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Signalling and transport through the nuclear membrane

Péter Csermely *, Tamás Schnaider, Ildikó Szántó

Institute of Biochemistry I., Semmelweis University, P.O. Box 260, H-1444 Budapest, Hungary

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Contents

1. Introduction

In eukaryotes the genetic material is segregated to the cell nucleus. This segregation is far from being complete: the nuclear membrane is a mediator of numerous transport

Abbreviations: ER, endoplasmic reticulum; grp, glucose regulated protein; hsp, heat shock protein; NLS, nuclear localization signal; PI, phosphatidylinositol; protein kinase A, cAMP-dependent protein kinase; protein kinase C; Ca^{2+}-phospholipid-dependent protein kinase

* Corresponding author. Fax: +36 1 266 6550; email: csermely@puskin.sote.hu.

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processes and the cell nucleus is actively involved in signal transduction. Nuclear signalling bears many similarities to the signal transduction in the cytoplasm, but various levels of nuclear organization give new possibilities for the regulation of nuclear processes and require the help of special 'distributors', molecular chaperones.

After a survey of the structure of nuclear membrane and transport mechanisms, we will briefly discuss these latter, special aspects of nuclear processes. In the last two parts of our review we give an overview of the regulation and pathology of nuclear signalling. For further information on nuclear membranes, transport and signal transduction numerous excellent recent reviews are available [2,13,108,120,122,127,138,158,295,301,367,386].

2. Structure of the nuclear membrane

The cell nucleus is surrounded by the nuclear envelope which is composed of the outer and inner nuclear membranes separated by the perinuclear space. The nuclear double-membrane is *not isolated*, its outer layer is continuous with the endoplasmic reticulum and has many contacts with cytoplasmic and intranuclear filamental networks. The membrane is *not uniform*, shows various structures and domains and lastly the nuclear membrane is *not steady*, displaying great changes during the cell cycle and after various extracellular stimuli.

2.1. Membrane lipids

Two-third of nuclear lipids is phosphatidylcholine, but phosphatidylethanolamine, phosphatidylinositol (PI), phosphatidic acid, phosphatidylserine and some other minor lipid fractions are also present. The diffusion-rate of lipids in both nuclear membranes is roughly the same [399]. The high similarity of the lipid composition of the two nuclear membranes may be derived from their connection via the peripheral channels of the nuclear pore complexes [196].

Nuclear lipids are mostly synthesized in the ER and travel to the nuclear membrane via diffusion. Phosphatidylinositol uptake may be facilitated by a PI-transfer protein [54] and the nuclear membrane may also communicate with other membranes by means of vesicle budding [187]. Phosphatidylserine can be synthesized in the nuclear membrane [216] and the interesting observations of Watkins and Kent [464], that activated CTP:phosphocholine cytidyltransferase translocates to the nuclear membrane raised the possibility that the nuclear membrane may also be a site of phosphatidylcholine synthesis. The existence of a matrix-bound phospholipase-A_2 has been reported [441], and numerous other enzymes, such as cyclooxygenases, lipoxygenases, cytochrome P450 participating in arachidonic acid metabolism are also present in the cell nucleus [367]. In agreement with this 'oxidative environment' around the cell nucleus, guanylate cyclase is also reported to be localized in the outer nuclear membrane [109]. 'Unwanted' peroxidation of nuclear lipids is prevented by a glutathione *S*-transferase associated with the nuclear envelope [445] and by the increased glutathione content of nuclei [22].

Enzymes of phosphatidylinositol metabolism, such as the inositol polyphosphate-1- and PI-4-phosphatases [424,498] also show a partial localization to the nuclear membrane. The nuclear translocation of PI-3-kinase after nerve growth factor treatment has been also reported [324]. Unfortunately, due to the technical difficulties in obtaining pure nuclear preparations using detergent-free methods,

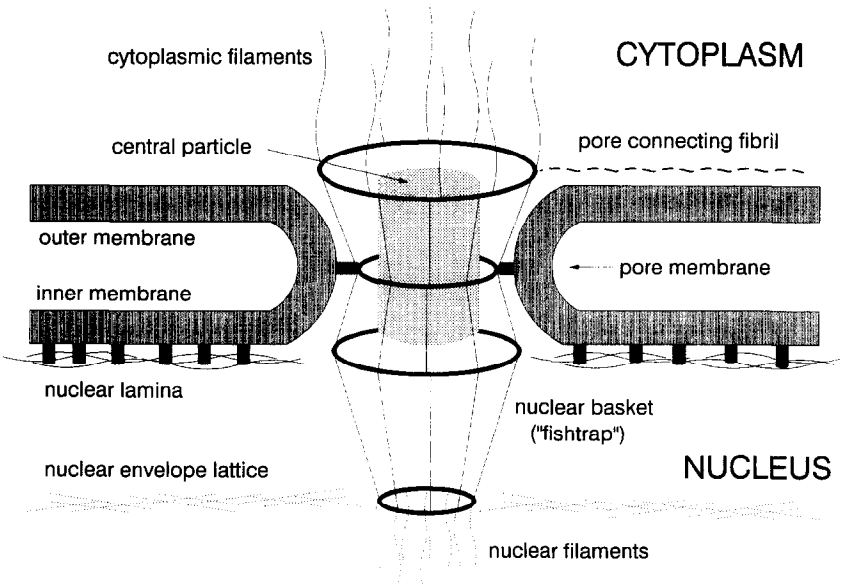

Fig. 1. Schematic structure of the nuclear pore complex.

few studies address the question of the localization of other enzymes of the PI-cycle to the nuclear membrane. However, available data indicate, that PI-4-kinase is attached to intranuclear structures of the nuclear pore complex [145,348] and many of the other PI-related enzymes, such as the PI-5-kinase, inositol polyphosphate-1-phosphatase, diacylglycerol kinase and phospholipase C-β1 are mostly localized in the inside of the nuclear matrix [60,75,112,348,498,512] (see Section 3.1 for further details). A few studies also indicate the presence of lipid structures (most probably in form of proteolipids) in the inside of the cell nucleus (see [111,140] and references therein) which may be related to (remnants of) the single nuclear membrane surrounding the chromatin mass during nuclear reassembly [413].

Some questions of further research: What is the significance and importance of nuclear lipid synthesis? Besides the well-known link between the outer nuclear membrane and the ER, how does de nuclear membrane communicate with other membranes and lipid structures outside and inside the cell nucleus? Is the lipid composition of the two layers of inner and outer nuclear membranes symmetrical? What is the structure of intranuclear lipids? Are they linked to the inner nuclear membrane? How are they formed? How is the nuclear PI-cycle activated?

2.2. Membrane proteins, the nuclear pore complex

Nuclear pore complexes are the largest (mol. wt. 124 000 kDa), and most notable structures of the nuclear envelope. Electron microscopic and atomic force microscopic studies [4,196,335] indicate, that the pore complex has a tripartite structure consisting of a central particle embedded between a cytoplasmic and a nuclear octagonal ring (Fig. 1.). The central particle contains a central plug and eight radiating spokes preserving the characteristic eightfold symmetry. High resolution electronmicroscopical analysis disclosed the existence of various pore-attached cytoplasmic filaments (including connecting fibrils between adjacent nuclear pores) and intranuclear basket-like structures (fish-traps) connecting the nuclear pore complex with the nuclear envelope lattice -which is distinct from the nuclear lamina- [157,158]. The intranuclear basket may be also linked to DNA by the constituting zinc-finger protein, nup153 [79,434]. Intranuclear actin filaments are also attached to the pore complex ([215] and references therein). The cytoplasmic and nuclear pore baskets and attached filaments may act as tracks guiding proteins and RNA in transit to the pore. Despite our exponentially increasing knowledge on the approx. 100 different proteins of the nuclear pore complex, the exact mechanism how this elaborate structure participates in nucleocytoplasmic transport is still largely unknown. Structural studies indicated, that the pore complex harbors eight central channels between the spokes of the central particle and may also accommodate another set of eight peripheral channels which serve as sites for the transport of proteins, RNA and smaller molecules [4,196].

Our knowledge of *other membrane proteins* of the nuclear envelope is mostly concentrated on the enzymes and receptors of intranuclear calcium-metabolism. Hence the existence of a transmembrane Ca-ATPase [249], IP3-receptor [282], PI-4-phosphatase [424] and a nuclear calnexin homologue [153] has been reported. A set of GTP-binding proteins preferentially bind to the inner nuclear membrane [389] and the presence of putative protein kinase C receptors in the nuclear envelope has been also proposed [214].

