REVIEW ARTICLE
Nucleocytoplasmic transport

Paul S. AGUTTER* and Detlef PROCHNOW†

*Department of Biological Sciences, Napier University, Colinton Road, Edinburgh EH10 5DT, U.K., and †Institut für Biochimie, Biozentrum der Universität, N220, Marie-Curie-Strasse 9, 60439 Frankfurt/Main 70, Germany

INTRODUCTION

The nucleus and cytoplasm in eukaryotes are physically separated throughout interphase. The two compartments differ markedly in composition and function, but constant communication between them is a precondition for the survival of the cell; therefore molecular traffic across the nuclear envelope must be precisely choreographed. Modulation of this traffic might play a role in regulating some cellular activities. Indeed, the obvious sites of exchange, the nuclear pore-complexes, can adopt 'open' or 'closed' forms [1,2], and some transport-related activities are sensitive to endogenous nuclear envelope protein kinases that respond to hormonal and other signals [3]. Moreover, nucleocytoplasmic transport processes change qualitatively and quantitatively during development, aging and carcinogenesis (for reviews see [4,5]). A process that is fundamental to the eukaryotic state, and is likely to play a significant role in cell regulation, is inherently interesting; so it is not surprising that nucleocytoplasmic transport has been the subject of a good deal of research over the past few years (as examples of the many reviews, see [6–10]).

During the past decade, the application of molecular-biological and other techniques to the study of nucleocytoplasmic transport has led to advances in two particular areas: (1) the biochemistry of the pore-complex and of its interactions with transported macromolecules; and (2) identification of factors responsible for the accumulation of particular molecular species in one or other compartment.

These foci of interest need some explanation. The pore-complex is responsible for the 'molecular sieving' properties of the nuclear envelope (e.g. [11]); low-M_r solutes can exchange freely and passively through them. It was established in the 1960s that macromolecules also migrate via the pore-complexes [12,13], but for all except the lowest-M_r proteins, the rate constant of passive movement is of the order of hours. Transportable molecules must therefore contain signals (location signals) that initiate specific, rapid, movement through the pore-complex, and much research has been devoted to identifying both these signals and the receptors that recognize them [7–9]. It is generally assumed that characterization of the location signals and knowledge of the functional organization of the pore-complex are crucial for elucidating nucleocytoplasmic transport mechanisms.

However, the location signals, the signal receptors and the activities of the pore-complex cannot account for all our knowledge of nucleocytoplasmic transport. Studies on amphibian oocytes and other cells show that most protein and RNA species are effectively immobilized within either nucleus or cytoplasm, or both [14–16]. Intracompartmental binding, and not translocation across the nuclear envelope, therefore determines the nucleocytoplasmic distribution ratios of many macromolecules [17,17a]. For at least some classes of macromolecules, both signal–receptor interaction at the pore-complex and extensive nuclear and cytoplasmic binding are relevant to the establishment of intracellular distributions. In these cases, nucleocytoplasmic transport is not simply a matter of crossing a barrier (the nuclear envelope) through specific channels (pore-complexes) between two aqueous compartments. It is more likely to be a 'solid-state' process, in which a negligible fraction of the transport substrate is 'in solution', and most or all of the material remains bound to intracellular structures, of which the pore-complex is only one example. In a solid-state transport process, either the transported molecules are transferred directly from one binding site to the next, or they move along a long-range fibrillar system, perhaps in a manner analogous to the axonal transport of neurotransmitter vesicles (cf. [18]). In either case, the transported substrate is presented to the pore-complex in an immobile, not a soluble, form. There is substantial evidence for such a solid-state transport system in the case of mRNA [19], minimally involving release from an intranuclear binding site, translocation through a pore-complex and binding to a cytoplasmic site [4,8], and there is suggestive evidence for similar systems for other classes of molecules. As yet, however, the mechanistic details remain obscure.

In short, the pore-complex and the location signals are important in nucleocytoplasmic exchanges, and compared with the rest of the transport machinery they are relatively well characterized. Most of this Review therefore concerns advances in the studies of pore-complexes and location signals, and the longer-range solid-state systems receive less emphasis. This asymmetry reflects the present state of knowledge and the main thrust of current research, not the relative importance of the topics for our ultimate understanding of nucleocytoplasmic transport.

There is continuing confusion in the field about a significant methodological implication of the solid-state transport concept. Since the advent of techniques that have enabled the movements of microinjected molecules to be studied in living cells, it has become widely accepted that *in situ* methods for studying nucleocytoplasmic exchanges are reliable and that *in vitro* methods, utilizing nuclei and other subcellular fragments, are not. The argument for this position is simple and at first sight seems incontrovertible. Microinjection does not disrupt the cell significantly, so molecular movements *in situ* take place in an essentially unperturbed system. In contrast, subcellular fractionation inflicts substantial damage, e.g. on nuclei, and *in vitro* studies are therefore unphysiological and their results correspondingly difficult to interpret. So far as export from the nucleus is concerned, this argument is valid if and only if the process is essentially identical with translocation through the pore-complex, the distribution of the transported species within the individual compartments (cytoplasm and nucleoplasm) being essentially a matter of 'diffusion'. However, if specific association with a solid-state apparatus within each compartment is a prerequisite for physiological transport, then *in situ* methods can

Abbreviations used: WGA, wheat-germ agglutinin; SV40, simian virus 40; RNP, ribonucleoprotein; NTPase, nucleoside triphosphatase.

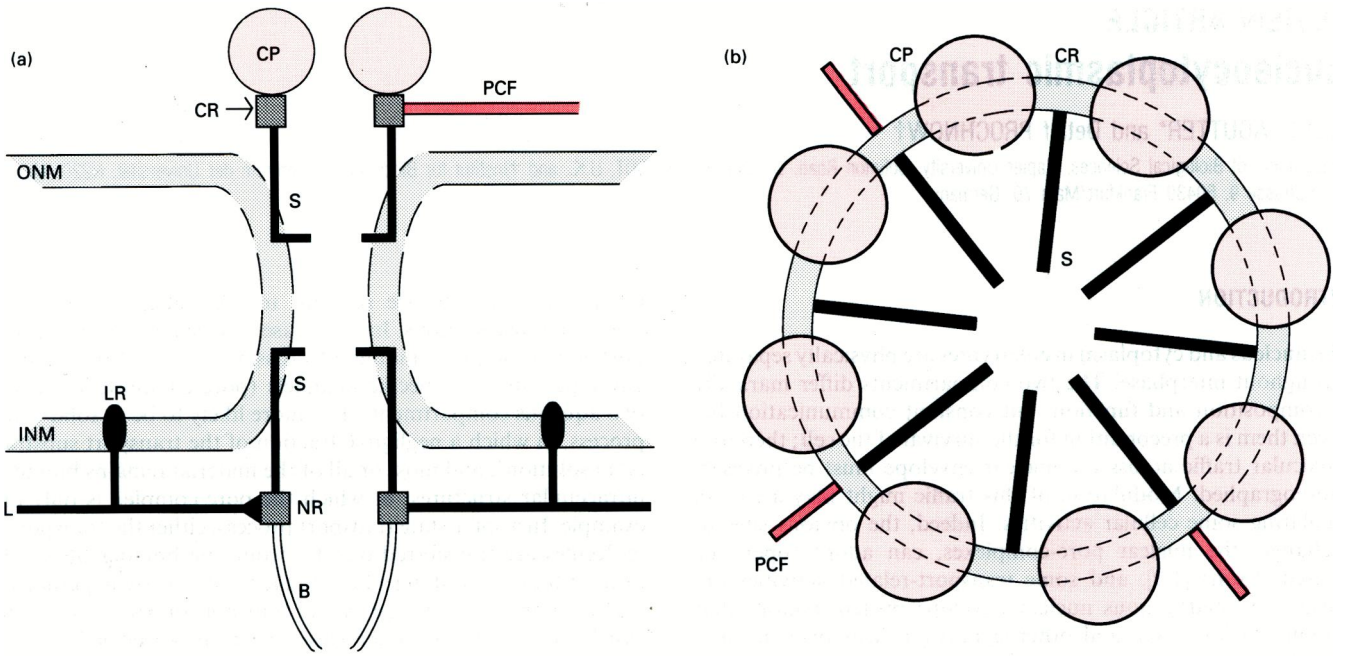

Figure 1 Schematic representation of a nuclear pore-complex

This Figure attempts to represent a consensus view of pore-complex architecture in the light of known functional properties. (**a**) Vertical section; (**b**) surface view. Some features (the octagonal symmetry, the existence of nucleoplasmic and cytoplasmic rings, the 'struts' and the 'basket' structure) are almost universally recognized, but details are more controversial; the number of pore-complex models that have been proposed is more or less commensurate with the number of researchers who have studied the structure. Key: CP, particle on cytoplasmic face (compacted fibril?); CR, cytoplasmic ring; NR, nucleoplasmic ring; S, strut; B, basket framework; ONM, outer nuclear membrane; INM, inner nuclear membrane; L, lamina; LR, lamin receptor; PCF, pore-connecting fibril.

be validated only if it can be shown that the microinjected material has made the requisite association; in practice, this has not been shown in any instance known to us. In contrast, if (say) nuclei are isolated and incubated under conditions where their internal solid-state apparatus remains functionally intact, and if efflux of endogenous molecules from such nuclei is studied, then physiologically interpretable results are obtained. Therefore, *in situ* methods are superior if and only if export is not a solid-state process. If export is a solid-state process, then superiority lies, at least potentially, with *in vitro* methods. For this reason, we place emphasis on *in vitro* rather than *in situ* studies of mRNA transport, which is almost certainly a solid-state process. In contrast, nuclear import of proteins cannot usefully be studied with isolated nuclei even if it is a solid-state process [4,8], because no solid-state cytoplasmic elements are present in the preparations.

