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A structure/function analysis of Rat7p/Nup159p, an essential nucleoporin of *Saccharomyces cerevisiae*

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SUMMARY

Rat7p/Nup159p is an essential nucleoporin of *Saccharomyces cerevisiae* originally isolated in a genetic screen designed to identify yeast temperature-sensitive mutants defective in mRNA export. Here we describe a detailed structural-functional analysis of Rat7p/Nup159p. The mutation in the *rat7-1* ts allele, isolated in the original genetic screen, was found to be a single base pair change that created a stop codon approximately 100 amino acids upstream of the actual stop codon of this 1,460 amino acid polypeptide, thus eliminating one of the two predicted coiled-coil regions located near the carboxyl terminus of the protein. These coiled-coil regions are essential since an allele lacking both coiled-coil regions was unable to support growth under any conditions. In contrast, no other region of the protein was absolutely required. The SAFG/PSFG repeat region in the central third of the protein was completely dispensable for growth at temperatures between 16°C and 37°C and cells expressing this mutant allele were indistinguishable from wild type. Deletion of the amino-terminal third of the protein, upstream from the repeat region, or the portion between the repeat region and the coiled-coils resulted in temperature-sensitivity, but the two alleles showed distinct phenotypes with respect to the behavior of nuclear pore complexes (NPCs). Taken together, our data suggest that Rat7p/Nup159p is anchored within the NPC through its coiled-coil region and adjacent sequences. In addition, we postulate that the N-terminal third of Rat7p/Nup159p plays an important role in mRNA export.

Key words: Nuclear pore complex, RNA transport, Yeast, Pore clustering

INTRODUCTION

Nuclear pore complexes (NPCs) are large, complex proteinaceous structures embedded in the nuclear envelope. Each metazoan NPC has a mass of 125 MDa while *S. cerevisiae* NPCs have a mass of approximately 66 MDa. NPCs act as macromolecular gates through which all nucleocytoplasmic transport occurs. High resolution electron microscopy combined with image reconstruction has been used to examine the structure of NPCs. These analyses indicate that NPCs possess eightfold rotational symmetry perpendicular to the plane of the nuclear envelope (NE) and can be divided into four major structural components. The first is the scaffold of the NPC, which contains a cytoplasmic ring, a nucleoplasmic ring and a central ring with spokes (Unwin and Milligan, 1982; Akey and Goldfarb, 1989; Jarnik and Aebi, 1991; Hinshaw et al., 1992; Akey and Radermacher, 1993). The second component is the central transporter, which is involved in active transport of macromolecules that move between cytoplasm and nucleus (Jarnik and Aebi, 1991; Gerace, 1992). The cytoplasmic fibrils that emanate from the cytoplasmic ring and are involved in substrate docking represent the third component. The fourth is the basket-like structure that is attached to the nucleoplasmic ring and protrudes into the nucleoplasm (Richardson et al., 1988; Jarnik and Aebi, 1991; Gerace, 1992; Goldberg and Allen, 1992; Ris and Malecki, 1993). By means of field emission in-lens scanning electron microscopy (FEISEM), this basket has been shown to act in docking of mRNP particles to the NPC and directing them towards the central channel of the pore (Kiseleva et al., 1996).

Approximately 25 nucleoporins have been identified in *S. cerevisiae* using either genetic or biochemical approaches. Based on their sequences, they can be divided into two major groups. The first group includes the repeat-containing proteins. Different *S. cerevisiae* nucleoporins contain XFXFG repeats (Nup1p, Nup2p, Nsp1p) (Hurt, 1988; Davis and Fink, 1990; Loeb et al., 1993) or GLFG repeats (Nup49p, Nup57p, Nup100p, Nup116p, Nup145p) (Wente et al., 1992; Grandi et al., 1993; Wente and Blobel, 1993, 1994; Fabre et al., 1994). In addition, Rat7p/Nup159p contains 22 XXFG repeats (primarily SAFG and PSFG) and these repeats along with the sequences between them can be organized into four tandem nearly perfect 26 amino acid repeats (Gorsch et al., 1995). Another protein, Rip1p (Stutz et al., 1995), contains repeats that are closely related to the repeat region of Rat7p/Nup159p. Rip1p was identified using the yeast two hybrid system, based

on its interactions with the nuclear export signal (NES) of HIV-1 Rev protein. It is uncertain whether Rip1p is a component of NPCs. Degenerate GSXS, GSSX, GSXF, and GFXS repeats have been found in Npl4p and several other nucleoporins, but there are generally only a few of these repeats in a specific nucleoporin and they are often widely separated from one another, so it is not known if these are functional repeats (DeHoratius and Silver, 1996). An interaction between the repeats of nucleoporins and factors involved in nucleocytoplasmic transport is suggested from two hybrid analyses and the direct biochemical interaction of karyopherin β with Nup116p (Iovine et al., 1995). The second broad group of nucleoporins lacks characteristic repeats and includes Rat2p/Nup120p, Rat3p/Nup133p, Rat9p/Nup85p, Nup170p, Nup188p, Nup157p, Nup82p, Nup84p, Seh1p, Nic96p, and Pom152p (Aitchison et al., 1995a,b; Goldstein et al., 1996; Grandi et al., 1995a; Heath et al., 1995; Hurwitz and Blobel, 1995; Li et al., 1995; Nehrbass et al., 1996; Pemberton et al., 1995; Wozniak et al., 1994). Many nucleoporins contain domains with a strong potential to form coiled-coils, a structure that is thought to mediate protein-protein interactions.

Under appropriate extraction conditions, discrete complexes of nucleoporins can be isolated and some appear to perform specific functions. The possible functions of these complexes have been elucidated mainly by determining how deletion or mutation of individual members of a complex affects NPC function (e.g. RNA export, protein import) or biogenesis. For example, Nup120p, Nup85p, Nup84p, Seh1p, and a small fraction of the total Sec13p in cells, along with other proteins, comprise a subcomplex of the NPC (Siniossoglou et al., 1996). When *NUP120, NUP85,* or *NUP84* is deleted, NPCs continue to form but are clustered into one or a few regions of the nuclear envelope, which also shows significant distortions (Aitchison et al., 1995a; Heath et al., 1995; Goldstein et al., 1996; Siniossoglou et al., 1996). The finding that several of these mutant strains have defects in mRNA export, but not in nuclear protein import, suggests that this subcomplex functions specifically in an RNA export pathway. Other complexes, such as the one including Nup188p, Pom152p and Nic96p, are thought to play more of a structural role because these proteins have been localized to the central core region of the pore, and mutations in the genes encoding some of these nucleoporins cause defects in NPC biogenesis and structure (Nehrbass et al., 1996; Zabel et al., 1996). Nsp1p is present in a complex with Nup49p, Nic96p and Nup57p, which is required for protein import into the nucleus (Grandi et al., 1995b).

Using a genetic screen to identify mutant yeast strains with temperature-conditional defects in RNA export at 37°C, our laboratory has isolated ts alleles of *RAT9/NUP85, RAT2/NUP120, RAT3/NUP133, RAT10/NUP145* and *RAT7/NUP159* (Gorsch et al., 1995; Heath et al., 1995; Li et al., 1995; Goldstein et al., 1996; Dockendorff et al., 1997). The *RAT7/NUP159* gene encodes a 1,460 amino acid protein essential for cell viability (Gorsch et al., 1995). Accumulation of poly(A)$^+$ RNA in nuclei occurs rapidly after cells carrying the temperature-sensitive *rat7-1* allele are shifted to the non-permissive temperature of 37°C. Temperature-shifted mutant cells also show severe fragmentation of the nucleolus, dramatically reduced rRNA synthesis, and substantial defects in rRNA processing. No protein import defects are seen when *rat7-1/nup159-1* cells are grown either at 23°C or 37°C. When this strain is grown at the permissive temperature, NPCs are clustered together and this clustering is partially reversed when mutant cells are shifted to the non-permissive temperature. Rat7-1p/Nup159-1p is rapidly lost from NPCs when mutant cells are shifted to 37°C (Gorsch et al., 1995). Immunoelectron microscopy using isolated nuclear envelopes indicates that Rat7p/Nup159p is located on the cytoplasmic side of the NPC (Kraemer et al., 1995).

In order to define the essential domain(s) of the protein and to determine how deletion of various domains would affect nucleocytoplasmic transport, NPC clustering, and retention of Rat7p/Nup159p in NPCs, we constructed a set of deletion mutants of *RAT7/NUP159*. We also determined that the mutation in the *rat7-1/nup159-1* strain creates a termination codon at amino acid 1,365, thereby truncating the protein by 96 amino acids and removing one of the two adjacent domains predicted to form coiled-coil structures. Completely deleting the coiled-coil region created a non-functional allele, indicating that the coiled-coil region is absolutely essential for the function of Rat7p/Nup159p. In contrast, the central repeat domain could be deleted without any phenotypic effects. By evaluating the effect of these mutations on growth, mRNA export, NPC distribution, and Rat7p/Nup159p localization and retention, we were able to establish that the N-terminal domain of Rat7p/Nup159p is important for efficient mRNA export at both 23°C and 37°C and the carboxyl third of Rat7p/Nup159p is involved in anchoring of Rat7p/Nup159p within NPCs.

