The NTF2 Gene Encodes an Essential, Highly Conserved Protein That Functions in Nuclear Transport in Vivo*

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The small protein p10/Ntf2p has been implicated in protein import in vitro (Moore, M. S., and Blobel, G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10212–10216; Paschal, B. M., and Gerace, L. (1995) J. Cell Biol. 129, 925–937). Here we present the first evidence that demonstrates an essential in vivo role for the NTF2 gene product in nuclear transport. The NTF2 locus was identified in a screen for temperature-sensitive Saccharomyces cerevisiae mutants defective in the localization of nuclear proteins. Genetic analysis demonstrates that the NTF2 gene is essential for viability in budding yeast. Two temperature-sensitive mutants, ntf2-1 and ntf2-2, that each contain single point mutations in highly conserved amino acid residues show defects in the localization of nuclear proteins but not in the export of poly(A)⁺ RNA following a shift to the nonpermissive temperature. An epitope-tagged version of Ntf2p was used to show that the protein is concentrated at the nuclear envelope. Finally, the human gene under the control of the yeast promoter fully substitutes for the deleted yeast gene. Taken together, these results demonstrate the exquisite functional conservation of this protein throughout evolution and indicate a critical role in nuclear transport.

Bi-directional transport of macromolecules across the nuclear envelope occurs through nuclear pores that are proteinaceous channels in the membrane bilayer (1, 2). In addition to the nuclear pores, a number of soluble factors are required for the movement of both proteins and RNA across the nuclear envelope. Recent work has identified a number of these factors and presented models for their roles in the transport reaction (3–5). GTP hydrolysis mediated by the small GTP-binding protein Ran is required both for the import and export of proteins and for the export of poly(A)⁺ RNA (6–9). In addition, a number of factors that regulate the GTP hydrolysis activity of Ran play critical roles in transport. These proteins include the cytoplasmic GTPase activating protein RanGAP (10–13), the cytoplasmic Ran binding protein 1 (RanBP1) (14–17), and the nuclear import of proteins to the nucleus, they were unable to address the nuclear import process. In a second study, Ntf2p was identified as the necessary transport component that was depleted from cytosols following incubation with the HeLa cell O-linked glycoprotein p62 (35), a component of the nuclear pore (36). These results suggested that not only is Ntf2p important for transport to the nucleus in vitro but that it also interacts with the nuclear pore. While these experiments led to the proposal that the NTF2 gene product was important for the import of proteins to the nucleus, they were unable to address their in vivo role of the protein. The data reported here is the first demonstration of an essential in vivo function for Ntf2p in the nuclear transport process.

EXPERIMENTAL PROCEDURES

Strains and Plasmids—The temperature-sensitive yeast library that was generated by UV mutagenesis of the parent strains FY86 (MATα, ura3-52, leu2-211, his3Δ200) and FY23 (MATa, ura3-52, leu2-211, trp1-63) was the generous gift of Dr. Charles Cole (Dartmouth University) (37). The plasmid pPS902 was isolated from a Ycp50 genomic library (URA3, CEN) (38) by complementation of the temperature-sensitive strain PSY54 (MATα, ura3-52, leu2-211, his3Δ200). Plasmid pPS882 was generated by cloning the 4.3-kb Sad-PstI genomic fragment from pPS902 into pRS315 (LEU2, CEN) (39)); plasmid pPS5930 was generated by cloning the NsiI-SacI genomic fragment from pPS902 into pRS315 (LEU2, CEN) (39)); plasmid pPS5883 was generated by cloning the 3.8-kb XbaI genomic fragment, containing the NTF2 coding region, from pPS902 into pRS316 (URA3, CEN) (39)); plasmid pPS916 was created by