The argument for *in vitro* rather than *in situ* approaches to the study of mRNA transport is important for interpreting the literature. For instance, *in vitro* studies have suggested that poly(A)$^+$ and poly(A)$^-$ mRNAs compete for export from the nucleus, but *in situ* studies have indicated the opposite (see below). Until the methodological issue is satisfactorily resolved, interpretation of these conflicting findings will remain controversial. We shall return to the question of methods at the end of this Review.

THE PORE-COMPLEX

Structure and relationship to other cell components

The nuclear pore-complex is an octagonally symmetrical cylinder about 80 nm in length and 100 nm in diameter [20]. Its protein M_r has been estimated at 1.25×10^8 by scanning electron microscopy [21]. The density of pore-complexes on the nuclear surface generally correlates with the metabolic activity of the cell [20]. Pore-complexes from all eukaryotic cells seem to be virtually identical in structure and are probably closely similar in composition. However, fine details of ultrastructure are sensitive to conditions of sample preparation and therefore remain controversial, and as yet only a few of the proteins making up the structure have been fully characterized.

Figure 1 is a schematic diagram of a 'consensus' view of pore-complex structure (cf. Figure 3 in [10] for a similar representation). The eight radial 'spokes' can occupy more or less of the area circumscribed by the rings, so the patent aperture in the centre can change from about 10 nm ('closed' state) to about 40 nm ('open' state); it is through this aperture that macromolecules are translocated [1,2]. Studies with colloidal gold particles suggest that translocation occurs via fibrils running along the length of the cylinder, orthogonal to the plane of the nuclear envelope [22]. The significance of the octagonal 'basket' structure on the nucleoplasmic face [23] is not yet clear, but it may link the pore-complex with the fibres of the nucleoskeleton [20]. The nucleoplasmic ring is attached to the lamina of the envelope [23,24]. Pore-connecting fibrils in the plane of the outer nuclear membrane are probably linked to the cytoplasmic rings [25]. The eight granules seen on the cytoplasmic face of the pore-complex in isolated nuclear envelopes may be the compacted remains of fine fibrils linked to the cytoskeleton [18,20]; the existence of such fibrils is revealed by high-energy transmission electron microscopy of thick resinless sections [26,27]. Thus, although many details of the organization have not yet been elucidated, there is considerable evidence for fibrillar connections between an individual pore-complex and (i) the nucleoskeleton,

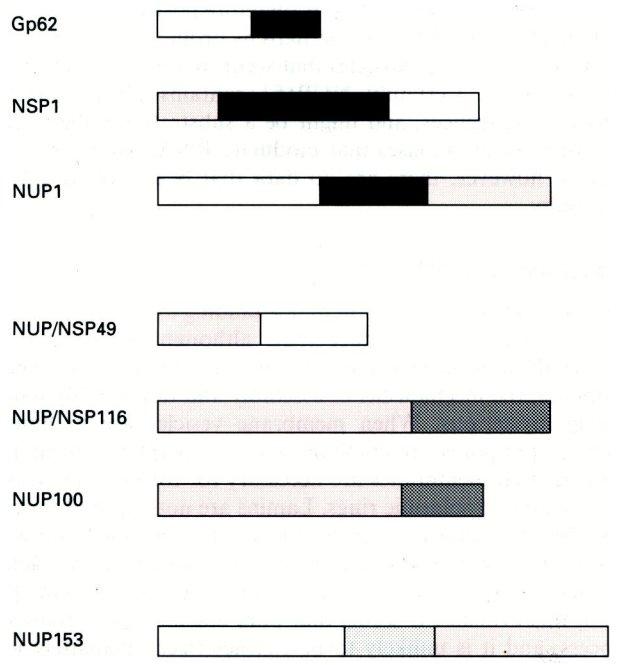

Figure 2 Comparison of domain structures of the known nucleoporins

Key: white, KPAFSFGAK; pink, GLFG or related repeat; black, conserved domain; dark grey, XFXFG repeat; light grey, zinc finger domain.

Table 1 Structural motifs in nucleoporins

The nucleoporins studied to date seem to fall into two main structural groups. These are characterized by the distinctive repeat units listed here; X represents any amino acid residue.

Group	Examples	Motif
1	Gp62 NSP1 NUP1	KPAFSFGAK
2	NUP/NSP49 NUP/NSP116 NUP100	GLFG
	NUP153	XFXFG $CX_2CX_{10}CX_2C$ (zinc finger)

(ii) the cytoskeleton and (iii) other pore-complexes. If this inference is valid, the lamina and the intranuclear fibrillar system (nucleoskeleton) are linked to the cytoskeleton via the pore-complex [23,24,26]. Such an arrangement could provide a basis for solid-state transport.

Pore-complexes are also firmly linked to the nuclear membranes, independently of the lamina–inner-membrane junctions. Chronologically the first pore-complex component to be characterized, the glycoprotein Gp210, is located on the periphery of the cytoplasmic ring and may be involved in linking the two nuclear membranes to the pore-complex. The bulk of this molecule lies in the perinuclear cisterna, between the two nuclear membranes; its C-terminal domain contains a membrane-spanning segment [28] which seems to be sufficient for sorting the glycoprotein into the membrane region contiguous to the pore [30]. Gp210 is rich in N-linked high-mannose oligosaccharides of the type found in the lumen of the endoplasmic reticulum; these are responsible for its binding to concanavalin A [29,31]. More recently, Hallberg et al. [31a] have described a wheat-germ agglutinin (WGA) binding protein of M_r 121000 which is also located in the pore membrane region (see below).

Nucleoporins

The other pore-complex glycoproteins that have been characterized to date, the nucleoporins, contain O-linked N-acetylglucosamine residues and therefore bind to WGA [32]. They are not membrane-associated, although they can form complexes with Gp210 that may partly determine their organization [30,33], and they probably make up some 5–10% of the mass of the pore-complex. Available sequence data, especially for the most abundant of these components in vertebrates (Gp62), indicate an α-helical C-terminal domain with the coiled-coil structure typical of intracellular structural proteins [34,35]. The N-terminal domain of Gp62, which contains the 10–20 glycosylation sites, comprises 15 copies of a conserved nine-residue motif interspersed with variable-sequence regions of similar size, probably forming a β-sheet [35]. Gp62 is similar in sequence and structure to two yeast-specific pore-complex proteins, NUP1 and nucleoskeleton-like protein (NSP) 1 [36,37], though these proteins contain more copies of the N-terminal motif. A distinct group of nucleoporins has been identified by work in several laboratories (see e.g. [38–40]). The structural features of these various proteins are shown schematically in Figure 2 and Table 1. The M_r-121000 protein described by Hallberg et al. [31a] (see above) also binds WGA and has the XFXFG motif; it is believed to anchor other pore components to the membranes.

Nucleoporins are certainly involved in translocation of macromolecules across the pore-complex; nuclear import of proteins, and export of mRNA, tRNAs, UsnRNAs and ribonucleoproteins (RNPs) and ribosomal subunits, can all be inhibited by WGA [41–43c], and at least some of them can be inhibited by monoclonal antibodies against nucleoporins [41,44]. Gp62 forms a hetero-oligomer, other components of which include the nucleoporins Gp58 and Gp54, and it is apparently this hetero-oligomer (stable in 2 M urea or 2 M NaCl) that functions in translocation [45]. Although the locations of the individual nucleoporins within the pore-complex have not been demonstrated, it is tempting to suggest that complexes such as the Gp62–Gp58–Gp54 oligomer constitute some part of the 'spokes' visible in the electron microscope (Figure 1); certainly Gp62 and NSP1 are accessible to monoclonal antibodies from both the nucleoplasmic and cytoplasmic surfaces and therefore are probably symmetrically distributed. To extend this speculation, the coiled-coil C-terminal domains might interact to form the orthogonal fibrils along which translocating macromolecules migrate, and hinges between the C-terminal coiled-coil domain and the glycosylated N-terminal domain might be involved in 'opening' and 'closing' the pore (Figure 3).

