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The Ran/TC4 GTPase-binding domain: Identification by expression cloning and characterization of a conserved sequence motif

(GTP-binding protein/nucleoporin/cell cycle)

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ABSTRACT **Ran/TC4 is an essential, nuclear GTPase implicated in the initiation of DNA replication, entry into and exit from mitosis, and in nuclear RNA and protein transport through the nuclear pore complex. This diversity of functions suggests that Ran interacts with a large number of downstream targets. Using an overlay assay, we detected a family of putative target proteins that associate with GTP-bound Ran. The sequence of only one such protein, HTF9a/RanBP1, is known. We have now cloned two additional Ran-binding proteins, allowing identification of a distinctive, highly conserved sequence motif of ≈150 residues. This motif represents a minimal Ran-binding domain that stabilizes the GTP-bound state of Ran. The isolated domain also functions as a coactivator of Ran-GTPase-activating protein. Mutation of a conserved residue within the Ran-binding domain of HTF9a protein drastically reduced Ran binding. Ran-binding proteins coimmunoprecipitated with epitope-tagged Ran from cell lysates, suggesting that these proteins may associate *in vivo*. A previously uncharacterized *Caenorhabditis elegans* gene could encode a protein (96 kDa) possessing two Ran-binding domains. This open reading frame also contains similarities to nucleoporins, suggesting a functional link between Ran and nuclear pore complexes.**

The Ran/TC4 GTPase is a highly conserved nuclear protein expressed in all eukaryotic cells examined to date (1–3). Disruption of Ran function, by either the introduction into cells of dominant gain-of-function mutants or removal of a regulatory factor, results in a pleiotropic phenotype characterized by cell-cycle arrest (4–8), premature chromosome condensation (2, 8) or exit from mitosis (9), and the accumulation of incorrectly processed nuclear RNA (10–12). In *Xenopus* oocyte preparations a dominant loss-of-function mutant of Ran blocks nuclear growth and the entry into S phase and inhibits dephosphorylation of the cdc2 p34 mitotic protein kinase (5), preventing the initiation of mitosis. *In vitro* studies also indicate that Ran may be an essential cytosolic component for the transport of karyophilic proteins into the nucleus through the nuclear-pore complex (13, 14).

This extraordinary diversity of functions suggests that Ran likely interacts with a large number of downstream target proteins. Using an overlay assay, we have previously identified a number of proteins that bind to and stabilize the GTP-bound state of Ran (15). The smallest of these proteins, Ran-binding protein 1 (RanBP1), has been cloned and is identical to a previously sequenced open reading frame called HTF9a (16, 17) that encodes a polypeptide of ≈24 kDa. Many of the other proteins that bind Ran in the overlay assay are >100 kDa in size. Here we report the cloning of two additional RanBPs from a human hippocampal cDNA library and the identification of a highly conserved sequence motif that functions as a Ran-binding domain (RanBD).† The isolated domain stabilizes the GTP-bound state of Ran and acts as a costimulator of Ran GTPase activity in the presence of Ran-GTPase-activating protein (GAP). A previously unidentified gene, *sfo1*, from *Saccharomyces cerevisiae* possesses one potential RanBD (J. Trueheart and J. Thorner, personal communication), and a *Caenorhabditis elegans* gene could encode a protein that posseses two RanBDs. This latter gene additionally shows similarities to nucleoporins NUP153p, NSP1, and NUP255p (also known as the protooncogene *CAN*) (18, 19).

