NUCLEAR PROTEIN TRANSPORT

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I. INTRODUCTION

The specific localization of molecules within the cell is an essential process. One set of basic localization problems concerns the nucleus: (1) what mechanisms select proteins to be localized to the nucleus; (2) how proteins cross the nuclear envelope; and (3) how these proteins are retained in the nuclear interior. We consider these questions and review nuclear envelope structure. In addition, we review what is known about protein transport into the nucleus and compare it with transport into the endoplasmic reticulum and mitochondria, two organelles which share many phenomenological similarities with each other and the nucleus.

II. STRUCTURE OF THE NUCLEAR ENVELOPE

Before discussing nuclear transport, we will review the structure and biosynthesis of the nucleus.[1,2] The nucleus of a typical mammalian cell has a diameter of 3 to 10 μm and is separated from the cytoplasm by the nuclear envelope.[3] The nuclear envelope is constructed of two lipid bilayers, the outer and inner nuclear membranes, which are separated by a gap of 10 to 60 nm called the perinuclear space.[4] The outer membrane is apparently continuous with the endoplasmic reticulum, making it appear that the endoplasmic reticulum lumen and perinuclear space are also continuous, although little is known about any chemical continuity between these membranes or spaces.[1,2]

The composition of nuclear and endoplasmic reticulum membranes is similar in terms of lipid pattern, many proteins, glycoproteins, and carbohydrates.[1,2] These results could be due to real similarities or cross-contamination between these two membrane fractions, which are very difficult to separate completely. Some cells, such as avain erythrocytes and certain species of green algae, do not show continuities between the endoplasmic reticulum and nucleus.[2]

The nucleoplasmic surface of the inner membrane is associated with the nuclear lamina, a fibrous network which appears to act as a "nucleoskeleton".[5,6] In addition to providing a structural framework for the nuclear envelope, the lamina is thought to participate in the organization of chromatin.[6,7] In mammals, this lamina has three major protein components, lamins A, B, and C, which form an insoluble network that remains after membranes and peripheral proteins have been removed with nonionic detergents and salt.[6,8,9] The nuclei of some higher eukaryotic cell types, such as clam oocytes, appear to have only one or two lamins.[10] McKeon et al.[11] and Fisher et al.[12] identified large regions of homology between

the A and C lamins and the large α-helical domain of cytoplasmic intermediate filaments. This domain is involved in coil-coiled assemblies of the intermediate filaments[11] and, hence, might play a similar structural role in the interactions between the lamins.

A. THE NUCLEAR PORE

One of the most striking features of the nuclear envelope is the nuclear pore. Pores are found throughout the nuclear membranes of all eukaryotes and, in typical mammalian cells, are found at a density of about 11 pores per square micrometer of membrane area (3000 to 4000 pores/nucleus).[3] This density varies in different organisms from 3 pores/μm^2 to 59 to 60 pores/μm^2.[1] The pore bridges the gap between the inner and outer nuclear membranes and may serve as a point of contact between these two membranes, which are otherwise topologically distinct.[1] The pore complex also interrupts the nuclear lamina and may be bound to it; pore complexes remain associated with the lamina after extraction with salt and nonionic detergent.[6,13]

The subunit structure of the pore has been characterized primarily by electron microscopy. It is a cylindrical complex consisting of two "coaxial annuli", one facing the cytoplasm and one facing the nucleoplasm, with each annulus being made of eight smaller subunits. Spokes emanate toward the center from the periphery. Sometimes a central plug is found.[14] The pore complex as a whole has a diameter of about 120 nm, but the actual channel through the center is much smaller, having an apparent diameter of approximately 9 nm.[4,14,15]

A few of the pore proteins have been identified. A 190-kDa glycoprotein is localized to the region of the membrane immediately adjacent to the pore and may act as an anchor for the pore.[16] Fisher and colleagues[12] have identified a 190-kDa ATPase and proposed that it may be a functional pore component.[17,18]

A 62-kDa wheat germ agglutinin (WGA)-binding protein is found associated with the pore.[19] This protein is probably the same as the 63- to 65-kDa WGA-binding protein identified by Finlay et al.[20] This protein is glycosylated in the cytoplasm prior to assembly into the nucleus and is part of a group of recently identified O-linked nuclear envelope glycoproteins containing multiple O-linked-*N*-acetylglucosamine residues.[19,21] More significantly, Finlay et al.[20] showed that WGA blocks nuclear transport *in vitro* transport assays. It appears that WGA does not cause this effect by blocking the pore opening, because it does not block the passive diffusion of dextrans (10 to 64 kDa) across the nuclear membrane.[20,22]

III. ASSEMBLY OF THE NUCLEUS

Little is known about the biosynthesis of the nuclear envelope. Its biosynthesis differs from that of other membrane-bound organelles in that for most organisms, the nuclear envelope is dispersed and reassembled during the course of each mitosis. (In fungi, nuclear envelope dissassembly during mitosis does not occur.[23]) During mitosis, the membrane breaks up into small vesicles, and the lamina becomes reversibly depolymerized.[7,8] The vesicles appear to remain associated with lamin B, and lamins A and C become soluble.[8,9] Mitotic, depolymerized lamins have a higher level of phosphorylation than do interphase lamins.[8,24] Reversible phosphorylation could control the polymerization of the lamina and, perhaps, also the assembly and disassembly of the nuclear envelope.[8,24] What happens to the pore complexes during mitosis remains unknown.

Studies of nuclear assembly have been assisted by the development of systems for *in vitro* nuclear assembly and transport. Addition of purified DNA (either from phage lambda or *Xenopus*) to *Xenopus* oocyte extracts triggers formation of nuclear envelopes around the added DNA.[25,26] At least some of these synthetic nuclei are functional reproductions of natural nuclei; many have a complete double membrane, a nuclear lamina, nuclear pores, and are active in nuclear transport.[27] The fact that lambda DNA triggers this response indicates

that specific eukaryotic DNA sequences are not required for assembly of the functional nuclear envelope.[27] Nucleus-like structures are also formed by seeding extracts from homogenized metaphase Chinese hamster ovary cells with DNA.[9] Transport is studied with these *in vitro* systems, as well as with extracts composed of rat liver nuclei and nuclear envelope vesicles.[20,28,29] Experiments with these systems have indicated that the lamins assemble into a network on which the rest of the nucleus is constructed and have demonstrated that ATP is not necessary for this assembly process.[9]

How does the nucleus increase in size? Materials stockpiled during interphase are incorporated into the envelope when it is reformed at the end of mitosis. However, this cannot completely explain nuclear membrane growth because the membrane can increase in size during S phase.[3] Furthermore, nuclei of yeast and other fungi do not visibly disassemble during mitosis,[23] making it unlikely that reformation could be the only means of adding material to the nuclear envelope.

The nucleus may grow by directly exchanging material with the endoplasmic reticulum.[3] Ribosomes are sometimes found on the outer nuclear membrane.[1] The inner membrane could grow by exchanging material with the outer membrane at the pores.[3] How these membranes would retain their functional identity is not made clear by this model.

A third possibility is that the nucleus grows by fusing with cytoplasmic vesicles, as do the Golgi apparatus and the plasma membrane.[30] Vesicles have been seen to fuse with the nuclear envelope in the electron microscope in a variety of cell types.[1] However, there is little other evidence to indicate that such fusion does occur or what role it might play in nuclear function. Since the vesicles appear to fuse only with the outer membrane, the question of how the inner membrane grows remains open. It may receive material from the outer membrane by exchanging it at the pores.

IV. PROTEIN LOCALIZATION

A. POSSIBLE MECHANISMS OF NUCLEAR PROTEIN IMPORT

What determines which proteins are localized to the nucleus? While some proteins are localized specifically to the nuclear interior, others apparently cannot gain access to the nuclear interior at all, and still others appear to partition equally between the nucleus and cytoplasm.[4] Two models have been proposed to explain this problem. The first proposes that molecules gain access to the nuclear interior by freely diffusing in and out of the nuclear pores and accumulate in the nucleus by binding to some factor inside (DNA, the lamina, possibly a soluble "retention protein").[31,32] Alternatively, some proteins might diffuse into the nucleus and accumulate there because of greater stability in the nuclear environment.[33] This diffusion and retention model of nuclear localization was widely accepted until recently. A more recent model is "selective transfer". This model postulates that nuclear transport is an active and specific process, triggered by the specific interaction of a nuclear localization sequence on the protein with a receptor, presumably in the pore.