YEPD, yeast extract plus dextrose; PCR, polymerase chain reaction.
Ntf2p Is Essential for Nuclear Transport in Vivo

cloning the 1.9-kb Xbal-BglII genomic fragment encoding NTF2 from pPS902 into pRS316, and plasmid pPS917 was made by cloning the 7.9-kb Nsi I-NcoI genomic fragment from pPS902 into pRS315 (see Fig. 1). The ntfl-1 (pPS919) and ntfl-2 (pPS920) plasmids were created by gap-repair ligation (40) of the mutant PCR products (see below) into pSP882 cut with XbaI and BglII. The diploid strain used for the deletion of the NTF2 open reading frame was engineered at the MATa (MATa/α) 3-52, ura3-52, leu2a1 containing pSP882) was generated by sporrulating the diploid strain PSY853 (NTF2/NTF2:His3, ura3-52/ ura3-52, leu2a1/leu2a1, his3a200/hihis3a200). The haploid strain PSY852 (MATa, NTF2::His3, ura3-52, leu2a1 containing pSP882) was generated by sporulating the diploid strain PSY853 (NTF2/NTF2:His3, ura3-52/ura3-52, leu2a1/leu2a1, his3a200/hihis3a200 containing pSP882). The plasmid for the expression of an N-terminal fusion to GFP (41) has been described previously (42). The epitope-tagged version of Ntf2p was expressed in-frame with 45 bp directly 5' of the NTF2 start site and a XhoI site 3' of the stop codon for expression of the GFP fusion protein (pSP886). Either pPS334 encoding the SV40-NLS fused to γ-galactosidase (43) was employed as a galactose-inducible reporter for the localization of nuclear proteins.

Mutant Screen—Strains were grown in YEFP at 25 °C to a concentration of 1 × 10^7 cells/ml and then shifted to the nonpermissive temperature, 36 °C, for 2 h. Cells were fixed and prepared for indirect immunofluorescence as described previously (44). Samples were stained with polyclonal antibodies directed against the nuclear protein Npl3p (45) or an artificial reporter protein, SV40-NLS, or invertase (1:10,000 dilution) (42) was followed by incubation with FITC-labeled anti-rabbit antibodies (Jackson ImmunoResearch) at a 1:50 dilution (50). DAPI was used to visualize chromatin.

RNA Localization—This assay was carried out as described by Amberg et al. (37) with a number of modifications. Cells were grown, fixed, and digested as described above for indirect immunofluorescence microscopy. After cells were adhered to slides they were permeabilized with 0.1% Triton X-100 in P solution for 10 min. Cells were washed once in P solution and equilibrated in 0.1 m triethanolamine (TEA), pH 8.0, for 2 min followed by incubation in 0.1 m TEA, 0.25% acetic anhydride for 2 min. The slides were then incubated in 0.1 m TEA, 0.25% acetic anhydride buffer (50 mmol deionized formamide, 4 × SSC, 1 × Denhardt's solution, 125 μg/ml RNA, 10% dextran sulfate, 500 μg/ml denatured salmon sperm DNA). The digoxigenin-labeled oligo(dT) probe (50-mer) was labeled with a Genius 6 kit (Boehringer Mannheim). Hybridization was carried out overnight at 37 °C. Slides were washed for 2 min in 0.1 × SSC, then in 0.1 × SSC, then incubated in antibody blocking buffer (0.1 m Tris, pH 9.0, 0.15 m NaCl, 5% heat-inactivated fetal calf serum, 0.3% Triton X-100). Samples were incubated in antibody (FITC-conjugated anti-digoxigenin) diluted 1:200 in Antibody blocking buffer for 2 h. Slides were washed twice (10 min and then 30 min) in Antibody wash 1 (0.1 m Tris, pH 9.0, 0.15 m NaCl) and then twice (10 min then 30 min) in Antibody wash 2 (0.3 m Tris, pH 9.5, 0.1 m NaCl, 50 mm MgCl2). As with indirect immunofluorescence microscopy, DAPI was used to visualize chromatin.