MATERIALS AND METHODS

Yeast strains, cell culture and growth curves

The yeast strains used in this study are listed in Table 1 and the plasmids used are listed in Table 2. Strains were cultured using standard methods (Sherman, 1991), using rich (YPD, yeast, peptone, dextrose) or defined media (SC, synthetic complete) lacking the appropriate amino acids. Temperature-sensitive mutants were grown at 23°C (permissive temperature) or shifted to 37°C (restrictive temperature). Genetic techniques and plasmid transformations using electroporation were performed using standard methods (Rose et al., 1989; Guthrie and Fink, 1991).

For temperature-shift experiments, cells cultured in liquid media at 23°C were shifted to a 37°C water bath, and incubation was continued as indicated in the figure legends. To prepare growth curves, single colonies were inoculated into 5 ml of YPD and allowed to grow overnight at room temperature. Cell density was determined by measuring the OD_{600} using a Beckman DU-30 spectrophotometer. Duplicate cultures of each strain were diluted to between OD_{600} 0.04 and 0.06 in YPD. One duplicate was incubated at 23°C and the other at 37°C. Duplicate samples were removed from each culture after various times up to 26 hours and cell density determined by measuring OD_{600}.

Mapping the mutation in the *rat7-1* ts allele

The mutation in the *rat7-1* allele was mapped using the gap repair technique (Rothstein, 1991). pLG4 was digested with *Eco*47III (5,341) and *Hpa*I (1,341) to delete the N-terminal domain, and with *Eco*47III (5,341) and *Eco*NI (6,930) to delete the C-terminal domain. Large linear plasmid fragments were purified by agarose gel electrophoresis and then transformed into the LGY101 (*rat7-1*) yeast strain. Growth on plates containing SC-Leu selected for repair of the plasmid using the chromosomal mutant copy of the *RAT7/NUP159* gene. A *rat7-1* strain harboring a plasmid where the C-terminal domain was derived from the genomic *rat7-1* allele was unable to

Table 1. Yeast strains

Strain	Genotype	Comments
FY23	*MATs ura3-52 trp1Δ63 leu2Δ1*	Wild type; derived from S288C, obtained from Dr Fred Winston
FY86	*MATα ura3-52 trp1Δ63 leu2Δ1*	Wild type; derived from S288C, obtained from Dr Fred Winston
LGY108	*MAT**a** ura3-52 his3Δ200 leu2Δ1 RAT7::HIS3* (pLG4)	*RAT7* null strain transformed with a plasmid expressing wild-type Rat7p/Nup159p
LGY109	*Matα RAT7::HIS3 his3Δ200 ura3-52 leu2Δ1* (pLG4)	*RAT7* null strain transformed with a plasmid expressing wild-type Rat7p/Nup159p
VDPY121	*Matα RAT7::HIS3 his3Δ200 ura3-52 leu2Δ1* (pVDP16)	*RAT7* null strain transformed with a plasmid expressing Rat7-ΔNp
AMY101	*Matα RAT7::HIS3 his3Δ200 ura3-52 leu2Δ1* (pAM1)	*RAT7* null strain transformed with a plasmid expressing Rat7-ΔRp
AMY102	*Matα RAT7::HIS3 his3Δ200 ura3-52 leu2Δ1* (pAM2)	*RAT7* null strain transformed with a plasmid expressing Rat7-ΔCp
VDPY106	*Matα RAT7::HIS3 his3Δ200 ura3-52 leu2Δ1* (pVDP6)	*RAT7* null strain transformed with a plasmid expressing Rat7-ΔC_Ip
VDPY104	*Matα RAT7::HIS3 his3Δ200 ura3-52 leu2Δ1* (pLG4/pVDP4)	*RAT7* null strain transformed with plasmids expressing wild-type Rat7p and Rat7-Δ_{cc}p
VDPY123	*Matα RAT7::HIS3 his3Δ200 ura3-52 leu2Δ1* (pSD3)	*RAT7* null strain transformed with a plasmid expressing rat7-1p
VDPY122	*Matα RAT7::HIS3 his3Δ200 ura3-52 leu2Δ1* (pVDP17)	*RAT7* null strain transformed with a plasmid expressing Rat7-Cp

Table 2. Plasmids

Plasmids	Markers	Comments
pLG7	*LEU2 CEN4 Amp^r*	YCplac111 [Δ(*Hin*dIII-*Sma*I)] containing the *RAT7/NUP159* gene
pVDP16	*LEU2 CEN4 Amp^r*	derived from pLG7 and encoding Rat7-ΔNp
pAM1	*LEU2 CEN4 Amp^r*	derived from pLG7 and encoding Rat7-ΔRp
pAM2	*LEU2 CEN4 Amp^r*	derived from pLG7 and encoding Rat7-ΔCp
pVDP6	*LEU2 CEN4 Amp^r*	derived from pLG7 and encoding Rat7-ΔC_Ip
pVDP4	*LEU2 CEN4 Amp^r*	derived from pLG7 and encoding Rat7-Δ_{cc}p
pSD3	*LEU2 CEN4 Amp^r*	YCplac111 containing the *rat7-1* allele
pVDP17	*LEU2 CEN4 Amp^r*	derived from pLG7 and encoding Rat7-Cp under control of the *RAT7/NUP159* promoter
pLG4	*URA3 RAT7 CEN*	YcpLac33 containing the *RAT7* gene

grow at 37°C, indicating that the copy of *RAT7/NUP159* created by gap repair carried the mutation that confers temperature sensitivity. This plasmid was retrieved from this strain, amplified in bacteria, and sequenced using an Applied Biosystems Model 373A DNA sequencer.

Construction of the *RAT7/NUP159* deletion mutants

The N-terminal deletion was made by using a PCR-based approach (Eisinger and Trumpower, 1997). A DNA fragment between *Stu*I (1,472) and the beginning of the *RAT7/NUP159* open reading frame (ORF) was amplified using as primers 5′-CATCCTGCAGAGAAGA-CATGTTATAATGGTTC-3′ and 5′-GCAGATTCAGGCCTGACTA-CATTGTCG-3′; in addition, a *Pst*I site was created at the beginning of *RAT7/NUP159* ORF. pLG7 was cut with *Pst*I (3,405) and *Stu*I (1,472) and ligated to the PCR fragment which had been previously digested with these two enzymes. The repeat domain deletion was made by cutting pLG7 with *Pst*I (3,405) and *Spe*I (4,730) and religating the resulting large DNA fragment using an oligonucleotide linker that introduced an *Eco*RI site. The C-terminal deletion was made by cutting pLG7 with *Bgl*II (6,031) and *Bam*HI (7,648) and religating the resulting plasmid. The C terminus internal deletion was made by cutting pLG7 with *Spe*I (4,730) and *Bst*II07I (5,811), isolating the large DNA fragment, filling in the overhanging ends using the Klenow fragment of DNA polymerase, followed by ligation. The deletion of the coiled-coil domains was made by digesting pLG7 with *Bst*II07I (5,811) and *Pml*I (6,209), isolating the large DNA fragment, followed by ligation. To make the construct where only the carboxy terminus of Rat7p/Nup159p is being expressed, a DNA fragment between *Stu*I (1,472) and the beginning of *RAT7/NUP159* ORF was amplified using as primers for PCR 5′-CCTTCAAAGGGC-CCATGTTATAATGGTTCTGTAATC-3′ and 5′-GCAGATTCAGGC-CTGACTACATTGTCG-3′; in addition an *Apa*I site was created at the beginning of the *RAT7/NUP159* ORF. pLG7 was digested with *Apa*I (4,745) and *Stu*I (1,472) and ligated to the PCR product which had been previously digested with these two enzymes.

In all the cases, the plasmids containing the deletion constructs were transformed into LGY108 (containing a disruption of *RAT7/NUP159* and containing *RAT7/NUP159* on a URA3*/CEN* plasmid). Colonies were selected on SC-Leu plates, and the transformants plated onto 5-FOA-containing medium. The nature of the deletions was confirmed by restriction endonuclease digestion patterns, DNA sequencing, and an analysis of the immunoreactivity of the mutant proteins with antibodies directed against distinct portions of Rat7p/Nup159p.

In situ hybridization and immunofluorescence assays

In situ hybridization assays for poly(A)$^+$ RNA localization and indirect immunofluorescence techniques for protein localization have been described previously (Amberg et al., 1992; Copeland and Snyder, 1993; Gorsch et al., 1995; Li et al., 1995). Antibody against Rat7p/Nup159p raised in guinea pigs (Gorsch et al., 1995) was used at a 1:3,000 dilution. FITC-conjugated goat anti-guinea pig IgG (Vector Laboratories Inc., Burlingame, CA) was used at 1:250 dilution. Antibody against the carboxyl third of Rat7p/Nup159p was raised in rabbits (Cocalico Biologicals, Reamstown, PA) and used at a 1:500 dilution. FITC-conjugated goat anti-rabbit IgG (Vector Laboratories Inc., Burlingame, CA) was used at a 1:1,000 dilution. RL-1 monoclonal antibody (a gift from Larry Gerace, Scripps Research Institute, La Jolla, CA) (Snow et al., 1987) was used at a dilution of 1:200. FITC-conjugated goat anti-mouse IgM (Vector Laboratories Inc.) was used at a dilution of 1:600. Monoclonal antibody 2.3B (which recognizes nucleolar Nsr1p in yeast) (a gift from M. Snyder, Yale University, New Haven, CT) was used at a dilution of 1:10. FITC-conjugated goat anti-mouse IgG (Vector Laboratories Inc.) was used at a dilution of 1:600.