Models of this kind might have some bearing on the controversial myosin-dependent translocation mechanism proposed by Berrios and Fisher [46–48]. Antibodies against *Drosophila* pore-complex material cross-react with the heavy chain of non-muscle myosin [46,47], and vice-versa [48]. It is possible that monoclonals recognizing epitopes on the coiled-coil domains of nucleoporins and myosin might cross-react; however, Berrios and Fisher note that the ATP hydrolysis associated with most translocation processes has many of the characteristics of ATP hydrolysis by myosin [46].

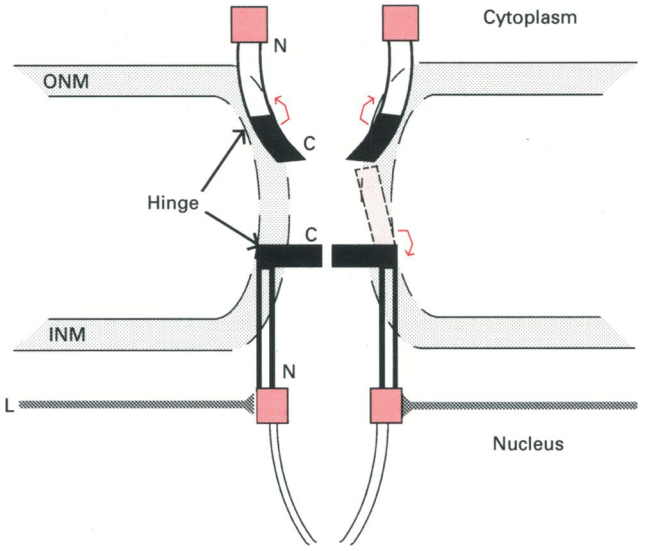

Figure 3 Possible organization of nucleoporins in the pore-complex struts

In this speculative model, the glycosylated N-terminal domains of the nucleoporins are assumed to lie at the periphery of the pore, directly or indirectly associated with the cytoplasmic and nuclear rings. The extended C-terminal coiled-coil domain is assumed to form the orthogonal fibrils of the pore-complex. The hinge of the nucleoporin molecule marks the boundary of the strut visible by electron microscopy.

A nucleoporin of M_r 153000, NUP153, is located exclusively on the nucleoplasmic face of the pore-complex, possibly in the 'basket' structure (see Figure 1). Its C-terminal region contains 'zinc finger' motifs characteristic of nucleic acid binding proteins [49] (see Table 1 and Figure 2). Although all four of these motifs are of the C_2–C_2 type found in DNA binding proteins, the identical spacing of 10 residues between each cysteine pair in all four fingers, and the probable independence of the motifs conferred by the long (approximately 40 residue) spacing between one finger and the next, suggest RNA binding capability (Figure 4; see the discussion in [50]). It is therefore possible that NUP153 participates in RNA export, perhaps by promoting the unfolding of ribonucleoprotein particles that seems to be a prerequisite for export [51,52]. Certainly NUP153 contains phosphorylation consensus sequences, and might be a substrate for the nuclear envelope protein kinases that modulate RNA export [3,52]. At present, however, there are no data that bear directly on this possibility.

Nuclear pore assembly

The limitations of our current understanding of the pore-complex are highlighted by the fact that, although the structure is disassembled at prophase in open mitosis and is reassembled at telophase, the mechanisms of assembly and disassembly remain largely mysterious. When membrane vesicles are in limiting supply, 'pre-pores' resembling 'spoke' complexes form [54], implying that membranes are necessary for forming the nucleoplasmic and cytoplasmic rings. Lamins are not required for pore assembly [55]. Pores formed in the absence of Gp62 and other translocation-related nucleoporins are translocationally inactive, but activity can be restored by adding the glycoproteins [56]. From this evidence it seems that pore assembly is a multi-step process, and it is unlikely to be characterized adequately until more of the components of the structure have been identified.

An interesting possibility is that NUP153 is necessary to organize nascent pore-complex material on the decondensing telophase chromosomes. This speculation assumes that the zinc fingers on NUP153 are DNA-binding rather than RNA-binding motifs. If this is the case, then transcribable regions of the genome might be linked closely to the pore-complexes in the daughter nuclei, facilitating the export of mRNAs to the cytoplasm, as required by the gene gating hypothesis of Blobel [57].

LOCATION SIGNALS AND TRANSLOCATION

The nature of location signals

A location signal is recognized by a specific receptor and permits the molecule that bears it to be translocated through the pore-complex. Location signals are parts of mature macromolecules,

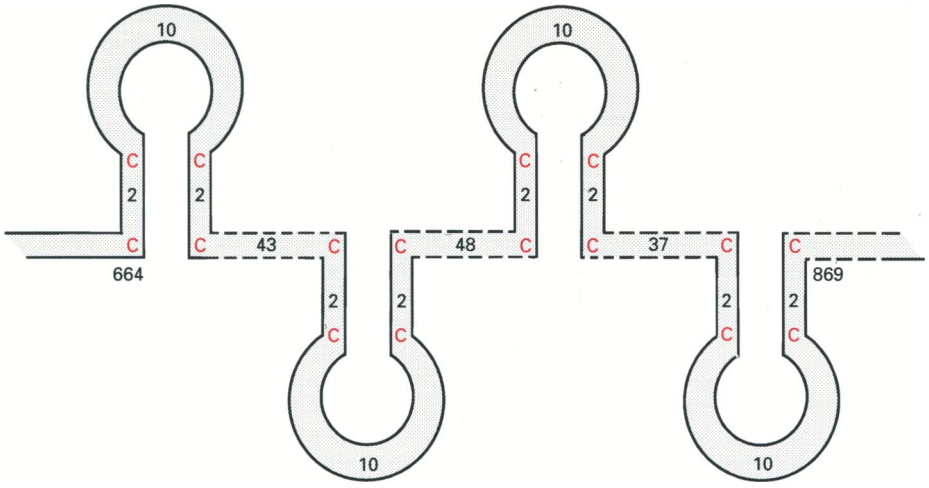

Figure 4 The zinc finger motifs of NUP153

Schematic summary of the zinc-finger-like domain of the most nucleoplasmically directed of the known nucleoporins. The key features are the 16 cysteine residues, occurring in four groups of four, which probably constitute four zinc binding sites.

Table 2 Some nuclear location signals in proteins

Protein	Location signal	Reference
SV40 large-T antigen	P_{126}KKKRKVE	57
Yeast MAT α_2	RPAATKK	58
Polyoma large-T	P_{280}KKARED V_{533}SRKRPR	6
N1	V_{531}SRKRPR A_{548}KKSKQE	59
Nucleoplasmin	R_{152}PAATKKAGNAKKKKLDKED	60
Lamin A	KEKRKRI	

in contrast, for example, to the signal sequences that permit sorting of proteins into endoplasmic reticulum or mitochondria, which are generally removed after translocation. In proteins, location signals are oligopeptide sequences that belong to one of two main classes, of which the paradigm examples are simian virus 40 (SV40) large-T antigen and MAT α_2 (Table 2). Some nucleus-targeted proteins have more than one location signal (see Table 2 and [58,59]) and although signals differ in efficiency, and more efficient ones dominate over less efficient ones, the effects of multiple signals are additive [62–64]. In nucleoplasmin there are three overlapping signals between residues 152 and 168; two resembling Mating Type α_2 (152–157 and 158–163) and one resembling SV40 (162–168) (Table 2 and [60]); nucleoplasmin uptake from cytoplasm to nucleus is very rapid [65,66].

In tRNAs there seems to be a common location signal around G^{58} in the highly conserved D loop [67]. In mRNAs there are various signals, one of which is an oligo(A) segment of at least 15 residues [53]; another is the 5′ cap [68,69]. It is possible that efficient export of mRNAs from the nucleus depends on the concerted action of the 5′ cap signal and another signal such as oligo(A). The latter need not be part of a 3′ poly(A) tail, as has often been supposed. On the other hand, spatial separation of the cap from the oligo(A) sequence is not evidence against their simultaneous involvement in translocation; the concerted action of two spatially separated signals appears to be necessary for the translocation of U_1snRNP [70]. Furthermore, involvement of the 3′ end of the mRNA in translocation is suggested by some *in situ* studies; for instance, histone mRNA export requires maturation of the 3′ end of the molecule [70a].

Any location signal lies on the surface of the molecule that bears it. If it is buried, or if it is surrounded by sequences that mask its effect, it will not function efficiently and the molecule might not be translocated [71]. Part of the nuclear location signal of U_2snRNP seems to be the AU_nG sequence that lies in a portion of the RNA between the two pairs of loops [72]. This region is apparently buried in the naked RNA, which is exported from the oocyte nucleus, but exposed in the RNP complex, which is nucleus-targeted [73]. Other snRNPs may have location signals in their protein rather than RNA constituents, but the exposure of the signal only when the mature complex is formed may be a common principle. This principle may also be exemplified by steroid receptors, the location signals of which are apparently exposed only when the steroid is bound [74]. However, experimental evidence in this area needs careful interpretation. For example, although import of most UsnRNAs to the nucleus is inhibited by an excess of the cap structure m_3GpppG, import of U6 is inhibited by excess of the SV40 large-T location signal peptide [43c]. In this instance, exposure of a location signal on a protein component is not a possible explanation.