The attraction of the diffusion and binding theory is its simplicity. It uses only statistical mechanics and principles of self-assembly and does not postulate any unknown factors. The only strong evidence supporting it consists of experiments showing that some particles (nonnuclear proteins and dextrans) can enter the nucleus with a rate based only on their size, indicating that diffusion across the nuclear envelope does occur.[4,15] But particles with a diameter greater than 9 nm cannot diffuse into the nucleus,[4] even though proteins much larger than this are found inside the nucleus. Several lines of more recent evidence have pointed out other problems with the diffusion model. Foremost among these is that nuclear proteins, such as nucleoplasmin, can accumulate in the nucleus very quickly, much faster than dextrans of similar size.[32,34] Additionally, proteins with multiple (up to six) nuclear localization sequences (see below for a more detailed definition) per molecule move more

rapidly from the cytoplasm to the nucleus than proteins with only one or two nuclear localization sequences.[34]

The selective transport model proposes selective translocation of proteins into the nucleus, rather than passive retention in the nuclear interior. It is supported by experiments demonstrating the existence of nuclear targeting sequences (also called nuclear localization signals or nuclear signal sequences) in some nuclear proteins. These sequences are necessary for transit across the nuclear envelope. Specific information for nuclear protein localization appears to reside both within the transported protein (see section below) and the transport apparatus.[28,35,36] Depletion of ATP blocks nuclear protein accumulation both *in vivo*[27] and *in vitro*[28,35] consistent with specific transport requiring energy.

B. PROTEIN TRANSPORT INTO THE ENDOPLASMIC RETICULUM AND MITOCHONDRIA

We will review the process of protein localization to the endoplasmic reticulum and mitochondria. The endoplasmic reticulum, unlike the nucleus, is partitioned from the cytoplasm by only a single lipid bilayer. No structures analogous to either the nuclear lamina or the pores are visible by electron microscopy. The endoplasmic reticulum functions as a place of early modification and protein sorting in the secretory pathway. Proteins to be secreted or localized to the plasma membrane, Golgi apparatus, or lysosomes first pass through the endoplasmic reticulum before moving on to other organelles. Proteins and lipids are modified in the endoplasmic reticulum by the addition of carbohydrates in the first steps of the maturation of glycoproteins and glycolipids.

One standard model of protein localization into the endoplasmic reticulum is as follows.[37,38] Translocation of a targeted protein into the endoplasmic reticulum is mediated by the "signal recognition particle" (SRP), a complex consisting of six polypeptide chains and a 7S RNA, and the docking protein, an integral component of the endoplasmic reticulum membrane.[39-41] SRP recognizes and interacts with the signal sequence of the targeted protein.[42] Translation of a secreted or membrane bound protein is initiated on cytoplasmic ribosomes. Then, after the signal sequence has cleared the ribosome, the SRP binds to the ribosome-nascent protein complex, halting translation until the complex interacts with the docking protein on the endoplasmic reticulum surface.[41,43-45] At this point the SRP is released, along with the translation inhibition, and the protein begins to be inserted into the lumen of the endoplasmic reticulum.[46] At some point the signal sequence is cleaved by a specific peptidase, although there are some proteins with internal signal sequences, such as ovalbumin, which have uncleaved signal sequences.[47,48] How the protein crosses the membrane remains unclear. There are a number of theories, but there is no consensus on a mechanism.[37,38]

Mitochondrial localization shares several characteristics with transport into the nucleus. The mitochondrion, like the nucleus and unlike the endoplasmic reticulum, is bounded by a double membrane layer. Also, unlike the endoplasmic reticulum, the mitochondrion contains proteinaceous pores in its outer membrane, although these pores are smaller than those found in the nucleus, having an estimated radius of 20 Å.[49] Discussion of translocation into the mitochondria is complicated by the fact that there are four compartments to which proteins can be translocated: the outer membrane, inner membrane, intermembrane space, and matrix (lumen).

Nuclear and mitochondrially localized proteins are initiated on free cytoplasmic ribosomes and can be translocated posttranslationally. Mitochondrial proteins made *in vitro* are imported into isolated mitochondria and are taken up equally well, regardless of whether the mitochondria are added posttranslationally or if they are added during protein synthesis.[38,50] Also, extramitochondrial pools of precursor mitochondrial proteins can be detected in the cytoplasm of yeast cells during pulse-chase experiments.[51] Mitochondria do have polysomes on their outer membranes enriched in nascent mitochondrial preproteins, but these

polysomes are only a small fraction of the total cellular polysomes translating a given mitochondrial protein.[38,52,53] As for both the nucleus and endoplasmic reticulum, many mitochondrial proteins contain an amino acid signal sequence which somehow specifically interacts with the translocation apparatus to localize the protein to the correct intramitochondrial compartment.[54] For instance, the cytoplasmic and mitochondrial histidine-tRNA synthetases of yeast are encoded by the same gene, but the mitochondrial form differs only by having a mitochondrial targeting sequence at its N-terminus.[55] Most of mitochondrial signal sequences are removed as the protein assembles into the mitochondria.[54] Miura et al.[56] and Argan et al.[57] have demonstrated that a cytoplasmic factor, which may be analogous to the endoplasmic reticulum SRP, is necessary for translocation to take place. Proteins must remain unfolded in order to assemble into mitochondria.[58,59] ATP is required to maintain proteins in an import competent conformation. An enzyme responsible for keeping proteins unfolded may be located in the cytoplasm or in the mitochondria.[60]

A large body of evidence supports these models of endoplasmic reticulum and mitochondrial localization.[37,38,61] It is generally accepted that the interaction between the SRP, translocated protein, and docking protein is best described by the theories above. However, a controversial aspect of this model is whether translocation into the endoplasmic reticulum is necessarily cotranslational. This idea derives from the observation that the endoplasmic reticulum is studded with ribosomes, and from experiments showing that proteins can be localized to microsomes *in vitro* if the membranes are present during protein synthesis, but not if they are added after the protein is complete.[62,63]

Thus, it may be that translocation to the endoplasmic reticulum need not be cotranslational. Then why is the endoplasmic reticulum studded with ribosomes? This conflict can be resolved by postulating that in the cell, translocation initiates during translation, but that this coupling is not a required part of the mechanism of translocation. Possibly the translocation apparatus has components, unrelated to the act of translocation itself, whose function is to ensure that endoplasmic reticulum-destined proteins are not translated until they are being translocated. Some proteins undergo translational arrest *in vitro* at distinct stages in their translation. For preprolactin, 75 amino acids are synthesized before translational arrest occurs.[64] Thus, enough of the protein may be exposed to allow the SRP interaction to occur.

However, Maher and Singer[65] have postulated that *in vitro* proteins cannot be translocated across the endoplasmic reticulum membrane posttranslationally because they have intrachain disulfide bonds, which do not allow the protein to unfold in a manner required for translocation.[66] In fact, human preprolactin can be posttranslationally translocated across the endoplasmic reticulum membrane *in vitro* if the medium is sufficiently reducing.[65] Thus, translocation of proteins would depend on an equilibrium between the folded and the unfolded state. Since the folded state is generally favored, posttranslational translocation would be slowed or not take place at all.

C. SIGNAL SEQUENCES

The most striking similarity between transport into the nucleus, the endoplasmic reticulum, and the mitochondria is that specific amino acid sequences are capable of targeting proteins to each organelle. In all three cases, these "signal sequences" can be attached by gene fusion to cytoplasmic proteins and will correctly localize these chimeric proteins to their respective organelles.[67-70]

Although signal sequences are necessary for the localization of proteins to these organelles, in no case has a consensus sequence for a signal sequence been found. Sequence analysis and study of point mutations has identified at least a general motif for endoplasmic reticulum signal sequences. An endoplasmic reticulum signal sequence is normally at the N-terminal end of the protein and usually consists of 16 to 26 residues with a short, basic stretch at its N-terminus (1 to 5 residues), a core of strongly hydrophobic amino acids (4

to 8 residues), and a third domain with small, helix-breaking amino acids that appears to be a leader peptidase recognition site.[38]

Mitochondrial signal sequences are not as well characterized, but are usually hydrophilic and free of acidic residues.[71] Roise et al.[72] showed that a chemically synthesized peptide corresponding to the cytochrome c oxidase subunit IV mitochondrial targeting sequence could form an amphiphilic helix sufficiently surface active enough to strongly perturb phospholipid bilayers. They also found that a membrane potential (such as the one across the mitochondrial inner membrane) enhanced the activity of the peptide. Von Heijne[71] showed that most of the known mitochondrial presequences fit the model for an amphiphilic helix. Amphiphilic helices have been proposed to be the common structural feature of many surface active peptides.[73-75] Possibly the membrane disruption caused by the helix of a signal sequence could create a pathway through which the protein could pass. This ability of an amphiphilic helix to create a membrane channel through which proteins might pass would dispense with the need for transit pores. In this way, mitochondrial protein import may differ from protein import into the endoplasmic reticulum and the nucleus.

