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The Nuclear Membrane

Colin Dingwall and Ronald Laskey

The nuclear membrane forms a major barrier within the cell, permitting levels of regulation not found in prokaryotes. The dynamics and diverse functions of the nuclear membrane and its associated structures are considered in this review. The role of the nuclear pore complex in selective transport across the nuclear membrane has been studied to a considerable degree; however, many crucial questions remain. Components of a signal transduction mechanism are associated with the nucleus, suggesting that nuclear functions may be influenced directly by this system. The involvement of the heat shock cognate protein Hsc70 in nuclear protein import is discussed, and a specific signal-presentation role for this protein is proposed.

The nuclear membrane is the hallmark of eukaryotic cells and a major landmark in evolution. As the most conspicuous boundary inside eukaryotic cells, the nuclear membrane functions to separate the genome from the cytoplasm. This separation permits types of regulation that are not found in prokaryotic cells. In this review, we consider the complex architecture of the nuclear membrane and its associated structures, which are collectively called the nuclear envelope, and we consider the mechanisms by which the nuclear envelope mediates and regulates selective traffic of molecules between the nucleus and cytoplasm. Because many features of the mechanism of nucleocytoplasmic exchange have been reviewed extensively (*1*–*12*), we have chosen to emphasize aspects that have been reviewed less thoroughly. In addition, we propose a specific role for Hsc70, a member of the heat shock class of proteins, in the presentation of the nuclear localization sequence.

Membrane Structure and Dynamics

The nuclear membrane actually consists of two concentric lipid bilayers; the outer lipid bilayer is continuous with the endoplasmic reticulum (ER). Both layers of membrane are perforated by nuclear pore complexes that serve as channels for molecular exchanges between the nucleus and cytoplasm. Nuclear pore complexes also appear to function as rivets that hold the inner and outer layers of membrane together.

The inner nuclear membrane is lined by the nuclear lamina, a layer that is composed of A and B type lamins, a specialized type of intermediate filament protein (*1*, *13*, *14*). The structure of the nuclear lamina varies in different organisms. In *Xenopus* oocytes, the lamina is a dense orthogonal meshwork of fibers (*15*). In contrast, the lamina of interphase cells in *Drosophila* is more disperse, such that areas of the inner nuclear membrane may be exposed directly to the nuclear interior and only a fraction of the chromatin is in contact with the lamins (*16*).

The nuclear envelope disassembles at the onset of mitosis and is reassembled at the end of mitosis. A number of experimental systems have been developed to investigate the mechanisms and to identify the components of these processes. The overall mechanism of assembly involves the attachment of vesicles to the chromatin followed by the fusion of vesicles to produce the

The authors are at the Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR, United Kingdom.

double membrane system. With the use of demembranated *Xenopus* sperm chromatin and an extract from *Xenopus* eggs it is possible to reconstruct these events in vitro (*17–19*). The egg extract provides both soluble factors and membrane vesicles. Different fractionation protocols have identified several distinct vesicle populations that are precursors of the nuclear envelope (*20, 21*). One class of vesicle binds to chromatin and appears to be involved both in targeting the membranes to the surface of the chromatin and in the assembly of nuclear pore complexes. Examination of decondensed sperm chromatin in the egg extract has shown that a population of vesicles of defined size bound to the chromatin (*22*). Another distinct vesicle population is thought to contribute much of the nuclear membrane lipid. The inhibition of pore complex assembly produces nuclear envelopes with the two layers of the nuclear membrane widely separated, demonstrating the rivet-like function of the nuclear pore complex (*23*).