The nuclear outer and inner membranes are connected via the nuclear pore complexes where the two membranes merge. This continuity allows the free diffusion of integral membrane proteins with small (< 60 kDa) cytoplasmic domains between the two membranes [448,478]. Some integral proteins of the inner membrane, such as the lamin B receptor [426,484,485], are anchored to the nuclear lamina and to DNA. This is reflected by their lateral diffusion, which is at least 500 times slower than that of outer membrane proteins [399].

The nuclear envelope consists of three major domains, the outer and inner membranes and the pore domain. The presence of various domains of the nuclear membrane is supported by the existence of at least two types of nuclear vesicles at mitosis [63,458]. Binding of integral membrane proteins to cytoplasmic and nuclear filaments as well as to DNA makes it very likely that the existence of other 'micro'-domains will be also detected within the three major domains of the nuclear envelope. The interesting observation, that nuclear lamina is discontinuous and there are large regions in the nuclear envelope where no lamins can be observed, supports this view further [344].

How the nuclear membrane is assembled? Due to the intensive studies on in vitro systems we have a quite detailed knowledge on the reassembly of nuclear membranes after mitosis. We will summarize the present view of this process in Section 5.2. Our knowledge about the continuous assembly of nuclear membranes and about targeting of the membrane proteins during interphase, where the nuclear envelope doubles in surface area in preparation for mitosis, is much more limited. Besides lateral diffusion from the rough endoplasmic reticulum [361], vesicle fusion to and budding from nuclear membranes [187] may also influence the protein composition. Damaged nuclear membrane is repaired by the help of the rough endoplasmic reticulum [135].

Besides their common nuclear localization signal (see in Section 3.2) integral proteins of the nuclear envelope may utilize various mechanisms for their targeting to the final destination. However, intramembrane protein-protein interactions seem to play an important role in this process. The C-terminal CAAX motif of lamins A/C and B allows the farnesylation of immature lamins A/C and lamin B, which

participates in their proper intranuclear sorting [198,310]. Targeting of gp210, an anchor of the nuclear pore to the nuclear envelope, to the pore membrane domain is achieved by its single transmembrane helix [486]. Similarly, the N-terminal first membrane spanning region of the lamin B receptor is involved in the sequestration the receptor to the inner nuclear membrane [426]. However, in case of the latter protein and the lamina-associated polypeptide 2 nucleoplasmic domains also play a role in final targeting [142,428].

Some questions of further research: What filaments are attached to the nuclear pore complex? Do the nuclear pore complexes move laterally? What are the constituents of the nuclear envelope lattice? Do the 'peripheral channels' of the nuclear pore complex exist? What are the differences in the protein pattern of the outer and inner nuclear membranes? Do these membranes contain 'micro'-domains? How are they changed and renewed in interphase?

2.3. Attachments from outside: the ER connection

The outer bilayer of the nuclear envelope is the continuation of the endoplasmic reticulum membrane. p55, an inner nuclear membrane protein is able to translocate between nuclei via the ER in fused cells, which gives an elegant proof of that the two membranes are not segregated [361]. However, the absence of the typical ER enzymes CTP:ethanolamine- and CTP:phosphocholine cytidyltransferases from 'resting' nuclear membranes (where no facilitated lipid synthesis occurs [216]), the transfer of nascent HMG-CoA reductase from the outer nuclear membrane to the smooth ER [347] and the uneven distribution of cytochrome *P*-450 isoenzymes between nuclear and ER membranes [123] make it unlikely, that the two membranes are in complete equilibrium.

The outer membrane is a major site of synthesis of membrane and secretory proteins [147]. Facilitated synthesis of endoplasmic reticulum proteins results in the proliferation of the nuclear membrane. These and other observations raise the possibility that the nuclear membrane is a possible site where the endoplasmic reticulum is formed [317,347,457]. Vica versa the endoplasmic reticulum membrane participates in the repair of nuclear membranes after damage [135].

The nuclear envelope interacts with various cytoskeletal elements of the cytoplasm, including actin filaments, microtubules, centrosomes and intermediate filaments [147]. The outer nuclear membrane is less rigid, than its inner counterpart which is reflected in the relatively large diffusion rate of its integral membrane proteins [399]. Consequently attachment of filamentous structures and ribosomes to components of the outer membrane [147] does not restrict lateral mobility of integral membrane proteins to such an extent as it occurs with inner membrane proteins.

Some questions of further research: is there any 'gating-mechanism' between the endoplasmic reticulum and the outer nuclear membrane? What type of cytoplasmic filaments attach to the outer nuclear membrane and how? What are the specificities of the protein composition of the outer nuclear membrane?

2.4. The perinuclear space

The perinuclear space, i.e. the lumen of the double membrane structure of the nuclear envelope is continuous with the lumen of the ER. This way the perinuclear space also sequesters calcium, and likely harbors the major lumenal calcium-binding ER proteins. Some of these putative ER/nuclear lumenal proteins behave as molecular chaperones: e.g. calreticulin, BiP (grp78), endoplasmin (grp94), and protein disulfide isomerase [349]. An interesting observation suggesting a discontinuity between the lumen of ER and of nuclear envelope, that in activated human leukocytes 5-lipoxygenase and its activating protein are exclusively localized in the perinuclear space [483].

Some questions of further research: Is the perinuclear space different from the lumen of the ER? If yes, how is the difference maintained? Do the lumenal proteins of the perinuclear space play any specific role?

2.5. Attachments from inside

The inner membrane of the nuclear envelope is rather rigid, which is reflected by a limited lateral diffusion of its integral membrane proteins [399]. Restricted mobility may be a consequence of the multiple anchors of the inner membrane to nuclear envelope lattice and to nuclear lamina. Lamins, the constituents of the laminal structure, are mostly associated in dimers, forming a filamental network, which further associates to thicker filamentous structures and/or paracrystal formations [101]. The closely related lamin A and C are expressed in differentiated cells and so may participate in the organization of heterochromatin and in transport processes ([306] and Section 3.3). The nuclear inner membrane is bound to the nuclear lamina via lamin B receptors [484] and by other lamina-associated polypeptides [137]. Mature lamin B (but not lamins A and C) possess a C-terminal farnesyl residue which may also participate in the attachment of the inner membrane to lamin filaments [101].

Lamin B binds to matrix attachment regions (MAR-s, or scaffold attachment regions, SAR-s) of DNA [267]. Matrix attachment regions are situated at the bases of 5–200 kb long DNA-loops, contain AT-rich DNA sequences, a novel ATPase, histone H1, nucleolin, topoisomerase II and other specific binding proteins participating in the enhancement of gene transcription in the interphase cell nucleus [42,106,211,395]. Lamin B may not be the only contact of the inner nuclear membrane with DNA, since the lamin B receptor also contains a DNA-binding

region [485] and DNA binding of the pore protein, nup153 has been reported by Sukegawa and Blobel [434].

Some questions of further research: What is the role of the nuclear envelope lattice? Are the associations of matrix attachment regions with the nuclear lamina / nuclear membrane rigid or are they restructured during interphase?

3. Nuclear transport mechanisms

3.1. Ion transport

The nuclear membrane is punctured by 2–60 pores/μm^2 [147]. Experiments of Paine et al. [345] and Lang et al. [247] indicated that nuclear pores act as molecular sieves allowing the free diffusion of molecules below 30–60 kDa [147]. As a logical consequence, the concentration of small molecules, such as various anions and cations should equilibrate in the intranuclear and cytoplasmic sides of the nuclear envelope rapidly.