Molecules without location signals can sometimes be translocated, if they can form sufficiently tight complexes with translocatable molecules without masking the location signals of the latter. For instance, if antibodies to nucleus-targeted proteins are injected into the cytoplasm they can be conveyed to the nucleus [75]. Conversely, molecules that do have location signals might not be translocated (a) if the signal is modified (e.g. by phosphorylation in the case of a protein [76]), or (b) if the molecule is attached to another cellular component so that it cannot travel to the pore-complex. For instance, a membrane-binding domain might prevent nuclear uptake of a protein with a nuclear location signal; and although oligo(A) is an export signal for some mRNAs, many adenylated RNAs remain restricted to the nucleus *in vivo*.

Criteria for demonstrating that a sequence of a molecule is a location signal include the following [71]. (1) When the signal is ablated or modified (e.g. lysine-128 of the SV40 large-T antigen is replaced by threonine) and the mutant molecule is microinjected into the cell, it remains restricted to the compartment into which it is injected. (2) If the putative signal is incorporated into a molecule that is usually not translocatable, the fusion product will be translocated. For instance, incorporation of the SV40 signal into pyruvate kinase or BSA [58,63] causes nuclear uptake after microinjection into the cytoplasm. However, if a defective signal is incorporated (e.g. the SV40 signal in which lysine-128 has been replaced by threonine), the fusion product will not be translocated, providing an important experimental control. (3) The kinetics of uptake need to be considered. It is likely that a molecule with a single relatively inefficient location signal will be translocated slowly, and incomplete transport from one compartment to the other might be observed [62,63]. Nucleocytoplasmic transport should not be construed as an 'all-or-nothing' process.

Receptors for location signals

For proteins, tRNAs and mRNAs, binding of the location signal to the receptor is rapid, ATP-independent and insensitive to WGA [41,53,77–80]. In all cases, the affinity of the receptor for the signal is of the order of 10^7 M. This value is strikingly low. It implies that if translocation is to be efficient, the number of receptors must be large, by analogy with extracellular matrix receptors, which typically have affinities 2–3 orders of magnitude lower than (say) hormone receptors, but are much more abundant. This in turn suggests that the receptors might not be confined to the pore-complexes (which presumably could house only limiting numbers of specific receptors [81]), and indeed there is evidence for widespread intracellular distributions of both protein [82–84] and mRNA [85] receptors, perhaps involving the cytoskeletal and nucleoskeletal fibres involved in solid-state transport. Visualization by fluorescence microscopy of proteins microinjected into the cytoplasm often reveals non-uniform distributions of material apparently in transit to the nucleus. This kind of microscopic evidence is difficult to interpret, but examination of some of the fluorescence micrographs (e.g. in [58–61]) might suggest that the transported protein is at least partly associated with cytoplasmic fibrils.

Identification of the signal receptors has proved difficult because (a) their affinities are of the same order as non-specific binding affinities, and (b) in many studies it has been assumed that they are restricted to the nuclear envelope, so samples containing small receptor populations have been studied. Nevertheless, it is now apparent that there are several receptors with different but overlapping specificities for protein location signals [82,86–88]. M_r values of 140, 100, 70–76, 67–70 and 55–60

(all $\times 10^3$) have been reported. A common feature of these molecules may be an acidic oligopeptide sequence, because antibodies specific for the sequence DDDED seem to block all nucleus-targeted protein receptors [89]. Present knowledge of protein import receptors has been reviewed by Yamasaki and Lanford [90].

Competitive binding studies have identified the putative monomethyl cap receptor (M_r 80000) involved in U_1snRNP translocation [91] and mRNA translocation [68]. As yet, the receptors for many transportable materials, e.g. other snRNPs and tRNAs, have not been identified. A crucial question concerns the structural relationships between these various receptors and other components of the transport apparatus, including the components of the pore-complexes. The relationship may be one of simple identity with a component such as myosin, as suggested by Berrios et al. [48]. Although this possibility is interesting in view of the possible role of actin fibrils in the solid-state transport system (see [85] and the discussion below), the consensus of current opinion is against it. The receptors are unlikely to be nucleoporins because WGA does not usually interfere with binding [79,80]. In the case of the mRNA oligo(A) receptor, the probable M_r is around 110000, though the material located on the nucleoplasmic face of the nuclear envelope by photoaffinity labelling seems to have been recovered as proteolytic fragments of this molecule [92]. The 110000-M_r protein does not correspond in size to any known nucleoporin. The question of the relationships between location signal receptors and the nucleoporins and other transport components remains unresolved at present, and it might be approached successfully first in yeasts, for which extensive gene libraries are available. In this respect, the work of Silver and her colleagues [93,94] is particularly promising.

The ATP-dependence of translocation

For historical reasons, most of our knowledge about mRNA translocation has been acquired through the use of cell-free systems, some of which have been shown to behave physiologically (see the Introduction to this Review; for a detailed discussion see [4]). In contrast, our knowledge of protein translocation has come from *in situ* studies or from use of nuclei resealed in egg extracts [95]. The intact systems used to study nuclear protein uptake are advantageous in physiological comparability, but disadvantageous in respect of kinetic analysis. The requirement for ATP hydrolysis was demonstrated for mRNA translocation long before it was found for protein translocation [8] and has only recently been corroborated by *in situ* studies [68], and the enzyme involved in ATP utilization has been identified in the former case but not the latter [96,97]. Also, by comparing the turnover rate of this enzyme, the nuclear envelope nucleoside triphosphatase (NTPase; EC 3.6.1.15), with the maximum rate of RNA export through the pore-complexes (which is in the order of 1 molecule/s per pore [81,98]), it can be estimated that approximately 10^3 ATP molecules are hydrolysed per average-sized poly(A)$^+$ RNA molecule translocated. As yet, no such estimate has been made in the case of protein translocation.

The NTPase, like the oligo(A) binding site, seems to be located on the inner face of the nuclear envelope [99,100]. Alteration of the NTPase activity results in proportionate changes in the mRNA translocation rate [96,98]. When the affinity of the binding site for poly(A) or poly(A)$^+$ RNA is increased by endogenous phosphorylation, the NTPase is concomitantly inhibited [53]; when the binding site is blocked by a monoclonal antibody the NTPase is markedly stimulated [85]. WGA and other general inhibitors of translocation, such as lumicolchicine at concentrations of 2–5 mM, do not inhibit the NTPase [98]. These results suggest that the NTPase activity is coupled not simply to mRNA translocation *per se*, but also to binding at the pore-complex (cf. [31,79]). Details at the molecular level, however, remain unclear.

Not all transported macromolecules have the same requirement for ATP hydrolysis. Translocation of poly(A)$^-$ mRNAs such as histone messengers seems in some systems to have a lower ATP-dependence than translocation of poly(A)$^+$ RNAs such as globin and albumin messengers ([101]; however, see [98]). Interestingly, tRNA translocation has no requirement for ATP at all, either *in situ* [67] or *in vitro* [98]; the *in vitro* model system used resealed nuclear envelope ghosts in which the RNA was entrapped; in contrast, mRNAs could not be exported from the ghosts without ATP [98]. Export of ribosomal subunits from *Xenopus* oocyte nuclei seems to be ATP-dependent [43a]. These differences in ATP requirements amongst transportable species have not been explained. Perhaps ATP ensures that translocation is vectorial by changing the probability of mRNA binding to open or closed states of the pore-complex, by analogy with its effect on the probability of cross-bridge formation in muscle contraction. In respect of mRNA export from nuclei, ATP does seem to contribute to unidirectionality, but so do other factors such as the cytoplasmic location of the major polysomal poly(A) binding protein [102]. It is at least feasible that vectorial translocation of tRNA is ensured by other means, and since tRNAs and mRNAs have different binding sites in the pore-complex, a translocation system has evolved that is not NTPase-coupled. This hypothesis is illustrated in Figure 5. The kinetic complexity of at least some translocation processes is well attested [103].

A difficulty for this model is the existence of proteins that shuttle between the two compartments [104]. These include the ribonucleosome core protein A1 [105] (though the C-group proteins of this structure have an almost exclusively nuclear location; see below) and the heat-shock protein hsp70, which might facilitate the nuclear import of other proteins [106,107]. How the translocation system accommodates this shuttling process is a challenging problem, which is unlikely to be resolved until the pore-complex is more fully characterized.

Modifiers of the translocation rate

Considerable attention has been paid to a family of cytosolic and polysome-associated proteins that stimulates mRNA efflux from isolated nuclei and probably accelerates translocation [85,108–110]. The proteins in this family might not be separate gene products; they are probably derived from degradation of the cytoplasmically located 110000-M_r oligo(A) receptor [98]. However, translocation does seem to respond to specific intracellular regulators that are fundamentally distinct from components of the solid-state system itself. The export of HIV-1 mRNA from the nucleus is promoted by the virally encoded rev protein, which interacts directly with the translocation apparatus [111,112]. A heterodimer of the adenovirus proteins E1B and E4 selectively promotes export of viral mRNA and inhibits export of cellular mRNAs, and the influenza virus protein NS1 selectively inhibits the transport of poly(A)$^+$ RNAs (for review, see [112a]). An endogenous glucose-binding protein isolated from mammalian nuclei increases the affinity of the oligo(A) receptor for poly(A)$^+$ RNAs [113].