For all fluorescence microscopy, images were obtained by using a cooled CCD (charge-coupled device) camera. Identical exposure con-

ditions were used for all comparable images within each figure; that is, all FITC images within a single figure were obtained using the same exposure conditions, as were all DAPI images, though the exposure conditions for FITC images and for DAPI images were not the same. Composites were prepared using Adobe Photoshop without altering the images.

Western blotting

Cells were pelleted by centrifugation at 2,500 rpm for 2.5 minutes and washed once with water. 200 mg of acid washed glass beads and 200 μl of hot sample buffer (62.5 mM Tris-Cl, pH 6.8, 2% SDS, 10% glycerol, 8 M urea, 0.72 M 2-mercaptoethanol and 0.05% Bromophenol Blue) were added to the cell pellets. After brief vortexing, cells were transferred to a 100°C water bath for 3 minutes. Cells were then lysed as follows: 10 seconds vortexing and 50 seconds incubation in a 100°C water bath, repeated 5 times during 5 minutes. The supernatant was then transferred to a clean Eppendorf tube and equal volumes of cell lysate were loaded onto a 10% SDS-polyacrylamide gel. The gel was run at 200 V for approximately 50 minutes. Proteins in the gel were transferred to a PVDF membrane by electroblotting overnight at 100 mA in a coldroom. The membrane was briefly washed with a 1× PBS, 0.1% Tween-20 solution (solution A) and then blocked for 1 hour with a 1× PBS, 5% nonfat milk solution, 0.1% Tween-20 (solution B). Membranes were incubated with anti-Rat7p/Nup159p antibodies raised against the repeat domain (1:40,000 dilution), the N-terminal domain (1:6,000 dilution), or the C-terminal domain (1:10,000 dilution) in solution B for 2 hours. Four washes of 10 minutes each were done with solution A. The membrane was then incubated for 1 hour with anti-guinea pig or anti-rabbit secondary antibody coupled to horseradish-peroxidase (Amersham Inc., Arlington Heights, IL), diluted 1:10,000 in solution B.

Electron microscopy

S. cerevisiae were examined by electron microscopy using previously described protocols (Byers and Goetsch, 1975; Wright and Rine, 1989; Goldstein et al., 1996). Sections were cut on a Sorvall MT5000 ultramicrotome with a section thickness of 90 nm and examined after staining on a JEOL 100CX electron microscope at 80 kV.

RESULTS

The coiled-coil domain of Rat7p/Nup159p is essential for its function

The *RAT7/NUP159* gene was identified previously in a genetic screen for temperature-sensitive yeast strains that accumulated poly(A)$^+$ RNA in their nuclei when shifted to 37°C (Gorsch et al., 1995). When cells carrying the *rat7-1/nup159-1* allele are shifted from 23°C to 37°C, a rapidly occurring defect in nucleocytoplasmic export of poly(A)$^+$ RNA occurs in 100% of the cells. Under these conditions, the mutant Rat7-1p/Nup159-1p protein is lost from the nuclear rim and NPCs, which are clustered together in mutant cells grown at 23°C, become considerably less clustered (Gorsch et al., 1995).

Rat7p/Nup159p can be divided into three distinct domains (Fig. 1). The N-terminal domain (amino acids 1-456) lacks characteristic motifs. The central domain (amino acids 456-900) contains 22 XXFG repeats (mainly SAFG and PSFG) as well as three of the XFXFG repeats that are also found in Nup1p, Nsp1p, and Nup2p (Davis and Fink, 1990; Nehrbass et al., 1990; Loeb et al., 1993). The C-terminal domain (amino acids 900-1,460) contains two nearby regions with a high probability of forming coiled-coil structures (Lupas et al., 1991).

Rat7p/Nup159p is essential for cell viability. In order to identify its essential domain(s), we constructed deletion mutants lacking various portions of *RAT7/NUP159* and transformed them (on *CEN/LEU2* plasmids) into a *RAT7/NUP159* null strain carrying wild-type *RAT7/NUP159* on a *CEN/URA3*

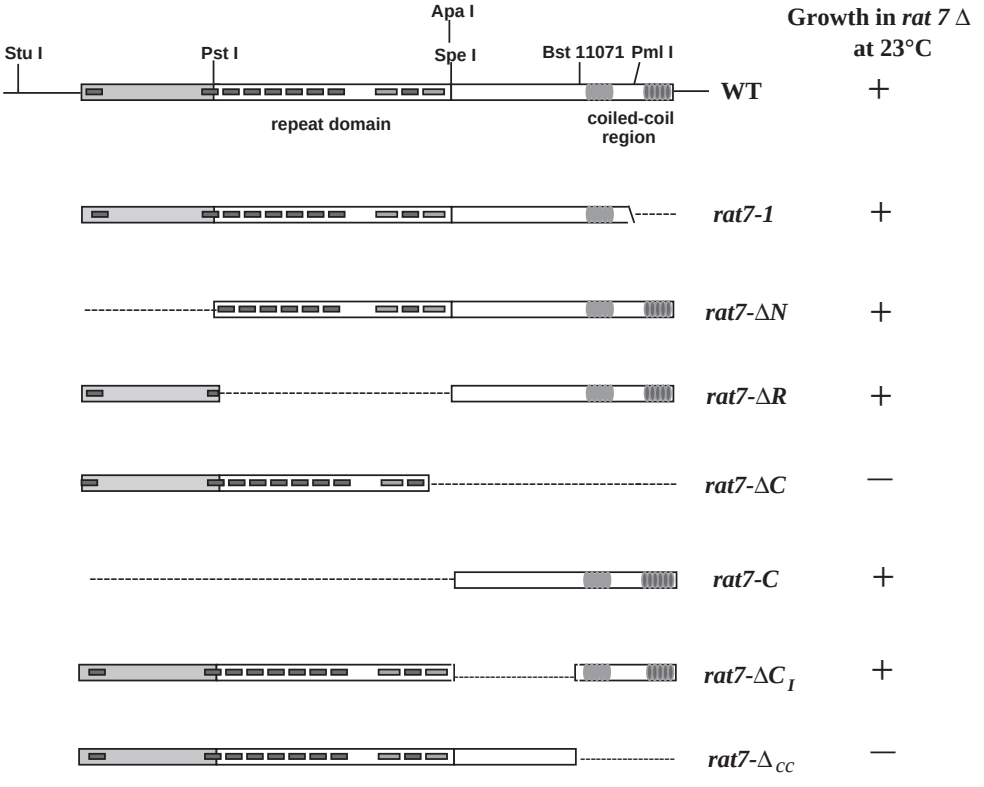


Fig. 1. The coiled-coil domain of Rat7p/Nup159p is essential for cell viability. The figure shows a diagram of the *RAT7/NUP159* ORF indicating the different domains and relevant restriction sites used to prepare mutant constructs, the different deletion alleles used in this study, and the growth of strains containing those alleles in the context of the *RAT7* null strain at 23°C. The black boxes located primarily in the central region represent the XXFG repeats (most often SAFG and PSFG, 22 repeats in all) while the lighter shaded boxes in the central region represent two of the three XFXFG repeats found in Rat7p/Nup159p. For simplicity, the precise locations of all 22 XXFG repeats and the 3 XFXFG repeats are not shown.

plasmid. Transformants were plated onto 5-FOA in order to select for loss of the plasmid-borne wild-type gene, and monitored for growth at 23°C. The results are shown in Fig 1. The strain containing the N-terminal deletion is referred to as *rat7-ΔN*, the strain containing the repeat domain deletion is referred to as *rat7-ΔR* and the strain containing the C-terminal deletion is referred to as *rat7-ΔC*. Strains containing either *rat7-ΔN* or *rat7-ΔR* were able to grow at 23°C. However, when the C-terminal third of the protein was deleted, no growth was detected, indicating that essential sequences are located within the carboxyl third of the protein.

To define more specifically the essential region of Rat7p/Nup159p, we constructed alleles in which either the coiled-coil region (*rat7-*Δ_{cc}) or the region between the repeat domain and the coiled-coils (*rat7-*ΔC_I) was deleted. The mutant lacking the coiled-coil region was unable to support growth of the null strain, but growth at 23°C was restored by a plasmid in which the region between the repeat domain and the coiled-coil region (*rat7-*ΔC_I) was deleted (Fig. 1). We conclude that the coiled-coil domain is the essential part of Rat7p/Nup159p. No other region of the protein is absolutely required for growth.

