP1 (Fig. 4, bars l and m).

In this assay, the RBD of Nup2p clearly interacts with yeast Ran, giving levels of β-galactosidase activity equivalent to those observed with the RBP (Fig. 4, compare bars f and k). However, only background levels of β-galactosidase were observed when mammalian Ran or the Q69L mutant was used in combination with the RBD of Nup2p (Fig. 4, bars i and j). This is not due to low levels of expression of mammalian Ran and the Q69L mutant as both can functionally interact with other proteins in the two-hybrid system (for Q69L with the yeast RBP1, see above, and for mammalian Ran with yeast Ran, see below). We observed no interaction between this domain of Nup2p and either the control Rab3a or *Drosophila* Bicoid fusions (Fig. 4, bars g and h). The interaction between the RBD of Nup2p and yeast Ran is very specific since mammalian Ran does not interact yet shows 82% identity with the yeast protein.

Surprisingly, the data also indicate that Ran–Ran interactions occur, as β-galactosidase activity was detected when two copies of yeast Ran were expressed in the same cell, one being fused to the DNA binding domain and the other to the activation domain (Fig. 4, bar a). In a parallel experiment, the same result was obtained when mammalian Ran was fused to both the DNA binding domain and the activation domain (data not shown). Interestingly, mammalian and yeast Ran are able to interact (Fig. 4, bar d); however, the Q69L mutant is unable to interact with either Ran (e.g., Fig. 4, bar e, and data not shown). Preliminary results indicate that another mutant Ran (T24N) that accumulates in the GDP-bound state (28) is also unable to interact with either the yeast or human Ran. This suggests that the interaction may involve the nucleotide-free or "empty" form of the Ran protein rather than the nucleotide-bound forms.

DISCUSSION

RBP1 from mouse was initially characterized as a protein of 203 amino acids (12). Through data base searches we have identified a number of proteins harboring related RBDs of ≈170 amino acids. Three of the RBP-containing proteins (RBP1 from human, mouse, and yeast) are likely to be homologues because they are largely composed of an RBD with only short extensions and, furthermore, share extensive sequence identity. Additionally, three RBDs were found in nucleoporins, one in the yeast nucleoporin Nup2p and two in a putative nucleoporin from *C. elegans* (F59A2.1). In these proteins the RBDs are associated with other domains that probably mediate the integration of the protein in the nuclear pore (14). Finally, a RBD was also found in the SC5610.10 protein. In this protein the RBD is preceded by a hydrophilic domain, suggesting that the protein might be soluble.

The RBDs found in mouse RBP1, yeast RBP1, and Nup2p are quite divergent (minimally 31% identical) yet all bind Ran (see below); therefore, we consider it likely that all the other proteins with RBDs also interact with Ran. In yeast we have identified three proteins that contain a RBD, and this number is likely to be equivalent or even larger in higher eukaryotes given the relative sizes of their respective genomes.

By biochemical methods and the two-hybrid system, we have demonstrated a direct interaction between yeast Ran and two of the proteins identified in these searches, namely, the yeast RBP1 and Nup2p proteins. In addition we have shown that the yeast RBP1 protein binds both yeast and mammalian Ran, which are extremely similar (82% identical) and differ significantly only in short sequences at their N and C termini. We suggest that these differences may be the basis for the species-specific interaction observed between Nup2p and yeast Ran.

In addition to being species specific, our biochemical data suggest that the interaction between Nup2p and Ran is weaker than the Ran–RBP1 interaction, since we were unable to detect it *in vitro* by using assays that detect Ran–RBP1 complexes. Alternatively, it is possible that this particular interaction is stabilized by additional factors, such as Nup1p or Srp1, that are absent from our *in vitro* assays. Srp1 was initially identified as a suppressor of mutations in the large subunit of RNA polymerase I in *S. cerevisiae* and is located at the nuclear envelope (30). It also interacts with Nup1p and Nup2p nucleoporins in a mutually exclusive manner (31). However, a *Xenopus* Srp1 homologue functions in the docking step of nuclear protein import (8). This suggests that Nup1p and Nup2p constitute at least part of the docking site for karyophiles at the nuclear pore complex, but in addition our data show that Nup2p also interacts with the essential factor for translocation through the nuclear pore complex.

The results from the two-hybrid analysis indicate that Ran–Ran interactions take place *in vivo* and that this interaction can occur between Ran proteins from different species. The data also suggest that this interaction may involve the nucleotide-free form of the protein, but we do not know whether this interaction is direct or involves other proteins. It may be

relevant that the nucleotide-free form of Ran forms a stable complex with RCC1 and RBP1 *in vitro* (32).

The function of the RBD in these various proteins is unclear but they could affect the Ran GTPase cycle by stabilizing one form of Ran and inhibiting or enhancing the activity of the GTPase-activating enzyme or of the nucleotide exchange factor RCC1 (32). Defects in the nuclear GTPase cycle have consequences for a wide range of cellular events that are not obviously related, such as transport into and out of the nucleus, RNA synthesis and processing, DNA replication, and cell cycle control (33, 34). Within the nucleus, factors associated with specific functions, such as splicing (35) and DNA replication (36), are located at specific sites that can be visualized by fluorescence microscopy. We suggest that the RBPs may also be located at specific sites within the nucleus, such as the nuclear pore in the case of Nup2p, and thereby may be associated with different nuclear functions. Consequently, they can act to locate Ran and, hence, be function-specific downstream effectors of the nuclear GTPase cycle. This would explain the pleiotropic effects of mutations that disrupt the nuclear GTPase cycle and predict that ablation of the function of specific RBPs would give rise to defects in specific nuclear processes.