With respect to protein import to the nucleus, separate soluble factors are required for binding to the nuclear envelope and for ATP-dependent translocation [114,115]. Some of these factors have been isolated and characterized, and their possible relationship to factors involved in protein uptake into mitochondria

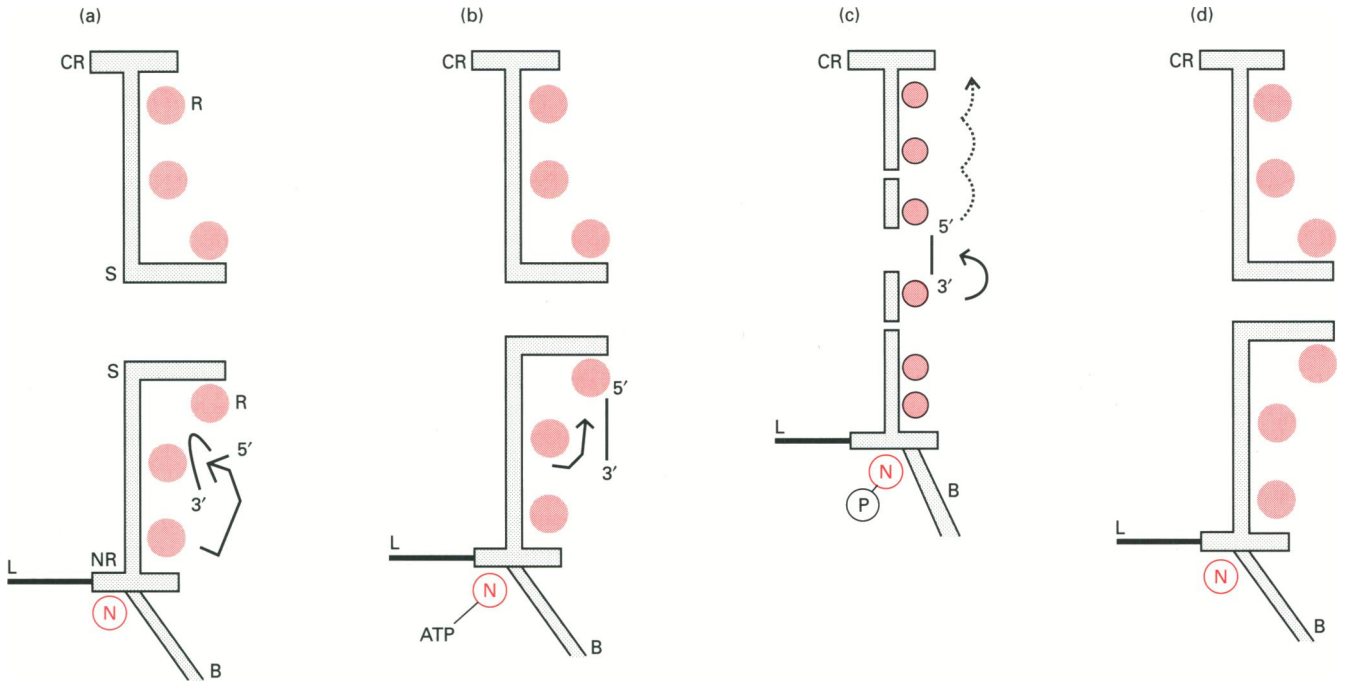

Figure 5 Possible scheme for ATP-coupled and uncoupled translocation

This speculative scheme shows half of a pore-complex in vertical section; the membranes are omitted. Key: CR, cytoplasmic ring; NR, nuclear ring; L, lamina; S, struts; B, basket structure; R, RNA receptors; N, NTPase. (**a**) An RNA molecule is passing from the first to the second of a series of receptors (not necessarily identical) aligned along the fibril/strut system (nucleoporin?). (**b**) The RNA reaches the receptor at the end of a strut. If the translocation is NTPase-coupled, the enzyme activity is likely to be initiated at this point. Note that a different signal sequence might be involved in binding to this receptor. (**c**) The pore is altered to its 'open' form (large patent radius) either as a result of allosteric coupling initiated by the binding of the ligand to the strut-terminal receptor (**b**) or by the activity of the NTPase. The RNA is then passed along the remaining receptors in the line and on to the cytoskeletal system. (**d**) The pore returns to its resting state because (i) no ligand is bound to the receptors and, if the process is NTPase-coupled, (ii) the NTPase has ceased to be active.

and endoplasmic reticulum has been considered [116]. However, it seems likely that the transport-facilitating factors so far identified represent only a small subset and that their modes of action are diverse [117]. Some of them may act as components of the solid-state transport system *in vivo*, or might partition between solid-state structures and a soluble form (e.g. [83,84]).

Extracellular effectors are also involved in the control of translocation [118], and the most widely studied of these include insulin and epidermal growth factor [119–122]. In physiological concentrations, both of these factors stimulate endogenous phosphorylation of the oligo(A) receptor and, apparently because of the consequent increase in poly(A)$^+$ RNA binding to the receptor, stimulate the NTPase. Although the biological relevance of these effects is controversial (how are the insulin and epidermal growth factor internalized so that they can interact with nuclear envelope components?), they might be related to the hormone-induced migration of protein kinase C to the nuclear envelope [123].

OTHER COMPONENTS OF THE SOLID-STATE SYSTEM

Nuclear structures

Abundant evidence that mRNA precursors are firmly attached to intranuclear structures has accumulated over the past two decades and has been reviewed in detail [4,8]. The evidence includes the fact that nearly normal restriction of RNAs is retained in isolated liver nuclei, so long as nuclear swelling and RNAase and proteinase activities are inhibited; when such nuclei are incubated in suitable buffers, the poly(A)$^+$ RNA exported to the supernatant in an ATP-dependent manner is intron-free, translatable, uncontaminated by hnRNA C-group proteins and divisible into the three main abundance classes of cytoplasmic mRNA. Since isolated nuclei are structurally damaged and lose unbound components by leakage, it follows that mRNAs and their precursors cannot leak from liver nuclei during isolation and are therefore not unbound. Puncturing of the amphibian oocyte nuclear envelope *in situ* also fails to cause nucleo-cytoplasmic RNA redistribution.

Recently, some specific transcripts have been shown to follow visible nucleoskeletal tracks to the pore-complex [124–126], and microinjected intron-containing RNA has been found to associate with the 'speckle' structures in which splicing components seem to be concentrated [127]. These observations accord with the solid-state perspective, but other recent observations have contradictory implications. For example, an extensively transcribed gene product in the salivary glands of *Drosophila* larvae showed a somewhat diffuse, web-like intranuclear distribution [128], and nascent RNA from the transcription of an individual gene has been located on several hundred, rather than just a few, intranuclear domains [129]. These apparent conflicts have led Rosbash and Singer [130] to propose that the visible tracks are formed not by completed but by nascent RNA, and that after processing the messenger may be free to 'diffuse'. In principle, this hypothesis could reconcile the more recent debate [124–129], but it is incompatible with the longer-established evidence [4,8] and assumes, contrary to this evidence, that diffusion would be a sufficiently rapid and efficient process to convey messengers to pore-complexes at a viable rate. An alternative explanation for

the apparently conflicting evidence on 'tracks' is that some mRNAs are targeted to one or a few pore-complexes, in accordance with the gene gating hypothesis [57], while others can be transported (perhaps at random) to most or all of these structures.

This explanation predicts differences in the attachments to the nucleoskeleton between the more and the less obviously track-restricted transcripts. Given the growth of knowledge about pre-mRNA and mRNA attachments to the matrix, it should soon be possible to test these alternatives. The structures involved in these attachments are located on the nucleoskeleton or nuclear matrix [4]; evidence in support of this includes the maintenance of RNA tracks, for instance from EB40, in operationally defined matrix preparations [131]. The RNA-binding structures co-localize with splicing factors [132,133] and may be enriched in them. Release of mRNA from spliceosome complexes *in vitro* requires an RNA helicase-like protein, PRP22 [134], so it is possible that this protein is required for release *in situ*. The C-group proteins of the ribonucleosome core seem to be intimately involved in anchoring the hnRNA to such nucleoskeletal fibrils [135]. Immature mRNA precursors seem to be bound to actin-containing fibrils, and mature mRNA within the nucleus to DNA topoisomerase II [136,137], and it is possible that migration of the RNAs from the internum of the nucleus to the pore-complex depends on actin fibrils and myosin motors [138,139]. It now appears that, in addition to mRNA and its precursors, at least some proteins move along solid-state structures in the nucleus. This has been clearly demonstrated in the case of the nucleolar protein Nopp 140 [140], a phosphoserine-rich nuclear location signal binding protein, which shuttles between cytoplasm and nucleolus along tracks that can be visualized by immuno-electron microscopy [141]. Structural linkages between the nucleolus and the pore-complex suggest that transport of ribosomal subunits might also be a solid-state process. Certainly the rate of efflux of ribosomal subunits from resealed nuclear envelope vesicles, in the absence of nucleoskeletal structures, is very slow (I. Hassell and H. Fasold, personal communication). On this evidence, it begins to seem that, at least within the nucleus, solid-state migration of macromolecules might be a general phenomenon.