Three types of experiments demonstrate the role of the nuclear targeting sequence: deletion analysis, mutation experiments, and gene fusions. Dingwall et al.[34] provided the first evidence for the existence of a nuclear targeting sequence by performing deletion analysis on nucleoplasm, a major nuclear protein of *Xenopus* oocytes. They proteolytically cleaved nucleoplasm into N-terminal "core" particles and C-terminal "tail" particles. Limited proteolysis also yielded multiple species consisting of "core" species to which zero to five "tails" were still attached. Particles still retaining the C-terminus were still capable of nuclear localization following injection into the oocyte cytoplasm. Particles with five tails were more efficiently imported than those with fewer tails. When "core particles" (nucleoplasm pentamers without tails) were injected into the nucleus, they could be retained there. However, they did not accumulate in the nucleus when cytoplasmically injected. These results indicated that something specific to the C-terminal tail region promoted protein entry into the nucleus. Similar deletion analysis has revealed regions of influenza virus NP[76] and NS1,[77] SV40 T-antigen,[78,79] SV40 VP,[80] polyoma large T,[81] adenovirus E1A,[82] rat glucocorticoid receptor,[83] human lamin A,[84] histone H1,[85] and yeast GAL4 protein[86] that are important for nuclear localization. Polyoma large T-antigen[81] and influenza NS1[77] proteins were shown to contain two regions important for nuclear localization. In the case of polyoma large T-antigen,[81] deletion of both sequences results in non-nuclear polyoma T-antigen, but deletion of either one or the other has only a partial effect on nuclear localization.

The mutation experiment is best exemplified by the work with large T-antigen of the simian virus SV40. SV40 T-antigen is a 94-kDa protein found predominantly in the nucleus of infected mammalian cells.[87] Lanford and Butel[88,89] and Kalderon et al.[78] found mutations in T-antigen that resulted in a single amino acid change at lysine 128. Conversion of T-antigen lysine 128 to either threonine[78] or asparagine[88] caused the normally nuclear protein to be cytoplasmically localized and excluded from the nucleus. These mutational analysis and deletion experiments led to the proposal that a short stretch of amino acids between 126 and 132 was important for T-antigen nuclear localization (see Table 1). A similar stretch of amino acids lies between amino acids 415 and 421 of human lamin protein (see Table 1). Conversion of the lysine at position 417 of lamin to isoleucine or threonine resulted in a mutant protein that, for the most part, did not assemble into the nuclear envelope.[84] Lamin lysine 417 corresponds to SV40 T-antigen lysine 128 in its position in the proposed nuclear localization sequence. A number of mutations that cause single amino acid changes in the N-terminus of the yeast GAL4 protein affect its nuclear localization to varying degrees.[90]

Gene fusions were first used to demonstrate the ability of a short stretch of amino acids to target a normally cytoplasmic protein to the nucleus in the yeast, *Saccharomyces cerevisiae*,[86,91] and in Vero cells.[79] Fusions between portions of genes encoding a nuclear protein

TABLE 1
Nuclear Localization Sequences

Viral	
SV40 T-antigen	^{126}PKKKRKV^{132}
SV40 VP1	^{1}APTKRKGS^{8}
Polyoma large T	^{189}VSRKRPRPA^{197}
	^{280}PKKARED^{286}
Influenza NP	^{327}QLVWMACNSAAFEDLRVLSFIR^{348}
Influenza NS1 NLS1	^{28}DAPFL*DRLRRD*QKSLRG^{44}
NLS2	^{203}WGSSNENGGPPLT*PKOKRKM*ARTARSKVRRDKMAD^{237}
Adenovirus E1A	^{285}KRPRP^{289}
Saccharomyces cerevisiae	
Mat α2	^{1}MN*KIPIK*DLLNPQ^{13}
Ribosomal L3	^{1}MSHRKYEAPRHGHLGFL*PRKR*^{22}
GAL4	^{1}MKLLSSIEQACDIC*RLKKLK*CSKEKPKCA^{29}
Histone H2B	^{1}MSAKAEKKPASKAOAEKKPAAKKTSTSTDP*GKKRSKA*^{37}
Other	
Xenopus nucleoplasm	^{153}AV*RPAATK*KAGQAKKK^{169}
Rat glucocorticoid receptor	^{497}YRKCLQAGMNLEARK*TKKKIK*GIQQATA^{524}
Human lamin A	^{407}SQTQGGG*SVTKKRKLE*STESRSSFSQHARTSGRVAVEE^{444}

Note: Italicized sequences are proposed consensus nuclear localization sequences. Numbers indicate where the amino acids occur in the protein indicated in the left column. For references, see citations in the text.

and a cytoplasmic protein are joined *in vitro*, and the resulting plasmid DNA is introduced into cells. When the gene fusion is expressed, a chimeric protein is produced that contains portions of both nuclear and cytoplasmic proteins. The intracellular location of the chimeric protein is determined by immunocytochemical and biochemical techniques. Hall et al.[91] showed that the first 13 amino acids of MATα2, a yeast DNA-binding protein, are sufficient to localize *Escherichia coli* β-galactosidase to the yeast nucleus. Similarly, Silver et al.[86] showed that the GAL4 nuclear targeting sequence is contained in the first 74 GAL4 amino acids by fusing different sections of GAL4 to the 5′ end of the *E. coli lacZ* gene. The intracellular location of the resulting chimeric proteins was determined by immunofluorescence with anti-β-galactosidase. The first 22 amino acids of the yeast ribosomal L3 protein[92] and yeast histone H2B[93] have also been shown to be sufficient to target *E. coli* β-galactosidase to the yeast nucleus. *E. coli* β-galactosidase was chosen as the nonnuclear component of the chimeric proteins because as a cytoplasmic protein, it is not localized to any intracellular compartment in eukaryotic cells and its size (116 kD) might limit its ability to diffuse through the nuclear pore. Interestingly, Moreland et al.[93] did find that some β-galactosidase did enter the nucleus but did not accumulate there.

Fusions between SV40 T-antigen and β-galactosidase also localize to the nucleus in animal cells.[79] However, pyruvate kinase is often used as the cytoplasmic portion of the chimeric proteins in animal cells because, as a true cytoplasmic protein, it is completely nuclear excluded.[79] Fusions containing only the SV40 T-antigen nuclear targeting sequence at the N-terminus of pyruvate kinase are sufficient to completely relocalize the chimeric protein to the nucleus.[79] Similar analyses have been used to define nuclear localization sequences in *Xenopus* nucleoplasm,[94,95] rat glucocorticoid receptor,[83] and polyoma large T-antigen.[81] Some gene fusion experiments have employed galactokinase[82] or α-globin[76] as the cytoplasmic portion of the fusion protein. A pentapeptide from adenovirus E1A is

sufficient to localize an E1A-galactokinase fusion protein to the nucleus.[82] Fusions between influenza NS1 amino acids 28 to 44 and α-globin were nuclear localized.[77] It is important to emphasize that the gene fusion experiment tests the *sufficiency* of a particular sequence to act as a nuclear targeting sequence. Only in the case of SV40 T-antigen, polyoma T-antigen, and influenza NP has the same sequence been shown to be important for localization of the native protein.

There is little obvious similarity between nuclear signal sequences, except for a preponderance of basic amino acids in some of the sequences (see Table 1). At least two consensus nuclear targeting sequences have been proposed. Hall et al.[91] found sequences similar to the peptide KIPIK in several yeast nuclear proteins and proposed that a short stretch of hydrophobic amino acids bordered on either side by positively charged amino acids could be a common structural theme for nuclear signal sequences. A second proposed sequence, PKKKRKV, is found in a number of viral proteins, as well as the *Drosophila* 70-kDa heat shock protein and the nucleoplasmin tail region.[96] Extensive study of protein sequences indicates that there is no pattern common to all nuclear proteins.[32] However, the nuclear localization sequence of large T-antigen from the SV40 virus can direct localization both to the nucleus of the *Xenopus* oocyte[97] and the yeast *S. cerevisiae*.[98] These results suggest that there is conservation of the recognition and translocation mechanisms across species.