Epitope-tagged Ntfl—In order to generate an epitope-tagged version of Ntflp, a unique Sall site was introduced into the coding region of NTF2 at the N terminus, which is the region of the protein that is least conserved. The altered protein (pPS950) was functional as it was able to complement the temperature-sensitive ntf2 mutant. The epitope-tagged version of Ntflp was made by inserting digonucleotides encoding the 11-amino acid myc epitope (EKLISEEDLN) engineered with in-frame Sall sites. This generated a functional epitope-tagged version of Ntflp (pPS951). The proper insertion of the myc epitope tag was verified by sequence analysis and immunoblotting (see Fig. 7A).

Immunoblotting—To confirm the presence of the myc epitope in the NTF2 coding region, immunoblotting of strains carrying pPS951 was carried out. Wild type strains were transformed with the plasmid and grown in synthetic media lacking uracil 2% glucose as the carbon source to a concentration of 3 × 10^7 cells/ml. Samples were prepared as described previously (13) and resolved on 15% polyacrylamide gels (47). Immunoblotting and immunoreactive protein bands were visualized with the ECL chemiluminescence kit (Amersham Life Sciences, Inc.). Localization of Ntflp—Ntflp was localized either by indirect immunofluorescence microscopy using the epitope-tagged version of the protein or by examining the localization of a functional GFP fusion protein in living cells. For indirect localization, cells were transformed with the wild type strain FY86 was transformed with pPS951 encoding the myc-tagged Ntf2p. Cultures were grown in synthetic media lacking leucine to maintain the plasmid. Cells were diluted with 0.5 mmol/glycine, fixed in ice-cold methanol for 30 min (13), and then processed as described above for indirect immunofluorescence microscopy. Proteins were visualized by incubation with rabbit anti-myc (9E10) tissue culture supernatant (48). Immunoblots were probed with the monoclonal anti-myc antibody 9E10 (1:200 dilution). Immunoreactive bands were visualized with the ECL chemiluminescence kit (Amersham Life Sciences, Inc.).
**RESULTS**

In order to isolate components of the nuclear transport system, we chose to screen for temperature-sensitive yeast mutants defective in the localization of nuclear proteins. The temperature-sensitive library used was generated by ultraviolet mutagenesis of the wild type strains FY86 and FY23 (37). Each temperature-sensitive mutant was grown in YEPD at the permissive temperature (25°C) and then shifted to the nonpermissive temperature (36°C) for 2 h. Following the shift to the nonpermissive temperature, cells were fixed for indirect immunofluorescence microscopy, and the localization of the nuclear protein, Npl3p, was examined. After screening approximately 1200 mutant strains, six strains that mislocalized the nuclear protein were identified. Each strain was crossed to strains containing mutations in genes that had previously been implicated in nuclear transport including \(\text{rna1–1} \) (12), \(\text{npl3–1} \) (19), and \(\text{prp20–1} \) (13, 19, 37) to identify mutants in these known genes. This approach identified an allele of \(\text{prp20} \) which confirmed the ability of this screen to identify mutants in the nuclear transport machinery. Therefore, further characterization of the unidentified mutants was undertaken.

Identification of the NTF2 Locus—One mutant was found to have a defect in the localization of both Npl3p and the reporter protein H2B-NLS-b-galactosidase (data not shown). The mutant strain was back-crossed three times to the parent strain (FY23) to yield the working strain PSY854. In all tetrads examined, temperature sensitivity and protein mislocalization co-segregated as a single locus suggesting that a single mutation was responsible for both phenotypes. The genomic DNA corresponding to the mutant locus was cloned by complementation of the temperature-sensitive phenotype by transforming the strain with a yeast genomic library (38). Several clones that rescued the temperature-sensitive phenotype were identified. Restriction digests demonstrated that all clones contained overlapping inserts. The clone that contained the smallest complementing fragment was chosen for further analysis. This clone, pPS902, contained a 13-kb insert that mapped to a previously sequenced region of chromosome V corresponding to approximately 7000–20,000 bp of cosmid 9537 (Genbank Accession U18778). Subcloning demonstrated that the fragment responsible for restoring both temperature resistance and correction localization of nuclear proteins was a 7.9-kb \(\text{NsiI-NcoI} \) fragment encoding two complete genes transcribed in opposite directions from one another (Fig. 1, panel A). The first gene corresponds to a 3.5-kb open reading frame encoding the \(\text{PSL1} \) gene that is allelic with the previously described \(\text{sec3} \) locus (51, and the other gene is the NTF2 gene.