We next identified the mutation in the *rat7-1/nup159-1* allele. For this purpose, gap-repair was used (see Materials and Methods), followed by DNA sequence analysis. The mutation is a single nucleotide change at the codon for amino acid 1365, which is changed from TTA to TAA, thereby creating a premature termination codon. The *rat7-1/nup159-1* mutant is predicted to encode a truncated protein lacking one of the two predicted coiled-coil domains (Fig. 1).

In a previous screen for high copy extragenic suppressors of the *rat7-1/nup159-1* ts allele, we identified the C terminus of *RAT7/NUP159* as an intragenic suppressor (Del Priore et al., 1996). Because it was unclear what promoter was being used to express the C-terminal portion of Rat7p/Nup159p, a construct was made in which the C terminus of Rat7p/Nup159p was expressed under control of the *RAT7/NUP159* promoter; this construct is called *rat7-C*. A plasmid expressing this fragment of Rat7p/Nup159p was able to complement a *RAT7/NUP159* null strain at 23°C, demonstrating that neither the N-terminal third nor the repeat domain was required for growth at 23°C.

We next examined the expression of the different Rat7p/Nup159p mutant proteins. Fig. 2 shows western blots using antibodies generated against the N-terminal third (Fig. 2B), the repeat domain (Fig. 2A), or the C-terminal third of Rat7p/Nup159p (Fig. 2C). Most of the mutant proteins were expressed at similar levels. Since cells expressing only Rat7p/Nup159p lacking its C-terminal or coiled-coil domain were not viable, this protein could be examined only in the presence of wild-type Rat7p/Nup159p. Only a very low level of expression of Rat7-Δ_{cc}p was detected (the major band in Fig. 2A, lane 5 is wild-type Rat7p/Nup159p), possibly reflecting a failure to incorporate this protein into NPCs and its rapid turnover (Fig. 2A, lane 5). Wild-type Rat7p/Nup159p was previously shown to migrate with an apparent molecular weight of 205 kDa. The data in Fig. 2 indicate that all of the mutant proteins migrated more slowly on SDS polyacrylamide gels than predicted from their molecular weights, indicating that no single domain of Rat7p/Nup159p is responsible for its unexpectedly slow electrophoretic mobility.

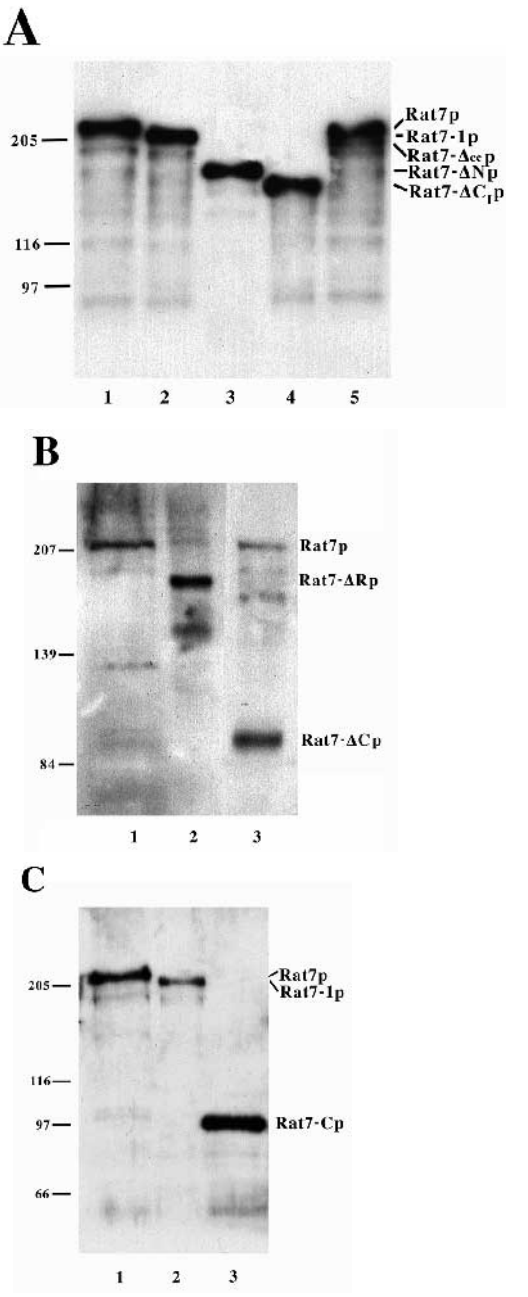


Fig. 2. Western blot analysis to examine the expression of the different *RAT7/NUP159* alleles at 23°C. (A) Western blot using an anti-Rat7p/Nup159p antibody against the repeat domain. (1) Rat7p; (2) Rat7-1p; (3) Rat7-ΔNp; (4) Rat7-ΔC_Ip; (5) Rat7p-Δ_{cc}p; note that the major band present in this lane is wild-type Rat7p/Nup159p. (B) Western blot using an anti-Rat7p/Nup159p antibody against the N-terminal domain. (1) Rat7p; (2) Rat7-ΔRp; (3) Rat7-ΔCp. (C) Western blot using an anti-Rat7p/Nup159p antibody against the C terminus. (1) Rat7p; (2) Rat7-1p; (3) Rat7-Cp.

We plated cells carrying these deletion alleles at 37°C in order to determine if they were temperature-sensitive (Fig. 3A). Growth of all strains was temperature-sensitive except the one producing Rat7-ΔRp, which grew as well as wild type (FY86) at both 23°C and 37°C. In the case of *rat7-ΔN* and r*at7-C*, limited growth was detected after 3-4 days of incubation at

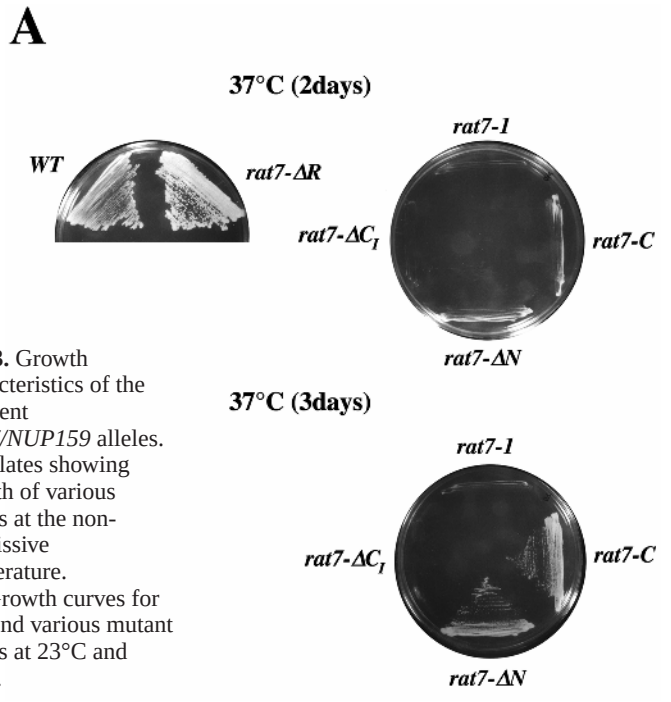
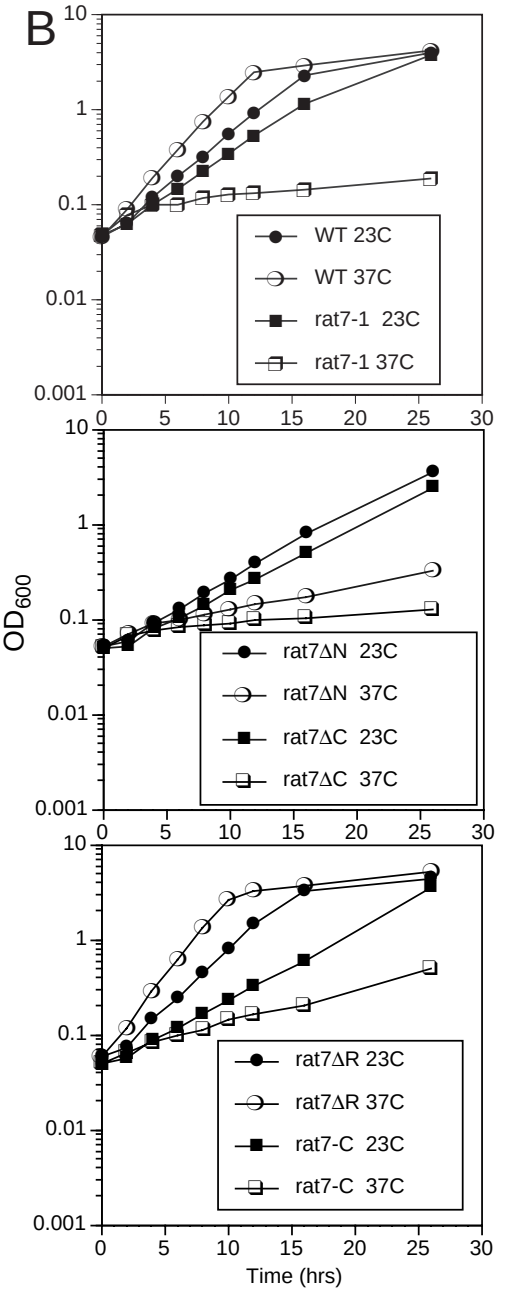


Fig. 3. Growth characteristics of the different *RAT7/NUP159* alleles. (A) Plates showing growth of various alleles at the non-permissive temperature. (B) Growth curves for WT and various mutant alleles at 23°C and 37°C.