The binding of vesicles to the chromatin requires both chromatin and membrane-bound proteins but does not require adenosine triphosphate (ATP). However, ATP is essential for the fusion of chromatin-bound vesicles to form the membrane (*22*). The fusion of vesicles is sensitive to reagents that react with thiol groups of proteins. It is not clear whether this sensitivity is related to a thiol-sensitive protein in the cytosol that is required for vesicle fusion events in the ER, Golgi complex, and early endosome (*22*). The fusion of the chromatin-bound vesicles to form the nuclear membrane system also requires guanosine triphosphate (GTP) hydrolysis, which occurs in membrane fusion events in exocytosis and endocytosis (*22, 24*). The nonhydrolyzable GTP analogue guanosine-5′-*O*-(3-thiotriphosphate (GTP-γ-S) inhibits vesicle fusion in these extracts by binding to a soluble factor, which may be a member of a family of small GTPases involved in vesicle fusion events in the endomembrane system (*25*). These results indicate that the activity of these proteins is not limited to the Golgi apparatus and provide evidence for a molecular link between secretory vesicles and vesicles that mediate postmitotic nuclear assembly. However, the general distribution of proteins and enzyme activities characteristic of the ER (*20, 21*) indicates that the vesicle subpopulations that contribute to nuclear envelope assembly are distinct from the majority of ER-derived vesicles. Therefore, although the mechanism of vesicle fusion in the two systems may be similar, the nuclear envelope is assembled from a subset of ER-derived vesicles.

Phosphorylation regulates lamin depolymerization and also regulates the association of membranes with chromatin (*26*). Dephosphorylation, most probably of the membrane-bound receptor, promotes binding to the chromatin, whereas phosphorylation during mitosis causes release of the chromatin-bound membranes. It is thought that this phosphorylation is not the result of the direct action of the kinase that regulates the cell cycle but the result of the components of a separate phosphatase-kinase regulatory system regulated by the cell cycle–specific kinase.

The receptor-coupled signaling system in the plasma membrane of eukaryotic cells transduces extracellular signals across the plasma membrane to stimulate a cascade of intracellular events leading to cell growth and proliferation. A principal component of this system is phosphoinositidase C, a receptor-activated enzyme that cleaves phosphatidylinositol-4,5-bisphosphate (PIP_2) to liberate diacyglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). The DAG stimulates protein kinase C, and the IP_3 stimulates calcium release from intracellular stores. This signaling system may be similar to an entirely separate nuclear phosphoinositide signaling system located in the nuclear membrane and possibly in the nuclear interior (*27, 28*). Evidence for a nuclear signaling system comes from several observations: Purified nuclei in vitro synthesize PIP_2 and phosphatidylinositol-4 phosphate, and a transient decrease in the mass of these lipids occurs when Swiss 3T3 cells are cultured in the presence of insulin-like growth factor–1 (IGF-1) and a coincident increase in nuclear DAG occurs (*29*). These data suggest that a signal reaches the nucleus as a result of the stimulation of the IGF-1 receptor and that this signal stimulates a nuclear phosphoinositidase C (PIC) enzyme (*30*). Support for this hypothesis comes from an analysis of the subcellular distribution of different PIC isozymes in which it has been shown that the nuclei of Swiss 3T3 cells contain the β isozyme whereas the γ isozyme is confined to the cytoplasm (*31*). PIC-β is regulated by a subclass of G proteins that is a family of heterotrimeric GTP-binding, membrane-bound proteins involved in cell signaling. The G proteins have been reported to be integral components of the nuclear lamina (*32, 33*).

As mentioned above, one product of the activity of phosphoinositidase C is DAG, which stimulates protein kinase C (PKC). Important substrates of PKC have usually been considered to be plasma membrane and cytoplasmic proteins, but translocation of certain PKC isozymes to the perinuclear region and the nuclear interior has been observed in a number of cell types after stimulation with growth factors or tumor promoters (*29, 34–37*). This translocation resulted in increased phosphorylation of a number of nuclear proteins, including lamin B (*36*). Although no nuclear localization sequence has been mapped in PKC, deletion of the NH_2-terminal regulatory domain of the α isozyme has shown that regions in the hinge and catalytic COOH-terminal domains are exposed in the truncated protein. In contrast to the wild-type protein (*38*), the truncated protein is located in the nucleus. These results are consistent with a model in which PKC activation exposes regions in the hinge and catalytic domains, allowing translocation to the nucleus.