MATERIALS AND METHODS

Expression Cloning. A human hippocampus cDNA expression library in Lambda ZAPII (Stratagene) was screened with [α-^{32}P]GTP-Ran, essentially as described by Coutavas *et al.* (16). Recombinant Ran protein loaded with [α-^{32}P]GTP and the complex was stabilized by addition of 10 mM $MgCl_2$ (15). Unincorporated nucleotide was removed by passage over a Pharmacia PD-10 column. Plaques were lifted after induction for 4 hr with isopropyl β-D-thiogalactoside and renatured overnight in 20 mM Hepes, pH 7.5/25 mM potassium acetate/10 mM magnesium acetate/5 mM dithiothreitol/50 μM GTP/50 μM GDP/4% nonfat dried milk/0.25% Tween 20. This was replaced with a similar buffer containing 0.05% Tween-20 and [α-^{32}P]GTP-Ran (10^6 cpm/ml), and filters were incubated at 4°C for 2 hr and then washed five times in 150 mM NaCl/20 mM Tris, pH 7.4/10 mM magnesium acetate. pBluescript SK plasmids were excised from tertiary plaques according to the manufacturer's instructions (Stratagene). As a final test for clones that express Ran-binding proteins, bacteria containing plasmids with putative positive inserts were incubated with isopropyl β-D-thiogalactoside to induce protein expression, and then bacterial extracts were run on SDS/PAGE and transferred to nitrocellulose. RanBPs were detected by a Ran overlay assay as described (15). Two independent clones were identified that expressed RanBPs of ≈50 kDa (clone AB1) and 90 kDa (clone AB2) (data not shown). Inserts were completely sequenced in both directions by using an ABI automated sequencer.

Expression and Analysis of the AB1 RBD. The putative Ran-binding domain of clone AB1 (390 bp, residues PHFE . . . CKFE) was amplified by PCR and subcloned into pGEX-2T. The resulting glutathione *S*-transferase (GST) fusion protein was expressed in *Escherichia coli* and purified over glutathi-

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Abbreviations: GST, glutathione *S*-transferase; RanBD, Ran-binding domain; RanBP, Ran-binding protein; GAP, GTPase-activating protein.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U19240 and U19248).

one-Sepharose. The size of the GST–RanBD protein is ≈40 kDa. A GST fusion of a second, unrelated region (Reg2) of clone AB1 was also constructed for use as a negative control. Ten to fifteen micrograms of each protein was loaded onto an SDS/polyacrylamide gel for analysis by the Ran overlay assay (15). To measure release of GTP, Ran was loaded with [α-^{32}P]GTP and added to buffer alone (10 mM glutathione, 1 mM GTP, 1 mM GDP, 1 mM EDTA, 1 mM dithiothreitol, 120 mM NaCl, 100 mM Tris, pH 8.0) or buffer plus $MgCl_2$ (10 mM), GST–RanBD (1–5 μg), or GST (10 μg) at 23°C. At intervals, aliquots were removed and assayed for [α-^{32}P]GTP remaining bound to Ran by binding to nitrocellulose filters (15). Hydrolysis of [α-^{32}P]GTP bound to Ran was measured over 20 min. Cytosolic extract containing Ran–GAP activity was prepared as described by Coutavas *et al.* (16) and added at a protein concentration of 0.067 mg/ml. GAP assays were done essentially as described (20). All assays were done at 23°C.

Analysis of Fragments and Mutants of HTF9a. Fragments of HTF9a were amplified by PCR and subcloned into pGEX-2T. Purified GST–fusion proteins were separated on SDS/12% polyacrylamide gels and quantitated for protein by the Bradford assay. Point mutants of HTF9a were selected from a random mutant library in pGEX-2T created by template-limited PCR (N.R.O. and I.G.M., unpublished data). Clones were sequenced to identify the mutated residues. One mutant, E37K, was selected for further study. To detect binding to the GST–HTF9a protein, equal quantities (0.1 μg) of wild-type or E37K GST–HTF9a or GST protein were bound to glutathione-Sepharose beads and incubated for 10 min at 4°C with [α-^{32}P]GTP-loaded Ran of known specific activity. After extensive washing, counts remaining bound to the beads were determined.