There is little similarity between the lengths of nuclear targeting sequences. While only seven amino acids of T-antigen are necessary for nuclear localization,[78,79] polyoma large T-antigen,[81] influenza NS1,[77] and MATα2[91] appear to contain two sequences necessary for complete nuclear import. These latter cases are intriguing because Dingwall et al.[34] found that nucleoplasmin pentamers containing a greater number of "tail" peptides (tails contain the nuclear targeting sequences) migrate more quickly into the nucleus. Smith and colleagues[96] obtained similar results, demonstrating that partially defective nuclear localization signals can cooperate to result in more complete nuclear accumulation.[99] Possibly "extra" nuclear targeting sequences are functional and cause a protein to be more quickly or efficiently taken up into the nucleus. Lanford et al.[100] demonstrated this directly. Ovalbumin to which only one or two nuclear location sequences were chemically cross-linked took up to 1 h to move from the cytoplasmic site of injection to the nucleus in TC-7 cells. On the other hand, ovalbumin with six peptides conjugated per molecule took only 15 min to migrate into the nucleus. There is also evidence that multiple localization signals can increase the efficiency of mitochondrial localization.[101]

D. RECEPTORS

The fact that specific peptide signals allow some proteins into the interior of the endoplasmic reticulum, mitochondria, and nucleus while cytoplasmic proteins lacking a signal sequence are excluded[79,100] suggests that transport is receptor mediated in all three systems. One could postulate a specific interaction between the signal sequence and another part of the transport mechanism, which would select only proteins with the correct signal sequences to be localized inside of these organelles.

The "receptor" system for the endoplasmic reticulum is well characterized. The first receptor is the signal recognition protein, which, as mentioned above, is a "cytoplasmic receptor" that interacts directly with the various endoplasmic reticulum signal sequences and binds to the ribosome/nascent protein complex. The docking protein in the endoplasmic reticulum membrane then acts as the receptor for the SRP/ribosome/nascent protein complex and allows translation to resume.

What do the (putative) receptors for organelle protein import recognize? Somehow the SRP recognizes and specifically interacts with the various secretion signal sequences, even though there is no primary sequence homology between them. There is at least vague

homology of charge between these sequences (see previous description of pattern); thus the signal sequence/SRP interaction may be, in part, an electrostatic one. Alternatively, receptors may recognize the secondary structure of the signal sequence. Nuclear localization sequences, on the other hand, can be short (the T-antigen signal sequence is only seven amino acids long) and can function in almost any protein environment (as shown by gene fusion experiments[99]). Recognition of secondary structure or charge may also be the cue for nuclear receptor interaction.

Less is known about receptors for transport into the mitochondria, although there is evidence that they do exist. Experiments indicate that mitochondrial precursors en route to the inner membrane or matrix specifically bind to the mitochondrial outer membrane.[102,103] The binding does not require an energized membrane and is diminished by protease treatment of the membranes.

Gillespie et al.[104] found that a synthetic peptide corresponding to amino acids 1 to 27 of preornithine carbamyltransferase (preOTC) can block import of preOTC into mitochondria, while another peptide of similar charge-to-mass ratio had no effect. The same peptide blocked import of precursor forms of other mitochondrial matrix and inner membrane proteins.

These results suggest the existence of a common proteinaceous receptor for mitochondrial protein import. However, outer mitochondrial membrane proteins appear to not require these same receptors and may employ a different mode of import.[105,106]

Though we have not addressed transport into chloroplasts, chloroplast import shares many similarities with that of the organelles discussed here. Chloroplast proteins are often encoded by the nucleus and have a transient NH_2 terminal signal sequence. Pain et al.[107] have identified a receptor for protein import into chloroplasts by isolating anti-idiotype antibodies to a 30-amino acid chloroplast signal sequence, and localized this receptor to envelope contact zones. Whether mitochondrial protein import receptors will be localized to similar areas of contact between inner and outer membranes remains to be seen.

These similarities suggest that if signal sequence receptors exist as part of the endoplasmic reticulum and mitochondrial (and even chloroplast) localization mechanisms, they may exist for the nucleus as well. None have yet been identified, although this could be due only to our poor knowledge of the biochemistry of the nuclear envelope. Nuclear receptors might be associated with the pores since it is through the pores that transport apparently takes place.[108]

Experimental evidence for the existence of a nuclear transport receptor was provided by Goldfarb et al.,[97] who cross-linked BSA to the T-antigen nuclear targeting sequence and found that the uptake rate of the complex was saturable. Further support for receptor-mediated uptake comes from *in vitro* experiments with isolated rat liver nuclei.[28] Isolated nuclei import SV40 T-antigen in a signal-dependent manner. The uptake is dependent on both temperature and ATP. The uptake of radioactively labeled SV40 T-antigen is reduced when a 50-fold chemical excess of unlabeled SV40 T-antigen is included in the reaction. These results suggest that nuclear import may be a receptor-mediated process.

There might be many nuclear targeting sequences if there were more than one type of receptor. However, the competition experiments described above, as well as the fact that *Xenopus* nucleoplasmin can be localized into the yeast nucleus,[109] make it unlikely that there are specific receptors for each protein to be nuclear-localized.[79]

E. AN ALTERNATIVE MECHANISM

The nuclear localization signal sequences could bind to DNA or some other component of the nuclear interior. Saturation of the uptake rate would then be due to the filling up of the appropriate binding sites. There may be no nuclear localization consensus sequence because there is no common binding factor — just different types of DNA binding sites. This idea is a restatement of the diffusion and binding model. In fact, the nuclear localization

signal of GAL4 contains the DNA binding regions.[110] Examination of the SV-40 T-antigen nuclear targeting sequence suggests that it might bind DNA due to its positive charge. However, the hypothesis that localization capabilities of these sequences are due only to their DNA binding properties can be rejected for the following reasons. Paucha et al.[111] demonstrated that T-antigen containing a mutated nuclear targeting sequence was capable of binding DNA but incapable of directing nuclear localization. Conversely, SV40 large T-antigen mutants that fail to bind to DNA can, nevertheless, localize to the nucleus by virtue of their respective nuclear localization sequences.[112] Similar results were obtained in yeast with LexA, a prokaryotic DNA-binding protein which nonspecifically binds to DNA but is not nuclear localized.[113] For DNA binding to work as a mechanism of retention, the binding must be nonspecific because there are too few specific DNA binding sites to localize the large amount of protein that exists in a normal cell nucleus.

F. UTILIZATION OF ENERGY

Signal-mediated localization of proteins to the endoplasmic reticulum, mitochondria, and nucleus requires ATP, while localization to the mitochondrial matrix requires both ATP and a transmembrane electrochemical potential.[26,27,114-116] Thus, all three events are active processes. No one yet knows how either the ATP or the transmembrane potential is utilized for translocation. "Active processes" could include both active transport (where the protein is pulled across the membrane) and facilitated diffusion (where the protein is somehow selectively allowed to diffuse through a pore or membrane). We still cannot distinguish between these possibilities for the nucleus. However, Newmeyer and Forbes[35] have shown that binding at the nuclear pore does not require ATP, but subsequent import through the pores does. Proteins could be pulled through the pores (or across other parts of the nuclear membrane), or the pore could be selectively widened so only certain proteins diffuse through the channel. The pore may work as a diaphragm and, under certain conditions, expand the central channel so that it is larger than its apparent diameter determined from diffusion experiments.

ATP is not necessary to retain proteins inside the nucleus.[27] Two possible mechanisms might retain proteins inside these organelles; they could bind to some interior component or they could be retained in the organelle interior because they cannot diffuse out through the pore. Passage through the pore is unidirectional. The second possibility may best explain retention of proteins in the endoplasmic reticulum since there is probably very little free diffusion of soluble proteins across the hydrophobic endoplasmic reticulum membrane. Both mechanisms could be utilized in the nucleus.

Size could prevent some molecules from diffusing out of the nucleus. When introduced into the cytoplasm, molecules above approximately 45 Å (60 to 70 kDa) cannot freely diffuse into the nucleus and remain in the cytoplasm. Proteins of 60 kDa or greater are retained when directly microinjected into the nucleus.[100] Furthermore, if large nuclear molecules cross the nuclear membrane in an energy-dependent process,[27] they will not be able to exit the nucleus by the same pathway because this would be unfavorable thermodynamically.

