Targeting of a Cytosolic Protein to the Nuclear Periphery

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Abstract. The yeast nuclear envelope protein NSP1 is located at the nuclear pores and mediates its essential function via the carboxy-terminal domain. The passenger protein, cytosolic dihydrofolate reductase from mouse, was fused to the 220 residue long NSP1 carboxy-terminal domain. When expressed in yeast, this chimeric protein was tightly associated with nuclear structures and was localized at the nuclear periphery very similar to authentic NSP1. Furthermore, the DHFR-C-NSP1 fusion protein was able to complement a yeast mutant lacking a functional NSP1 gene showing that DHFR-C-NSP1 fulfills the same basic function as compared to the endogenous NSP1 protein. These data also show that the NSP1 protein is composed of separate functional moieties: a carboxy-terminal domain that is sufficient to mediate the association with the nuclear periphery and an amino-terminal and middle repetitive domain with an as yet unknown function. It is suggested that heptad repeats found in the NSP1 carboxy-terminal domain, which are similar to those found in intermediate filament proteins, are crucial for mediating the association with the nuclear pores.

The nuclear pores are the sites of transport of proteins and nucleic acids between the nuclear and cytoplasmic compartments (for review, Dingwall and Laskey, 1986; Gerace and Burke, 1988). The transport through the pores is mediated by specific nuclear targeting signals that are found in many nuclear proteins and recognized by receptors presumably located at the nuclear periphery and the nuclear pores (Kalderon et al., 1984; Newmeyer and Forbes, 1988; for review see also, Gerace and Burke, 1988). Nuclear pores are composed of supramolecular complexes, the nuclear pore complexes, which reveal an octagonal symmetry in the electron microscope and have an estimated molecular mass of approximately $100 \times 10^6$ D (Unwin and Milligan, 1982; Reichelt et al., 1990). Several components of the nuclear pores have been identified and a role in nuclear transport has been proposed for a few of them (Gerace et al., 1982; Davis and Blobel, 1986, 1987; Park et al., 1987; Snow et al., 1987). Recently, cDNA probes for nuclear pore proteins were cloned and the DNA sequence and the deduced amino acid sequence of nuclear pore proteins is now available (D’Onofrio et al., 1988; Wozniak et al., 1989; Starr et al., 1990). Nuclear pore proteins have been identified in yeast (Aris and Blobel, 1989; Nehrbass et al., 1990; Davis and Fink, 1990) and an essential role in nuclear envelope functions has been implied (Nehrbass et al., 1990). By immunological cross-reactivity, it has been shown that the yeast nuclear pore proteins NSPI and NUP1 are evolutionarily related to the mammalian nucleoporins of which p62 is the most prominent member (Davis and Blobel, 1986).

So far, nothing is known about how nuclear pore proteins reach their final destination. This information, however, is crucial to understand the assembly of nuclear pore complexes and to piece together the mechanism by which nuclear proteins are targeted to the nuclear pores and imported into the nucleus. To address this problem, we analyzed the different domains of NSPI, a yeast nuclear pore protein, for their role in nuclear targeting and nuclear pore association.

Here, we demonstrate that the 220 amino acid long carboxy-terminal domain of NSPI contains signals to guide a murine cytosolic protein, dihydrofolate reductase, to the nuclear periphery, most likely to the nuclear pores. This is the first demonstration of a sequence mediating nuclear envelope association.

Materials and Methods

Yeast Strains and Media

The diploid yeast strain JU4.2 × JR26-19B (a/α, ade2-1/ade2-1, ade8/ADE8, can1-100/can1-100, his4/HIS4, his3/HIS3, leu2-3/leu2-3, lys1-l/lys1-l, ura3-52/ura3-52) and TF2 heterozygous for NSPI (a/α, ade2-1/ade2-1, ade8/ADE8, can1-100/can1-100, his4/HIS4, his3/HIS3, leu2-3/leu2-3, lys1-l/lys1-l, ura3-52/ura3-52) have been used (Hurt, 1988). Strains were grown in rich YPD (yeast extract, peptone, D-glucose) medium or minimal medium containing glucose or galactose as described in Hurt, 1988.