The existence of separate nuclear and cytoplasmic signaling pathways suggests that separate nuclear and cytoplasmic calcium fluxes should be detectable. An exclusively cytoplasmic or nuclear flux of calcium has been demonstrated in at least two cases (*39, 40*). It is not clear how these calcium fluxes can be kept separate by the nuclear membrane; this poses several problems because in our current model of nuclear pore structure, the pore contains a passive channel in addition to the specific transport channel.

The association of growth factor molecules and polypeptide hormones (which normally act at the cell surface) with the nucleus and the nuclear membrane has been documented. In some cases this association has been shown to stimulate pore-mediated macromolecular transport in isolated nuclei (*41*). Platelet-derived growth factor (PDGF) is a potent mitogen composed of two related polypeptides linked by disulfide bonds. The B chain contains a nuclear localization sequence that can target heterologous proteins to the nucleus. The A chain nuclear localization sequence is encoded by an exon that is subject to alternative splicing, which suggests that control of its nuclear localization may occur by regulation of splicing (*42*).

In two other cases, that of basic fibroblast growth factor (bFGF) and int-2, the choice of initiation codon determines the subcellular localization of the protein. In bFGF, a nuclear localization sequence is present in an amino acid segment between two alternative start codons (*43*). The mouse *int-2* gene encodes a fibroblast growth factor (FGF)–related product and is a frequent target of oncogene activation in mouse mammary tumor virus (MMTV)–induced carcinomas. Similar to bFGF, an NH_2-terminally extended int-2 protein is localized to the nucleus (*44*). These two examples of post-transcriptional regulation of subcellular localization suggest that these factors may influence cellular behavior by two distinct mechanisms, determined by the subcellular localization of the proteins.

The presence of a nuclear-associated signaling pathway and the association of growth factors with the nucleus and the nuclear membrane constitute an additional level of biochemical complexity and may provide additional mechanisms for the regulation of nuclear activities.

The Nuclear Pore Complex

The nuclear pore complex is well named (*45, 46*). It has a molecular mass estimated at 10^8 daltons and is five times the mass of an SV40 virus particle or about 30 times the mass of a ribosome, both of which it can transport (*47*). It spans both the inner and the outer layers of the nuclear membrane and is organized with eightfold symmetry around a central channel.

A three-dimensional map of the nuclear pore complex has been published recently (*48*) that confirms the eightfold radial symmetry and identifies interconnected rings and columns (Fig. 1). It is unlikely that the central channel in the density map is open space, but instead it may be the site of the proteins that function in selective transport. An attractive feature of this model is the existence of eight smaller channels between the inner annulus subunits and the outer rings (Fig. 1). Whereas signal-mediated selective transport occurs through the center of the pore complex, the more peripheral small channels may be the route of passive diffusion of smaller molecules.

Until recently, some features of the nuclear pore complex have remained controversial. For example, electron microscopy studies of sectioned material (*49*) reported fibrils extending discrete, but different, distances away from nuclear pore complexes into the nucleus and cytoplasm. These structures, however, were rarely seen in isolated nuclear envelopes. Recently, the fibrils extending into the nucleus have been shown to form basketlike structures (*50, 51*) of unknown composition and function (Fig. 2). It is unclear if the set of fibrils that extend into the cytoplasm forms part of a larger, more organized structure, but these fibrils selectively bind particles coated with nuclear proteins (Fig. 3), implicating them in an early stage of nuclear protein import (below).

The Mechanism of the Nuclear Pore Complex

The design of the nuclear pore complex and the identity of nuclear localization sequences have become much clearer in recent years (*2, 3, 9, 45, 46*). These advances focus attention on the mechanism of pore complex function. The pore complex might act as a regulated gate or it might physically propel molecules into the nucleus.