Coimmunoprecipitation of RanBPs with Epitope-Tagged Ran. A V19 mutant of Ran, equivalent to the V12 dominant gain-of-function mutant of Ras, was generated by megaprimer PCR. This Ran protein behaves biochemically, as expected for a constitutively activated mutant (S.A.R. and I.G.M., unpublished data). The V19 and wild-type Ran open reading frames were subcloned into eukaryotic vector pKH3 (21), which adds a triple HA1 epitope tag to the N terminus. These constructs were transfected by coprecipitation with calcium phosphate into 293 cells. After 16 hr the cells were labeled with [^{35}S]methionine (250 μCi; 1 Ci = 37 GBq). After 24 hr cell extracts were immunoprecipitated with anti-HA1 antibody 12CA5 (21). The immunoprecipitated proteins were separated by SDS/PAGE and analyzed by a Ran overlay assay and by fluorography to detect heterologously expressed Ran.

RESULTS

Expression Cloning of Additional RanBPs. Expression screening of a human hippocampal cDNA library with [α-^{32}P]GTP-labeled Ran identified two previously unidentified Ran-binding proteins, here called AB1 and AB2. A highly conserved region in both clones is also present in HTF9a/RanBP1 and in a *Saccharomyces cerevisiae* gene, *sfo1*. A search of GenBank identified a previously uncharacterized open reading frame in the *C. elegans* genomic cosmid F59A2.1 (22) that possesses two copies of a similar sequence. Alignment of these six sequences (Fig. 1) indicates the existence of a specific motif extending over ≈150 residues, which we have named the RanBD. Both the AB1 and AB2 clones contain long open reading frames that, outside of this RanBD, possess no similarity to HTF9a. The N-terminal domain in F29A2.1 contains a 23-amino acid residue insert in the middle of the conserved region. There are five stretches of almost identical residues in the consensus RanBD: (*i*) **FEPVVPLPDXXEVKTGEEXE**; (*ii*) **RAKL(F/Y)R(F/Y)**; (*iii*) **KEWKERGXG**; (*iv*) **MRRDQV**, and (*v*) **LKØCANH**, bounded by isolated conserved residues at characteristic positions within the RanBD (where **Ø** = aliphatic residues and X = any residue).

To determine whether this sequence motif functions as a RanBD, residues 7–165 of the AB1 clone (numbering as in Fig. 1) were expressed as a GST fusion protein (Fig. 2*A*). After electrophoresis and transfer to nitrocellulose, this protein bound [α-^{32}P]GTP-labeled Ran in an overlay assay (Fig. 2*A*). A GST fusion protein of another sequence from clone AB1, outside of the RanBD, did not bind [α-^{32}P]GTP-Ran. Therefore, the isolated domain is capable of binding the Ran GTPase.

The RanBD Stabilizes the GTP-Bound State of Ran. The rate of release of [α-^{32}P]GTP from Ran was measured in the presence or absence of GST–AB1–RanBD. As expected, in the presence of Mg^{2+} the rate of release was negligibly small; addition of excess EDTA destabilizes the Ran–GTP complex, and release occurred with a $t_{1/2}$ of ≈1 min. Addition of GST–AB1–RanBD reduced this rate ≈10-fold (Fig. 2*B*). Addition of an equivalent concentration of GST had no effect on release. Therefore, Ran–GTP can bind undenatured AB1–RanBD, and the interaction stabilizes the GTP-bound state of Ran.

The RanBD Is a Coactivator of Ran–GAP. The remarkable stability of the Ran–GTP–RanBD complex (15) suggests the necessity for a mechanism to terminate association. We therefore tested the ability of the AB1 RanBD to modulate the stimulation of GTP hydrolysis on Ran by Ran–GAP (16, 23). Addition of cytosolic extract containing Ran–GAP activity