Construction of a Haploid Yeast Strain Expressing a Fusion Protein Between Dihydrofolate Reductase from Mouse and the NSPI Carboxy-Terminal Domain

DNA recombinant work was performed according to published procedures (Maniatis et al., 1982). The DNA coding for the COOH-terminal domain of NSPI was isolated by restriction as described (Nehrbass et al., 1990) and its 5'-end was fused in frame to the 3'-end of the mouse DHFR gene whose stop codon has been altered by introduction of a Bgl II restriction site (Hochuli et al., 1988). This fusion gene, which encoded a chimeric protein...
protein NSP2 (Nehrbass et al., 1990), which is very prominent in the extract of TF2 transformed with plasmid YEP13-ADHI-DHFR-C-NSP1. Lane 2, yeast control strain (TF2); lane 3, yeast strain expressing the DHFR-C-NSP1 fusion protein (TF2 transformed with plasmid YEP13-ADHI-DHFR-C-NSP1); lane 4, extract from 
Escherichia coli


eXpressed NSPI (serum EC7-3 and EC9-3; see also Hurt, 1988) and mouse dihydrofolate reductase (DHFR). Details for construction of the chimeraic gene DHFR-C-NSP1 and its expression in yeast are outlined under Materials and Methods. Amino acid numbers are indicated below the schematic drawings.

Figure 1. Construction of a fusion protein consisting of the cytosolic protein dihydrofolate reductase and the NSPI carboxy-terminal domain. Schematic representation of the authentic NSPI protein, the NSPI carboxy-terminal domain alone (C-NSP1), the fusion protein between cytosolic dihydrofolate reductase from mouse and the NSPI carboxy-terminal domain (DHFR-C-NSP1) and authentic dihydrofolate reductase (DHFR). Details for construction of the chimeraic gene DHFR-C-NSP1, and its expression in yeast are outlined under Materials and Methods. Amino acid numbers are indicated below the schematic drawings.

1. Abbreviation used in this paper: ADH, alcohol dehydrogenase.

Immune sera, Affinity Purification of Antibodies, Indirect Immunofluorescence, and Immunoelectron Microscopy

Immune sera raised against 
Escherichia coli


eXpressed NSPI (serum EC7-3 and EC9-3; see also Hurt, 1988) and mouse dihydrofolate reductase (UM15-3; kindly provided by Dr. G. Schatz, Basel, Switzerland) were used. Antibodies against NSPI were affinity purified using purified mouse DHFR (Sigma Chemical GmbH, Munich, Federal Republic of Germany), which was immobilized by blotting onto nitrocellulose. For indirect immunofluorescence, affinity-purified antibodies from five consecutive elutions were concentrated to ~50 µl by ultrafiltration.

Indirect immunofluorescence on yeast cells (Nehrbass et al., 1990) and isolated nuclei (Hurt et al., 1988) was performed as described earlier; in particular, yeast cells were fixed in 3% formaldehyde before spheroplasting and nuclei were also fixed in 3% formaldehyde before the latter were centrifuged on polylysine-coated coverslips. For indirect immunofluorescence, affinity-purified anti-NSPI and anti-DHFR antibodies in a 1:5 dilution were used followed by a second antibody, Texas red-labeled goat anti-rabbit IgG, in a 1:100 dilution. For DNA staining, 0.5 µg/ml Hoechst 33258 was used. Labeled cells were inspected in a Zeiss Axiophot fluorescence microscope using the Plan-Neofluar 100x/1.3 objective and pictures were taken with

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mouse DHFR (left) and the immune serum EC9-3 made against NSP1 (right). Lane 1, purified mouse DHFR (1 µg; Sigma Chemical GmbH); lane 2, yeast control strain (TF2); lane 3, yeast strain expressing the DHFR-C-NSP1 fusion protein (TF2 transformed with plasmid YEP13-ADHI-DHFR-C-NSP1); lane 4, extract from 
Escherichia coli


eXpressed NSPL (serum EC7-3 and EC9-3; see also Hurt, 1988) and mouse dihydrofolate reductase (DHFR). Details for construction of the chimeraic gene DHFR-C-NSP1, and its expression in yeast are outlined under Materials and Methods. Amino acid numbers are indicated below the schematic drawings.

Figure 2. Expression of the DHFR-C-NSP1 fusion protein in yeast. Expression in yeast and 
Escherichia coli

, of the fusion protein composed of mouse DHFR and the carboxy-terminal domain of NSPI was carried out as described in Materials and Methods. Yeast cells were grown in minimal glucose medium supplemented with the appropriate nutrients and total protein extract was prepared. Aliquots of the yeast and 
Escherichia coli



Expressed NSPI (serum EC7-3 and EC9-3; see also Hurt, 1988) and mouse dihydrofolate reductase (DHFR). Details for construction of the chimeraic gene DHFR-C-NSP1, and its expression in yeast are outlined under Materials and Methods. Amino acid numbers are indicated below the schematic drawings.