Contrary to this expectation, numerous studies showed, that a significant concentration gradient of calcium ions is built up or preserved between the cytoplasm and the nucleus of several resting or stimulated cell types, such as smooth muscle cells [194,195,480,481], neurons [26,191,366], erythroblasts [496], hepatocytes [465], mammary adenocarcinoma cells [21], B lymphocytes [143], mast cells [228] and basophilic leukemia cells [199]. Recent experiments of Connor [78], Al-Mohanna et al. [7] and Gerasimenko et al. [148] indicated that confocal microscopy of Ca-sensitive dyes may report artefactual changes of nuclear calcium concentration. Badminton et al. [15] were able to demonstrate transient Ca^{2+} gradients between the nucleus and the cytoplasm using a nucleus-targeted aequorin suggesting that not all the previously observed differences between nuclear and cytoplasmic calcium were artefacts. The cell nucleus of primary rat sensory neurones is reported to be insulated from larger changes of cytoplasmic calcium [7] and in resting oocytes a persistent ooplasm/nuclear calcium gradient seems to exist [257,397]. IP_3, cyclic-ADP-ribose and ryanodine may induce local intranuclear increases in free calcium concentration independent of the cytosolic calcium-level by releasing calcium from the nuclear envelope [148]. In other experiments the existence of a nuclear barrier to Ca^{2+} ions has been questioned by several groups [6,43,155,342,511] using fluorescent calmodulin, targeted aequorin, Calcium Green dextran, microinjected Ca-sensitive dyes and whole cell-patch clamp. The observed differences may arise from the differences of nuclear calcium permeability in different cell types, changes in permeability in different physiological or developmental conditions and/or by inherent limitations of the various methods used [305].

Other small molecules, such as saccharose [200], inuline [201] and glutathion [22] also show an uneven distribution between the intranuclear compartimentum and the cytoplasm. The membrane-potential of nuclear membranes [156,263,264,285] indicates a similar concentration gradient of Na^+ and K^+ ions. These observations can not be explained solely by the different concentration of water in the intranuclear space and cytoplasm [200,201] and indicate a reduced permeability of nuclear pores towards certain small molecules and the existence of active transport mechanisms across the nuclear membrane.

3.1.1. Ca^{2+} ions

As we have described earlier (Section 2.1) most enzymes of the phosphatidylinositol (PI) cycle have been also localized in the cell nucleus (Fig. 2). Nuclear uptake of phosphatidylinositol is partially mediated by a PI-transfer protein [54]. Its phosphorylation is achieved by PI-4-kinase attached to intranuclear structures of the nuclear pore complex [144,348] and by the matrix-bound PI-5-kinase [348]. Phosphorylated PI is cleaved by phospholipase C-β which is also localized in the inner proteolipid-like structures of the nuclear matrix [348,512] and may be regulated by beta-gamma subunits of G-proteins [52,229]. Phospholipase C-delta has been also localized in the nucleus [12]. As a product of phospholipase C action, nuclear diacylglycerol is produced which may participate in the translocation and activation of nuclear protein kinase C [279,383]. Inactivation of nuclear diacylglycerol is mediated by the diacylglycerol-kinase of nuclear matrix [348]. The other product of the cleavage of phosphatidylinositol-4,5-bisphosphate, IP_3, binds to its specific nuclear receptor [148,190,270–272,282,330,387] and triggers a release of Ca^{2+} ions from the lumen of the nuclear envelope [148,190,270–272,282,330]. The exact localization of nuclear IP_3 receptors is not consistently clear, so their participation in the generation of *intranuclear* calcium signals has been questioned [113,305]. Nuclear calcium release can also be achieved by cyclic ADP-ribose or ryanodine [148]. Local nuclear Ca-gradients help the formation and fusion of nuclear membrane vesicles in nuclear breakdown and reassembly both in somatic cells and in oocytes [397,431,435,436], activate gene transcription, nuclear protein transport, several nuclear protein kinases (such as the calcium-calmodulin-dependent protein kinase and protein kinase C), protein phosphatases (such as calcineurin), proteases, the chromatin structure, specific endonucleases in apoptosis and nucleotide triphosphatases [13,49,167,224,328,331].

Uptake of nuclear Ca^{2+} to the lumen of the nuclear envelope, which may function as a Ca^{2+} reservoir, is achieved by calmodulin-dependent nuclear Ca-pumps [329]. The existence of nuclear IP_4 (inositol-1,3,4,5-tetrakis-phosphate) receptors has been reported by Köppler et al [239], which participates in the ATP-independent uptake of nuclear Ca^{2+} [271]. The identity of nuclear Ca-ATPases with their counterparts in the ER [13,46,249,328,331], as well as their exact localization to the outer or inner mem-

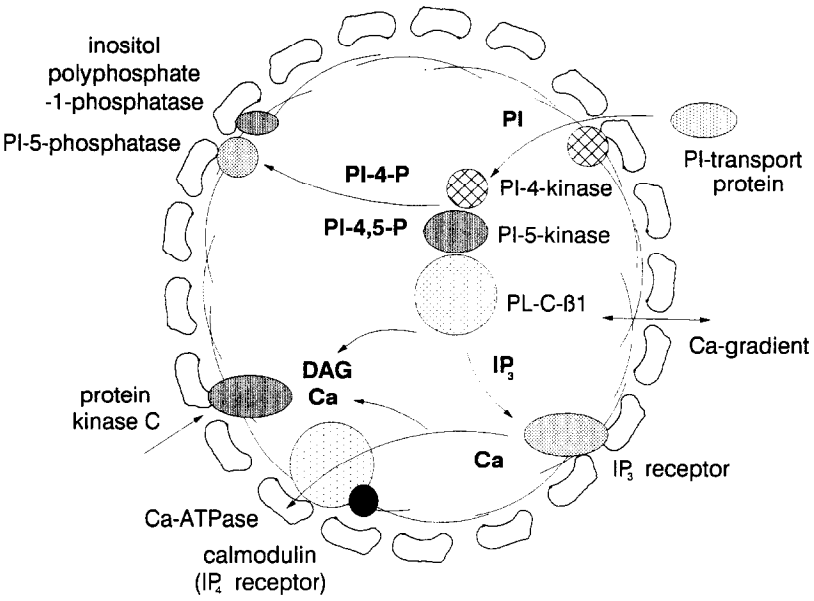

Fig. 2. Calcium metabolism of the cell nucleus. Abbreviations from the top to the bottom of the figure: PI, phosphatidylinositol; PI-4-P and PI-4,5-P, phosphatidyl-inositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate, respectively; PL-C-β1, phospholipase-C β1 isoform; IP_3 and IP_4, inositol-1,4,5-trisphosphate and inositol-1,3,4,5-tetrakisphosphate, respectively; DAG, diacyl-glycerol.

brane of the nuclear envelope is not entirely clear at present. Our present knowledge about the generation of intranuclear Ca^{2+} gradients [26,330] is also very limited. The regulation of nuclear calcium metabolism after various stimuli will be discussed in Section 5.1.

Some questions of further research: How permeable is the nuclear pore for small molecules? What is the significance of the fact, that a part of the nuclear PI-cycle is localized in the nuclear matrix? How does the nuclear envelope participate in nuclear PI metabolism?

3.1.2. Na^+ and K^+ ions

The cell specific, maximally 10 mV inside-negative membrane potential of the nuclear membrane [49,263,264,156,285,335] indicates the presence of a nuclear concentration gradient of Na^+ and K^+ ions. Recently the existence of nuclear K^+-selective ion channels was also demonstrated using patch clamp technique. The conductivity of the channels varied between 200 and 1000 pS due to the different isolation procedures and experimental conditions inducing a variability in the number of open channels. The total number of channels correlated with the number of nuclear pores, the upper limit of their conductivity was close to the theoretical upper limit of the nuclear pore conductivity [283,284,286,335]. The opening of nuclear K^+ channels is induced by ATP and deoxynucleotides, blocked by the macromolecular transport inhibitor, wheat germ agglutinin and requires GTP hydrolysis and cytosolic factors [48,287], which is similar to the characteristics of nuclear protein and RNA transport (see Sections 3.2 and 3.3). A co-activation of macromolecular transport and ion channel activity has been also reported [49]. These observations suggest that nuclear ion transport is mediated by the nuclear pore complex. Nuclear ion channels can also be activated by intranuclear cAMP-dependent phosphorylation [47], and most probably co-exist in different, voltage-gated and ligand-gated forms [49].

In agreement with the classical experiments of Langendorf [248,418] Oberleithner et al. [334] reported, that Na^+ influx is transduced directly into the nucleus by supranuclear sodium-transporters during depolarization of Madin-Darby canine kidney cells.