**Fig. 1. Cloning of NTF2 and comparison of predicted amino acid sequences of human PP15 and yeast NTF2.** A, a schematic of the region of \(\text{S. cerevisiae} \) chromosome V containing the NTF2 gene is shown. The open reading frames including \(\text{PSL1/SC3}, \text{NTF2}, \) and \(\text{SRP1} \) (Serine Rich Protein) are indicated by the arrows. Important restriction endonuclease sites are noted. The subclones used to identify the defects in the PSY854 mutant are flanked by the letters indicating the restriction enzymes used to create them. The \(\text{NsiI-NcoI} \) fragment corresponds to pPS917; the \(\text{NsiI-SaiI} \) fragment corresponds to pPS903; the \(\text{XbaI-XbaI} \) fragment corresponds to pPS853; and the \(\text{XbaI-BglII} \) fragment corresponds to pPS916. The ability of these plasmids to complement the temperature-sensitive (TS) and protein localization (Localization) phenotypes are shown on the right. The SpeI sites used to create the probe for Southern blotting are indicated. The deletion found in the PSY845 mutant strain is indicated at the top. B, the amino acid homology between the human PP15 protein and Ntf2p from \(\text{S. cerevisiae} \) is shown. Identical amino acids are shaded. The site of insertion of the myc epitope is indicated by the underlined amino acid (L). This is also the amino acid altered in the epitope-tagged version of Ntf2p (L to V). The mutations found in ntf2–1 and ntf2–2 are indicated by asterisks. The mutation in ntf2–1 changes amino acid 83 from methionine (M) to threonine (T). The ntf2–2 mutation changes amino acid 91 from aspartic acid (D) to glycine (G).
S2). The second gene (YER009w) encodes a small protein identified by homology to the small human protein PP15 (Fig. 1, panel B) (33) and later shown to encode a homologue of p10/NTF2 (34, 35, 53). In order to determine which gene was mutated in strain PSY854, further subcloning was undertaken to separate the two open reading frames. This analysis revealed that the temperature sensitivity and the nuclear localization defect were separable. A 4-kb NsiI-ScaI fragment encoding only the PSL1/SEC3 gene (pPS903) was able to restore temperature resistance; however, the same probe was unable to rescue the protein localization defect (Fig. 1, panel A). In contrast, either a 3.8-kb XbaI fragment (pPS883) or a 1.9-kb XbaI-BglII fragment (pPS916) was able to restore protein localization to the mutant but was unable to restore temperature resistance (Fig. 1, panel A). Thus, it seemed that the two phenotypes resulted from changes in both genes. In order to determine the nature of the mutation in the mutant strain PSY854, Southern blotting was carried out. A 900-bp SphI fragment corresponding to the N terminus of PSL1/SEC3 and a portion of the NTF2 open reading frame was used to probe genomic DNA prepared from either the mutant strain, PSY854, or the parent strain, FY86. Results indicate that the mutant strain contains a deletion of approximately 250 bp in this region (see Fig. 1, panel A). Subsequent mapping with smaller probes indicates that the small deletion eliminates the start codon of the PSL1/SEC3 gene and a portion of the NTF2 promoter. Since one phenotype of deletion of the PSL1/SEC3 gene is temperature sensitivity on rich media (52) and no defect in nuclear transport is observed, we conclude that the temperature-sensitive phenotype results from deletion of the functional PSL1/SEC3 gene and that the nuclear transport defect results from a deletion of a portion of the promoter region of the NTF2 gene.