It is known that ATP hydrolysis is required for translocation through the pore complex (*52, 53*); however, the molecular mechanism of translocation remains unknown. One model for pore complex function proposes a double iris mechanism similar to the airlock on a spacecraft (*54*). In this model the steps would be (i) a signal-dependent opening of the iris on the cytoplasmic face, (ii) the entry of the signal-bearing particle, (iii) the closure of the outer iris, and (iv) the opening of the inner iris to release the particle into the nucleus. The opening and closing of the iris in this model would be energy dependent. In order for the nucleus to achieve selected protein accumulation rather than equilibration, only the cytoplasmic face of each iris should respond to an import signal, whereas only the nuclear face should respond to an export signal.

Alternatively, ATP hydrolysis may be required for the physical propulsion of imported molecules through the nuclear pore complex. The fibrils on the cytoplasmic face of the pore complex may have a function in this model. Maul (*55*) proposed that the fibrils may serve as static rails along which molecular motor molecules move nuclear proteins; alternatively, the fibrils themselves may move into the nucleus, similar to the propulsion cable in a cable car system. Although ATP hydrolysis is not required for the binding of nuclear proteins to these pore fibrils (*53*), it is not known whether ATP hydrolysis allows movement of the fibrils themselves or of other molecules along the fibrils. Identification of the proteins that make up the fibrils would give clues to the nature of transport mechanisms.

One adenosine triphosphatase (ATPase) in the nuclear pore complex may be a myosin-like protein isolated from nuclear envelopes of *Drosophila* cells. Although immunochemical studies suggest that there is a form of myosin in the pore complex itself, this myosin has not been characterized in detail (*56, 57*). An ATPase with the dynamic properties of myosin could play a crucial role in any of the pore mechanisms proposed above.

Selective Import

The import of proteins into the nucleus is one of the most studied areas of cell biology (*2–12*). Many details have become known particularly in the characterization of nuclear localization sequences. The first such sequence to be mapped in detail was the seven amino acid nuclear localization sequence of SV40 large T antigen (*2*). However, the bipartite motif found in *Xenopus* nucleoplasmin, in which two clusters of basic residues are separated by a spacer segment, may be a more general and widespread type of nuclear localization sequence (*58*).

An increasing number of proteins bind to nuclear localization sequences in a sequence-specific manner and are therefore candidates for receptors in nuclear protein import (*10*). The criteria that define a nuclear import receptor are: specific recognition of the nuclear localization sequence, interaction with the pore complex, and stimulation of import. The majority of the candidate receptors that have been identified only partly fulfill these criteria, and the binding studies have almost exclusively used the SV40 large T nuclear localization sequence. Therefore, it remains to be determined whether these proteins bind other sequences with similar specificity and whether they are involved directly in transport.

With the use of a permeabilized cell transport assay (*59*), it has been shown that purified proteins that are nuclear localization sequence binding proteins can stimulate the nuclear uptake of a protein–nuclear localization sequence peptide conjugate (*60, 61*). However, cytosolic factors are

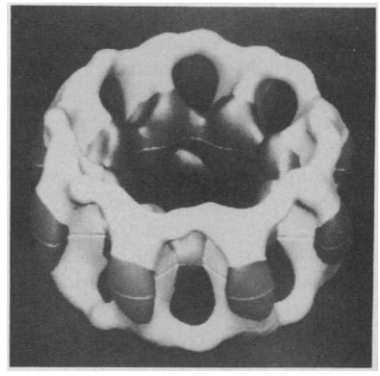

Fig. 1. A three-dimensional model of the nuclear pore complex. [Reprinted from (*48*) with permission]