- *Some questions of further research: How uniform are the nuclear pore complexes / ion channels? Are the ion fluxes fully symmetrical around the nucleus?*

3.1.3. Zn^{2+}, Cl^- ions and other small molecules

Total (free + bound) zinc concentration can be comparable to that of calcium in the cell nucleus of some cells [88,495]. Zinc ions may serve as a second messenger [86,170], are constituents of several transcription factors and nuclear enzymes [23,453], may play a significant role in the stabilization of DNA [422] and a drop in intranuclear zinc concentration may contribute to the activation of specific endonucleases in apoptosis [117,152,415,501, 502].

However, our knowledge about the nuclear transport of Zn^{2+} is rather limited, which is mostly due to the few experimental methods available in the field. Experiments of Weser and Bischoff [472,473] and Coussins et al. [84] indicated a temperature-dependent uptake of ^{65}Zn to intact nuclei. Hechtenberg and Beyersmann [186] showed that the levels of intranuclear Ca^{2+} and Zn^{2+} are controlled

differentially and the latter is independent of ATP. The zinc-specific fluorescent indicator, zinquin developed by the group of Ian J. Forbes [502] may be a great help in further investigations of the field. However, care should be exercised in planning experiments with the cell nucleus, since the dye seems to show a largely extranuclear distribution [85,502].

Nuclear chloride channels with conductances of 150 and 58 pS were described by Tabares et al. [439]. In contrast to the ATP-dependent cation channels, the latter, small conductance chloride channel was blocked by ATP. The mechanisms maintaining the concentration gradient of other small molecules, such as reduced glutathion [22] are not known.

Some questions of further research: Does the intranuclear free zinc concentration differ from that of the cytosol? Does it change after various stimuli? Are chloride ions transported through the nuclear membrane via nuclear pores as well? Do other molecules, such as ATP, GTP, NADH, NADPH also show an uneven distribution between the cell nucleus and the cytoplasm?

3.2. Transport of proteins

As we have mentioned in Section 3.1, the nuclear pore was regarded as a molecular sieve allowing the free diffusion of molecules below 30–60 kDa [147]. However, the rate constant of passive diffusion is of the order of hours [2] and there are several proteins entering the cell nucleus during interphase, which are larger than the upper size limit for free diffusion, such as the large T antigen product of SV40 virus (92 kDa), or nucleoplasmin (110 kDa pentamer) [144,147,177,382]. Facilitated diffusion of small proteins, such as histone H1 [40] and calmodulin [365], also occurs. These observations suggest the presence of a selective, quick, active transport through the nuclear pore complex. Limited proteolysis and point mutations of the above two proteins helped to localize a short, basic segment, a so-called *nuclear localization signal* which responsible for selection for intranuclear active transport. Many nuclear localization signals correspond to the consensus sequence of

KK*xxxxxxxxxx*K*xx*KK

where 'K' denotes a lysine or arginine and 'x' stands for any amino acids. In the bipartite consensus sequence two sets of basic amino acids are separated by approximately ten residues [107,381]. The N-terminal basic group contains at least two, while the C-terminal pentamer accommodates at least three arginins or lysins. In other nuclear proteins only the C-terminal basic group exists preceded by a helix-breaking proline or glycine [37,107].

Besides the larger nuclear proteins (e.g.: lamins, c-myc, steroid receptors, hsp70, p53, etc. [144,382]) nuclear localization signals facilitate the transport of some small nuclear proteins, such as histone H1 [40], which have a free (but slow) access to the cell nucleus via diffusion. The nuclear localization signal (NLS) is the only signal which is required for nuclear transport. With an NLS-peptide originally non-nuclear proteins become translocated to the cell nucleus [161].

Besides the NLS there are some other signals which govern the intranuclear targeting of certain proteins. A glutamine residue surrounded by basic amino acids results a nucleolar localization [144]. An arginine/serine-rich domain targets proteins to the speckled intranuclear spliceosomes [430], while the C-terminal CAAX-group of immature lamin A/C molecules and lamin B leads to farnesylation, and subsequent transfer to the inner nuclear membrane [198]. Other multi-basic nucleolar localization signals have been also described [452]. The correct localization of integral membrane proteins in the nuclear envelope is helped by their transmembrane helices (see Section 2.2). Parallel with the elucidation of new and new intranuclear domains [149,430] the discovery of new intranuclear localization signals is expected.

The investigation of the *mechanism of nuclear protein transport* is far from being complete and has a high priority among cell biologists [2,122]. The transport process consists of two major steps: a fast binding of the target protein to the NLS-receptor and a relatively slow, energy requiring transfer of the protein through the nuclear pore (see Fig. 3. [325,326,375]).

Nuclear protein transport requires the participation of various cytoplasmic factors [362]. The first set of these proteins ('binding proteins') binds of NLS-containing proteins to their receptors and the second set ('co-transporters') allows the transport of pre-bound NLS-proteins through the nuclear pore.

NLS-proteins bind to the *NLS-receptor*(s) with low affinity ($K_a = 10^7$). This is not surprising, if we consider that their association is transient, the NLS-receptor has to release the NLS-protein to allow its transport through the nuclear pore. To compensate for the low affinity binding, the number of NLS-receptors has to be high. The high abundance may explain why the receptors are not exclusively confined to the pore complexes and found widely distributed inside the cell [2].

The search for the NLS-receptors by affinity chromatography, photoaffinity probes, ligand-blotting, and by antibodies against its putative complementary acidic region uncovered several proteins in the molecular weight range of 55 to 140 kDa [138]. Some of the putative NLS-receptors are predominantly nucleolar proteins, like nucleolin, numatrin and nopp140, suggested to participate in rRNA transport [203,292,490]. The functional significance of the NLS-binding site of these proteins is not known. The relatively poor definition of the nuclear localization signal, the low affinity binding, and the variable intracellular distribution may all explain the difficulties in the search for NLS-receptors. Recent data of Görlich et al [165] identified six closely related importin molecules

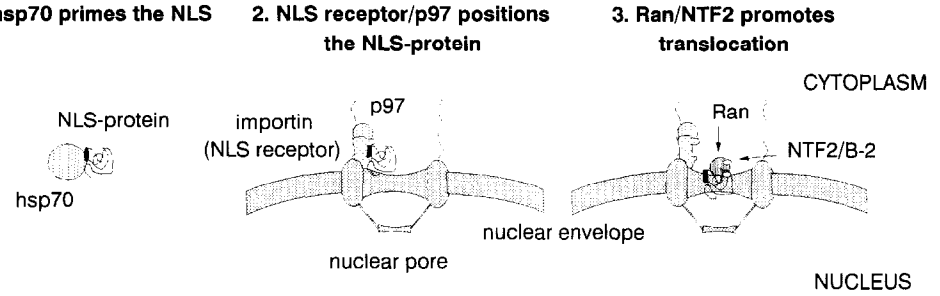

Fig. 3. Initial steps of protein transport through the nuclear pore. (The role of hsp70 is not entirely clear yet.) Abbreviations: hsp70, 70 kDa heat shock protein; NLS, nuclear localization signal; p97, 97 kDa NLS-receptor stimulating protein; Ran, ras-related nuclear GTP-binding protein; NTF2/B-2, 10-15 kDa nucleoporin-binding homodimer, acting in concert, or after Ran.

(karopherin alpha in [369]) which are good candidates for the 'true' NLS-receptor and together with a 97 kDa protein [1] (kariopherin beta in [369]) participate in positioning of the NLS-protein to the nuclear pore (Fig. 3. [362]). p97 might play a role in stabilizing the ligand/NLS receptor/nuclear pore complex [295].

Besides the NLS-receptor an important 'binding protein' is the 70 kDa heat shock protein (hsp70). hsp70 may prime the NLS-containing protein for binding to its receptor and requires ATP for its function ([414], see Section 4.2).

The small GTPase Ran (ras-related nuclear protein) together with a 10–14 kDa binding protein, NTF2 belongs to the 'co-transporters', and may function in the passage of the NLS-protein from its receptor to the channels of the nuclear pore complex [296,311,312,343]. Ran is a very abundant (0.4% of total protein), primarily intranuclear protein, which may play a general role in all phases of nucleocytoplasmic transport (Fig. 3 [313]). Locking Ran in its GTP-bound state blocks the nuclear transport of proteins [400]. Bischoff et al. [25] proposed a tempting hypothesis for the shuttling of Ran across the nuclear pore between its cytoplasmic GTPase activator, RanGAP1, and intranuclear GDP/GTP exchanger, RCC1. However, the exact localization of RanGAP1 is not entirely clear yet [25,81], and an intranuclear murine Ran GTPase activator homologue has been also described [182]. A co-factor of RanGAP1, RanBP1 forms a complex with Ran, the exchanger RCC1, hsc70 and a 340–360 kDa protein, RanBP2 [295,394]. Clearly, the characterization of the 'Ran-related complex' will give us a better understanding on the mechanism of nuclear transport in the near future.