37°C. We next examined the growth properties of these strains in liquid culture at 23°C or following a shift to 37°C. The results are shown in Fig. 3B. At 23°C, strains containing the *rat7-ΔN*, *rat7-ΔC$_I$*, and *rat7-C* alleles grew more slowly than either wild-type or *rat7-1* cells. At 37°C, strains containing *rat7-1* and r*at7-ΔC$_I$* did not show any growth, whereas those containing *rat7-ΔN* and *rat7-C* showed limited growth, consistent with the formation of very small colonies on plates after 3-4 days of incubation at 37°C. The strain containing *rat7-ΔR* grew slightly more rapidly than wild type at both 23°C and 37°C.

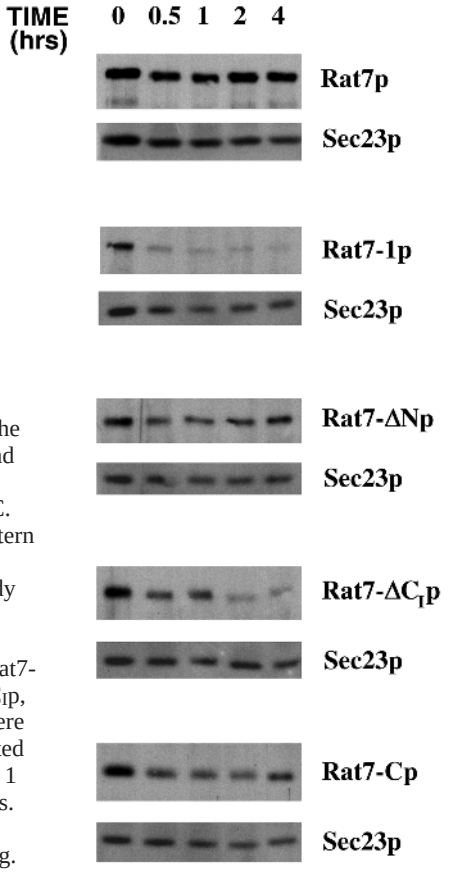


Fig. 4. Comparison of the stability of wild-type and mutant forms of Rat7p/Nup159p at 37°C. The figure shows a western blot using an anti-Rat7p/Nup159p antibody against the C terminus performed on extracts expressing wild type, Rat7-1p, Rat7-ΔNp, Rat7-ΔC$_I$p, Rat7-Cp. The strains were grown at 23°C and shifted to 37°C for 30 minutes, 1 hour, 2 hours, or 4 hours. Sec23p was used as a control for equal loading.

To examine the fate of the temperature sensitive mutant proteins following a shift to the non-permissive temperature, samples of cells expressing wild-type or various mutant alleles were grown at 23°C, shifted to 37°C for 0.5, 1, 2, or 4 hours, and processed for western analysis. In all cases, the amount of extract examined by electrophoresis came from the same number of cells. The results are shown in Fig 4. No significant decrease in the levels of Rat7p/Nup159p was detected for WT, *rat7-ΔN* and *rat7-C*, even after 4 hours at 37°C. Rat7-1p was

extremely unstable; even after 30 minutes at 37°C very little protein could be detected. Rat7-ΔC_Ip was somewhat more stable than Rat7-1p, but most of the protein was gone after 2 hours of incubation at 37°C. Sec23p was used as a control to monitor the loading of equivalent amounts of cell extracts.

Mutations of Rat7p/Nup159p affect RNA export but not protein import

rat7-1 cells have a dramatic defect in mRNA export when shifted to 37°C (Gorsch et al., 1995). To determine how these other mutations affected mRNA export, an in situ hybridization assay was performed on wild-type cells (FY86) and on cells containing the *rat7-1*, *rat7-ΔN*, *rat7-ΔR*, *rat7-*ΔC_I, and *rat7-C* alleles. The results are shown in Fig. 5. In WT cells, poly(A)$^+$ RNA was evenly distributed throughout the cell both at 23°C and following a one hour shift to 37°C. In *rat7-1* cells, 20-30% showed some nuclear accumulation of poly(A)$^+$ RNA at 23°C, while 100% of cells showed nuclear accumulation along with a dark cytoplasm after a shift to 37°C, as reported previously (Gorsch et al., 1995). In both *rat7-ΔN* and *rat7-C* cells grown at 23°C, 100% of the cells showed nuclear poly(A)$^+$ RNA accumulation, although a cytoplasmic signal was still observed. When both strains were shifted to 37°C for 1 hour, all cells showed increased nuclear accumulation of poly(A)$^+$

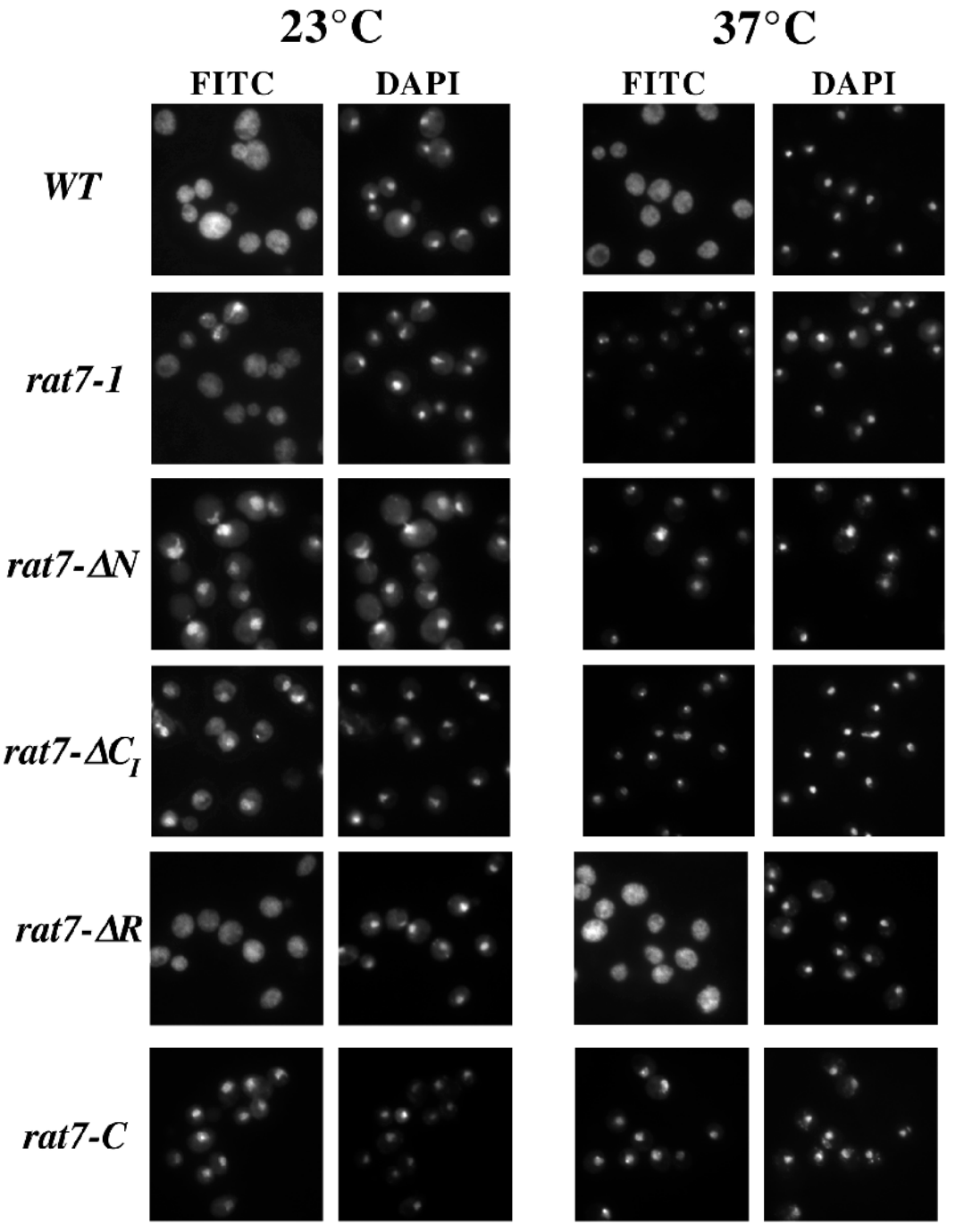


Fig. 5. In situ hybridization to examine trafficking of poly(A)$^+$ RNA in strains carrying mutant alleles of *RAT7/NUP159*. The figure shows an in situ hybridization assay performed for WT, *rat7-1*, *rat7-ΔN*, *rat7-*ΔC_I, *rat7-ΔR*, and *rat7-C*, grown at 23°C or shifted to 37°C for 1 hour. The FITC channel shows the poly(A)$^+$ RNA signal and the DAPI channel shows the signal for DNA.