The experiments with LexA (see Section E preceding) demonstrated that DNA binding was not sufficient to localize this diffusable DNA-binding protein to the nucleus. But, somehow, small proteins are retained in the nucleus. Histones are small molecules below the diffusion radius of the nucleus and so, theoretically, are small enough to diffuse out. Histone retention may be due to incorporation into nucleosomes, but there is not enough DNA in the *Xenopus* oocyte nucleus to bind all of the histones stockpiled there.[117-119] One of the functions of nucleoplasmin, the most abundant protein in a *Xenopus* oocyte nucleus, might be to retain the unassembled histones in the nuclear interior.[117-121]

G. DIFFERENCES BETWEEN NUCLEAR PROTEIN IMPORT AND TRANSPORT OF PROTEINS INTO THE ENDOPLASMIC RETICULUM AND MITOCHONDRIA

Although there are intriguing similarities between mechanisms of localization to the nucleus, the endoplasmic reticulum, and the mitochondria, there are also fundamental differences. Nuclear proteins are apparently synthesized on free cytoplasmic polysomes, while proteins that are localized inside the endoplasmic reticulum are initiated on free ribosomes, but are often completed on the rough endoplasmic reticulum. Mitochondrial proteins can apparently be synthesized either on free ribosomes or on the mitochondrial outer membrane.[38,52,53,122]

Many secreted, membrane-bound, and mitochondrial proteins are proteolytically modified after translocation. Nuclear-localized proteins are not, to our knowledge, modified; there are no differences between molecular weights and isoelectric points of "precursor proteins" (*in vitro* translated from mRNA) and "mature proteins" (isolated from nuclei or microsomes).[123] Proteins isolated from nuclei can be relocalized to the nucleus when microinjected,[124] but proteins removed from the endoplasmic reticulum or mitochondria generally cannot return, except for those with uncleaved signal peptides such as ovalbumin.[125]

This dissimilarity need not have any implications for the translocation mechanism. Since the nucleus dissociates during each mitosis in most organisms (in fungi it does not), nuclear proteins must be relocalized after each mitosis, while endoplasmic reticulum and mitochondrial proteins are translocated only once.[126] Removal of endoplasmic reticulum and mitochondria signal peptides accompanies the translocation process, which need only occur once. Relocalization may be a continual process in cells where the nucleus must reassemble.

Transport across the nuclear envelope is bidirectional. Many chemical species are transported in one or both directions. Some proteins cross only to the inside of the nucleus. But mature mRNAs are selectively exported, and other molecules such as snRNAs cross the membrane at different times in different directions.[127,128] Ribosomal proteins enter the nucleus, assemble into ribosomal particles in the nucleolus, and then return to the cytoplasm. The nucleus of a rapidly growing cell must import approximately 100 histone molecules per minute per pore, and at the same time export 3 newly assembled ribosomes per minute per pore.[4,33] This traffic is only a small portion of the total nuclear cytoplasmic exchange. Mechanisms controlling transport out of the nucleus may be independent of those controlling transport in. However, in at least one case, the same pore was shown to accommodate both protein import and RNA export.[129]

1. Translocation Conformations

While the experiments cited above imply that some mitochondrial proteins must remain unfolded to cross both the endoplasmic reticulum and mitochondrial membranes, there is evidence which indicates that proteins (and other particles) can cross the nuclear membrane in their native conformations.[65,114,125,130] The finding that nuclear pores have a functional radius of about 90 Å led to the suggestion that large proteins must either unfold or at least deform to pass through the pores. However, Feldherr et al.[108] were able to show that gold-conjugated nucleoplasmin particles with diameters between 50 and 200 Å can pass through nuclear pores, demonstrating that large particles can fit through pores without deforming.

While endoplasmic reticulum and mitochondrial signal sequences can target cytoplasmic proteins to their organelles when attached via gene fusion, nuclear targeting sequences can function not only as a continuous part of a protein, but also when artificially conjugated to normally nonnuclear proteins. When the seven-amino acid nuclear targeting sequence of T-antigen is chemically conjugated to ovalbumin, BSA, IgG, and ferritin, the proteins are localized to the nucleus.[97,100] Nuclear localization signals can even localize nonproteins such as the gold-coated nucleoplasmin mentioned above. These results show that molecules with

with molecular weights of up to 465 kDa[100] and diameters up to 200 Å[108] can be imported into the nucleus. Experiments of this type have not been done for endoplasmic reticulum and mitochondrial signal sequences.

In gene fusions, signal sequences are integrated into cytoplasmic proteins by splicing the DNA encoding the signal sequence to DNA encoding a cytoplasmic protein and so are connected to the proteins by normal peptide bonds. In cross-linked protein conjugates, proteins and other particles are attached by a bond provided by a chemical cross-linker. This connection differs in chemical composition from a normal peptide bond. The particle to be transported is not a continuous string of amino acids.

While a nuclear targeting sequence can function in diverse molecular environments, it is not true that it can function in any environment. Efficiency of a nuclear targeting sequence does depend on its surroundings because in some positions in a protein its activity is masked. Insertion of the T-antigen nuclear targeting sequence into five places within chicken muscle pyruvate kinase has varying effects on nuclear localization.[99] The function of nuclear targeting sequences is not limited to a particular region of the protein (i.e., N-terminal or C-terminal). Even when placed at the N-terminus, the ability of a nuclear localization sequence to function depends on to what protein it is fused. The first 29 GAL4 amino acids will efficiently localize a GAL4-invertase fusion protein to the nucleus in *S. cerevisiae*, but not a GAL4-β-galactosidase fusion protein.[98] Similarly, a single SV40 T-antigen nuclear location sequence targets a SV40-invertase fusion protein to nucleus more efficiently that a SV40-β-galactosidase fusion protein in *S. cerevisiae*.

2. The Role of Pores in Transport

The nuclear membrane has large visible pores containing an apparently aqueous channel, while the mitochondria has smaller, proteinaceous pores, and the endoplasmic reticulum membrane contains no similar structure. Since the endoplasmic reticulum apparently does not have pores, it would seem that the basic mechanisms for crossing its membrane must be very different from those of the other two organelles.

One compelling piece of evidence that proteins do enter the nucleus through the pores is from direct observation. Feldherr et al.[108] injected *Xenopus* oocytes with gold-conjugated nucleoplasmin and followed it by electron microscopy from the cytoplasm, through the actual pore opening, and into the nucleus. This evidence indicates that at least some proteins are selectively transported through the nuclear pores.

One interesting, and as yet unexplained, observation from this experiment was that the gold-conjugated particles seemed to line up outside the nucleus. This behavior implies a cytoplasmic/cytoskeletal system for directing proteins into the nucleus. There may be cytoplasmic receptors around the pores. Indeed, Georgatos et al.[131] have demonstrated specific binding of the nuclear envelope to particular segments of the intermediate filaments vimentin and desmin. They were able to show that these fragments recognized specifically lamin B, but not other protein constituents of the nuclear envelope. Possibly, these filaments somehow participate in localization.

V. CONCLUSIONS

How does the information in this review reflect on the three questions we initially posed? The mechanism by which a nuclear protein is selected for localization most likely is that a receptor recognizes a signal sequence within the protein. The evidence for a receptor is circumstantial; no nuclear receptor has been identified, either biochemically or genetically. That signal sequences mediate nuclear localization has been established.

Proteins presumably cross the nuclear membrane through the nuclear pore. The first direct evidence for this was the experiments of Feldherr,[108] showing that nucleoplasmin-

coated gold particles can be seen within the pore. The primary reason for the acceptance of this idea is that the pores exist and serve no other obvious function besides transport.

Two plausible mechanisms exist for retaining proteins within the nucleus: binding to DNA or other components of the nuclear interior and trapping of proteins too large to diffuse out through the pore. From the structure of the nucleus, mitochondria, and endoplasmic reticulum, one might imagine that different mechanisms would be used for protein localization. It is, therefore, perhaps surprising that localization to all three organelles involves signal sequences. For localization of proteins to the endoplasmic reticulum, receptors in the form of SRP exist which recognize signal sequences. This suggests that analogous receptors will recognize nuclear signal sequences and mediate nuclear localization. On the basis of this analogy, the experiments of Goldfarb et al.[97] are readily interpreted that nuclear localization is a saturable process. Identification of the nuclear receptor will clearly be a goal of future research.

Within the last 10 years, a broad outline of the process of protein localization to the nucleus has been established. Several questions remain. What is the mechanism by which the pore opens? How are proteins translocated through the pore? How is the direction of traffic determined for a given pore? How is the signal sequence recognized? How far can the analogy between mechanisms of localization to the endoplasmic reticulum and the nucleus be extended? In the future, *in vitro* nuclear localization systems and possibly mutations affecting the localization mechanism itself should shed light on these problems.