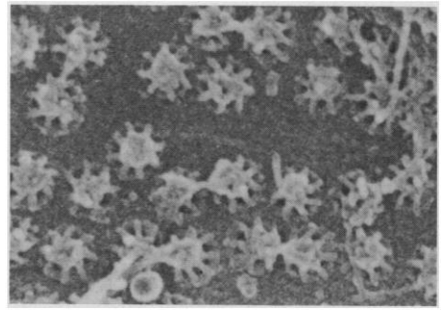

Fig. 2. Baskets of fibrils on the nuclear faces of nuclear pore complexes in the *Xenopus* oocyte nucleus visualized by scanning electron microscopy (magnification, ×30,000). [Photograph provided by T. D. Allen and M. W. Goldberg]

required in order to detect the stimulatory effect, indicating that additional factors are required for nuclear protein uptake.

In vitro transport systems have been used to identify a number of factors essential for pore function and nuclear protein import. Wheat germ agglutinin depletion of *Xenopus* egg extracts removes glycoproteins essential for pore function; these glycoproteins can then be added back to a depleted extract to restore activity (*62, 63*). Fractionation of *Xenopus* oocyte extracts has detected soluble factors that mediate both steps of the protein import process (*64*).

A number of nuclear pore proteins have been identified with the use of antibodies; a few of these proteins have been cloned and sequenced (Table 1). The presence of a heptad repeat sequence in the NUP1 and NSP1 proteins from yeast appears to be unique to these proteins and may therefore represent a diagnostic feature of this class of nuclear pore proteins (*65, 66*). A number of the nuclear pore proteins, or nucleoporins, are modified by a cytoplasmic modifying enzyme with *O*-linked *N*-acetylglucosamine residues (*67, 68*). In contrast, gp210, a glycoprotein that anchors the pore complex into the nuclear membrane and projects into the perinuclear cisterna, has modifications characteristic of Golgi glycoproteins (*69*).

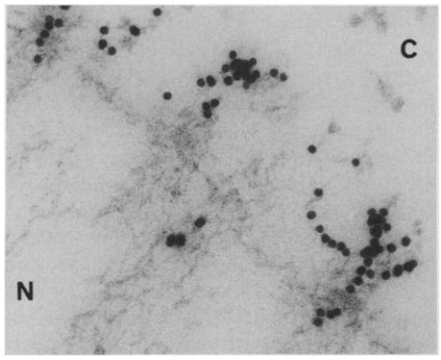

Fig. 3. Fibrils on the cytoplasmic surface of the nuclear pore complex bind colloidal gold particles that have been coated by a nuclear protein, nucleoplasmin. N, nucleus; C, cytoplasm. [Photograph provided by A. D. Mills; reprinted from (*53*) with permission]

Evidence for the existence of biochemically distinct pathways for import into the nucleus has come from competition experiments (*70*). It has emerged that the import of U snRNAs (U1, U2, U4, and U5) that contain a 5′ trimethyl G cap and are complexed by Sm proteins is competed by free cap analog but not by bovine serum albumin–conjugated with the SV40 nuclear localization sequence (BSA-NLS). In contrast, the import of U6 RNA, which lacks the trimethyl G cap and is not complexed by Sm proteins, is competed by BSA-NLS but not by cap analog. The import of U3 snRNA, which contains a trimethyl G cap but does not bind Sm proteins, is not competed by either free cap or BSA-NLS.

However, these biochemically distinct pathways must converge at the nuclear pore complex because they are all inhibited, to different extents, by the coinjection of wheat germ agglutinin and antibodies to the nucleoporins. These competition experiments lead to the important conclusion that under conditions in which one pathway is saturated, the nuclear pore complex channel remains unsaturated and available for the translocation of the members of the other classes of transport substrates. Thus, the saturable step must occur before the occupation of the translocation channels, a concept consistent with the identification of soluble factors that mediate the import of proteins into the nucleus.