The identification of the *transporters* among the proteins of the nuclear pore complex, the nucleoporins seems to be a rather difficult task, if we consider that the pore complex has a molecular weight of 124 MDa and contains more than hundred different proteins (see Section 2.2). Nuclear protein transport can be blocked by the lectin wheat germ agglutinin (WGA) and requires ATP. Recently a subset of nucleoporins could be identified which serve as docking sites for the transported protein complex both on the cytoplasmic and nucleoplasmic side of the nuclear pore complex. These nucleoporins all seem to incorporate repetitive peptide motifs [370].

Morphological studies suggest two basic models, the 'continuous' and the 'gating' hypothesis for *protein translocation* across the nuclear pore complex. According to the continuous model ATP is utilized for the partial unfolding and subsequent refolding of the transported protein in the cytoplasmic and nuclear face of the pore complex. The gating hypothesis describes the nuclear pore complex as a dynamic entity where the periodic opening and closure of the cytoplasmic and nuclear rims are responsible for most of the ATP consumption [108,177]. Some observations describing 'open' and 'closed' states of the nuclear pore complex as well as the regulation of nuclear protein transport by the lumenal calcium concentration [167] seem to favor the latter mechanism. Recurrent findings indicate the participation of various cytoplasmic and intranuclear filaments (including actin, myosin and intermediate filaments) in the transport process, which may associate with the outer rims of the pore complex, or traverse the nuclear pore longitudinally [2,226].

Most of the transported proteins are retained in the cell nucleus by binding to DNA, RNA or to proteinaceous nuclear structures. However, several proteins intensively shuttle between the nucleus and the cytoplasm (for a list of some examples see Table 1) . RNA-binding proteins and protein-binding proteins may participate in RNA and protein transport, respectively, requiring the escort of a 'helper' molecule. The escorting helper participates in a shuttle and it is 'recycled' for the next round of macromolecular transport. Outward movement of proteins from the nucleus requires the same nuclear localization signal like their import, but it is not energy-dependent [174]. The recent finding of Moroianu and Blobel [316] implicating the requirement of Ran (and GTP hydrolysis) for nuclear protein export is a little contradictory to the previous general view. Shuttling seems to be a general phenomenon, which mostly depends on intranuclear protein retention [402]. However, addition of an extra nuclear localization signal to the glucocorticoid receptor confines the shuttling molecule to the nucleus [269]. The extra NLS may interfere with the export, but may help the retention

Table 1
Shuttling proteins between the nucleus and cytoplasm

	Protein	References
1.	*RNA-binding proteins*	
	ribosomal L3 protein	[315]
	nucleolin (C23)	[35]
	numatrin (nucleophosmin, B23)	[35]
	hnRNA-binding A1 protein	[357]
	AU-binding protein A	[230]
	U1 nRNA-binding U1A protein	[225]
	yeast Np13	[134]
2.	*'Protein-binding' proteins*	
	70 kDa heat shock protein	[259]
	NLS-binding protein, nopp140	[292]
	NLS-binding protein, numatrin	[35]
3.	*DNA-binding proteins*	
	HMG1	[371]
	progesterone receptor	[173]
	glucocorticoid receptor	[269]
	estrogen receptor	[98]

and may also facilitate the re-uptake of the modified receptor. Shuttling DNA-binding proteins usually have a reduced affinity for DNA, because of their association with other proteins, such as heat-shock proteins (progesterone receptor [240]) or other DNA-binding proteins (HMG1 [33]). Shuttling proteins may be modified during their transit through the cytoplasm and may also provide a feedback mechanism for regulating cytoplasmic processes, such as translational efficiency or mRNA stability [402].

Some questions of further research: How many intranuclear targeting signals exist? How do the numerous NLS-receptors participate in the transport process? What is the exact molecular mechanism of protein transport across the nuclear pore complex?

3.3. RNA transport

There are some similarities and major differences between protein and RNA transport systems. The most important similarity is that both the protein and RNA transport proceeds through the nuclear pore complex [404]. However, most proteins, with the exception of those participating in a shuttle, go into the nucleus, while RNA is transported out of the cell nucleus. The small ribonucleoproteins (snRNP-s) participating in hnRNA-splicing provide a rare example, where a co-transport of a protein and RNA can be observed. Since the transport of mRNA, rRNA, nRNA and tRNA differ from each other quite substantially, we will discuss these transport processes separately.

3.3.1. mRNA transport

The mechanism of mRNA transport -similarly to that of the protein transport- involves two major steps: the recognition of RNA molecules to be transported and their transfer through the nuclear pore.

Recognition of mRNA. mRNA transport takes place after the maturation of hnRNA, since partially spliced or unspliced hnRNA seldom leaves the cell nucleus [177,404]. It is still subject of debate if the recognition of mRNA requires any special signal at all. When we talk about the recognition of a certain molecule we usually think about a diluted protein solution in our test-tube where after diffusion the target protein binds to its receptor with high affinity. This simplified view is not fully valid even in blood serum or in the cytoplasm, but completely looses its applicability when we talk about the highly ordered structure of the cell nucleus. We should rather talk about an 'organized transfer' or 'solid-state transport' of the mRNA molecule to the nuclear pore, than about its recognition [2,57].

In agreement with this general assumption, hnRNA has a rather 'organized tour' inside the cell nucleus. Splicing takes place most probably in the close proximity of the given gene [462,506]. Some of the small ribonucleoproteins, which participate in the splicing, however, form a highly organized speckled structure in the cell nucleus

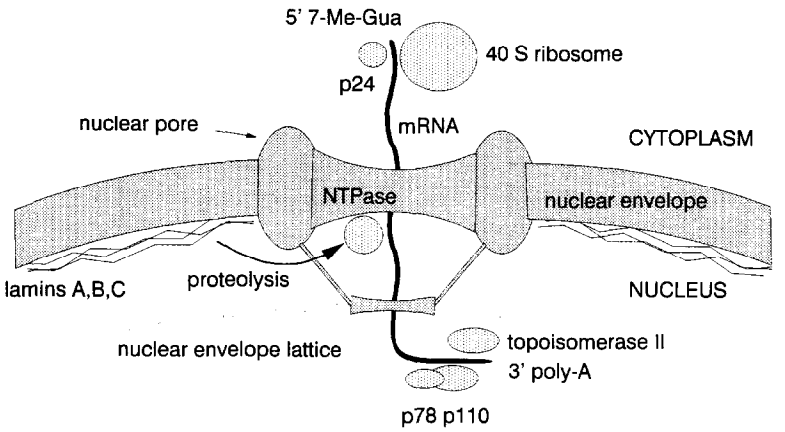

Fig. 4. mRNA transport. Abbreviations from the top to the bottom of the figure: 5' 7-Me-Gua, 7-methyl-guanosine cap of the 5' end of mRNA; p24, cytoplasmic 7-methyl-guanosine-binding protein participating in recruiting the 40 S ribosomal subunit; NTPase, the N-terminal nucleotide-triphosphatase fragment of lamins A and C participating in mRNA transport; p78, p110, nuclear poly-A-binding proteins.

[149,429,430] concentrated in interchromatin granule clusters, along perichromatin fibrils and in 'nuclear bodies' (coiled bodies) [38,58,147,398,462]. Many of these 'nuclear spots' may also function as ribonucleoprotein reservoirs [462,506].

hnRNA binds very strongly to the nuclear matrix prior maturation, while approximately 70% of spliced mRNA dissociates from the matrix [404]. hnRNA is anchored to the matrix via the 39–45 kDa C proteins and released by an RNA-helicase like protein, PRP22 [70,77,357]. Mature mRNA may be guided towards the nuclear pore by the nuclear envelope lattice (see Section 2.2, [157]).