REFERENCES

1. **Franke, W. W., Scheer, U., Krohne, G., and Jarasch, E.,** The nuclear envelope and the architecture of the nuclear periphery, *J. Cell Biol.*, 91, 39s, 1981.
2. **Franke, W. W. and Scheer, U.,** Structures and functions of the nuclear envelope, in *The Cell Nucleus*, Vol. 1., Busch, H., Ed., Academic Press, New York, 1974, 220.
3. **Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D.,** *Molecular Biology of the Cell*, Garland Publishing, New York, 1983, 17.
4. **Bonner, W. M.,** Protein migration and accumulation in nuclei, in *The Cell Nucleus*, Vol. 6 (Part C), Busch, H., Ed., Academic Press, New York, 1978, 97.
5. **Fawcett, D. W.,** On the occurrence of a fibrous lamina on the inner aspect of the nuclear envelope in certain cells of vertebrates, *Am. J. Anat.*, 119, 129, 1966.
6. **Dwyer, N. and Blobel, G.,** A modified procedure for the isolation of a pore complex-lamina fraction from rat liver nuclei, *J. Cell Biol.*, 70, 581, 1976.
7. **Gerace, L., Blum, A., and Blobel, G.,** Immunochemical localization of the major polypeptides of the nuclear pore complex-lamina fraction, *J. Cell Biol.*, 79, 546, 1978.
8. **Gerace, L. and Blobel, G.,** The nuclear envelope lamina is reversibly depolymerized during mitosis, *Cell*, 19, 277, 1980.
9. **Burke, B. and Gerace, L.,** A cell-free system to study reassembly of the nuclear envelope at the end of mitosis, *Cell*, 44, 639, 1986.
10. **Gerace, L., Comeau, C., and Benson, M.,** Organization and modulation of nuclear lamina structure, *J. Cell Sci.*, 1 (Suppl.), 137, 1984.
11. **McKeon, F. D., Kirchner, M. W., and Caput, D.,** Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins, *Nature*, 319, 463, 1986.
12. **Fisher, D. Z., Chaudhary, N., and Blobel, G.,** cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 6450, 1986.
13. **Aaronson, R. P. and Blobel, G.,** Isolation of nuclear pore complexes in association with a lamina, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 1007, 1975.
14. **Unwin, P. N. T. and Milligan, R. A.,** A large particle associated with the perimeter of the nuclear pore complex, *J. Cell Biol.*, 93, 63, 1982.
15. **Paine, P. L., Moore, L. C., and Horowitz, S. B.,** Nuclear envelope permeability, *Nature*, 254, 109, 1975.

16. **Gerace, L., Ottaviano, Y., and Kondor-Koch, C.,** Identification of a major polypeptide of the nuclear pore complex, *J. Cell Biol.*, 95, 826, 1982.
17. **Berrios, M., Blobel, G., and Fisher, P. A.,** Characterization of an ATPase/dATPase activity associated with the *Drosophila* nuclear matrix-pore complex-lamina fraction, *J. Biol. Chem.*, 258, 4548, 1983.
18. **Berrios, M. and Fisher, P. A.,** A myosin heavy chain-like polypeptide is associated with the nuclear envelope in higher eukaryotic cells, *J. Cell Biol.*, 103, 711, 1986.
19. **Davis, L. I. and Blobel, G.,** Identification and characterization of a nuclear pore complex protein, *Cell*, 45, 699, 1986.
20. **Finlay, D. R., Newmeyer, D. D., Price, T. M., and Forbes, D. J.,** Inhibition of *in vitro* nuclear transport by a lectin that binds to nuclear pores, *J. Cell Biol.*, 104, 189, 1987.
21. **Holt, G. D. and Hart, G. W.,** The subcellular distribution of terminal *N*-acetylglucosamine moieties, *J. Biol. Chem.*, 261, 8049, 1986.
22. **Jiang, L. W. and Schindler, M.,** Chemical factors that influence nucleocytoplasmic transport: a fluorescence photobleaching study, *J. Cell Biol.*, 102, 853, 1986.
23. **Byers, B.,** Cytology of the yeast life cycle, in *Life Cycle and Inheritance*, Strathern, J. N., Jones, E. W., and Broach, J. R., Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1981, 59.
24. **Ottaviano, Y. and Gerace, L.,** Phosphorylation of the nuclear lamins during interphase and mitosis, *J. Biol. Chem.*, 260, 624, 1985.
25. **Forbes, D. J., Kirschner, M. W., and Newport, J. W.,** Spontaneous formation of nucleus-like structures around bacteriophage DNA microinjected into *Xenopus* eggs, *Cell*, 34, 13, 1983.
26. **Newmeyer, D. D., Finlay, D. R., and Forbes, D. J.,** *In vitro* transport of a fluorescent nuclear protein and exclusion of non-nuclear proteins, *J. Cell Biol.*, 103, 2091, 1986a.
27. **Newmeyer, D. D., Lucocq, J. M., Burglin, T. R., and DeRobertis, E. M.,** Assembly *in vitro* of nuclei active in nuclear protein transport: ATP is required for nucleoplasmin accumulation, *EMBO J.*, 5, 501, 1986b.
28. **Markland, W., Smith, A. E., and Roberts, B. L.,** Signal-dependent translocation of simian virus 40 large-T antigen into rat liver nuclei in a cell-free system, *Mol. Cell Biol.*, 7, 4255, 1987.
29. **Riedel, N. and Fasold, H.,** Nuclear-envelope vesicles as a model system to study nucleocytoplasmic transport, *Biochem. J.*, 241, 213, 1987.
30. **Lucocq, J. M., Pryde, J. G., Berger, E. G., and Warren, G.,** A mitotic form of the golgi apparatus in HeLa cells, *Cell*, 104, 1987.
31. **Feldherr, C. M. and Ogburn, J. A.,** Mechanism for the selection of nuclear polypeptides in *Xenopus* oocytes. II. Two dimensional gel analysis, *J. Cell. Biol.*, 87, 589, 1980.
32. **Dingwall, C. and Laskey, R. A.,** Protein import into the cell nucleus, *Annu. Rev. Cell Biol.*, 2, 367, 1986.
33. **Silver, P. and Hall, M.,** Transport of proteins into the nucleus, in *Protein Transport and Organelle Biogenesis*, Das, R. C. and Robbins, P. W., Eds., Academic Press, New York, 1988, 747.
34. **Dingwall, C., Sharnick, S. V., and Laskey, R. A.,** A polypeptide domain that specifies migration of nucleoplasmin into the nucleus, *Cell*, 30, 449, 1982.
35. **Newmeyer, D. D. and Forbes, D. J.,** Nuclear import can be separated into distinct steps *in vitro:* nuclear pore binding and translocation, *Cell*, 52, 641, 1988.
36. **Richardson, W. D., Mills, A. D., Dilworth, S. M., Laskey, R. A., and Dingwall, C.,** Nuclear protein migration involves two steps: rapid binding at the nuclear envelope followed by slower translocation through nuclear pores, *Cell*, 52, 655, 1988.
37. **Hortsch, M. and Meyer, D. I.,** Transfer of secretory proteins through the membrane of the endoplasmic reticulum, *Int. Rev. Cytol.*, 102, 215, 1986.
38. **Wickner, W. T. and Lodish, H. F.,** Multiple mechanisms of protein insertion into and across membranes, *Science*, 230, 400, 1985.
39. **Walter, P. and Blobel, G.,** Purification of a membrane-associated protein complex required for protein translocation across the ER, *Proc. Natl. Acad. Sci. U.S.A.*, 27, 7112, 1980.
40. **Walter, P. and Blobel, G.,** Signal recognition particle contains a 7s RNA essential for protein translocation across the endoplasmic reticulum, *Nature*, 299, 691, 1982.
41. **Meyer, D. I., Krause, E., and Dobberstein, B.,** Secretory protein translocation across membranes — the role of the "docking protein", *Nature*, 297, 647, 1982.
42. **Wiedmann, M., Kurzchalia, T. V., Bielka, H., and Rappaport, T. A.,** Direct probing of the interaction between the signal sequence of nascent preprolactin and the SRP by specific cross-linking, *J. Cell Biol.*, 104, 201, 1987.
43. **Walter, P., Ibrahimi, I., and Blobel, G.,** Translocation of proteins across the endoplasmic reticulum. I. Signal recognition protein (SRP) binds to *in-vitro*-assembled polysomes synthesizing secretory protein, *J. Cell Biol.*, 91, 545, 1981.
44. **Walter, P. and Blobel, G.,** Translocation of proteins across the endoplasmic reticulum. II. Signal recognition protein (SRP) mediates the selective binding to microsomal membranes of *in-vitro*-assembled polysomes synthesizing secretory protein, *J. Cell Biol.*, 91, 551, 1981.