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Expressed NSPI (serum EC7-3 and EC9-3; see also Hurt, 1988) and mouse dihydrofolate reductase (DHFR). Details for construction of the chimeraic gene DHFR-C-NSP1, and its expression in yeast are outlined under Materials and Methods. Amino acid numbers are indicated below the schematic drawings.
In vivo complementation of a nspl" yeast mutant by the fusion protein between mouse DHFR and the NSPI carboxy-terminal domain. (A) Tetrad analysis \( \text{(top)} \) and growth of a tetrad on YPD and selective plates \( \text{(bottom)} \). (Top) Yeast TF2 heterozygous for NSPI was transformed with plasmid YEPI3-ADHI-DHFR-C-NSPI and the corresponding diploid strain was sporulated and tetrads were analyzed. Tetrads progeny were grown for 3 d on YPD plates at 30°C. Four viable tetrad spores were generally recovered, all of which exhibited similar growth rates. A scheme for the meiotic segregation of the intact and disrupted NSPI gene and the plasmid YEPI3-ADHI-DHFR-C-NSPI is also shown. (Bottom) Two out of the four viable haploid progeny (called Bi-B4) grown on YPD (left plate) were always URA+/LEU+ (right SD [ura-leu] plate) indicating that they carried the disrupted nspl gene (disrupted by the URA3 gene) and plasmid YEPI3-ADHI-DHFR-C-NSPI (containing the LEU2 gene). Tetrad C only gave two viable spores which were ura-. Most likely this tetrad had lost the complementing plasmid YEPI3-ADHI-DHFR-C-NSPI during growth on the sporulation plate (YPA plate). (B) Immunoblot analysis. Protein extracts derived from a complete tetrad from strain TF2 heterozygous for NSPI and transformed with plasmid YEPI3-ADHI-DHFR-C-NSPI were analyzed by SDS–12% polyacrylamide gel electrophoresis and immunoblotting using anti-NSPI antibodies. Immune serum EC7-3 was used, which reacts only with NSPI and the DHFR-C-NSPI fusion protein, but not with NSP2. Lane 1, diploid yeast strain expressing the DHFR-C-NSPI fusion protein (TF2 transformed with plasmid YEPI3-ADHI-DHFR-C-NSPI); lanes 2-5, haploid progeny from a tetrad derived from TF2 transformed with plasmid YEPI3-ADHI-DHFR-C-NSPI; lanes 2 and 3, haploid progeny expressing only the DHFR-C-NSPI fusion protein (URA+/LEU+); lanes 4 and 5, haploid progeny expressing only authentic NSPI (ura-/leu-); plasmid YEPI3-ADHI-DHFR-C-NSPI was segregated out from these two haploid progeny; lane 6, protein extract from \textit{E. coli} expressing the DHFR-C-NSP1 fusion protein under a bacteriophage promoter. The position of the DHFR-C-NSPI fusion protein and NSP1 is indicated by arrows. The molecular masses of a protein standard are also given.

**Extraction of Proteins from Yeast Cells, Subcellular and Subnuclear Fractionation and Immunoblotting**

Total protein extracts from yeast were prepared by resuspending freshly harvested cells from a 50-ml culture (OD 600 nm of 1) in 1.5 ml hot SDS sample buffer. The sample was vortexed together with 400 micron glass beads followed by a 20-min incubation at 96°C with occasional vortexing. The centrifuged extract corresponding to 1 OD (600 nm) was applied on a SDS–polyacrylamide gel and separated proteins were blotted onto nitrocellulose. Filters were blocked by 2% nonfat milk powder in PBS and immune sera were used in a 1:500 dilution. The protein bands were detected using protein A coupled to horseradish peroxidase.

Spheroplasting of yeast cells and subcellular fractionation including purification of nuclei was done as described earlier (Hurt et al., 1988).
Differential extraction of purified yeast nuclei with DNAse/RNase, Triton X-100, and 500 mM NaCl was essentially as described (Aris and Blobel, 1989). Aliquots of the different subcellular fractions (one-fold cell aliquot of the homogenate and postnuclear supernatant; a 10-fold cell aliquot of the crude nuclear pellet and the sucrose gradient fractions), as well as equal equivalents of the subnuclear fractions, were analyzed by SDS-PAGE and immunoblotting as described above.