Note. The gene encoding the yeast RBP1 protein has been named *YRB1* (for yeast Ran binder 1) in the *S. cerevisiae* registry of gene names according to the recommendations of M. Cherry, Stanford University (personal communication).

We thank Roger Brent for supplying the vectors and strains for the two-hybrid assay and the following for supplying clones and strains: SH3, G. Superti-Furga; Rab3a, J. Johannes; Ran clones, C. Klebe and A. Wittinghofer; Nup2 mutant yeast strains, J. D. J. Loeb. We thank Joe Lewis for help with the purification of the Ran proteins. We thank Iain Mattaj and members of his laboratory, Toby Gibson, and Karsten Weis for critical reading of the manuscript. C.D. gratefully acknowledges the financial support of the Cancer Research Campaign.

1. Newmeyer, D. D. & Forbes, D. J. (1988) *Cell* **52,** 641–653.
2. Richardson, W. C., Mills, A. D., Dilworth, S. M., Laskey, R. A. & Dingwall, C. (1988) *Cell* **52,** 655–664.
3. Moore, M. S. & Blobel, G. (1992) *Cell* **69,** 939–950.
4. Moore, M. S. & Blobel, G. (1993) *Nature (London)* **365,** 661–663.
5. Melchior, F., Paschal, B., Evans, J. & Gerace, L. (1993) *J. Cell Biol.* **123,** 1649–1659.
6. Moore, M. S. & Blobel, G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 10212–10216.
7. Adam, E. J. H. & Adam, S. (1994) *J. Cell Biol.* **125,** 547–555.
8. Gorlich, D., Prehn, S., Laskey, R. A. & Hartmann, E. (1994) *Cell* **79,** 767–778.
9. Bischoff, F. R. & Ponstingl, H. (1991) *Nature (London)* **354,** 80–82.
10. Bischoff, F. R., Klebe, C., Kretschmer, J., Wittinghofer, A. & Ponstingl, H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2587–2591.
11. Hattori, M., Tsukamoto, N., Nur-E-Kamal, M. S. A., Rubinfeld, B., Iwai, K., Kubota, H., Maruta, H. & Minato, N. (1995) *Mol. Cell. Biol.* **15,** 552–560.
12. Coutevas, E., Ren, M., Oppenheim, J. D., D'Eustachio, P. & Rush, M. G. (1993) *Nature (London)* **366,** 585–587.
13. Lounsbury, K. M., Beddow, A. L. & Macara, I. (1994) *J. Biol. Chem.* **269,** 11285–11290.
14. Loeb, J. D. J., Davis, L. I. & Fink, G. R. (1993) *Mol. Biol. Cell* **4,** 209–222.
15. Fields, S. & Song, O. (1989) *Nature (London)* **340,** 245–246.
16. Belhumeur, P., Lee, A., Tam, R., DiPaolo, T., Fortin, N. & Clark, M. W. (1993) *Mol. Cell. Biol.* **13,** 2152–2161.
17. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
18. Gribskov, M., McLachlan, A. D. & Eisenberg, D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4355–4358.
19. Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) *Nucleic Acids Res.* **22,** 4673–4680.
20. Hagemeier, C., Caswell, R., Hayhurst, G., Sinclair, J. & Kouzarides, T. (1994) *EMBO J.* **12,** 2897–2930.
21. Gyuris, J., Golemis, E., Chertkov, H. & Brent, R. (1993) *Cell* **75,** 791–803.
22. Frohnhofer, H. G. & Nusslein-Volhard, C. (1986) *Nature (London)* **324,** 120–125.
23. Kandels-Lewis, S. & Seraphin, B. (1993) *Science* **262,** 2035–2039.
24. Vojtek, A. B., Hollenberg, S. M. & Cooper, J. A. (1993) *Cell* **74,** 205–214.
25. Kopp, M., Muller, H. & Holtzer, H. (1993) *J. Biol. Chem.* **268,** 4766–4774.
26. Rout, M. P. & Wente, S. R. (1994) *Trends Cell Biol.* **4,** 357–365.
27. Sukegawa, J. & Blobel, G. (1994) *Cell* **72,** 29–38.
28. Klebe, C., Bischoff, F. R., Ponstingl, H. & Wittinghofer, A. (1995) *Biochemistry* **34,** 639–647.
29. Zerial, M. & Stenmark, H. (1993) *Curr. Biol.* **5,** 613–620.
30. Yano, R., Oakes, M., Yanaghishi, M., Dodd, J. & Nomura, M. (1992) *Mol. Cell. Biol.* **12,** 5604–5651.
31. Belanger, K. D., Kenna, M. A., Wei, S. & Davis, L. I. (1994) *J. Cell Biol.* **126,** 619–630.
32. Bischoff, F. R., Krebber, H., Smirnova, E., Dong, W. & Ponstingl, H. (1995) *EMBO J.* **14,** 705–715.
33. Dasso, M. (1993) *Trends Biochem. Sci.* **18,** 96–101.
34. Tartakoff, A. M. & Schneiter, R. (1995) *Trends Cell Biol.* **5,** 5–8.
35. Spector, D. L. (1993) *Annu. Rev. Cell Biol.* **9,** 265–315.
36. Adachi, Y. & Laemmli, U. K. (1992) *J. Cell Biol.* **119,** 1–15.