Chaperoning the Chaperone: A Role for Hsp70 in Presentation of Nuclear Localization Signals

Bipartite nuclear localization signals of the nucleoplasmin class pose a paradoxical problem. Many signals have 10 or 11 amino acids separating the two clusters of basic residues (*9*). However, with the exception of frequently occurring proline residues, these spacer sequences appear to be unrelated, and they presumably fold differently. If so, it is difficult to understand why signal spacers of 10 or 11 amino acids appear to be preferred.

A possible explanation of this paradox comes from two studies that show that the heat shock protein Hsp70, or its cognate Hsc70, is required for protein import into the nucleus (*63, 71*). Members of the Hsp family bind and stabilize unfolded conformations of short regions of peptide chain (*72–74*). The heat shock proteins bind peptide backbone rather than to amino acid side chains. Furthermore, peptides of ten amino acids can induce maximal ATP hydrolysis by members of the hsp family.

The significance of the length of the signal spacer can be explained by a model in which Hsc70 (or Hsp70) binds to and stabilizes a locally unfolded nuclear localization signal, thus presenting the nuclear localization signal to a second receptor in an unfolded conformation. In this model, Hsc70 would be the first of at least two sequential signal receptors; the second receptor would recognize the unfolded signal presented by Hsc70. This model is presented in Fig. 4. This proposed role for Hsc70 is analogous to the role of HLA proteins of the major histocompatibility complex in antigen presentation (*75*) and is consistent with recent proposals that the COOH-terminal domain of Hsc70 folds into a binding cleft similar to that of the HLA antigen binding site (*76, 77*).

It is important to note that this model does not propose the unfolding of the whole protein during nuclear transport. Such a possibility was excluded for nucleoplasmin because the subunits are not exchanged between pentamers during transport (*78*). Instead, only local unfolding of the signal region is required. If Hsc70 plays such a role, then there are parallels with its function in protein transport into other organelles (such as mitochondria and chloroplasts) in spite of the different problems encountered in transport through pores when compared to transport through membrane bilayers.

A further irony that emerges from this possibility is that of chaperoning the chaperone. The action of the Hsp70 family is frequently referred to as that of a molecular chaperone (*79*), a concept first defined for the action of nucleoplasmin itself (*80*).

Selectivity of Export

In general, both mRNA and snRNAs are transported across the nuclear envelope in the form of protein complexes (*81*). Like protein transport, RNA transport is signal-dependent, carrier-mediated, occurs through the nuclear pore complex, and may occur through multiple biochemically distinct pathways.

Table 1. Nuclear pore proteins.

Protein	Organism/ tissue	Molecular mass (kD)	Notable features*	Reference
NUP1	*S. cerevisiae*	130	T/S GFSFG heptad repeat	(*65*)
NSP1	*S. cerevisiae*	100	T/SGFSFG heptad repeat	(*66*)
Nucleoporins	Rat/liver	210, 180, 145, 100, 63, 58, 54, 45	*O*-linked *N*-acetylglucosamine	(*67*)
p62	Rat/liver	62 (54.3)	High Ser/Thr content *O*-linked *N*-acetylglucosamine	(*68*)
gp210	*Drosophila*	210	Asp-linked high mannose oligosaccharides	(*69*)

*Abbreviations for the amino acid residues are: F, Phe; G, Gly; S, Ser; T, Thr.

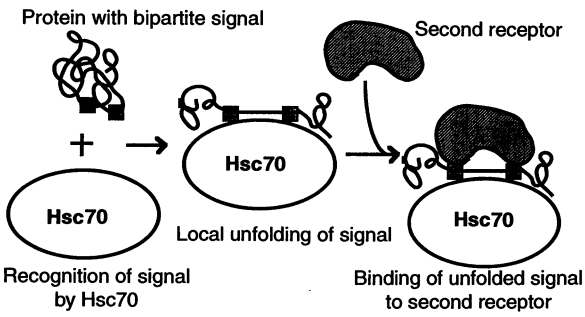

Fig. 4. A model for a signal presentation function for Hsc70 in nuclear protein import. The nuclear localization signal is bound and locally unfolded by Hsc70 for presentation to a second receptor. This model can explain the frequency of spacers of 10 or 11 amino acids between two clusters of basic residues (square boxes) in signals of the nucleoplasmin type (see text for details).