Results

In Vivo Expression of the Fusion Protein Between Dihydrofolate Reductase and the NSP1 Carboxy-Terminal Domain (DHFR-C-NSPI) in Yeast

The carboxy-terminal domain of NSP1 contains all the information to complement a yeast mutant lacking a functional nspl gene (Nehrbass et al., 1990). Since antibodies against this domain did not stain the yeast cells by indirect immunofluorescence, we could not determine its exact location within the nuclear compartment. We therefore decided to tag this domain with a new epitope and attached to its amino terminus a reporter protein, cytosolic dihydrofolate reductase from mouse. We then analyzed whether the fusion protein can be targeted to the nuclear periphery. The behavioral properties of the dihydrofolate reductase protein make it an excellent marker for the study of intracellular organelle targeting (Hurt et al., 1984). If expressed in yeast, DHFR exclusively partitions into the cytosolic fraction whereas if linked to a mitochondrial presequence, it is targeted to the mitochondrial matrix (Hurt et al., 1985). The fusion protein consisting of the entire dihydrofolate reductase coding sequence (187 residues), a short spacer sequence (7 residues), and the NSPI carboxy-terminal domain from residues 606–823 (Fig. 1) was expressed in yeast under the control of the alcohol dehydrogenase promoter. Expression was demonstrated by immunoblotting using affinity-purified antibodies against mouse DHFR or an immune serum made against yeast NSP1 (Fig. 2, lane 3, left and right). A fusion protein of ~53 kD on SDS–polyacrylamide gels was seen on the immunoblot; the predicted molecular mass according to the amino acid sequence was calculated to be 47 kD. Thus, the fusion protein migrates slightly abnormal on SDS–poly-
acrylamide gels. No band at the position of the fusion protein was seen in a control yeast strain (Fig. 2, lane 2). The fusion protein reacted with both the anti-DHFR and anti-NSP1 antibodies. The immunoreaction with anti-DHFR antibodies was highly specific for the DHFR-C-NSP1 fusion protein giving only one protein band on the immunoblot with no apparent proteolytic breakdown (Fig. 2, lane 3, anti-DHFR). Thus, the DHFR-C-NSP1 fusion protein is stably expressed in yeast. In contrast, the authentic NSP1 protein is susceptible to proteolysis even in living cells (see also, Fig. 3 B and Hurt, 1988). The observed proteolytic sensitivity of authentic NSP1 is mainly due to the presence of repetitive sequences within the central NSP1 domain. If the DHFR-C-NSP1 is expressed in *E. coli*, the fusion protein is proteolytically cleaved to a significant extent and a breakdown product corresponding roughly in size to the DHFR moiety becomes evident (Fig. 2, lane 4).

The Fusion Protein Between Dihydrofolate Reductase and the NSP1 Carboxy-Terminal Domain Can Complement the Nonviable Yeast Mutant Lacking a Functional NSP1 Gene

We wanted to test whether the DHFR-C-NSP1 fusion protein, likewise the NSP1 carboxy-terminal domain alone, can complement a yeast mutant lacking a functional NSP1 gene. In the case of complementation we would argue that this domain folds and acts independently from the rest of the NSP1 protein. The gene coding for the fusion protein was therefore placed under the control of the constitutively active yeast alcohol dehydrogenase promoter, inserted into a 2μ plasmid

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*Figure 4.*

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Figure 5. The DHFR-C-NSPI fusion protein cofractionates with nuclei. Expression of DHFR-C-NSPI and authentic DHFR in yeast and subcellular fractionation was performed as described in Materials and Methods. Subcellular fractionation of a haploid yeast strain devoid of authentic NSPI, but expressing the DHFR-C-NSPI fusion protein (YEP13-ADHI-DHFR-C-NSPI) and of a yeast strain expressing authentic DHFR (pLGSD5-DHFR).

Aliquots of total cell homogenate (H), postnuclear supernatant (PNS), crude nuclear pellet (NP), and fraction I to V of a final sucrose gradient were analyzed by SDS–12% polyacrylamide gel electrophoresis and immunoblotting using an immune serum against NSPI and NSP2 (EC9-3, top), against the nuclear marker NOP1 (bottom inset in top) and against mouse DHFR (lower panel). Nuclei, nuclear markers and the DHFR-C-NSPI fusion protein were recovered mainly in the crude nuclear pellet (NP) and in fraction IV of the final sucrose gradient. The positions of the fusion protein, NSP2, and NOP1, and of authentic DHFR are indicated by arrows. The molecular masses of a protein standard are also shown.