If this is so, then particular species may be targeted to particular pore-complexes and thence to particular areas of the cytoplasm, as seems to be the case with the pair-rule transcripts in *Drosophila* [142] (see also [57]). Similarly, the solid-state movement of proteins might ensure that they are targeted via specific pore-complexes to defined sites within the nucleus. The implications of such arrangements for the co-ordination of cellular activity are potentially far-reaching.

Cytoskeletal structures

There is abundant evidence for association between some cytoplasmic polysomes and the actin-containing microfilaments (for review see [143]). The evidence includes electron microscopy [144], immunohistochemistry [85,145], release of polysomes with cytochalasin B or D [144,146,147] and with 130 mM NaCl [147,148], inhibition of such release with phalloidin [147,149], and correlation of microfilament organization with cytomegalovirus infection [150]. Differential extraction studies using non-ionic detergents and deoxycholate have also led to the same conclusions. These experiments seem to imply that attachment to the actin cytoskeleton is a precondition for mRNA translation [147,151,152], assuming that the actin-containing protein gel of the rough endoplasmic reticulum membrane can be counted as part of the cytoskeleton [151,152]. However, the results of such experiments may be difficult to interpret (for reviews see [8,143]). The balance of evidence at present suggests that around 30% of the translationally active polysomes may be 'free', in the sense that they are associated with neither the microfilaments nor the endoplasmic reticulum [143]. Alterations in the size of this pool when the protein synthesis rate of the cell is increased may imply that the free polysomes contain the older mRNAs [153].

Colchicine does not affect polysome anchoring, implying that active polysomes are not associated with microtubules or intermediate filaments [146,147]. However, it is possible that translationally inactive messengers are bound to intermediate filaments, to judge from immunohistochemical and other studies on the distributions of untranslated messenger particles [154]. This raises the possibility that polysome redistribution between active and inactive pools in response to extracellular signals [153,155] occurs by means of messenger migration from one binding site to another.

The immobilization of much of the mRNA and many ribosomes in the cytoplasmic compartment is consistent with the solid-state transport concept and with the observed partitioning of different mRNAs to different parts of the cytoplasm [142,156]. On present evidence, it is difficult to say whether tRNAs and snRNPs are transported by solid-state mechanisms, but the possibility remains open. If nucleocytoplasmic transport of mRNAs, ribosomes and some proteins [16,17,140] is at least in part a solid-state process, then there is an intuitive likelihood that tRNA and snRNP transport are similar in kind.

FUTURE PROSPECTS

Paine [17a] has observed that both intracompartmental binding and translocation events at the pore complex influence nucleocytoplasmic protein distributions. Irrespective of whether transport processes are solid-state, there is no doubt that these two factors are potentially important in the distributions of any class of macromolecules in nucleus and cytoplasm. The future development of this field therefore depends on improved characterization of both translocation and intracompartmental binding.

Characterization of translocation depends on better understanding of the structure and dynamics of the pore-complex. This will increase as (a) interactions between nuclear envelope constituents during interphase and the mechanisms of assembly and disassembly during mitosis are more fully understood [157], (b) the structures and interactions of nucleoporins are known in more detail [10,158], and (c) more is learned about location signals and about the wealth of factors that modulate nuclear envelope binding and translocation [159]. Two crucial stages in the future advancement of our understanding will be (a) characterization of the relationships between location signal receptors and nucleoporins, and (b) establishment of a mechanism for the switching of pore-complexes between open and closed states. To reach both these stages will require detailed studies of a variety of systems.

It seems likely that characterization of intracompartmental binding will improve first in respect of pre-mRNA attachments in the nucleus. Identification of the nucleoskeletal components involved in establishing track-like and non-track-like transcript distributions, and the relationships between these and the distributions of splicing components, seems to be nascent in the recent publications reviewed in this article [125–139]. Details about RNA attachments in the cytoplasmic compartment and about intranuclear binding sites of karyophilic proteins may be slower to emerge, because they are not currently major foci of research interest, although the potential significance of such details for our understanding is already apparent [17a].

Potentially, both translocation and intracompartmental binding could determine the changing distributions of proteins and other macromolecules during the cell cycle. We have referred to the nucleocytoplasmic shuttling of a number of proteins during the course of this article (A-group hnRNP proteins, hsp70 and Nopp 140). These shuttling processes challenge our understanding of the underlying mechanisms, and if an improved understanding could be applied to the behaviour of cyclins, it would elucidate the control of the cell cycle itself. Recent studies on the complex interplay of intermolecular associations, binding at specific cellular locations and control by phosphorylation and dephosphorylation of the cyclins [160–162] support this view.

The time is now ripe for a fuller theoretical development of the 'solid-state transport' concept. As we have argued in the present review, there is now abundant evidence that not only mRNA transport, but also the transport of some proteins and probably of other macromolecules and complexes, are at least partly solid-state processes. However, apart from distinguishing the mechanism from a 'diffusion'-dependent process, the description 'solid-state' is not particlarly informative. There are several possible models for solid-state transport [8,18], and quantitatively articulated versions of these models, from which experimentally testable predictions can be obtained, would be valuable. Amongst other advantages, such developments should lead to more critical assessments of rival methods of study of nucleocytoplasmic transport processes. This model articulation is a biophysical problem; only when it has been satisfactorily addressed will it be possible to proceed to a more general, and more satisfactory, concept of nucleocytoplasmic transport, and to exclude naive models in which the sole regulator of intercompartmental exchange is assumed to be the openness of the pore-complex [163]. We are currently developing such models via a critical analysis of the application of diffusion theory in biology.