In order to confirm that there was no mutation in the coding region of the NTF2 gene, this portion of the genome was cloned by gap-repair from the mutant strain (40, 54) and completely sequenced. No mutations were identified. Consistent with a deletion in the region between the two genes the NTF2/PSL1 promoter could not be gap-repaired. Finally, to demonstrate that this locus was genetically linked to the observed phenotypes, a strain carrying a complete deletion of PSL1 was crossed to the mutant strain, PSY854. The resulting diploid strain was temperature-sensitive, and all spores generated from the diploid were also temperature-sensitive. These findings confirmed that the temperature sensitivity of the PSY854 strain is linked to the loss of a functional PSL1/SEC3 gene. However, the nuclear import defect associated with the promoter mutation in the NTF2 locus suggested that Ntf2p might be important for nuclear transport and led us to investigate further the in vivo function of Ntf2p.

NTF2 Is Essential for Viability in Saccharomyces cerevisiae—In order to determine whether the NTF2 gene was essential for viability, the entire NTF2 open reading frame was amplified at the ATG and ending at the stop codon was replaced with the HIS3 gene in the diploid strain PSY845. This strain was sporulated, and tetrads were dissected. In all cases only two viable spores were recovered, and they were never HIS’. (data not shown). Furthermore, when the diploid strain was transformed with pPS853 (URA3, CEN) encoding NTF2, four viable spores were occasionally obtained, and the spores that were HIS’ were never able to grow on FOA demonstrating that they were unable to live without the wild type plasmid encoding NTF2. These results indicate that the NTF2 gene is essential for viability in S. cerevisiae. An identical finding was recently reported by Nehrbass and Blobel (53).

Novel Temperature-sensitive Alleles of ntf2—To confirm the results obtained with the mutant strain, PSY854, and demonstrate that NTF2 is required for nuclear transport in vivo, we generated new conditional alleles of NTF2 using the PCR mutagenesis and plasmid shuffling technique described under "Experimental Procedures" (41). Five plasmids that allowed growth at 25 °C but not at the restrictive temperature of 36 °C were chosen for further analysis. The plasmids were retransformed into the deletion strain by a plasmid shuffle, and in all five cases the temperature-sensitive phenotype was found to be plasmid-linked. The inserts encoding NTF2 on all five plasmids were completely sequenced. In all cases, single base changes were identified within the coding region of NTF2 that resulted in a change in a single conserved amino acid residue. In four of the plasmids the same mutation was identified. This mutation changed base 248 from a T to a C and resulted in the change of aspartic acid (D) to glycine (G) and was designated ntf2–2 (Fig. 1, panel B).

To characterize the growth of the mutant strains, the mutant plasmids, pPS919 and pPS920, were retransformed into the NTF2 deletion strain, PSY852, by plasmid shuffle. As a control the plasmid pPS882 encoding wild type NTF2 was employed. Cultures were grown at the permissive temperature to a concentration of 0.2 \times 10^7 cells/ml in media lacking leucine or in YEPD. Cultures were then split, and half was maintained at the permissive temperature (25 °C), and half was shifted to the nonpermissive temperature (36 °C) as described under "Experimental Procedures." The relative increase in cell number (Fold Cell Growth) is plotted versus time. The point at which cells were shifted to the nonpermissive temperature is indicated by the arrow. Results are indicated for the wild type (○), ntf2–1 (●), and ntf2–2 strains (□).
of the SV40-NLS fused to invertase (42). Both wild type and mutant strains were first grown at the permissive temperature to a concentration of 1 × 10^7 cells/ml in media lacking leucine with raffinose (2%) as the carbon source. Galactose was then added to a final concentration of 2%, and cultures were induced for 2 h at 25 °C. Following the induction, cultures were split, and half was maintained at 25 °C (panel A), and half was shifted to the nonpermissive temperature, 36 °C (panel B). The reporter protein was detected with a polyclonal antibody against invertase (A, D, G) and Nomarski images are shown (C, F, G).