and transformed into the diploid yeast strain TF2 heterozygous for NSPI. Expression of the fusion protein could be shown by immunoblotting using antibodies against NSPI and dihydrofolate reductase (Fig. 3B, lane 1; see also Fig. 2, lane 3). This diploid transformant was sporulated to obtain haploid progeny and after tetrad dissection, four viable tetrad spores could be generally recovered (Fig. 3A). Haploid progeny from complete tetrads that contained the disrupted nsp1 gene copy and the 2μ plasmid with the chimeric gene coding for the DHFR-C-NSPI fusion protein showed no evident growth difference as compared to spores with authentic NSPI (Fig. 3A). Therefore, complementation by the DHFR-C-NSPI fusion protein was not different from control strains with endogenous NSPI. Haploid progeny with disrupted nsp1 transformed with the chimeric gene expressed the fusion protein of ~53 kD on SDS–polyacrylamide gels, but no NSPI protein (Fig. 3B, lanes 2 and 3). Again the fusion protein was very stable in the complemented haploid cells and proteolytic breakdown products could not be seen. This clearly demonstrates that a full-length DHFR-C-NSPI fusion protein and not a proteolytic fragment corresponding to the NSPI carboxy-terminal domain caused the in vivo complementation of the yeast mutant lacking a functional NSPI.

The DHFR-C-NSPI Fusion Protein Is Located at the Nuclear Periphery

From the complementation studies performed above it was concluded that the carboxy-terminal domain of NSPI can target a cytosolic protein to the nuclear periphery and nuclear pores. To directly demonstrate this result, we performed indirect immunofluorescence to determine the exact location of the DHFR-C-NSPI fusion protein within the yeast cell using DHFR-specific antibodies (Fig. 4). Affinity-purified anti-DHFR antibodies, which on immunoblots reacted only with the fusion protein, but not with NSPI (see also Fig. 2), predominantly stained the nuclear periphery of yeast cells complemented by the NSPI carboxy-terminal moiety of the chimeric protein (Fig. 4A). The staining of the nuclear boundary was often punctate or patchy, which was highly similar to the authentic NSPI immunolabeling and thus typical for nuclear pore labeling in yeast (see also Aris and Blobel, 1989; Nehrbass et al., 1990; Davis and Fink,
The DHFR-C-NSP1 Fusion Protein Cofractionates with Nuclei and the Insoluble Nuclear Fraction

We were interested to analyze by biochemical means the efficiency of nuclear targeting and the association of the DHFR-C-NSP1 fusion protein with nuclear envelopes in the complemented yeast strain. We recently reported a subcellular fractionation protocol for Saccharomyces cerevisiae that allows the efficient and reliable purification of yeast nuclei (Hurt et al., 1988). Using this technique, we performed a subcellular (Fig. 5) and subnuclear fractionation (Fig. 6) of strains expressing authentic dihydrofolate reductase or the DHFR-C-NSP1 fusion protein. Whereas mouse DHFR was exclusively found in the postnuclear supernatant of yeast cells, but not in the crude nuclear pellet or purified nuclei (Fig. 5, bottom), the fusion protein consisting of DHFR linked to the NSP1 carboxy-terminal domain cofractionated with nuclei and the nuclear markers NOP1 (Fig. 5, top) and histone H2B (data not shown).

After subnuclear fractionation, which allows a distinction between loosely bound and tightly associated nuclear proteins (Aris and Blobel, 1989), the DHFR-C-NSP1 fusion protein was not significantly extracted from purified nuclei by DNAse, RNAse, or detergent, and was only partially released by high salt treatment, a condition that completely releases the nucleolar protein NOP1 (Fig. 6). Applying the same subnuclear fractionation protocol, NSP1 is to a similar extent enriched in the salt-resistant nuclear fraction that is often referred to as the "pore complex/lamina" preparation (data not shown).

In summary, biochemical data, indirect immunofluorescence, and in vivo complementation studies demonstrate that the biochemical properties, as well as the subcellular and subnuclear location of the DHFR-C-NSP1 fusion protein, are comparable to those of authentic NSP1. We therefore conclude that the chimeric protein is associated with the nuclear periphery, can be found at the nuclear pores and fulfills the same essential function as the endogenous NSP1.