Insight into the mechanism of RNA translocation has come from structural studies of Balbiani ring granules in *Chironomus* salivary glands with the use of electron microscope tomography (*82*). Balbiani ring granules are pre-mRNA ribonucleoprotein (RNP) particles, each containing a 35- to 40-kb message encoding a secretory polypeptide. Within the particle an RNP fiber or ribbon is tightly folded into a ring. Transport through the pore is preceded by specific orientation of the particle at the pore entrance, such that the 5′ end of the RNA passes through the pore complex first, indicating that some structural feature of the RNP particle is recognized by the pore complex. The bent ribbon is gradually straightened, and certain proteins are shed from the particle, as shown by a reduction in its mass. This is consistent with observations that the protein composition of pre-mRNA particles in the nucleus is different from that of mRNP particles in the cytoplasm (*83*). The elementary RNP fiber is thus gradually unpacked and appears in the cytoplasm in a more or less extended conformation. The unfolding fiber has repeatedly been found associated with ribosomes. Furthermore, Balbiani ring RNA rapidly enters polysomes and is loaded onto the ER. This suggests that immediately upon translocation through the pore, the mRNA molecule becomes engaged in polysome formation, which enables a translation initiation complex to be formed directly (*82*).

The signals involved in RNA transport have been analyzed in detail for the small RNA molecules U1 snRNA, tRNA, and 5S RNA (*84–86*). For U1 snRNA, export from the nucleus is specified by the monomethyl-inverted guanosine cap. Transport back into the nucleus of the assembled snRNP particle requires a two-component signal composed of the Sm proteins and a trimethyl G cap (a modification of the monomethyl G cap by a cytoplasmic methylase). The binding of U snRNP–specific protein U1A is not required for the migration of the RNA, which suggests the existence of an independent pathway for the nuclear accumulation of the protein.

For the transport of 5S RNA, the newly transcribed RNA transiently interacts with La antigen, and La is then replaced with either ribosomal protein L5 or the 5S gene–specific transcription factor TFIIIA. Each of these two RNPs is transported out of the nucleus and accumulates in the cytoplasm. Since RNA molecules impaired in their ability to interact with either L5 or TFIIIA can be exported, there appear to be two equivalent but independent pathways for nuclear export of 5S RNA (*85*).

For tRNA, all of the processing and base modification steps must be completed before the molecule can be exported. The regions of the human $tRNA_i^{Met}$ molecule that are most critical for recognition by the transport system involve the highly conserved D stem loop and the T stem loop. Nucleotide changes that influence tertiary base pairing affect processing and transport, which indicates that the conformation of the molecule is critical for recognition in these processes (*86*). Unlike the two previous examples, no proteins have been identified that are involved in tRNA export.

The dependence of tRNA transport on the completion of processing contrasts with the transport of mRNA (*81*). Although the vast majority of mutant RNAs that do not complete splicing are retained and destroyed in the nucleus, the common occurrence of constitutive introns that undergo splicing in a tissue-specific or temporally regulated manner indicates that intron removal is not an obligatory requirement for transport.

Insight into the mechanism of mRNA export, consistent with the structural observations described above, have come from a number of studies (*87, 88*). The presence of frameshift and nonsense mutations within the gene that cause translation to terminate prematurely (within the first two-thirds of the mRNA) results in decreased levels of mRNA. Nonsense mutations positioned in the final third of the mRNA, however, do not cause premature termination. A possible explanation for the effect of such mutations is that protein synthesis itself may be the driving force for mRNA transport across the nuclear envelope; translation of the RNA by ribosomes on the cytoplasmic face of the nuclear pore complex may pull the RNA out of the nucleus. Early termination of translation stops transport when the bulk of the RNA is still within the nucleus, thus exposing the RNA to degradative enzymes. Termination of translation at later stages, when most of the RNA has been transported into the cytoplasm and has been translated, has less dramatic effects. A similar protein synthesis mechanism has also been proposed for the transport of tRNA molecules from the nucleus to the cytoplasm (*86*).