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                1          11         21         31         41         51         61         71         81
      HTF9a     DESNHDPQFE PIVSLPEQ.E*IKTLEEDEEE LFKMRAKLFR FASENDLPEW KERGTGDVKL LKHKEKGTIR LLMRRDKT.. ..........
clone AB1-RBD   DDDDDGPHFE PVVPLPDKIE VKTGEEDEEE FFCNRAKLFR F..DVESKEW KERGIGNVKI LRHKTSGKIR LLMRREQV.. ..........
clone AB2-RBD   EEERDGQYFE PVVPLPDLVE VSSGEENEQV VFSHRAKLYR YD..KDVGQW KERGIGDIKI LQNYDNKQVR IVMRRDQV.. ..........
CelF59A2-RBD1   EDYEPEGEFK PVIPLPDLVE VKTGEEGEQT MFCNRSKLYI YA..NETKEW KERGTGELKV LYNKDKKSWR VVMRRDQVTI SEETLPSNIE
CelF59A2-RBD2   GEYEPEVEFK PVIPLPDLVE VKTGEEDEEV MFSARCKLYK YY..SDLKEN KERGLGDIKL LKSNDNK.YR IVMRREQVH. ..........
      Sfolp     PE.SPDIHFE PVVHLEKV.D VKTMEEDEEV LYKVRAKLFR F..DADAKEW KERGTGDCKF LKNKKTNKVR ILMRRDKT.. ..........

Consensus:      -E------FE PVVPLPD-VE VKTGEEDEE- -F--RAKL-R -----D-KEW KERG-GD-K- L--K-----R --MRRDQV-- ----------

                91         101        111        121        131        141        151        161
      HTF9a     .......... ..LKICANHY ITPMMELKPN AGSDRAWVWN THADFADECP KPEL..LAIR FLNAENAQKF KTKFEECRKE
clone AB1-RBD   .......... ..LKICANHY ISPDMKLTPN AGSDRSFVWH A.LDYADELP KPEQ..LAIR FPTPEEAALF KCKFEEAQSI
clone AB2-RBD   .......... ..LKLCANHR ITPDMTLQNM KGTERVWEWT A.CDFADGER KVEH..LAVR FKLQDVADSF KKICDEAKTA
CelF59A2-RBD1   NGTEELCWNI QVLKVCANFP ILGSMTIQQM KSNEKAYTWF CE.DFSED.. QPAHVKLSAR FANVDIAGEF KTLFEKAVAE
CelF59A2-RBD2   .......... ...KLCANFR IEKSMKLSPK PNLPNVLTFM CQ.DFSEDAS NADPAIFTAK FKDEATAGAF KT....AVQD
      Sfolp     .......... ..LKICANHI IAPEYTLKPN VGSDRSWVYA CTADIAEGEA EAFT..FAIR FGSKENADKF KEEFEKAQEI

Consensus:      ---------- --LK-CANH- I-P-M-L-P- -G--R---W- ---DF----- ------LA-R F-----A--F K--FE-A---
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FIG. 1. Amino acid residue sequence alignment of RanBDs. Regions of significant similarity are displayed for HTF9a/RanBP1 (16, 17), clones AB1 and AB2 (GenBank accession numbers U19240 and U19248), two sequences from a previously uncharacterized *C. elegans* open reading frame, F59A2.1 (ref. 22; GenBank accession no. Z34801) and the *sfo1* gene from *S. cerevisiae* (accession no. L38489). The consensus sequence lists residues that are identical in four of six of the sequences shown. Asterisk indicates residue mutated to lysine (see Fig. 3 *B* and *C*). Underlined residues indicate boundaries of GST fusion fragments H2–H4 (see Fig. 3*A*).