Maturation of hnRNA provides special markers for the RNA molecule, such as the 5′ 7-methyl-GpppG cap and the 3′ poly-A-tail (see Fig. 4). These mRNA-markers may participate in the correct positioning of mRNA on the nuclear side of the nuclear pore complex before its transport [333,404]. Among the several poly-A-binding nuclear proteins a 78 kDa and a 110 kDa protein are suspected to participate in the transport of mRNA. Several mRNA-s, such as many neuronal mRNA-s, histone mRNA-s and beta-actin mRNA have no poly-A-tail, hence p78 and p110 can not participate in their 'organized transfer' to the nuclear pore [404]. The identification of the 7-methyl-GpppG-binding proteins is in progress [333,404].

mRNA reaches the nuclear pore complex migrating along curvilinear tracks by consecutive binding to various components of the 'splicing and maturation machinery', and/or mRNA may also use the intrachromosomal channel network for its migration inside the nucleus [462].

Transport of mRNA. The diameter of the mRNA-protein complex is approx. 20 nm, while the inner diameter of the nuclear pore is roughly 10 nm. Because of the substantial size difference, the mRNA-protein complex may partially 'unfold', dissociate during its transfer through the nuclear pore [293,294,430]. Unfolding of mRNA may be mediated by the topoisomerase II which is localized at the matrix attachment regions: close to the nuclear pore complex [404].

Transport begins with the 5′ end of mRNA which is consistent with the fact, that one of the most important transport signals, the 7-methyl-GpppG cap is also located at the 5′ end of the mRNA molecule [333]. The 3′ end may be attached to the nucleoplasmic ring of the nuclear pore complex until the entire mRNA molecule has been transported [294]. Recent observations identified at least five nucleoporins (NUP1p, NUP49p, NUP116p, NUP133p and NUP 159p [166]) constituting proximal or distal elements of the nuclear pore mRNA transport channel. RCC1, a nucleotide exchanger associated with the inner nuclear membrane and with the GTP-binding protein, Ran, is also involved in mRNA export. Locking Ran in its GTP-bound state blocks the export of poly-A-mRNA [400]. Ran may participate in the early steps of intranuclear 'organized tour' of mRNA as well [122,313]. According to some reports intranuclear actin filaments (myosin motors?) and a nuclear envelope-associated RNA-helicase are also involved in the translocation of mRNA [2,406].

mRNA transport is an energy-consuming process. The translocation of an average size poly-A-mRNA requires the hydrolysis of approximately 1000 ATP molecules (translocation of mRNA-s without poly-A tail seems to require less energy) [2]. The energy requirement is associated with the functioning of topoisomerase II and with a nucleotide-triphosphatase, which binds to the nuclear pore and reported to be identical with the N-terminal 46 kDa segment of lamins A and C [73,404]. The role of lamins in mRNA transport is further substantiated by the fact, that the transport can be blocked by anti-lamin B antibodies [16].

Cytoplasmic mRNA is also not freely diffusible. The 5′ end of mRNA is already associated with ribosomes and actively participates in the translation when the 3′ end of the same mRNA molecule is still intranuclear. Association of mRNA to the 40 S ribosome subunit is mediated by several initiation factors and by a 24 kDa 7-methyl-GpppG-binding protein [[96], p. 310]. Mutational studies suggest that the completion of the mRNA transport is helped by the driving force of concomitant protein synthesis which 'pulls out' the 3′ end of the mRNA molecule from the nucleus [108]. The mRNA molecule 'exchanges' most of its nuclear proteins to cytoplasmic ones during the transport process and associates with the cytoskeleton and actin filaments besides the ribosomes. The association helps the correct positioning of the newly synthesized proteins, hence the mRNA of many cytoskeletal proteins (e.g. actin, tubulin and vimentin) was shown to be associated with the corresponding filaments. The exchange of nuclear mRNA-binding proteins is not complete, since, e.g. the 36 kDa A1 protein, which helps RNA strand-annealing follows the mRNA to the cytoplasm [243,357]. The cytoplasmic 7-methyl-GpppG- and poly-A-binding proteins may also assure the irreversibility of mRNA transport [404].

The 'gene-gating' hypothesis of Günter Blobel [28], which was supported by several recent experimental findings [57,149,429,430] predicts, that a given nuclear pore transports the mRNA-s of mostly those genes, which are coded by the adjacent DNA-loops. This way the transport of many mRNA-s (and as a consequence most of the protein translation) shows an extremely high asymmetry in the cell [301].

3.3.2. rRNA transport

Ribosomal RNA is synthesized in the nucleolus. After the splicing of the primary 35–47 S pre-rRNA the transport of the 5, 17 and 28 S products is most probably facilitated by separate, but cooperating mechanisms which are relatively unknown. Ribosomal subunits are more or less assembled inside the nucleus and transported by the help of various carrier proteins through the nuclear pore [19,231]. 5 S rRNA first transiently associates with the La

antigen, which also participates in the termination of transcription. The La protein is exchanged to the transcription factor TFIIIa and to the L5 ribosomal protein. Association of rRNA with the latter two proteins is mandatory for the transport of 5 S rRNA [171]. Transport of 28 S rRNA is mediated by numatrin (ribocharin, B23) [203]. Similarly to numatrin the nucleolar nucleolin (C23) and the L3 ribosomal protein also shuttles between the nucleus and the cytoplasm [35,315], which suggests the participation of these proteins in the transport of rRNA.

3.3.3. nRNA transport

Formation of small nuclear RNA-s (snRNA-s, U RNA-s, in this review: nRNA-s) participating in the splicing of heteronuclear RNA differs from the synthesis of all the other RNA-s, since their maturation and association with proteins takes place in the cytoplasm. Mature nRNA is transported back to the nucleus [333]. The transport of nRNA-precursor from the nucleus to the cytoplasm is mediated by a carrier protein which recognizes an mRNA-like 7-methyl-GpppN-cap at the 5′ end of the nRNA molecule [281,333]. In the cytoplasm the 5′ guanosine of the cap is hypermethylated to a 2,2,7-trimethyl derivative and the nRNA molecule associates with several cytoplasmic proteins [280]. The newly formed nRNA complex is transported back to the nucleus by an other carrier protein. The 5′ 2,2,7-trimethyl-guanosine cap serves as a special nuclear localization signal for this latter, 'retrograde-transport'[175].

nRNA transport proceeds via at least three different mechanisms. Transport of U3 nRNA from the cytoplasm to the nucleus differs from that of the U1, U2, U4 and U5 nRNA-s, since it does not bind to cytoplasmic Sm proteins and can not be competed by trimethyl-guanosine. Nuclear transfer of U6 nRNA requires a monomethyl 5′-cap and is the only nRNA transport, which seems to proceed via similar mechanisms to those of the nuclear protein import [132,298,333].

3.3.4. tRNA transport

The mechanism of tRNA transport is less known, than the other RNA transport processes. The early works of Zasloff [504,505] demonstrated that the transport is saturable, so it may also involve a carrier protein, and the 'D' and 'T-psi-C' loops of tRNA plays an important role in the binding of the putative carrier [446]. Transport of tRNA (similarly to that of mRNA) takes place only after the full maturation of the RNA molecule [147]. Some steps of the tRNA transport process may be shared with mRNA molecules [94] but tRNA transport seems to have no requirement for ATP [363]. Interestingly, tRNA transport seems to differ from all the other RNA transport mechanisms by not requiring the involvement of the Ran GDP/GTP-exchanger, RCC1 [67].

Some questions of further research: How are the hnRNA and mRNA guided to the pore complex? How does gene-gating affect the asymmetry of the cytoplasm? How do the mRNA-s unfold prior their translocation? How do numatrin, nucleolin and the other carriers help the rRNA translocation? What are the differences between the transport-mechanism of various nRNA-s and tRNA-s?

4. Role of molecular chaperones in nuclear transport

4.1. Molecular chaperones in the cell nucleus

A recent definition describes molecular chaperones as "proteins that bind to and stabilize an otherwise unstable conformer of another protein — and by controlled binding and release of the substrate protein, facilitate its correct fate in vivo: be it folding, oligomeric assembly, transport to a particular subcellular compartment, or controlled switching between active/inactive conformations" [188]. Thus 'classical' molecular chaperones prevent the aggregation of folding intermediates ('unstable conformer'-s) of other protein molecules.

The cell nucleus differs from the cytoplasm by its highly organized structure (see Sections 3.2 and 3.3). Molecular crowding (which may be particularly pronounced in the cell nucleus) promotes the aggregation of macromolecules [510]. Partly because of this driving force, nuclear proteins and RNA are bound to intranuclear structures. Once this structure is established, free diffusion is very limited. In the cell nucleus the 'classical chaperones' preventing *random* molecular aggregation are rather jobless.