REFERENCES

1 Dworetzky, S. I. and Feldherr, C. M. (1988) J. Cell Biol. **106**, 575–584
2 Akey, C. W. and Goldfarb, D. S. (1989) J. Cell Biol. **109**, 971–982
3 Schröder, H.-C., Rottmann, M., Wenger, R., Bachmann, M., Dorn, A. and Müller, W. E. G. (1988) Biochem. J. **252**, 777–790
4 Agutter, P. S. (1984) Subcell. Biochem. **10**, 281–357
5 Müller, W. E. G., Agutter, P. S., Bernd, A., Bachmann, M. and Schröder, H.-C. (1985) in Thresholds in Aging; 1984 Sandoz Lectures on Gerontology (Bergener, M., Erminci, M. and Stahlin, H. B., eds.), pp. 21–58, Academic Press, London
6 Peters, R. (1986) Biochim. Biophys. Acta **864**, 305–359
7 Dingwall, C. and Laskey, R. A. (1986) Annu. Rev. Cell Biol. **2**, 367–390
8 Agutter, P. S. (1991) Between Nucleus and Cytoplasm, Chapman and Hall, London
9 Feldherr, C. M. (ed.) (1991) Nuclear Trafficking, Academic Press, New York
10 Stewart, M. (1992) Semin. Cell Biol. **3**, 267–277
11 Paine, P. L., Moore, L. C. and Horowitz, S. B. (1975) Nature (London) **254**, 109–114
12 Feldherr, C. M. (1962) J. Cell Biol. **14**, 65–72
13 Stevens, B. J. and Swift, H. (1966) J. Cell Biol. **31**, 55–57
14 Feldherr, C. M. and Pomerantz, J. (1978) J. Cell Biol. **78**, 168–175
15 Feldherr, C. M. and Ogburn, J. A. (1980) J. Cell Biol. **87**, 589–593
16 Paine, P. L. (1984) J. Cell Biol. **99**, 188s–195s
17 Paine, P. L. (1988) Cell Biol. Int. Rep. **12**, 691–708
17a Paine, P. L. (1993) Trends Cell Biol. **3**, 325–329
18 Maul, G. G. (ed.) (1982) Wistar Symp. Ser. **2**, 1–11
19 Agutter, P. S. (1988) Prog. Mol. Subcell. Biol. **10**, 15–96
20 Maul, G. G. (1977) Int. Rev. Cytol. Suppl. **6**, 75–186
21 Reichelt, A., Holzenburg, A., Bühle, E. L., Jarnik, M. and Aebi, U. (1990) J. Cell Biol. **110**, 883–894
22 Feldherr, C. M., Kallenbach, E. and Schültz, N. (1984) J. Cell Biol. **99**, 2216–2222
23 Jarnik, M. and Aebi, U. (1991) J. Struct. Biol. **107**, 237–249
24 Georgatos, S. D. and Blobel, G. (1987) J. Cell Biol. **105**, 117–125
25 Whytock, S., Moir, R. D. and Stewart, M. (1990) J. Cell Sci. **97**, 571–580
26 Fey, E. G., Wan, K. M. and Penman, S. (1984) J. Cell Biol. **98**, 1973–1984
27 Carmo-Fonesca, M., Cicadao, A. J. and David-Ferreira, J. F. (1987) Eur. J. Cell Biol. **45**, 282–290
28 Gerace, L., Ottoviano, Y. and Kondor-Koch, C. (1982) J. Cell Biol. **95**, 826–837
29 Wozniak, R. W., Bartnik, E. and Blobel, G. (1989) J. Cell Biol. **108**, 2083–2092
30 Wozniak, R. W. and Blobel, G. (1992) J. Cell Biol. **119**, 1441–1449
31 Greber, U. F., Senior, A. and Gerace, L. (1990) EMBO J. **9**, 1495–1502
31a Hallberg, E., Wozniak, R. W. and Blobel, G. (1993) J. Cell Biol. **122**, 513–521
32 Snow, C. M., Senior, A. and Gerace, L. (1987) J. Cell Biol. **104**, 1143–1156
33 Greber, U. F. and Gerace, L. (1992) J. Cell Biol. **116**, 15–30
34 Starr, C. M., D'Onofrio, M., Park, M. K. and Hanover, J. A. (1990) J. Cell Biol. **110**, 1861–1871
35 Cordes, V., Waizenegger, I. and Krohne, G. (1991) Eur. J. Cell Biol. **55**, 31–47
36 Davis, L. I. and Fink, G. R. (1990) Cell **61**, 965–978
37 Loeb, J. D. J., Davis, L. I. and Fink, G. R. (1993) Mol. Biol. Cell **4**, 209–222
38 Carmo-Fonesca, M., Kern, H. and Hurt, E. C. (1991) Eur. J. Cell. Biol. **55**, 17–30
39 Wimmer, C., Dove, B., Grandi, P., Nehrbass, U. and Hurt, E. C. (1992) EMBO J. **11**, 5051–5061
40 Wente, S. R., Rout, R. P. and Blobel, G. (1992) J. Cell Biol. **119**, 705–723
41 Baglia, F. A. and Maul. G. G. (1982) Proc. Natl. Acad. Sci. U.S.A. **80**, 2285–2298
42 Davis, L. I. and Blobel, G. (1986) Cell **50**, 699–709
43 Finlay, D. R., Newmayer, D. D., Price, T. M. and Forbes, D. J. (1987) J. Cell Biol. **104**, 189–200
43a Bataille, N., Helser, T. and Fried, H. M. (1991) J. Cell Biol. **111**, 1571–1582
43b Neumann de Vegvar, H. E. and Dahlberg, J. E. (1990) Mol. Cell. Biol. **10**, 3365–3371
43c Michaud, N. and Goldfarb, D. S. (1992) J. Cell Biol. **116**, 851–861
44 Featherstone, C., Darby, M. K. and Gerace, L. (1988) J. Cell Biol. **107**, 1289–1298
45 Finlay, D. R., Meier, E., Bradley, P., Horecka, J. and Forbes, D. J. (1991) J. Cell Biol. **114**, 169–183
46 Berrios, M., Blobel, G. and Fisher, P. A. (1983) J. Biol. Chem. **258**, 4548–4555
47 Berrios, M. and Fisher, P. A. (1986) J. Cell Biol. **103**, 711–724
48 Berrios, M., Fisher, P. A. and Matz, E. C. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 219–223
49 Sukegawa, J. and Blobel, G. (1992) Cell **111**, 5051–5061
50 Schwabe, J. (1992) Curr. Biol. **2**, 237–239
51 Mehlin, H., Daneholt, B. and Skoglund, U. (1992) Cell **69**, 605–613
52 Mattaj, I. (1990) Curr. Opin. Cell Biol. **2**, 528–538
53 McDonald, J. and Agutter, P. S. (1980) FEBS Lett. **116**, 145–148
54 Sheehan, M. A., Mills, A. D., Sleeman, A. M., Laskey, R. A. and Blow, J. J. (1988) J. Cell Biol. **106**, 1–12
55 Newport, J. W., Wilson, K. L. and Dunphy, W. G. (1990) J. Cell Biol. **111**, 2247–2259
56 Finlay, D. R. and Forbes, D. J. (1990) Cell **60**, 17–29
57 Blobel, G. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 8527–8529
58 Kalderon, D., Richardson, W. D., Markham, A. F. and Smith, A. E. (1984) Nature (London) **311**, 33–38
59 Hall, M. N., Hereford, L. and Herskowitz, I. (1984) Cell **36**, 1057–1065
60 Kleinschmidt, J. A. and Seiter, A. (1988) EMBO J. **7**, 1605–1614
61 Burglin, T. R. and de Robertis, E. M. (1987) EMBO J. **6**, 2617–2625
62 Lanford, R. E., Kanda, P. and Kennedy, R. C. (1986) Cell **46**, 575–582
63 Lanford, R. E., White, R. G. and Dunham, R. G. (1988) Mol. Cell. Biol. **8**, 2722–2729
64 Dworetzky, S. I., Lanford, R. E. and Feldherr, C. M. (1988) J. Cell Biol. **107**, 1279–1288
65 de Robertis, E. M., Longthorne, R. F. and Gurdon, J. B. (1978) Nature (London) **272**, 254–256
66 Dingwall, C., Sharnick, S. V. and Laskey, R. A. (1982) Cell **30**, 449–458
67 Tobian, J. A., Drinkard, I. and Zasloff, M. A. (1985) Cell **43**, 415–422
68 Dargemont, C. and Kuhn, L. C. (1992) J. Cell Biol. **118**, 1–9
69 Hamm, J. and Mattaj, I. W. (1990) Cell **63**, 109–118
70 Hamm, J., Darzinkiewicz, E., Tahara, S. M. and Mattaj, I. W. (1990) Cell **62**, 569–577
70a Eckner, R., Ellmeier, W. and Birnstiel, M. L. (1991) EMBO J. **10**, 3513–3522
71 Roberts, B. C., Richardson, W. D. and Smith, A. E. (1987) Cell **50**, 465–475
72 Könings, D. A. M. and Mattaj, I. W. (1987) Exp. Cell Res. **172**, 329–339
73 Mattaj, I. W. and de Robertis, E. M. (1985) Cell **40**, 111–118
74 Picard, D. and Yamamoto, K. R. (1987) EMBO J. **6**, 3333–3340
75 Madsen, M. P. F., Nielsen, S. and Celis, J. E. (1983) J. Cell Biol. **103**, 2083–2089
76 Newmayer, D. D. and Forbes, D. J. (1988) Cell **52**, 641–653
77 Rihs, H.-P. and Peters, R. (1989) EMBO J. **8**, 1479–1484
78 Richardson, W. D., Mills, A. D., Dilworth, S. M. Laskey, R. A. and Dingwall, C. (1988) Cell **52**, 655–664
79 Zasloff, M. A. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 6436–6440
80 Dabauville, M.-C., Schültz, B., Scheer, U. and Peters, R. (1988) Exp. Cell Res. **174**, 291–296
81 Garcia-Bustos, J. F., Wagner, P. and Hall, M. N. (1991) J. Biol. Chem. **266**, 22303–22309
82 Yamasaki, L., Kanda, P. and Lanford, R. E. (1989) Mol. Cell. Biol. **9**, 3028–3036
83 Breeuwar, M. and Goldfarb, D. S. (1990) Cell **60**, 999–1008