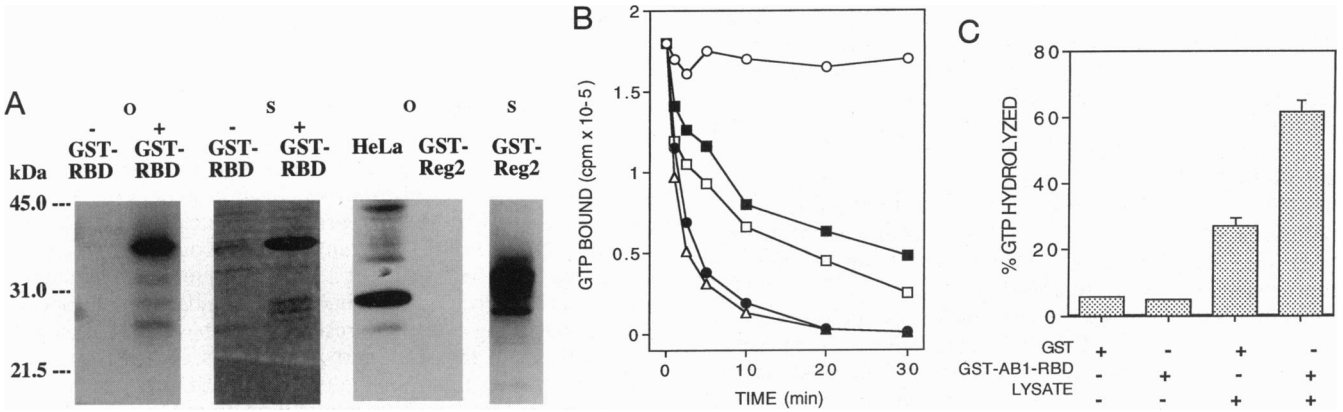

FIG. 2. The conserved sequence in clone AB1 binds Ran, stabilizes the Ran–GTP complex and coactivates Ran–GAP. (*A*) Overlay (O) of GST fusion proteins purified from uninduced (−) (lane 1) or isopropyl β-D-thiogalactoside induced (+) (lane 2) bacteria expressing pGEX-AB1RanBD. Lanes: 3 and 4, Coomassie-stained gel (S) of GST–AB1RanBD from uninduced and induced cells; 5 and 6, overlay (*O*) of Hela cell extract (positive control for Ran binding) and 12 μg of GST–Reg2, a fusion protein of a 35-amino acid residue fragment of clone AB1, C-terminal to the RanBD (RBD); 7, Coomassie-stained gel (*S*) of 12 μg of GST–Reg2. (*B*) Inhibition of [α-^{32}P]GTP release from Ran by GST–AB1RanBD. Lines show [α-^{32}P]GTP remaining bound to Ran in the presence of 5 mM $MgCl_2$ (○), excess EDTA (●), EDTA plus GST at 29 μg/ml (△), EDTA plus GST–AB1RanBD at 3 μg/ml (□), or EDTA plus GST–AB1RanBD at 14 μg/ml (■). (*C*) Hydrolysis of Ran-bound [α-^{32}P]GTP over 20 min in the presence of GST, GST–AB1–RanBD, cytosolic extract containing Ran–GAP activity plus GST, and extract plus GST–AB1–RanBD. Values are ±1 SD (n = 3). RBD, RanBD.

increased the hydrolysis of GTP bound to Ran by ≈5-fold. The presence of the AB1–RanBD together with Ran–GAP further increased GTP hydrolysis to ≈12-fold over the basal level (Fig. 2*C*). Hydrolysis of GTP bound to Ran was very low in the absence of Ran–GAP (k_{cat} = 0.002 min^{-1}), and the AB1–RanBD had no detectable intrinsic GAP activity. Thus, the AB1–RanBD can coactivate Ran–GAP, providing a possible mechanism for triggering dissociation of the Ran–RanBD complex.

Structure–Function Analysis of HTF9a RanBD. To delimit the boundaries of the RanBD, we created and affinity-purified GST fusion proteins of HTF9a residues 27–160, 27–112, 65–160, and 65–112 (HTF9a residue numbering), designated as H1, H2, H3, and H4, respectively. H1 encompasses the entire RanBD. Residue 65 corresponds to P48 in the alignment in Fig. 1, just before the EWKERG sequence; and residue 112 corresponds to P119 (Fig. 1) after the CANH conserved region. These proteins were analyzed by the overlay assay alongside GST and full-length GST–HTF9a (Fig. 3*A*). H1 associated with Ran–GTP as efficiently as full-length HTF9a, but binding was not detectable to fragments H2–H4, even after prolonged exposure. (These fragments were partially proteolyzed, perhaps because they do not fold correctly, and other proteins, possibly heat-shock proteins, associated nonspecifically with some of the fragments.) These data indicate that the entire conserved region in the RanBD is essential for high-affinity association with Ran–GTP.