To examine further the role of Ntf2p in protein transport, the localization of an endogenous nuclear protein Npl3p (45) was examined. Wild type cells were able to efficiently localize this endogenous nuclear protein Npl3p (45) was efficiently localized to the nucleus in wild type cells (A) but is lost throughout the cell in ntf2–1 (D) and ntf2–2 (G) strains. The corresponding DAPI (B, E, H) and Nomarski (C, F, I) images are shown.
culture supernatant (Fig. 6, panel A). A band of approximately 14-kDa molecular mass is observed only in the strain carrying the epitope-tagged version of Ntf2p. The predicted molecular mass of *S. cerevisiae* Ntf2p is 14.3 kDa. These results confirm the expression of a functional epitope-tagged Ntf2p.

The epitope-tagged Ntf2p was employed to determine the intracellular localization of the protein using indirect immunofluorescence microscopy. Ntf2p was also localized in living cells using a functional Ntf2p fusion protein consisting of the NTF2 open reading frame with GFP fused to the N terminus. Previous work has shown that GFP fusion proteins are useful for the intracellular localization of proteins in living cells because in the absence of any other protein moieties the GFP protein is localized diffusely throughout the cell (44, 58). Similar results were obtained regardless of whether Ntf2p was localized by indirect immunofluorescence or in live cells as a GFP fusion protein (Fig. 6).

In living cells expressing the Ntf2p-GFP fusion protein a punctate fluorescent pattern is observed (Fig. 6, panel B). This pattern is strikingly reminiscent of staining observed with numerous anti-nucleoporin antibodies (1). To determine whether Ntf2p co-localizes with nucleoporins, wild type cells were co-stained with anti-myc and anti-Nup159p antibodies (49). Results indicate that Ntf2p is localized in a pattern similar to that observed for Nup159p (Fig. 6, compare panels C and D). Since Nup159p has previously been shown to be a component of the nuclear pore (49, 59), the anti-Nup159p staining defines the nuclear rim. Thus, Ntf2p is concentrated at nuclear pores, the site at which bi-directional transport into and out of the nucleus occurs. In control experiments, no anti-myc staining was observed in cells transformed with vector alone or with a plasmid encoding untagged Ntf2p.

The NTF2 Gene Is Highly Conserved—Yeast Ntf2p and the human PP15 protein are 45% identical to one another at the amino acid level. In order to determine whether the two proteins are functionally conserved, we tested whether the human gene could replace the yeast gene. The human PP15 (NTF2) gene was amplified from a Jurkat cell cDNA library as described under "Experimental Procedures." To confirm that the myc-tagged protein was expressed, the plasmid encoding it, pPS951, was transformed into wild type cells. As controls, the wild type strain was either transformed with a vector, with an untagged version of Ntf2p (pPS950), or with a plasmid encoding another myc-tagged protein, Prp20p-myc. Lysates from each strain were made, and 10 µg of protein from each was resolved by polyacrylamide gel electrophoresis through 15% gels. An immunoblot is shown. The lanes are as follows: vector alone (1), untagged-Ntf2p (2), myc-tagged Ntf2p (3), and myc-tagged Prp20p (4). The positions of the bands for Ntf2p and Prp20p are indicated on the right. The molecular masses in kDa are indicated to the left. B, Ntf2p was localized either in living cells using the GFP fusion protein or by indirect immunofluorescence using the myc-tagged Ntf2p. Ntf2p was expressed as a fusion protein with GFP in wild type cells. The living cells viewed either under the FITC filter (A) or by Nomarski optics (B) are shown. Methanol-fixed cells stained with anti-myc antibodies (1:200 dilution) show a pattern of punctate nuclear rim staining (C) that is similar to that observed with the GFP fusion protein in living cells. This punctate staining is identical to that obtained in the same cells with an anti-nucleoporin antibody, anti-Nup159p (D) which defines the nuclear envelope (49).