Regulated Transport

Regulation of molecular transport across the nuclear envelope presents a number of advantages for the control of nuclear activities. Presynthesized transcription factors and cell cycle regulators can be maintained in the cytoplasm and only transported into the nucleus at specific times and in response to specific signals. This permits an extremely rapid response to such signals. The mechanisms that are used to achieve regulated entry include (i) conformation change after ligand binding, (ii) covalent modification of the nuclear localization signal, (iii) attachment to a cytoplasmic structure, and (iv) binding of regulatory subunits that mask the nuclear localization signal (*89*). In addition to these examples in which control is exerted at the level of the transported protein, there is evidence for differences in selectivity of the nuclear envelope in *Tetrahymena*. In this organism, the micronuclei and macronuclei accumulate specific subsets of nuclear proteins from the same cytoplasm. Thus, the macronuclei accumulate macronuclear histone H1, calf thymus histone H1, and the SV40 large T antigen NLS linked to bovine serum albumin. In contrast, histone H4 is accumulated in whichever nucleus is replicating, but the differential specificity of import of the other nuclear proteins is maintained. Therefore, the selectivity of transport can differ between two pore-containing nuclei present in the same cytoplasm (*90*).

A Role in Regulating DNA Replication

There is an increasing amount of evidence that the nuclear membrane plays multiple roles in the regulation of DNA replication. Perhaps it is not coincidental that the only eukaryotic cell-free systems that initiate DNA replication efficiently in vitro also assemble DNA into pseudonuclei surrounded by a functional nuclear envelope (*91, 92*). In extracts of *Xenopus* eggs, DNA that is assembled into pseudonuclei replicates under cell cycle control, whereas DNA that is excluded from pseudonuclei fails to replicate

(93). Interference with the assembly or the transport function of the nuclear envelope similarly prevents DNA replication (94). Furthermore, when multiple nuclei are incubated together in *Xenopus* egg extract the DNA within each nucleus replicates independently; the nuclear envelope both defines the unit of replication and determines the timing of replication (95, 96).

Integrity of the nuclear membrane is also required to prevent reinitiation of DNA replication within a single cell cycle. Treatments that reversibly permeabilize the nuclear membrane of a replicated nucleus allow DNA replication to recur without passage through mitosis (97, 98). These observations can be explained by a licensing factor model (97) in which an essential initiation factor for replication lacks a nuclear localization signal. This hypothetical factor can bind to chromatin during mitosis when the nuclear envelope breaks down.

Although the nuclear membrane does not break down during mitosis in yeast and other lower eukaryotes, a family of proteins has been discovered that shows similar behavior to that predicted for the hypothetical licensing factor. Two genes that are required for DNA replication in *Saccharomyces cerevisiae*, *CDC46* and *MCM3*, both encode gene products that remain cytoplasmic until anaphase, when they enter the nucleus (99–101). These proteins persist in the nucleus until the start of S phase and then disappear. Although there is no evidence that CDC46 and MCM3 limit DNA replication to one round per cell cycle, their behavior makes them candidates for this function. Not only are CDC46 and MCM3 partly homologous, but MCM3 shows strong sequence similarity to a mammalian protein P1 that associates with DNA polymerase α (102). It is not known whether proteins of this class are responsible for the observation that damage to the nuclear membrane causes reinitiation of DNA replication. However, both phenomena focus attention on roles for the nuclear membrane and regulated protein import in the coupling of DNA replication to the cell cycle. This concept suggests that the eukaryotic mechanism that limits DNA replication to one round per cell cycle may have coevolved with the distinguishing feature of eukaryotic cells, the nuclear membrane.

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