To determine whether specific conserved residues in the RanBD are required for high-affinity interaction with Ran–GTP, we created a random mutant library of HTF9a in pGEX and screened the library for mutants with a reduced ability to bind Ran–GTP. One example of such a mutant is E37K, in the first highly conserved region of the RanBD (residue 20 in Fig. 1). The binding of [α-^{32}P]GTP–Ran to the E37K mutant of HTF9a was at least 10-fold lower than that to wild-type HTF9a (Fig. 3*B*). To confirm the mutant phenotype, overlay assays were done on the wild-type and E37K GST–HTF9a proteins (Fig. 3*C*). To ensure that binding to the mutant would be detectable, a 15-fold-higher concentration of mutant than wild-type protein was applied to the gel. Quantitation revealed that even under these conditions the amount of [α-^{32}P]GTP–Ran bound to the E37K mutant was only ≈75% of that to wild-type HTF9a. Therefore at least some of the highly conserved residues in the RanBD are essential, either for structural integrity of the domain or for binding to Ran–GTP, and support the hypothesis that the high degree of similarity between the sequences identified in Fig. 1 reflects their similar function in binding the Ran GTPase.

RanBPs Coimmunoprecipitate with Epitope-Tagged Ran. The high affinity of the RanBD for Ran–GTP suggests that RanBPs should associate with Ran in the intact cell. To begin to approach this question, we transiently transfected 293 cells with either wild type or a constitutively activated mutant of Ran (V19) to which an HA1 epitope was attached at the N terminus. The cell extracts were immunoprecipitated with anti-tag antibody and analyzed for expression of the tagged Ran (by [^{35}S]methionine labeling) and for associated RanBPs by an overlay assay. Fig. 4 shows that a 28-kDa protein corresponding to HTF9a coprecipitated with the V19 mutant of Ran and, to a lesser extent, with wild-type Ran. This result is expected because the V19 mutant will be predominantly GTP-bound, whereas the wild-type protein may be largely GDP-bound. Both Ran proteins were expressed at equal levels in the 293 cells, as determined by [^{35}S]methionine labeling and immunoprecipitation.

DISCUSSION

The cloning of two additional RanBPs has provided sufficient information to identify a previously unrecognized, highly conserved sequence motif, which we have shown to encode a RanBD. This domain possesses a remarkably high affinity for Ran, stabilizes the GTP-bound state, and acts as a costimulator of GTP hydrolysis by Ran in the presence of Ran–GAP. This last effect is unique among known regulators of small GTPases, and its cellular function is unknown. However, an intriguing possibility is that the GAP costimulation activity provides an essential release mechanism. The off-rate of Ran–GTP from the RanBD is so slow that an efficient trigger to dissociate the complex may be essential to prevent all of the RanBPs from being permanently tied up in complexes with Ran (15). This idea is supported by the fact that the activated V19 mutant of Ran, which is unresponsive to Ran–GAP (S.A.R. and I.G.M., unpublished data) coimmunoprecipitates with HTF9a to a greater extent than does wild-type Ran. Hydrolysis of the GTP on Ran would immediately cause dissociation from the RanBD because the RanBD has no detectable affinity for Ran in the GDP-bound state (S.A.R.