RNA and the cytoplasmic signal was weaker, suggesting cessation or further reduction in RNA export. When the *rat7-ΔC$_I$* strain was grown at 23°C, 50-60% of the cells showed nuclear poly(A)$^+$ RNA accumulation; after a shift to 37°C for 1 hour, 100% showed strong nuclear accumulation and the cytoplasm was completely dark. In the case of the *rat7-Δ*R strain, there was little or no difference in the pattern of distribution of mRNA between this strain and wild type, though nuclei were slightly brighter in this strain shifted to 37°C than in wild-type cells treated identically. In conclusion, the alleles can be divided into three distinct groups. The first includes *rat7-ΔN* and *rat7-C*, where all cells accumulated poly(A)$^+$RNA in their nuclei at both 23°C and 37°C; while some cytoplasmic signal persisted in cells grown at the permissive temperature, this cytoplasmic signal was much weaker following a shift to 37°C. *rat7-ΔR* belongs to a separate group because it did not show any export defect at either temperature. The third group includes *rat7-ΔC$_I$* and *rat7-1*, which showed a more limited mRNA export defect at 23°C than the alleles in group 1, and a complete block in poly(A)$^+$ RNA export at 37°C.

Another phenotype associated with the *rat7-1* mutation is disruption of the nucleolus after cells are shifted to 37°C (Gorsch et al., 1995). To determine how these different *rat7* alleles would affect the patterns of nucleolar antigen distribution, an indirect immunofluorescence assay was performed on wild-type and mutant cells incubated at 23°C or shifted to 37°C, using an anti-fibrillarin antibody. At 23°C, all showed a nucleolar staining pattern very similar to that of wild type. At 37°C, all of the mutants showed some degree of nucleolar disruption except for *rat7-ΔR*, which showed a wild-type pattern (data not shown).

No defects in the nuclear import of an NLS-reporter protein was seen in *rat7-1* cells either grown at 23°C or shifted to 37°C (Gorsch et al., 1995). To determine if any of the other mutant alleles caused defects in import of proteins containing canonical NLSs, we examined the location of a nuclear GFP-reporter protein, in which GFP is fused to a nuclear localization signal and other sequences and expressed constitutively (Saavedra et al., 1996; Shulga et al., 1996). Under normal conditions, this 42 kDa reporter protein is entirely nuclear. By treating cells with sodium azide, cellular metabolism and generation of ATP are inhibited leading to diffusion of the reporter out of the nucleus and its accumulation throughout the cell. After washing cells and resuspending them in glucose-containing medium, ATP levels are restored and cellular growth resumes. Under these conditions, the NLS-GFP reporter is reimported rapidly (within 10 minutes) into the nucleus (Saavedra et al., 1996; Shulga et al., 1996). We performed this assay on wild-type cells and cells carrying each of the mutant *rat7* alleles. In all cases, cells were grown at 23°C, treated with sodium azide to permit diffusion of the reporter, and subsequently incubated in normal medium at 23°C or 37°C. No protein import defects were detected in any of the mutant strains under any conditions, including shifting them to 37°C for 2 hours prior to treatment with sodium azide (data not shown). That is, close to 100% of the cells reimported the GFP

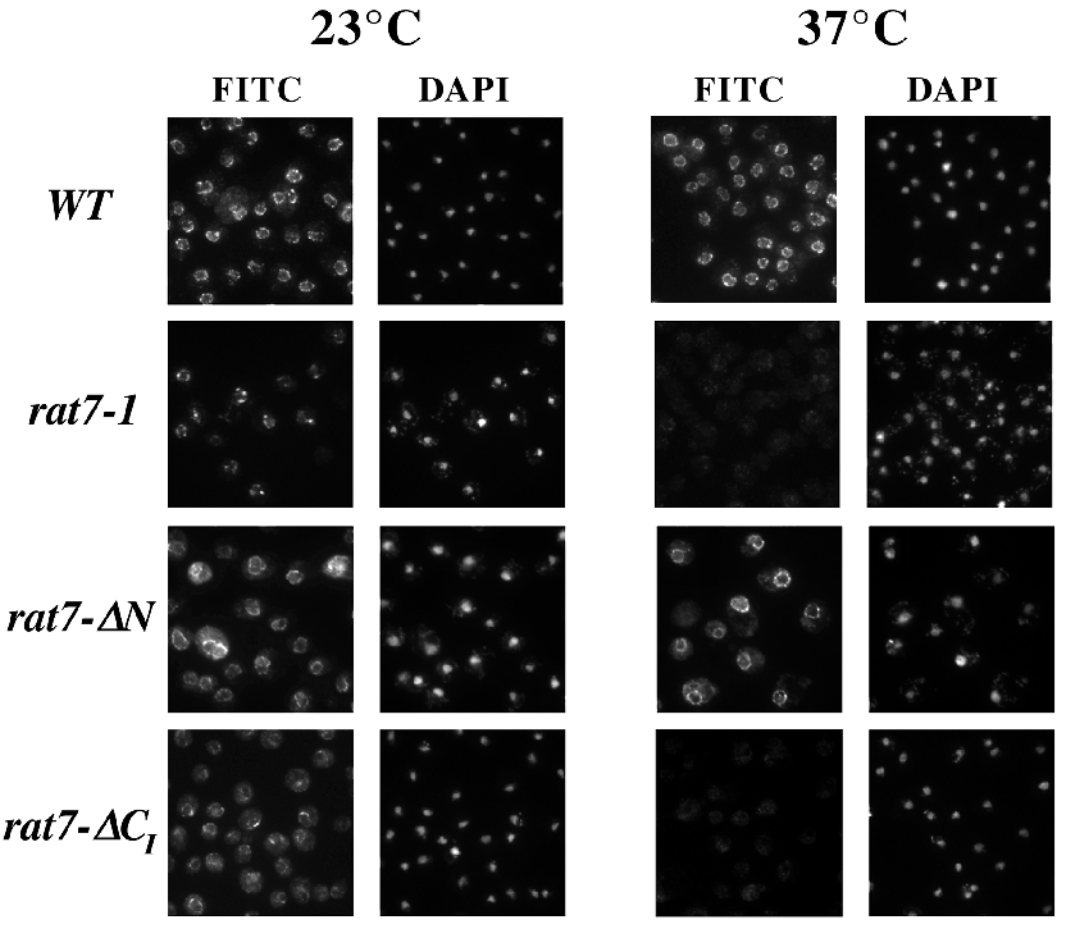


Fig. 6. Indirect immunofluorescence to examine Rat7p/Nup159p. WT, *rat7-1*, *rat7-ΔN*, and *rat7-ΔC$_I$* cells were grown either at 23°C or shifted to 37°C for 1 hour. Cells were then fixed and subjected to an immunofluorescence assay using an anti-Rat7p/Nup159p antibody. The DAPI images are of the same field as the corresponding FITC images.

reporter under non-permissive conditions. These results indicate that Rat7p/Nup159p does not play an essential role in import of proteins containing canonical NLSs.

The C-terminal domain of Rat7p/Nup159p is necessary to maintain Rat7p/Nup159p at the pore and for a normal distribution of NPCs

In the *rat7-1* strain, Rat7-1p dissociates from NPCs when cells are shifted to 37°C. To examine the behavior of the various mutant forms of Rat7p/Nup159p, we examined the protein in each mutant strain by indirect immunofluorescence, both at 23°C and following a shift to 37°C, using a polyclonal anti-Rat7p/Nup159p antibody. The results are shown in Fig. 6. In wild-type cells grown at 23°C or shifted to 37°C, a normal punctate nuclear rim staining pattern was observed that completely surrounds the nucleus (stained with DAPI). In *rat7-1* cells grown at 23°C, Rat7-1p is at the pore and the pores are clustered whereas following a shift to 37°C, no visible signal for Rat7-1p can be detected at the pore, as previously reported (Gorsch et al., 1995). When *rat7-ΔN* cells were grown at 23°C or shifted to 37°C, Rat7-ΔNp was present at the nuclear rim and NPC distribution was very similar to that seen in WT cells. The same wild-type distribution of Rat7p/Nup159p was seen in *rat7-ΔR* and *rat7-C* cells (data not shown). In the case of *rat7-*ΔC_I, Rat7-ΔC_Ip was present at the nuclear rim and NPCs were clustered when cells were grown at 23°C. When these cells were shifted to 37°C for one hour, the mutant protein was lost from NPCs as is the case for Rat7-1p. In this case the different alleles can be divided into two distinct groups. The first includes *rat7-ΔN*, *rat7-ΔR*, and *rat7-C*, which exhibited a wild-type distribution of Rat7p/Nup159p at both temperatures. The second group includes *rat7-1* and *rat7-*ΔC_I, where the protein was at the pore and pores were clustered at 23°C, while the protein was lost from the nuclear rim following a shift to 37°C.