However, the enormous task to establish the nuclear structures and superstructures still remains. The high level of organization confers rigidity to the nuclear structure, while the changes during interphase require high flexibility. This apparent contradiction is resolved and the reorganization of nuclear structure after mitosis is achieved with the help of numerous proteins assisting in the dissociation and association of protein-DNA, protein-RNA and protein-protein complexes. The conformation of long DNA and RNA molecules is also adjusted continuously [114]. Most of the assisting nuclear proteins (such as nucleoplasmin, HMG1, topoisomerases, helicases etc.) fit the general characteristcs of chaperones (Table 2, [449]). The controlled binding and release, the relative instability of the targets is present, the same definition of molecular chaperones gains a novel meaning in the cell nucleus.

'Classical chaperones' have a low specificity, and bind to many types of folding intermediates. Binding is mostly transient, and binding affinity shows ATP-dependent fluctuations [20,150,188,202,467]. The reduced stability of the chaperone-complex is essential, since the bound target must enjoy a relative flexibility for assisted restructuring. In the cell nucleus there is an obvious class of proteins, which bind to their target transiently. Some of these pro-

Table 2
Molecular chaperones in the cell nucleus

Name	Nuclear chaperone function	References
A. nuclear molecular chaperones		
nucleoplasmin	histone-DNA assembly	[252]
HMG1 (s)	histone-DNA assembly	[33,371]
nucleolin (n,s)	ribosome assembly NLS-binding protein	[35,121,421,490]
numatrin (n,s)	ribosome assembly NLS-binding protein	[35,160,203]
fibrillarin	ribosome assembly	[447]
L3 protein (n,s)	ribosome assembly	[315]
nopp140 (n,s)	ribosome assembly NLS-binding protein	[292]
DRS1 RNA-helicase	ribosome assembly	[377]
DRS2 "Ca-ATPase"	ribosome assembly	[378]
A1 protein (s)	hnRNA assembly general RNA chaperone	[193,357,359]
U1A protein (s)	U1 nRNA assembly	[225]
B. 'classical' molecular chaperones		
phospho-hsp27 (*)		[233]
B-crystalline (*)		[236,459]
hsp40 (dnaJ) (*,n)		[181]
hsp56 ppi	steroid receptor	[146]
59 kDa ppi	in Sf9 cells	[9]
TRiC-P5 (hsp60)	nuclear actin filaments (?)	[221]
70 kDa ppi (n)	NLS-binding protein ribosome assembly	[410]
hsp70 (*,n,s)	steroid receptor nuclear protein transport topoisomerase I protection	[72,206,360,414, 468]
grp78 (BiP)		[254]
hsp90	steroid receptor chromatin restructuring transcriptional activation	[76,92,146,209]
calnexin		[153]
grp94 (*)		[470]
hsp110 (n)		[433]

(n) = nucleolar localization.
(s) = participates in nucleocytoplasmic shuttle.
hsp** = ** kDa heat shock protein.
(*) = translocates to the cell nucleus after heat shock.
ppi = peptidyl-prolyl isomerase.
grp** = ** kDa glucose regulated protein.

teins are released from the nucleus and participate in a nucleo-cytoplasmic shuttle [402]. Thus shuttling proteins can be regarded as potential molecular chaperones and indeed, many of these proteins assist the assembly of various RNA/DNA-protein complexes (Table 2).

Noteworthy that several steroid receptors have been also established as shuttle-proteins [173,269]. In vitro studies indicate, that one action of steroid receptors may be to facilitate assembly of functional initiation complexes at hormone-regulated promoters [234,372]. Receptor action is also accompanied by alterations in local chromatin-structure [234,260,351,355,372,503]. These findings imply that steroid receptors function as nuclear chaperones besides their role as transcription factors. The interactions of steroid receptors with other transcription factors, such as AP1 [105,494], CREB [205] and others [407,432,477] may indicate a broad binding-specificity - an other hallmark of chaperone function.

Besides the above-mentioned established or proposed nuclear chaperones there are numerous 'classical' chaperones, which reside in the cell nucleus (such as the 110 kDa heat shock protein), or translocate to the nucleus after cellular stress. The intranuclear function of these latter proteins is far from being elucidated (Table 2).

We recognize, that loosening a definition is a slippery path which may lead to the loss of its original meaning. However, besides the fact that they fit the definition, the proposed 'nuclear' molecular chaperones (such as nucleoplasmin, nucleolin and numatrin) show many similarities with 'classical' chaperones, such as the 70 and 90 kDa heat shock proteins: 1. The mRNA level of nucleolin and the 70 kDa heat shock protein (hsp70) changes parallely in cell cycle, during liver regeneration and cell differentiation [266,320,337]. A parallel increase in the synthesis of numatrin and hsp70 can be observed at the activation of B and T lymphocytes, and after stimulation with growth factors [128–130,444]. 2. Nucleolin (amino acids: 141–167, 188–213 [251]), numatrin (amino acids: 120–132 [61,401]) and the 90 kDa heat shock protein (hsp90) (amino acids: 229–255, 257–274 [314]) all contain a

Table 3
Molecular chaperones in the initiation of eucaryotic gene transcription and replication

Name of chaperone	Interacting protein/DNA sequence	References
A. DNA conformation in transcription		
HMG1 (DNA-bending)		[449]
TFIIF helicase		[427]
TFIIIA gyrase		[238]
B. Nucleosome restructuring or positioning		
nucleoplasmin	GAL4-AH, USF, Sp1	[64]
GAGA/heat shock factor	heat shock element	[460]
HMG14/HMG17	5S rRNA	[451]
C. Promotion of transcription factor binding		
HMG I(Y)	NF-kappa-B	[443]
HMG I(Y)	ATF-2	[115]
numatrin	YY1	[210]
numatrin	p50	[62]
hsp90	E12/MyoD	[411,417]
hsp90	estrogen receptor	[209]
SWI2 helicase	glucocorticoid receptor	[499]
SWI/SNF complex	GAL4, TATA-binding protein	[80,207,245,352]
thioredoxin	NF-kappa-B	[183]
redox factor Ref-1	c-fos/c-jun (AP1)	[487,488]
TFIIF	serum response factor	[509]
homeodomain proteins	serum response factor	[169]
D. Prevention of transcription factor binding		
calreticulin	steroid receptors	[99,323]
E. Promotion of replication		
numatrin	DNA polymerase alpha	[440]
hsp60	DNA polymerase epsilon	[423]

hsp = heat shock protein.

nucleoplasmin-like, long, Glu/Asp-rich amino acid stretch [119], which harbors some of the major phosphorylation sites of the proteins [51,61,255], participates in binding of nuclear localization signal [160,490], reorganization of chromatin structure, and generally, in chaperone function [92,121,203,304,421,476]. 3. Nucleolin and numatrin possess nucleotide binding sites, showing structural and functional similarities with the ATP-binding sites of hsp70 and hsp90 [45,89,90,131,322,341,469]. 4. Except nucleolin, the oligomerization of all the other proteins has been reported [250,252,500].

Besides the general help of nuclear chaperones in the assembly of various RNA/DNA-protein complexes, they can also assist in the association of specific transcription factors with their response elements by bending the respective or distal regions of DNA, by restructuring/positioning the interfering nucleosomes or by direct promotion of the binding process. Nuclear chaperones also participate in the regulation of DNA replication (Table 3). In the next section we will summarize the effect of various cytoplasmic and nuclear molecular chaperones on nucleocytoplasmic transport processes.

Some questions of further research: What is the function of 'classical' molecular chaperones, such as hsp27, hsp70, hsp110 in the cell nucleus? What is the functional significance of the extensive analogy between nuclear and 'classical' molecular chaperones? How generally does eukaryotic transcription need the help of molecular chaperones?