45. **Walter, P. and Blobel, G.,** Translocation of proteins across the endoplasmic reticulum. III. Signal recognition protein (SRP) causes signal sequence-dependent and site-specific arrest of chain elongation that is released by microsomal membranes, *J. Cell Biol.*, 91, 557, 1981.
46. **Gilmore, R. and Blobel, G.,** Transient involvement of signal recognition particle and its receptor in the microsomal membrane prior to protein translocation, *Cell*, 35, 677, 1983.
47. **Palmiter, R. D., Gagnon, J., and Walsh, K.,** Ovalbumin: a secreted protein without a transient hydrophobic leader sequence, *Proc. Nat. Acad. Sci. U.S.A.*, 75, 94, 1978.
48. **Lingappa, V. R., Lingappa, J. R., and Blobel, G.,** Chicken ovalbumin contains an internal signal sequence, *Nature*, 281, 117, 1979.
49. **Columbini, M.,** Pore size and properties of channels from mitochondria isolated from *Neurospora crassa*, *J. Membr. Biol.*, 53, 79, 1980.
50. **Schleyer, M. and Neupert, W.,** Transport of ADP/ATP carrier into mitochondria, *J. Biol. Chem.*, 259, 3487, 1984.
51. **Reid, G. A. and Schatz, G.,** Import of proteins into mitochondria, *J. Biol. Chem.*, 257, 13056, 1982.
52. **Ades, I. A. and Butow, R. A.,** The products of mitochondria-bound cytoplasmic polysomes in yeast, *J. Biol. Chem.*, 255, 9918, 1980.
53. **Suissa, M. and Schatz, G.,** Import of proteins into mitochondria, *J. Biol. Chem.*, 257, 13048, 1982.
54. **Pfanner, N., Hartl, F.-U., and Neupert, W.,** Import of proteins into mitochondria: a multi-step process, *Eur. J. Biochem.*, in press.
55. **Natsoulis, G., Hilger, F., and Fink, G. R.,** The HTS1 gene encodes both the cytoplasmic and mitochondrial histidine tRNA synthetases of *S. cerevisiae*, *Cell*, 46, 235, 1986.
56. **Miura, S., Mori, M., and Tatiban, M.,** Transport of ornithine carbamoyltransferase precursor into mitochondria, *J. Biol. Chem.*, 258, 6671, 1983.
57. **Argan, C., Lusty, C., and Shore, G.,** Membrane and cytosolic components affecting transport of the precursor for ornithine carbamoyltransferase into mitochondria, *J. Biol. Chem.*, 258, 6667, 1983.
58. **Eilers, M. and Schatz, G.,** Binding of a specific ligand inhibits import of a purified precursor protein into mitochondria, *Nature*, 322, 228, 1986.
59. **Schleyer, M. and Neupert, W.,** Transport of proteins into mitochondria: translocational intermediates spawning contact sites between outer and inner membranes, *Cell*, 43, 339, 1985.
60. **Pfanner, N., Tropschug, M., and Neupert, W.,** Mitochondrial protein import: nucleoside triphosphates are involved in conferring import-competence to precursors, *Cell*, 49, 815, 1987.
61. **Schatz, G. and Butow, R. A.,** How are proteins imported into mitochondria?, *Cell*, 32, 316, 1983.
62. **Blobel, G. and Dobberstein, B.,** Transfer of proteins across membranes, *J. Cell Biol.*, 67, 835, 1975.
63. **Rothman, J. E. and Lodish, H. F.,** Synchronised transmembrane insertion and glycosylation of a nascent membrane protein, *Nature*, 269, 775, 1977.
64. **Wolin, S. L. and Walter, P.,** Ribosome pausing and stacking during translation of a eykaryotic mRNA, *EMBO J.*, in press.
65. **Maher, P. A. and Singer, S. J.,** Disulfide bonds and the translocation of proteins across membranes, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 9001, 1986.
66. **Singer, S. J., Maher, P. A., and Yaffe, M. P.,** On the translocation of proteins across membranes, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 1015, 1987.
67. **Lingappa, V. R., Chaidez, J., Yost, C. S., and Hedgpeth, J.,** Determinants for protein localization: B-lactamase signal sequence directs globin across microsomal membranes, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 456, 1984.
68. **Hurt, E. C., Pesold-Hurt, B., and Schatz, G.,** The amino-terminal region of an imported mitochondrial precursor polypeptide can direct cytoplasmic dihydrofolate reductase into the mitochondrial matrix, *EMBO J.*, 3, 3149, 1984.
69. **Hurt, E. C., Pesold-Hurt, B., Suda, K., Oppliger, W., and Schatz, G.,** The first twelve amino acids (less than half of the pre-sequence) of an imported mitochondrial protein can direct mouse cytosolic dihydrofolate reductase into the yeast mitochondrial matrix, *EMBO J.*, 4, 2061, 1985.
70. **Keng, T., Alani, E., and Guarente, L.,** The nine amino-terminal residues of δ-aminolevulinate synthase direct β-galactosidase into the mitochondrial matrix, *Mol. Cell Biol.*, 6, 355, 1986.
71. **Von Heijne, G.,** Mitochondrial targeting sequences may form amphiphilic helices, *EMBO J.*, 5, 1335, 1986.
72. **Roise, D., Horvath, S. J., Tomich, J. M., Richards, J. H., and Schatz, G.,** A chemically synthesized pre-sequence of an imported mitochondrial protein can form an amphiphilic helix and perturb natural and artificial phospholipid bilayers, *EMBO J.*, 5, 1327, 1986.
73. **Kaiser, E. T. and Kezdy, F. J.,** Secondary structures of proteins and peptides in amphiphilic environments (a review), *Proc. Natl. Acad. Sci. U.S.A.*, 80, 1137, 1983.
74. **Kaiser, E. T. and Kezdy, F. J.,** Amphiphilic secondary structure: design of peptide hormones, *Science*, 223, 249, 1984.

75. **Eisenberg, D.,** Three-dimensional structure of membrane and surface proteins, *Annu. Rev. Biochem.*, 53, 595, 1984.
76. **Davey, J., Dimmock, N. J., and Colman, A.,** Identification of the sequence responsible for the nuclear accumulation of the influenza virus nucleoprotein in *Xenopus* oocytes, *Cell*, 40, 667, 1985.
77. **Greenspan, D., Palese, P., and Krystal, M.,** Two nuclear location signals in the influenza virus NS1 nonstructural protein, *J. Virol.*, 62, 3020, 1988.
78. **Kalderon, D., Richardson, W. D., Markham, A. F., and Smith, A. E.,** Sequence requirements for nuclear localization of SV40 large-T antigen, *Nature*, 311, 33, 1984.
79. **Kalderon, D., Roberts, B. L., Richardson, W. D., and Smith, A. E.,** A short amino acid sequence able to specify nuclear location, *Cell*, 39, 499, 1984.
80. **Wychowski, C., Berichou, D., and Girard, M.,** A domain of SV40 capsid polypeptide VP1 that specifies migration into the cell nucleus, *EMBO J.*, 5, 2569, 1986.
81. **Richardson, W. D., Roberts, B. L., and Smith, A. E.,** Nuclear location signals in polyoma virus large T, *Cell*, 44, 77, 1986.
82. **Lyons, R. H., Ferguson, B. Q., and Rosenberg, M.,** Pentapeptide nuclear localization signal in adenovirus, E1A, *Mol. Cell Biol.*, 7, 2451, 1987.
83. **Picard, D. and Yamanoto, K. R.,** Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor, *EMBO J.*, 6, 3333, 1987.
84. **Loewinger, L. and McKeon, F.,** Mutations in the nuclear lamin proteins resulting in their aberrant assembly in the cytoplasm, *EMBO J.*, 7, 2301, 1988.
85. **Dingwall, C. and Allan, J.,** Accumulation of the isolated carboxy-terminal domain of histone H1 in the *Xenopus* oocyte nucleus, *EMBO J.*, 3, 1933, 1984.
86. **Silver, P. A., Keegan, L. P., and Ptashne, M.,** Amino terminus of the yeast GAL4 gene product is sufficient for nuclear localization, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 5951, 1984.
87. **Pope, J. H. and Rowe, W. P.,** Detection of specific antigen in SV40-transformed cells by immunofluorescence, *J. Exp. Med.*, 120, 121, 1964.
88. **Lanford, R. E. and Butel, J. S.,** Intracellular transport of SV40 large tumor antigen: a mutation which abolishes migration to the nucleus does not prevent association with the cell surface, *Virology*, 119, 169, 1982.
89. **Lanford, R. E. and Butel, J. S.,** Construction and characterization of an SV40 mutant defective in nuclear transport of T-antigen, *Cell*, 37, 801, 1984.
90. **Silver, P., Chiang, A., and Sadler, I.,** Mutations that alter both localization and production of a yeast nuclear protein, *Genes Dev.*, 2, 707, 1988.
91. **Hall, M. N., Hereford, L., and Herskowitz, I.,** Targeting of *Escherichia coli* β-galactoside to the nucleus in yeast, *Cell*, 36, 1057, 1984.
92. **Moreland, R. B., Nam, H. G., Hereford, L. M., and Fried, H. M.,** Identification of a nuclear localization signal of a yeast ribosomal protein, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 6561, 1985.
93. **Moreland, R. B., Langevin, G. L., Singer, R. H., Garcea, R. L., and Hereford, L. M.,** Amino acid sequences that determine the nuclear localization of yeast histone 2B, *Mol. Cell Biol.*, 7, 4048, 1987.
94. **Burglin, T. R. and DeRobertis, E. M.,** The nuclear migration signal of *Xenopus laevis* nucleoplasmin, *EMBO J.*, 6, 2617, 1987.
95. **Dingwall, C., Robbins, J., Dilworth, S., Roberts, B., and Richardson, W. D.,** The nucleoplasmin nuclear location sequence is longer and more complex than that of SV40 large-T antigen, *J. Cell Biol.*, in press.
96. **Smith, A. E., Kalderon, D., Roberts, B. L., Colledge, W. H., Edge, M. et al.,** The nuclear location signal, *Proc. R. Soc. London Ser. B.*, 226, 43, 1985.
97. **Goldfarb, D. S., Gariepy, F. J., Schoolnik, G., and Kornberg, R. D.,** Synthetic peptides as nuclear localization signals, *Nature*, 322, 641, 1986.
98. **Nelson, M. and Silver, P.,** Context affects nuclear protein localization in *Saccharomyces cerevisiae*, submitted.
99. **Roberts, B. L., Richardson, W. D., and Smith, A. E.,** The effect of protein context on nuclear location signal function, *Cell*, 50, 456, 1987.
100. **Lanford, R. E., Kanda, P., and Kennedy, R. C.,** Induction of nuclear transport with a synthetic peptide homologous to the SV40 T-antigen transport signal, *Cell*, 46, 575, 1986.
101. **Bedwell, D. M., Klionsky, D. J., and Emr, S. C.,** The yeast F1-ATPase B subunit procursor contains functionally redundant mitochondrial protein import information, *Mol. Cell Biol.*, 7, 4038, 1987.
102. **Riezman, H., Hay, R., Witte, C., Nelson, N., and Schatz, G.,** Yeast mitochondrial outer membrane specifically binds cytoplasmically synthesized precursors of mitochondrial proteins, *EMBO J.*, 2, 1113, 1983.
103. **Zwizinski, C., Schleyer, M., and Neupert, W.,** Proteinaceous receptors for the import of mitochondrial precursor proteins, *J. Biol. Chem.*, 259, 7850, 1984.