Nuclear pore clustering is a common phenotype in yeast strains carrying a disruption or mutant allele of a gene encoding any one of several nucleoporins (see references Aitchison et al., 1995a; Heath et al., 1995; Goldstein et al., 1996, for examples). The *rat7-1* strain has a novel NPC distribution phenotype in which pores are clustered at 23°C and this clustering is dramatically reduced following a shift of cells to 37°C. To examine the NPC distribution pattern in cells carrying these mutant alleles, we performed electron microscopy studies on cells grown at 23°C or shifted to 37°C for 1 hour. Fig. 7 summarizes the findings from examining NPC distribution in thin sections from 50 cells carrying each allele. NPCs were considered to be clustered when a group of pores with a 'grape-like' organization was seen. Pores were considered to be grouped when several pores were found very close to each other and in the plane of the NE. For wild-type cells, grouping of NPCs was seen in approximately 20% of the sections examined, both at 23°C and 37°C. In *rat7-1* cells grown at 23°C, NPCs were either grouped or clustered in more than 80% of the sections examined. When these cells were shifted to 37°C, NPCs were grouped or clustered in 34%. In *rat7-ΔN* cells grown at 23°C, 25% showed grouped NPCs, but there was little clustering. A similar distribution was seen when these cells were shifted to 37°C. For *rat7-ΔR* and *rat7-C* cells grown at 23°C and 37°C, the percentages were very close to those seen for *rat7-ΔN* and wild type. In the case of *rat7-*ΔC_I, when

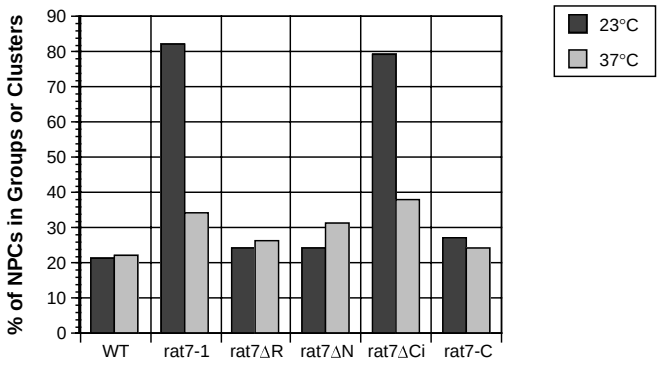


Fig. 7. Summary of electron microscopic studies to examine NPCs of *RAT7/NUP159* mutant strains. WT, *rat7-1*, *rat7-ΔN*, *rat7-ΔR*, *rat7-*ΔC_I, and *rat7-C* were examined. The strains were grown at 23°C and shifted to 37°C for 1 hour. Thin sections from 50 cells of each strain were examined and the percentage of NPCs that were grouped or clustered was determined. The dark bars show the percentage grouped or clustered at 23°C and the shaded bars represent the percentage grouped or clustered at 37°C.

cells were grown at the permissive temperature, 79% showed either grouped or clustered pores; when these cells were shifted to 37°C, the clustering phenotype was dramatically reduced, with only 39% showing clustering or grouping of NPCs. Immunofluorescence assays using the anti-nucleoporin antibody RL1 confirmed the results about grouping and clustering obtained using electron microscopy (data not shown). In conclusion, we can again divide the different *rat7* alleles into two distinct groups. The first includes *rat7-ΔN*, *rat7-ΔR* and r*at7-C*, which all show a wild-type distribution of NPCs at both temperatures. The second group includes *rat7-1* and *rat7-*ΔC_I, which both showed extensive clustering and grouping of NPCs at 23°C and a partial reversal of this phenotype when cells were shifted to the non-permissive temperature. Interestingly, reversal of the NPC clustering phenotype was tightly correlated with whether or not the mutant Rat7p/Nup159p was lost from NPCs when cells were shifted to 37°C.

DISCUSSION

Domain organization of Rat7p/Nup159p

A major goal of our studies is to identify and characterize factors essential for nucleocytoplasmic export of mRNA. Rat7p/Nup159p is an NPC component identified previously in our genetic screen for temperature-sensitive yeast mutants defective in mRNA export. Its domain organization is shown in Fig. 8, along with the portions of Rat7p/Nup159p encoded by each mutant allele and a summary of several of the properties of each mutant strain. Since Rat7p/Nup159p is one of the few essential yeast nucleoporins, one of our goals was to identify its essential domain(s). Our results show unambiguously that the putative coiled-coil domain is absolutely essential for this protein to perform its function. We also demonstrated that Rat7p/Nup159p contains three different domains that appear to be involved in distinct functions. These are: (1) the N terminus of Rat7p/Nup159p, upstream from the central repeat region, and extending from the beginning of the protein through amino acid 416; (2) the central repeat domain,

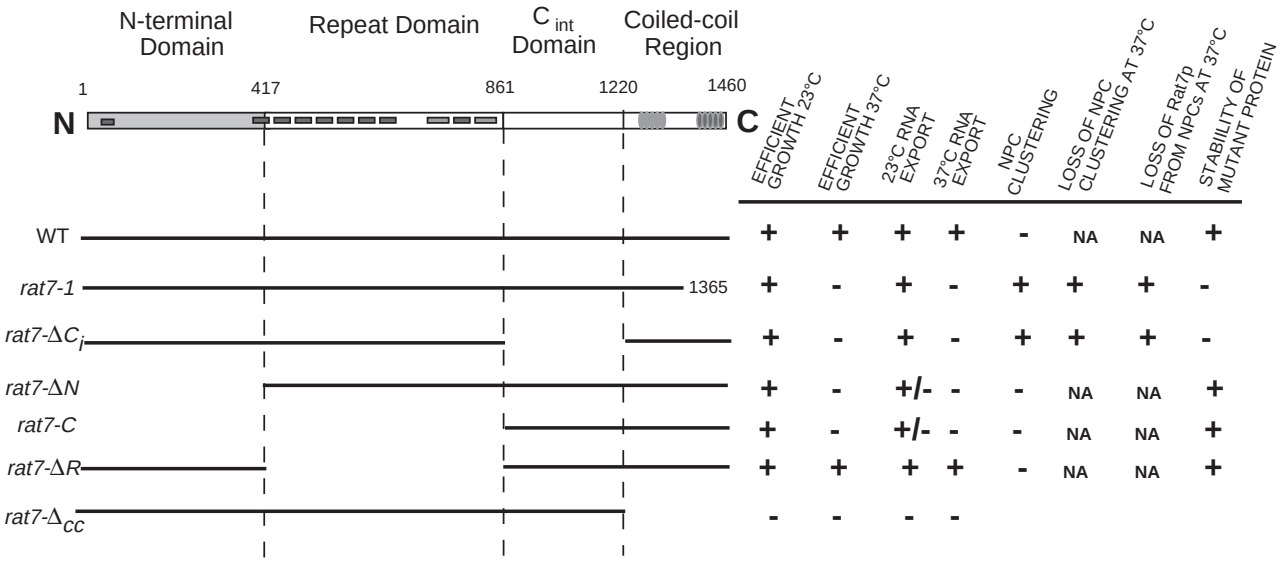


Fig. 8. Properties of *RAT7/NUP159* mutant alleles. The figure shows a schematic diagram of Rat7p/Nup159p, indicating the N-terminal, repeat, C-internal, and coiled-coil domains. The bars beneath the domain diagram represent the sequences present in the various mutant alleles studied. To the right are listed the properties of wild-type cells and cells disrupted for *RAT7/NUP159* and carrying the various mutant alleles. These include efficient growth at 23°C and 37°C, export of poly(A)$^+$ RNA at 23°C and 37°C, and effects on clustering of NPCs.

extending from amino acid 417-861; (3) the region located C-terminal to the repeat domain, extending from amino acid 862 through the end of the protein, and including the putative coiled-coil regions. The ability of the Rat7p/Nup159p to function in mRNA export appears to depend substantially on the presence of the N-terminal domain. In cells expressing Rat7p/Nup159p mutant proteins that lacked this domain, all cells showed nuclear accumulation of poly(A)$^+$ RNA even at room temperature, but no defects in NPC distribution. These defects in mRNA export became more severe following a shift to 37°C. Thus, the N-terminal domain appears to be required for efficient mRNA export, but it is not clear yet what the role of this domain in the export process actually is. This domain is not required for proper NPC distribution.

Some nucleoporins including Nup100p, Nup116p, and Nup145p contain putative RNA-binding domains within their sequences. Rat7p/Nup159p does not contain sequences related to those of known RNA binding domains, but the protein could contain a novel RNA-binding domain. However, since RNA is exported from the nucleus as a protein/RNA complex (mRNP), and some proteins that bind nuclear RNA contain nuclear export signals, RNA export may not depend on the presence of RNA binding domains within NPC components. Instead, interactions between proteins bound to mRNA and nucleoporins may mediate nucleocytoplasmic transport. Perhaps the N-terminal domain of Rat7p/Nup159p interacts with proteins directly bound to mRNAs, or with nucleoplasmic proteins that recognize the NESs in mRNP proteins and travel with mRNPs to the nuclear periphery. Since Rat7p/Nup159p is located at the cytoplasmic side of the pore, direct interactions between it and mRNP would have to occur as one of the later steps in RNA export.