Discussion

In this work we have addressed the question of how a protein can associate with the nuclear periphery and nuclear pores. The targeting of macromolecules to the nuclear pores can occur by different mechanisms. Proteins destined for the nuclear interior and initially synthesized in the cytosol carry specific nuclear targeting signals that guide them first to the nuclear pores and further mediate translocation through the nuclear pore complexes (Dingwall and Laskey, 1986; Newmeyer and Forbes, 1988). It was shown that nuclear proteins can bind to components located at the nuclear periphery and the nuclear pores (Newmeyer and Forbes, 1988; Akey and Goldfarb, 1989) and these nuclear envelope constituents may be receptors of the nuclear localization signals. In addition,
though the fusion protein was found inside the nucleus, a peripheral nuclear location of NSP1, which is located at the nuclear pores (Hurt, 1988; Nehrbass et al., 1990). However, this B-galactosidase, although functional, even when the globular and compactly folded dihydrofolate reductase is localized at the nuclear periphery and the nuclear pores (Nehrbass, U., unpublished data).

What Is the Molecular Basis for the Association of the NSP1 Carboxy-Terminal Domain with the Nuclear Pores?

The entire sequence of the NSP1 carboxy-terminal domain is organized into heptad repeats, i.e., a periodic disposition of apolar residues at positions a and d within a heptad repeat of the form a b c d e f g (Fig. 7). This type of heptad repeat is typically found in intermediate filament proteins such as lamins and cytokeratins which can form alpha-helical coiled-coils (Steinert and Roop, 1988). Interestingly, the existence of yeast lamin A and B analogous has been recently suggested (Georgatos et al., 1989). Secondary structure predictions based on the algorithmic program of Chou and Fasman (1978) suggest that several alpha-helical segments occur throughout the entire NSP1 carboxy-terminal domain that may be formed through interactions of the hydrophobic residues of heptad repeats either with each other or with another protein containing similar sequence repeats.

Consistent with this model is a mutation in one of the NSP1 carboxy-terminal heptad repeats (glutamic acid 706 in proline; see also Fig. 7), which could interfere with the alpha-helical organization, resulting in severe impairment of the NSP1 function and temperature-sensitive growth of the mutant (Nehrbass et al., 1990). In this mutant, NSP1 was no longer strictly associated with the nuclear periphery, but was also found in numerous aggregates in the cytosol (Nehrbass et al., 1990).

Together, these results and those using the gene fusion approach suggest that the NSP1 carboxy-terminal domain contains signals that can specifically bind to component(s) located at the nuclear boundary, presumably the nuclear pores. This docking process may depend on alpha-helical interactions between amphipathic heptad repeats. Whether NSP1 first dimerizes through its carboxy-terminal domain and then binds to a receptor-like component at the nuclear pores, or whether the monomeric NSP1 carboxy-terminal directly interacts with a component of similar heptad repeat organization and located at the nuclear pores requires further clarification.

It is interesting to note that the carboxy-terminal domain of rat liver p62 nucleoporin (D’Onofrio et al., 1988), a protein located at the nuclear pores in higher eucaryotes (Davis and Blobel, 1986) and implied to be involved in nuclear transport (Featherstone et al., 1988; Finlay and Forbes, 1990), shows a distinct homology to the yeast NSP1 carboxy-terminal domain (Carmo-Fonseca, M., and E. Hurt, manuscript in preparation) as well as a similar heptad repeat organization (Starr et al., 1990). Thus, this type of sequence motif may also mediate nuclear pore association in higher eucaryotes, and is conserved during evolution from yeast to man.

It is surprising that the carboxy-terminal domain is still functional, even when the globular and compactly folded di-

![Table: NSP1 Heptad Repeats vs. Lamin Heptad Repeats](image-url)
hydrofolate reductase protein is attached to it. This strongly argues that authentic NSP1 is split up into different functional domains that fold and function independently from each other. We have identified at least one function of the carboxy-terminal domain that is involved in nuclear pore association. We will exploit the DHFR characteristics to identify putative docking components of NSP1 located at the nuclear pores by affinity ligand purification using methotrexate affinity columns. The function of the second domain, the highly repetitive central part of NSP1, is not known and functional analysis is more difficult to perform because it is not essential for cell growth. Interestingly, NSP1 repetitive sequences are also found in other nuclear proteins such as NUP1 (Davis and Fink, 1990) and NSP2 (Nehrbass et al., 1990). NUP1 was shown to be localized to the nuclear pores. Thus, proteins with this new type of NSP1-like repetitive sequences seem to occur preferentially at the nuclear pores implying a pore-specific function. It remains to be shown whether NSP1 and NSP1-like proteins form a complex that associates with the nuclear pores.

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