**FIG. 5.** *ntf2–1* and *ntf2–2* mutant cells do not accumulate poly(A)+ RNA in the nucleus under conditions where protein localization to the nucleus is defective. A fluorescence in situ assay (37) was used to localize poly(A)+ RNA in wild type (A–C), prp20–1 (D–F), ntf2–1 (G–I), and ntf2–2 (J–L) cells following a 2-h shift to the nonpermissive temperature. Poly(A)+ RNA localization is shown in A, D, G, and J, DAPI in B, E, H, and K, and Nomarski in C, F, I, and L.

**FIG. 6.** Ntf2p is concentrated at nuclear pores. A, an epitope-tagged version of Ntf2p was generated as described under "Experimental Procedures." To confirm that the myc-tagged protein was expressed, the plasmid encoding it, pPS951, was transformed into wild type cells. As controls, the wild type strain was either transformed with a vector, with an untagged version of Ntf2p (pPS950), or with a plasmid encoding another myc-tagged protein, Prp20p-myc. Lysates from each strain were made, and 10 µg of protein from each was resolved by polyacrylamide gel electrophoresis through 15% gels. An immunoblot is shown. The lanes are as follows: vector alone (1), untagged-Ntf2p (2), myc-tagged Ntf2p (3), and myc-tagged Prp20p (4). The positions of the bands for Ntf2p and Prp20p are indicated on the right. The molecular masses in kDa are indicated to the left. B, Ntf2p was localized either in living cells using the GFP fusion protein or by indirect immunofluorescence using the myc-tagged Ntf2p. Ntf2p was expressed as a fusion protein with GFP in wild type cells. The living cells viewed either under the FITC filter (A) or by Nomarski optics (B) are shown. Methanol-fixed cells stained with anti-myc antibodies (1:200 dilution) show a pattern of punctate nuclear rim staining (C) that is similar to that observed with the GFP fusion protein in living cells. This punctate staining is identical to that obtained in the same cells with an anti-nucleoporin antibody, anti-Nup159p (D) which defines the nuclear envelope (49).

DISCUSSION

This study provides the first evidence that the product of the NTF2 gene plays an important role in nuclear transport in vivo. We have shown that the NTF2 gene product is essential for viability in *S. cerevisiae* and that temperature-sensitive yeast strains bearing conditional alleles of NTF2 are unable to efficiently localize proteins to the nucleus but are still able to export poly(A)+ RNA. Furthermore, two different approaches demonstrate that Ntf2p is concentrated at the nuclear envelope in vivo. Finally, Ntf2p function is exceedingly well-conserved as the human NTF2 gene can functionally substitute for the deleted yeast NTF2 gene.
Previous work has shown that Ntf2p is important for the transport of proteins into the nucleus in vitro (34, 35). However, neither of these studies was able to address the in vivo role of Ntf2p. It is only through the use of a genetic system such as S. cerevisiae and the generation of conditional alleles of the gene that an in vivo role can be inferred. Thus, genetic studies provide the essential complement to the biochemical studies that elucidate protein function.

In the past several years a combination of biochemical and genetic approaches has been used to demonstrate that bidirectional transport through the nuclear pores involves a cycle of highly regulated GTP hydrolysis (3, 4, 60). Several proteins that regulate the GTP hydrolysis activity of the small GTP-binding protein Ran have been identified including the exchange factor RCC1/Prp20p (18), the GTPase activating protein RanGAP1/Rna1p (11, 61), and the GAP activating protein RCC1/Prp20p (18). It is likely that the NTF2 gene also functions in the regulation of the Ran-GTP cycle. This hypothesis is consistent with the fact that the Xenopus Ntf2 protein (p10) was isolated in a complex with Ran (34).