Our data also suggest that the carboxy-terminal domain performs predominantly a structural role. Mutants containing various deletions in the C-terminal third displayed defects in nuclear pore distribution as well as in mRNA export, and the Rat7p/Nup159p mutant proteins were lost from NPCs at elevated temperatures. We do not know whether these mutant forms of Rat7p/Nup159p become unstable after they dissociate from NPCs at 37°C or, alternatively, whether they refold at 37°C such that they become targets for proteolysis, even when still part of nuclear pore complexes. In cells expressing only the C-terminal portion of Rat7p/Nup159p (*rat7-C*), the mutant protein was still inserted into NPCs and remained with NPCs even when cells were shifted to 37°C; the distribution of NPCs was normal at both 23°C and 37°C. This indicates that the C terminus of Rat7p contains sufficient information for targeting to NPCs and for normal NPC distribution. Thus, the C terminus of Rat7p/Nup159p appears to play a role in the association of Rat7p/Nup159p with NPCs or NPC subcomplexes, and may also play an important role in the stability of the protein. In *rat7-C* cells at 23°C, RNA export was considerably less efficient than in wild-type cells, since strong nuclear accumulation of poly(A)$^+$ RNA was readily detected.

Deletion of either the carboxyl third of Rat7p/Nup159p or the complete coiled-coil domain was lethal, while partial deletions in this region created temperature-sensitive alleles whose retention in NPCs was one of the temperature-sensitive phenotypes. We suspect that proteins attached to NPCs through the N terminus of Rat7p/Nup159p would also be lost from NPCs when Rat7p/Nup159p is lost, thereby explaining the strong temperature-dependent defects in mRNA export associated with mutations in the carboxyl third of the protein. Studies are in progress to identify components of this putative Rat7p/Nup159p subcomplex.

Partially reversible defects in NPC distribution were also seen in temperature-sensitive strains containing alleles altered in the carboxyl-third of Rat7p/Nup159p, suggesting that insertion of mutant Rat7p/Nup159p into NPCs resulted in an altered NPC distribution that was actually dependent on the presence of these mutant proteins. Perhaps in the absence of part of the carboxyl third of Rat7p/Nup159p, proteins that

would interact with that region form inappropriate interactions leading to clustering. When the subcomplex dissociates after a shift to 37°C, these abnormal interactions could be interrupted, permitting NPCs to assume a more normal distribution. Recent studies indicate that NPCs move quite rapidly within the NE (Belgareh and Doye, 1997; Bucci and Wente, 1997).

These findings suggest a model wherein the coiled-coil region of Rat7p/Nup159p is essential for its insertion and retention in NPCs. If only one of the two predicted coiled-coil domains is present (for example, in the *rat7-1* allele), then the protein can be assembled into NPCs at room temperature but is lost upon a shift to higher temperatures. If both of the coiled coil domains are absent, then the protein cannot be assembled into NPCs and the mutation is lethal. The phenotypes seen when sequences just upstream from the coiled-coil were deleted were identical to those of the *rat7-1* allele. This could indicate that the region of interaction between the carboxyl-third of Rat7p/Nup159p and other NPC components includes both the coiled-coil portion and upstream sequences. Alternatively, the coiled-coils may fold incorrectly or not be optimally oriented for interactions if the immediately upstream region is missing.

We were unable to assign any function to the Rat7p/Nup159p repeat domain. Cells lacking this domain were indistinguishable from wild-type cells in all of the analyses described in this paper. In the case of the other classes of repeat-containing nucleoporins, the repeat region can often be deleted from one member of the set without causing defects in growth or transport. Most likely, different proteins containing related repeats have redundant functions to some extent, although the GLFG region of Nup116p cannot be replaced by the shorter GLFG repeats of other yeast nucleoporins, or by the XFXFG repeats of another class of yeast nucleoporins (Iovine et al., 1995). Perhaps some other protein may be able to provide the function of the repeat domain of Rat7p/Nup159p. To test the hypothesis that the closely related Rip1p protein was performing such a function, we mated strains carrying the *rat7-ΔR* allele and a strain containing a complete disruption of *RIP1*, induced sporulation of diploids, and dissected tetrads. We found that these two mutations were not synthetically lethal (our unpublished results). Therefore, if the repeat domain of Rat7p/Nup159p performs some essential function, another nucleoporin (or some other factor) that lacks the type of repeats found in Rat7p/Nup159p must also be capable of performing its function. A synthetic lethal screen using *rat7-ΔR* is in progress and should provide insight into the functions of this repeat domain.

Mutation or deletion of several other nucleoporins results in defects in RNA export. These include Nup116p, Nup145p, Nup85p, Nup120p, Nup133p, Nup82p, and Npl4p (Wente and Blobel, 1993, 1994; Fabre et al., 1994; Aitchison et al., 1995a; Goldstein et al., 1996; Grandi et al., 1995a; Heath et al., 1995; Hurwitz and Blobel, 1995; Li et al., 1995; Pemberton et al., 1995; DeHoratius and Silver, 1996, Dockendorff et al., 1997). Nup85p and Nup120p are present in a complex with Nup84p, Seh1p and Sec13p and this complex appears to be important for RNA export and for NPC biogenesis (Siniossoglou et al., 1996).

We have preliminary evidence that Rat7p/Nup159p is present in another complex that also functions in RNA export. In preliminary studies, we found that Rat7p/Nup159p can be extracted from NPCs as a component of a small complex which sediments in sucrose gradients as if it had a size of approximately 300-400 kilodaltons (S. Dagher and C. N. Cole, unpublished results). Further analyses indicate that Nup82p and Nsp1p are two other members of this complex (N. Belgareh, V. Doye, S. Dagher, C. A. Snay and C. N. Cole, unpublished results). In this light, it is interesting to note that strong defects in mRNA export were also found in strains carrying mutant alleles of *NUP82* (Grandi et al., 1995a; Hurwitz and Blobel, 1995).

We suggest that the N terminus of Rat7p/Nup159p may be important for anchoring other components in this subcomplex. Since the association of Rat7p/Nup159p itself with NPCs was unaffected by deletion of the N terminus, this cannot be a region critical for anchoring Rat7p/Nup159p in NPCs. However, it is easy to imagine that other proteins could depend on Rat7p/Nup159p for normal insertion or retention in NPCs. This would explain the defects in mRNA export in *rat7-ΔN* cells grown at 23°C, where there could be reduced levels of Rat7p/Nup159p-associated proteins in NPCs or less efficient functioning of a suboptimally arranged subcomplex. It is also quite possible that proteins which associate with the NPC at least in part through sequences in the N terminus of Rat7p/Nup159p might be readily lost from NPCs when the temperature was shifted to 37°C, even though Rat7-ΔNp did not appear to dissociate from NPCs when cells were shifted to 37°C. Experiments are in progress to examine the fate and stability of this complex in the presence of various mutant alleles of *RAT7/NUP159*, *NUP82*, and *NSP1* and to determine how the amounts of Nup82 and Nsp1p associated with NPCs are altered when different mutant alleles of *RAT7/NUP159* are used. However, the fact that Nsp1p is also part of at least one other complex may complicate analyses of how mutations affecting Rat7p/Nup159p alter the levels of Nsp1p within NPCs.

Nuclear transport functions of Rat7p/Nup159p

In the studies reported here, we examined the roles of various domains of Rat7p/Nup159p in mRNA export and import of NLS-containing proteins. In general, nucleoporins essential for mRNA export do not appear to play an important role in the import of NLS-containing proteins, suggesting that they may be specialized for roles in mRNA export. Methods to examine the export of rRNA, 5S RNA, and tRNA in yeast have not yet been developed, so no information is available concerning whether nucleoporins required for mRNA export also play roles in export of other classes of RNA.

It is possible that Rat7p/Nup159p, and other nucleoporins essential for RNA export (e.g. Rat9p/Nup85p, Nup145p, Nup82p, Rat2p/Nup120p, Rat3p/Nup133p) also play roles in protein export and in nuclear import of proteins containing non-canonical NLSs. So far, only a few nuclear proteins lacking canonical NLSs and/or containing NESs have been described in *S. cerevisiae*. Many of these proteins are involved in nucleocytoplasmic exchange (e.g. Gle1p/Rss1p, Npl3p, Hrp1p, Kap95p) such that their localization and probable shuttling may be altered in yeast cells experiencing a strong block in RNA export. For example, Npl3p lacks a canonical NLS and has been shown to shuttle between the nucleus and the cytoplasm but it exits the nucleus only when bound to RNA and is retained in nuclei whenever RNA synthesis or export are

blocked by mutations in other gene products or treatment with drugs that inhibit RNA polymerase II transcription (Lee et al., 1996). Therefore, using these proteins to determine the role of Rat7p/Nup159p in NES-mediated protein export or import of nuclear proteins lacking canonical NLSs is not straightforward. Determining whether any nucleoporins required for RNA export are involved directly in NES-mediated protein export and import of proteins lacking canonical NLSs will require the development of appropriate reporter proteins and assays that can distinguish between indirect effects that occur as a consequence of inhibition of RNA export and direct effects implicating specific nucleoporins in other types of transport.

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