104. **Gillespie, L. L., Argan, C., Taneja, A. T., Hodges, R. S., Freeman, K. B., and Shore, G. C.,** A synthetic signal peptide blocks import of precursor proteins destined for the mitochondrial inner membrane or matrix, *J. Biol. Chem.*, 260, 30, 16045.
105. **Hennig, B. and Neupert, W.,** Assembly of cytochrome c. Apocytochrome c is bound to specific sites on mitochondria before its conversion to holocytochrome c, *Eur. J. Biochem.*, 121, 203, 1981.
106. **Gasser, S. M. and Schatz, G.,** Import of proteins into mitochondria, *J. Biol. Chem.*, 258, 3427, 1983.
107. **Pain, D., Kanwar, Y. S., and Blobel, G.,** Identification of a receptor for protein import into chloropasts and its localization to envelope contact zones, *Nature*, 331, 232, 1988.
108. **Feldherr, C. M., Kallenbach, E., and Schultz, N.,** Movement of a karyophilic protein through the nuclear pores of oocytes, *J. Cell Biol.*, 99, 2216, 1984.
109. **Sadler, I. and Silver, P.,** Nuclear location signals from *Xenopus* nucleoplasmin function in *Saccharomyces cerevisiae*, in preparation.
110. **Keegan, L., Gill, G., and Ptashne, M.,** Separation of DNA binding from the transcription-activating function of a eukaryotic regulatory protein, *Science*, 231, 699, 1986.
111. **Paucha, E., Kalderon, D., Richardson, W. D., Harvey, R. W., and Smith, A. E.,** The abnormal location of cytoplasmic SV40 large-T is not caused by failure to bind to DNA or to p53, *EMBO J.*, 4, 3235, 1985.
112. **Paucha, E., Kalderon, D., Harvey, R. W., and Smith, A. E.,** Simian virus 40 origin DNA-binding domain on large T antigen, *J. Virol.*, 57, 50, 1986.
113. **Silver, P., Brent, R., and Ptashne, M.,** DNA binding is not sufficient for nuclear localization of regulatory proteins in *Saccharomyces cerevisiae*, *Mol. Cell Biol.*, 6, 4763, 1986.
114. **Rothblatt, J. A. and Meyer, D. I.,** Secretion in yeast: translocation and glycosylation of prepro-α-factor *in vitro* can occur via an ATP-dependent post-translational mechanism, *EMBO J.*, 5, 1031, 1986.
115. **Eilers, M., Oppliger, W., and Schatz, G.,** Both ATP and an energized inner membrane are required to transport a purified precursor protein into mitochondria, *EMBO J.*, 6, 1073, 1987.
116. **Pfanner, N. and Neupert, W.,** Transport of F1-ATPase subunit B into mitochondria depends on both a membrane potential and nucleoside triphosphates, *FEBS Lett.*, 209, 152, 1986.
117. **Laskey, R. A., Honda, B. M., Mills, A. D., and Finch, J. T.,** Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA, *Nature*, 275, 416, 1978.
118. **Earnshaw, W. C., Honda, B. M., Laskey, R. A., and Thomas, J. O.,** Assembly of nucleosomes: the reaction involving *X. laevis* nucleoplasmin, *Cell*, 21, 373, 1980.
119. **Dingwall, C., Dilworth, S. M., Black, S. J., Kearsey, S. E., Cox, L. S., and Laskey, R.,** Nucleoplasmin cDNA sequence reveals polyglutamic acid tracts and a cluster of sequences homologous to putative nuclear localization signals, *EMBO J.*, 6, 69, 1987.
120. **Krohne, G. and Franke, W. W.,** Immunological identification and localization of the predominant nuclear protein of the amphibian oocyte nucleus, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 1034, 1980.
121. **Mills, A. D., Laskey, R. A., Block, P., and DeRobertis, E. M.,** An acidic protein which assembles nucleosomes *in vitro* is the most abundant protein in *Xenopus* oocyte nuclei, *J. Mol. Biol.*, 139, 561, 1980.
122. **Kellems, R. E., Allison, V. F., and Butow, R. A.,** Cytoplasmic type 80S ribosomes associated with yeast mitochondria, *J. Cell Biol.*, 65, 1, 1975.
123. **Dabauvalle, M. C. and Franke, W. W.,** Karyophilic protein. Polypeptides synthesized *in vitro* accumulate in the nucleus on microinjection into the cytoplasm of amphibian oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 5302, 1982.
124. **DeRobertis, E. M., Longthorne, R. F., and Gurdon, J. B.,** Intracellular migration of nuclear proteins in *Xenopus* oocytes, *Nature*, 272, 254, 1978.
125. **Waters, M. G. and Blobel, G.,** Secretory protein translocation in a yeast cell-free system can occur posttranslationally and requires ATP hydrolysis, *J. Cell Biol.*, 102, 1543, 1986.
126. **Beck, J. S.,** The behaviour of certain nuclear antigens in mitosis, *Exp. Cell Res.*, 28, 406, 1962.
127. **DeRobertis, E. M.,** Nucleocytoplasmic segregation of proteins and RNAs, *Cell*, 32, 1021, 1983.
128. **Zeller, R., Nyffenegger, T., and DeRobertis, E. M.,** Nucleocytoplasmic distribution of snRNPs and stockpiled snRNA-binding proteins during oogenesis and early development in *Xenopus laevis*, *Cell*, 32, 425, 1983.
129. **Dworetzsky, S. I. and Feldherr, C. M.,** Translocation of RNA coated gold particles through the nuclear pores of oocytes, *J. Cell Biol.*, 106, 575, 1988.
130. **Eilers, M. and Schatz, G.,** Binding of a specific ligand inhibits import of a purified precursor protein into mitochondria, *Nature*, 322, 228, 1986.
131. **Georgatos, S. D., Weber, K., Geisler, N., and Blobel, G.,** Binding of two desmin derivatives to the plasma membrane and the nuclear envelope of avian erythrocytes: evidence for a conserved site-specificity in intermediate filament-membrane interactions, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 6780, 1987.