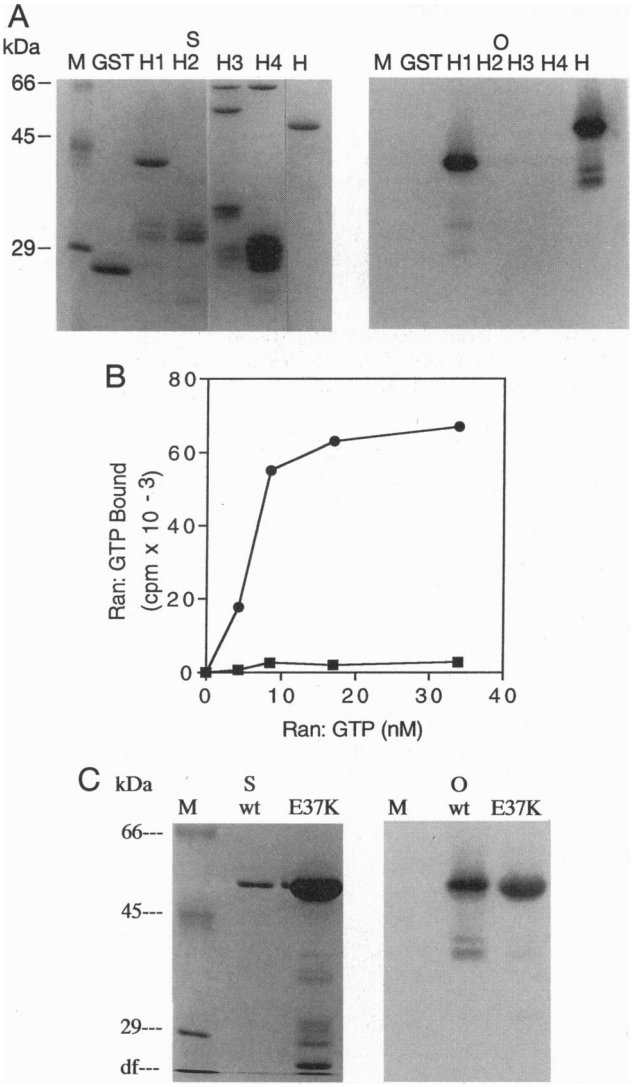

FIG. 3. Binding of Ran–GTP to fragments of the HTF9a RanBD expressed as GST fusion proteins. (*A*) (*Left*) Coomassie-stained gel (S) of purified GST fusion proteins. Lanes from left: M, molecular weight markers; GST, unfused GST protein; H1, GST–HTF9a residues 27–160; H2, 65–160; H3, 27–112; H4, 65–112; and H, full-length HTF9a, residues 1–203. (*Right*) Ran overlay assay (O) of the same samples as in *Left*. (*B*) A point mutation (Glu-37 → Lys) in the RanBD of HTF9a inhibits Ran binding. Binding of [α-^{32}P]GTP–Ran to equal quantities (0.1 μg) of wild-type (●) or E37K GST–HTF9a (■) protein attached to glutathione-Sepharose beads. Background counts (bound to GST alone) were subtracted for each sample. Data are representative of three similar experiments. (*C*) Wild-type (2 μg) or mutant E37K–HTF9a (30 μg) GST fusion proteins were analyzed by Coomassie staining (*Left*, S) or the Ran overlay assay (*Right*, O). Lanes from left: M, molecular weight markers; wt, wild-type GST–HTF9a; E37K, mutant GST–HTF9a. df, Dye front.

and I.G.M., unpublished data). The fact that Ran–GAP is apparently cytosolic (23) while the Ran exchange factor and many of the RanBPs are nuclear (15) suggests that these binding proteins may have to exit the nucleus to be released from a Ran–GTP complex. The mechanism by which the RanBD coactivates Ran–GAP remains to be determined. Presumably, however, the RanBD either prevents the association of a GAP inhibitor to Ran or forms a tertiary, activated complex with Ran. The recent description of the purification of Ran–GAP protein (23) should allow these two possibilities to be distinguished.