84 Stochaj, U. and Silver, P. A. (1992) J. Cell Biol. **117**, 473–482
85 Schröder, H.-C., Diehl-Seifert, B., Rottmann, M., Aitken, S. J. M., Bryson, B. A., Agutter, P. S. and Müller, W. E. G. (1988) Arch. Biochem. Biophys. **261**, 394–404
86 Benditt, J. O., Meyer, C., Fasold, H., Barnard, F. and Riedel, N. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 9327–9331
87 Adam, S. A., Lobl, T. J., Mitchell, M. A. and Gerace, L. (1989) Nature (London) **337**, 276–279
88 Lee, W.-C. and Melese, T. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 8808–8812
89 Imamoto-Sonebe, N., Matsuoka, Y., Semba, T., Okada, Y., Uchida, T. and Yoneda, Y. (1990) J. Biol. Chem. **265**, 16504–16508
90 Yamasaki, L. and Lanford, R. E. (1992) Trends Cell Biol. **2**, 123–127
91 Izaurralde, E., Stepinski, J., Darzynkiewicz, E. and Mattaj, I. W. (1992) J. Cell Biol. **118**, 1287–1295
92 Prochnow, D., Riedel, N., Agutter, P. S. and Fasold, H. (1990) J. Biol. Chem. **265**, 6536–6539
93 Silver, P., Sadler, I. and Osborne, M. A. (1989) J. Cell Biol. **109**, 983–989
94 Silver, P. (1991) Cell **64**, 489–497
95 Newmayer, D. D., Lucocq, J. M., Burglin, T. R., de Robertis, E. M. and Forbes, D. J. (1986) EMBO J. **5**, 501–510
96 Agutter, P. S., McArdle, H. J. and McCaldin, B. (1976) Nature (London) **263**, 165–167
97 Schröder, H.-C., Rottmann, M., Bachmann, M. and Müller, W. E. G. (1986) J. Biol. Chem. **261**, 663–668
98 Prochnow, D., Thomson, M., Benson, M., Seve, A.-P., Schröder, H.-C., Müller, W. E. G. and Agutter, P. S. (1994) Arch. Biochem. Biophys., in the press
99 Vorbrodt, A. and Maul, G. G. (1980) J. Histochem. Cytochem. **28**, 27–35
100 Kondor-Koch, C., Riedel, N., Valentin, R., Fasold, H. and Fischer, H. (1982) Eur. J. Biochem. **127**, 285–290
101 Schröder, H.-C., Friese, U., Bachmann, M., Carmo-Fonesca, M., Zaubitzer, T. and Müller, W. E. G. (1987) Eur. J. Biochem. **181**, 149–158
102 Bernd, A., Schröder, H.-C., Zahn, R. K. and Müller, W. E. G. (1982) Eur. J. Biochem. **129**, 43–49
103 Michaud, N. and Goldfarb, D. S. (1991) J. Cell Biol. **112**, 215-223
104 Goldfarb, D. S. (1991) Curr. Biol. **1**, 212–214
105 Pinol Roma, S. and Dreyfuss, G. (1992) Nature (London) **355**, 730–732
106 Shi, Y. and Thomas, J. O. (1992) Mol. Cell Biol. **12**, 2186–2192
107 Imamoto, N., Matsuoka, A., Semba, T., Okada, Y., Uchida, T. and Yoneda, Y. (1992) J. Cell Biol. **119**, 1047–1061
108 Schumm, D. E., Morris, H. P. and Webb, T. E. (1973) Cancer Res. **33**, 1821–1828
109 Webb, T. E., Schumm, D. E. and Palayoor, T. (1981) in The Cell Nucleus, volume 9 (Busch, H., ed.), pp. 199–247, Academic Press, New York
110 Moffett, R. B. and Webb, T. E. (1983) Biochim. Biophys. Acta **740**, 231–242
111 Malim, M. H., Hauber, J., Lee, S.-Y., Maizel, J. V. and Cullen, B. R. (1989) Nature (London) **338**, 254–257
112 Pfeifer, K., Weiler, B. E., Ugarkovic, D., Bachmann, M., Schröder, H.-C. and Müller, W. E. G. (1991) Eur. J. Biochem. **199**, 53–64
112a Krug, R. M. (1993) Curr. Opin. Cell Biol. **5**, 944–949
113 Schröder, H.-C., Facy, P., Monsigny, M., Pfeifer, K., Andreas, B. E. K. and Müller, W. E. G. (1992) Eur. J. Biochem. **205**, 1017–1025
114 Newmayer, D. D. and Forbes, D. J. (1990) J. Cell Biol. **110**, 547–557
115 Moore, M. S. and Blobel, G. (1992) Cell **69**, 939–950
116 Goldfarb, D. S. (1992) Cell **70**, 185–188
117 Sterne-Marr, R., Blevitt, J. M. and Gerace, L. (1992) J. Cell Biol. **116**, 271–280
118 Maquat, L. E. (1991) Curr. Opin. Cell Biol. **3**, 1001–1012
119 Schumm, D. E. and Webb, T. E. (1981) Arch. Biochem. Biophys. **210**, 275–279
120 Goldfine, I. D., Clawson, G. A., Smuckler, E. A., Purrello, F. and Vigneri, R. (1982) Mol. Cell. Biochem. **48**, 3–14
121 Schindler, M. and Jiang, L.-W. (1987) J. Cell Biol. **104**, 849–853
122 Schröder, H.-C., Wenger, R., Ugarkovic, D., Friese, K., Bachmann, M. and Müller, W. E. G. (1990) Biochemistry **29**, 2368–2378
123 Divecha, N., Banfic, H. and Irvine, R. F. (1991) EMBO J. **20**, 3207–3210
124 Lawrence, J. B., Singer, S. L. and Marselle, L. M. (1989) Cell **57**, 493–502
125 Huang, S. and Spector, D. L. (1991) Genes Dev. **5**, 2288–2302
126 Xing, Y., Johnson, C. V., Dobner, P. R. and Lawrence, J. B. (1993) Science **259**, 1326–1330
127 Wang, J., Cao, L. G., Wang, Y.-L. and Pederson, T. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 7391–7395
128 Zachar, Z., Kramer, J., Mims, I. P. and Bingham, P. M. (1993) J. Cell Biol. **121**, 729–742
129 Jackson, D. A., Hasson, A. B., Errington, R. J. and Cook, P. R. (1993) EMBO J. **12**, 1059–1065
130 Rosbash, M. and Singer, R. H. (1993) Cell **75**, 399–401
131 Xing, Y. and Lawrence, J. B. (1991) J. Cell Biol. **112**, 1055–1064
132 Spector, D. L., Fu, X.-D. and Maniatis, T. (1991) EMBO J. **10**, 3467–3477
133 Sui, H. and Spector, D. L. (1991) Genes Dev. **5**, 2288–2302
134 Company, M., Arenas, J. and Abelson, J. (1991) Nature (London) **349**, 487–493
135 van Eekelen, C. A. G. and van Venrooij, W. J. (1981) J. Cell Biol. **88**, 554–563
136 Schröder, H.-C., Trölltsch, D., Wenger, R., Bachmann, M. and Müller, W. E. G. (1987) Eur. J. Biochem. **167**, 239–245
137 Schröder, H.-C., Trölltsch, D., Friese, U., Bachmann, M. and Müller, W. E. G. (1987) J. Biol. Chem. **262**, 8917–8925
138 Schindler, M. and Jiang, L.-W. (1986) J. Cell Biol. **102**, 859–862
139 Ueyama, H., Nakayasu, H. and Ueda, K. (1987) Cell Biol. Int. Rep. **11**, 671–677
140 Zeitlin, S., Parent, A., Siverstein, S. and Efstratiadis, A. (1987) Mol. Cell. Biol. **7**, 111–120
141 Meir, U. T. and Blobel, G. (1992) Cell **70**, 127–138
142 Davis, I. and Ish-Horowicz, D. (1991) Cell **67**, 927–940
143 Hesketh, J. E. and Pryme, I. F. (1991) Biochem. J. **277**, 1–10
144 Ramaekers, F., Benedetti, E., Dunia, I., Vorstenbosch, P. and Bloemendal, H. (1983) Biochim. Biophys. Acta **740**, 441–448
145 Hesketh, J. E., Campbell, G. P. and Reeds, P. J. (1986) Biosci. Rep. **6**, 797–804
146 Lenk, R., Ransom, L., Kaufmann, Y. and Penman, S. (1977) Cell **10**, 67–78
147 Vedeler, A., Pryme, I. F. and Hesketh, J. E. (1991) Mol. Cell. Biochem. **100**, 183–193
148 Hesketh, J. E. and Pryme, I. F. (1988) FEBS Lett. **231**, 62–66
149 Adams, A., Fey, E. G., Pike, S. F., Taylorson, C. J., White, H. A. and Rabin, B. R. (1983) Biochem. J. **216**, 215–226
150 Jones, N. L. and Kirkpatrick, B. A. (1987) Eur. J. Cell Biol. **46**, 565–575
151 Cervera, M., Dreyfuss, G. and Penman, S. (1981) Cell **23**, 113–120
152 van Venrooij, W. J., Sillekens, P. T. G., van Eekelen, C. A. G. and Reinders, R. T. (1981) Exp. Cell Res. **135**, 79–91
153 Moon, R. T., Nicosea, R. F., Olsen, C., Hille, M. B. and Jeffery, W. R. (1983) Dev. Biol. **95**, 447–458
154 Scherrer, K. (1990) Mol. Biol. Rep. **14**, 1–9
155 Vedeler, A., Pryme, I. F. and Hesketh, J. E. (1990) Cell Biol. Int. Rep. **14**, 211–218
156 Singer, R. H., Langevin, G. L. and Lawrence, J. B. (1989) J. Cell Biol. **108**, 2343–2353
157 Dessev, G. N. (1992) Curr. Opin. Cell. Biol. **4**, 430–435
158 Forbes, D. J. (1992) Annu. Rev. Cell Biol. **8**, 495–527
159 Davis, L. I. (1992) Curr. Opin. Cell Biol. **4**, 424–429
160 Jessus, C. and Beach, D. (1992) Cell **68**, 323–332
161 Bailley, E., Pines, J., Hunter, A. and Bornens, M. (1992) J. Cell Sci. **101**, 529–545
162 Elledge, S. J., Richman, R., Hall, F. L., Williams, R. T., Lodgson, N. and Harper, J. W. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 2907–2911
163 Akey, C. W. (1990) Biophys. J. **58**, 341–355