The evidence collected thus far suggests that the regulation of Ran function mediated by Ntf2p occurs at the stage of translocation of macromolecules through the nuclear pore. In fact, Ntf2p is not required for targeting of NLS-containing substrates to the nuclear rim in vitro and has previously been shown to interact with the nucleoporin p62 in HeLa cell extracts (35). Our observation that Ntf2p is localized at the nuclear rim in vivo provides further evidence that the function of Ntf2p is carried out at the nuclear pore. Given this information, several models for Ntf2p function can be proposed. For instance, Ntf2p may be involved in recycling the Ran-GTP complex away from the nuclear pore complex after NLS proteins have been appropriately docked or it may function in the dissociation of the Ran-NLS-receptor complex at the pore (32). Further in vivo and in vitro studies will be needed to test these models for Ntf2p function.

Our results suggest that NTF2 plays an important role in the translocation of proteins into the nucleus. Recent work demonstrates that the critical components of the nuclear transport apparatus have been extensively conserved throughout evolution (4). It had been known for some time that the ultrastructure of the nuclear pore complex is conserved (36, 62), and it is now becoming clear that this is also the case for individual components of the pore complex (63). Conservation of structure and function is also the rule for the soluble factors that mediate the transport reaction. For example, human RAN is 82% identical to yeast RAN (Gsp1p) at the amino acid level. Functional conservation is apparent for both the GAP, Rna1p, and the exchange factor, RCC1/Prp20p. Rna1p from the yeast Schizosaccharomyces pombe complements the temperature-sensitive rna1-1 mutant in S. cerevisiae (64) and the human RCC1 protein is able to complement the temperature-sensitive S. cerevisiae mutant prp20-1 (65). Our findings demonstrate that Ntf2p is yet another conserved component of nuclear import machinery.

The data presented in this article suggest that the primary role of Ntf2p is in the import of proteins to the nucleus rather than in the export of poly(A)+ RNA. Under conditions where protein transport into the nucleus is clearly compromised, no nuclear accumulation of poly(A)+ RNA is observed. The same phenotype has been associated with mutants in other components of the nuclear transport system. Thus far mutations in either subunit of the NLS receptor have only been reported to cause defects in the localization of nuclear proteins but not in the export of poly(A)+ RNA (13, 66). It is possible that yet unidentified alleles of these two genes or even of NTF2 may have primary defects in RNA export; however, the current interpretation is that these components contribute to the movement of proteins into the nucleus, whereas other factors function in the export of RNA and/or proteins.

Experiments carried out with Npl3p do suggest that export of protein from the nucleus (at least Npl3p) continues in ntf2 mutants. Npl3p is a poly(A)+ RNA binding protein that shuttles rapidly between the cytoplasm and the nucleus (44, 45, 67). The current model for Npl3p function suggests that Npl3p exits the nucleus in conjunction with poly(A)+ RNA and then is re-imported into the nucleus (41). Therefore the localization of Npl3p is dynamic in nature. The nuclear localization reported for the protein (44, 45) simply reflects the fact that this highly abundant protein is recycled rapidly from the cytoplasm to the nucleus. Npl3p accumulates in the cytoplasm in ntf2 mutants (see Fig. 4). This finding is consistent with a model in which Npl3p continues to exit the nucleus at the nonpermissive temperature (otherwise no cytoplasmic pool would be observed) but re-import is slowed. Although recycling of Npl3p to the nucleus...
is slowed, there is a sufficient nuclear pool to maintain wild type levels of poly(A)\(^+\) RNA export at least until the cells begin to lose viability. Similar results have been obtained with other mutants defective in nuclear transport including the npl3–1 mutant (41, 45). When this mutant strain is grown at the semi-permissive temperature of 30 °C, Npl3p accumulates in the cytoplasm, but no defect in the export of poly(A)\(^+\) RNA from the nucleus is observed (41).

Our results demonstrate that the NTF2 gene product is essential for the appropriate localization of nuclear proteins in vivo, that the protein is concentrated at the site of translocation across the nuclear envelope, and that the function of the protein has been extremely well-conserved throughout evolution. These data complement the previous in vitro results and provide the first evidence for an in vivo role for NTF2 in the import of proteins to the nucleus.

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