The [α-^{32}P]GTP-labeled Ran overlay assay reveals a large family of RanBPs in all eukaryotic cells that have been

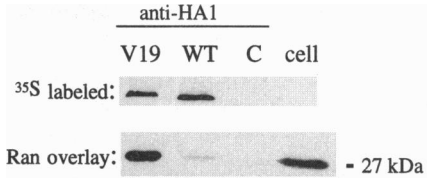

FIG. 4. Coimmunoprecipitation of a 28-kDa RanBP with Ran. (*Upper*) Immunoprecipitates from [^{35}S]methionine-labeled 293 cells transfected with HA1-tagged V19Ran (lane V19), wild-type Ran (lane WT), or vector control (lane C). (*Lower*) Ran overlay assay of anti-HA1 immunoprecipitates from 293 cells transfected with V19 Ran (lane V19), wild-type Ran (lane WT), vector control (lane C), or of total 293 cell extract (lane cell).

examined (15), and from a comparison of their properties with those of the cloned proteins, it is likely that they all contain similar RanBDs. However, in addition to the budding yeast *sfo1* gene, a search of the GenBank data base using the BLAST network server (24) uncovered only one related sequence. The open reading frame derived from this *C. elegans* genomic sequence F59A2.1 (22) contains two RanBDs, the first of which possesses a 23-residue insert. The overall structure of this gene is shown in Fig. 5. In addition to the RanBDs, there is an N-terminal domain rich in glutamine and alanine residues (QA domain), similar to regions in several transcriptional factors, such as zeste, gal11, and TFIID. Finally there are regions with similarities to nucleoporins. The region from residues 445–680 possesses two copies of the XFXFG nucleoporin "signature" motif and three copies of the FGG plus two copies of the SVFG repetitive peptide motifs found in the CAN/NUP215 nucleoporin (18). The region from residues 170–223 also contains one FGG motif that overlaps with an XFXFG motif, and the sequence SIFG, which is very similar to the SVFG motif. Overall, using the BLAST network server to identify proteins with similarities to residues 445–650, the smallest sum probabilities, $P(n)$, are 6.9×10^{-17} ($n = 3$) for rat NUP153 (18), 2.1×10^{-10} ($n = 5$) for nucleoporin NSP1, and 3.6×10^{-5} ($n = 3$) for NUP1 (GenBank accession nos. P20676, P14907, A44345, respectively). For this reason we suggest the name RANUP96p for the protein encoded by F59A2.1.

Given the possibility that the Ran GTPase functions in both RNA and protein nuclear transport through the nuclear pore complex (10, 25), it is tempting to speculate that RANUP96p may act as a nucleoporin that regulates transport in a Ran–GTP-dependent fashion. It will be of interest to identify mammalian homologs of RANUP96p. The identification of the RanBD sequence motif will also facilitate the cloning of related members of the family of RanBPs and provides a foundation for mutational analysis of the structure and cellular functions of these proteins.

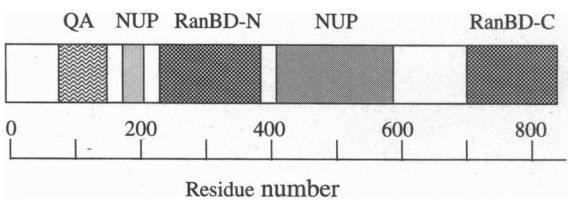

FIG. 5. Structure of *C. elegans* F59A2.1 open reading frame. The open reading frame encodes 877-amino acid residues. Two RanBD motifs are present, the first between residues 248–424 and the second between residues 723–870. Residues 90–154 are extremely rich in glutamine residues and 40–50% identical to similar regions in transcription factor TFIID, zeste, and gal11 (QA domain). NUP, residues 170–223 and 445–680 possess similarities to nucleoporins (19, 20). The region 445–680 is 42–47% identical to repetitive regions in rat NUP153 (residues 712–751, 781–829, and 841–897). We propose the name RANUP96p for the protein encoded by F59A2.1.

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