4.2. Molecular chaperones in nuclear transport

4.2.1. Protein transport

After the 1988 discovery, that the 70 kDa heat shock protein (hsp70) is involved in protein transport to the endoplasmic reticulum and to mitochondria [69,100] it was quite plausible to hypothesize, that it must also play a role in nuclear protein transport. The first evidence to prove this hypothesis came when Jeoung et al. [217] described the help of hsp70 in translocation of a cytoplasmic SV40 mutant to the nucleus. Later experiments showed, that hsp70 is actually required for nuclear transport being one of the many cytoplasmic factors necessary to the process (see Section 3.2, [206,414]). The eukaryotic dnaJ homologue, which helps the ATPase activity of hsp70 may also participate in the protein transport [31,391]. It is expected, that the other co-chaperone of hsp70, the nucleotide-exchanger eukaryotic grpE-homologue, is also part of the protein-priming chaperone complex.

hsp70 binds to the nuclear localization signal (NLS) sequence [206] and may prime it for binding to the NLS-receptor or may participate in the disassembly of the nuclear translocation complex following its entry to the cell nucleus. Involvement of hsp70 in nuclear protein transport does not seem to be universal, since in contrast to the SV40 large T antigen and nucleoplasmin, the nuclear transport of glucocorticoid receptors does not require the heat shock protein [493]. It is less clear at present, what is the significance of the NLS-binding of other chaperones, such as nucleolin, numatrin, nopp140 and the 70 kDa nucleolar peptidyl-prolyl-isomerase (see Table 2).

The recent observation of Saitoh and Dasso [394] identifying hsp70 as a part of the Ran/RanBP1/RanBP2/RCC1 complex indicate that hsp70 may also participate in later phases of protein (and probably RNA) translocation across the nuclear pore complex.

Some questions of further research: What is the exact function of hsp70 and other NLS-binding proteins in nuclear protein transport? How do proteins find their intranuclear place after translocation through the nuclear pore?

4.2.2. RNA transport

RNA transport is characterized as a 'solid-phase' process (see Section 3.3) meaning that the transported RNA molecule is bound to intranuclear or cytoplasmic structures along all its way. Therefore the transport process can not be restricted merely to the translocation but can only be envisaged as a complete set of individual steps from synthesis of RNA till its final utilization. hnRNA assembly requires the help of A1 protein, which may also function as a general RNA chaperone. Several heat shock proteins have also been reported to associate with hnRNA probably participating in restructuring of unspliced RNA after heat stress [237]. Translocation of mature mRNA through the nuclear pore is mediated by a nuclear envelope-associated RNA helicase [406].

Ribosomal RNA is transported as a bulky protein-complex, whose assembly and restructuring needs an almost continuous help of various chaperones, such as nucleolin, numatrin, fibrillarin, the L3 protein, nopp140, the DRS1 RNA-helicase and the 70 kDa nucleolar peptidyl-prolyl-isomerase. The details of the process and the 'order' and cooperation of various chaperones have not been addressed so far. Assembly of U1 nRNA is mediated by the U1A protein (for references see Table 2).

Some questions of further research: How do the various chaperones cooperate in the transport of different RNA classes? How is the cooperativity between 'protein' and 'RNA' chaperones achieved?

5. Regulation of nuclear transport after various stimuli

5.1. Hormones, growth factors, cytokines and other external agents

In this section we will discuss the general mechanism of the regulation of nuclear transport processes taking the different extracellular agonists as examples for certain

Table 4
Specific regulation of nuclear protein transport

Regulation mechanism	Effector molecule	References
A. Activation		
more NLS in one protein		
serum albumin-gold (model)		[118]
glucocorticoid receptor		[269]
phosphorylation		
SV40 large T	casein kinase II	[376]
rNFIL-6	protein kinase A	[297]
STAT family	Janus tyrosine kinases	[97,227,390]
borrowed NLS ('piggyback')		
adenovirus DNA polymerase	preterminal protein	[507]
cdc2 kinase	cyclin	[34]
de-masking of NLS		
NF-kappa-B/rel, phosphorylation of masking I-kappa-B	protein kinase A/C, eIF2-kinase	[151,416]
NF-kappa-B/rel, dephosphorylation of masking I-kappa-B	calcineurin	[141]
NF-kappa-B/rel precurzor C-terminus proteolysis	proteasome	[176,189,346]
B. Inhibition		
phosphorylation		
xnf7, SWI5	cdc2 kinase	[300,307]
HMG1	protein kinase C	[482]
masking of NLS		
steroid receptor	hsp90	[354]
aryl hydrocarbon receptor	hsp90	[339]
c-fos	?	[388]
protein kinase A	regulatory subunit	[332]
NF-kappa-B/rel	I-kappa-B, NF-kappa-B precurzor, its cleaved C-terminus, bcl-3	[180,258]

types of regulation. In later sections we will describe changes in nuclear transport processes in the cell cycle and after cellular stress.

5.1.1. Regulation of ion transport

Relatively few studies exist on the molecular mechanisms regulating nuclear ion transport. Nuclear protein kinase C seems to play an important role in the regulation of nuclear calcium metabolism. Hepatic nuclear inositol-trisphosphate receptor is phosphorylated by protein kinase C which leads to an increased Ca^{2+} release [282]. On the contrary, in smooth muscle cells activation of protein kinase C does not affect resting Ca^{2+} levels, while inhibits ATP-induced nuclear Ca^{2+} release. The effect of protein kinase C in this latter case can be reversed by nuclear protein phosphatase 1 or 2A [195]. These studies indicate that the regulation of nuclear calcium metabolism may show both intranuclear compartmentalization and cell-type dependent variations. Activation of phospholipase C-β [60,110,275], the calcium binding protein, regucalcin [491] and nicotinamide adenine dinucleotides [338] have been also shown to influence nuclear Ca^{2+} uptake and release. Examining nuclear K^+-fluxes, the patch-clamp studies of Bustamante [47] indicated the involvement of intranuclear cAMP-dependent protein kinase in activation of the process, while phorbol ester treatment induced the translocation of zinc from the nucleus to the cytoplasm [87,88].

Some questions of further research: What is the regulatory role of protein kinase C in nuclear calcium-metabolism? Is there any intranuclear compartmentalization in the regulation of nuclear ion-fluxes?

5.1.2. Regulation of protein transport

Regulation of nuclear protein transport can be achieved at two levels: the general regulation -involving the assembly and modulation of nuclear pores- influences the transport-rate of all proteins (and RNA-s), while the specific regulation -dealing mostly with the recognition of nuclear localization signals- determines the nucleocytoplasmic distribution of individual proteins.

Insulin, EGF, IGF-1, PDGF, testosterone and aldosterone are known to *generally* stimulate intranuclear protein transport [56,126,218,335]. The active diameter of nuclear pores may also increase from 10 nm to 23 nm upon stimulation [126,138]. The exact mechanism of agonist-induced assembly of new nuclear pores and/or stimulation of translocation through existing pores has not been cleared yet. Recent observations of Greber and Gerace [167] indicate that the depletion of calcium from the lumen of endoplasmic reticulum reversibly inhibits passive diffusion and active protein transport into the nucleus. Protein phosphorylation seems to play a general role in regulating intranuclear protein transport as well [303].

Specific regulation of protein transport plays a key role

in signal transduction, since most transcription factors have a signal-dependent access to the cell nucleus [97,204,227]. Some possible mechanisms of specific regulation are summarized in Table 4. Regulation can be achieved by post-translational modification (phosphorylation, dephosphorylation, limited proteolysis) and by formation or dissociation of protein-complexes. The nuclear localization signal can be amplified or attenuated, may be borrowed or masked (for examples see Table 4).

In case of the presence of multiple localization signals the co-translational signal, — e.g. hydrophobic membrane spanning region — 'wins' over the posttranslational nuclear localization signal. The posttranslational mitochondrial localization signal scores neck and neck: the signal closer to the N-terminus of the protein will dominate, or the protein will migrate to both cellular organelle [144,177].

Some questions of further research: What is the mechanism of signal dependent nuclear pore assembly in interphase? How are the translocation rate and pore diameter regulated? How general is the cytoplasmic docking of various transcription factors?

5.1.3. Regulation of RNA transport

Among the various processes of the field, the regulation of mRNA transport is the best studied. As it is mentioned in Section 3.3 unfolding of mature mRNA-protein particle is helped by topoisomerase II. The activity of the enzyme can be stimulated by both protein kinase C and casein kinase II [55,393,404]. The major regulatory element of mRNA transport is the complex of a 40 kDa nucleotide-triphosphatase (NTPase) and a 110 kDa poly-A-binding protein (p110). The NTPase is activated by phosphatydilinositol-phosphates [425] and inhibited by protein kinase C-induced phosphorylation [405]. p110 is inhibited by a not identified nuclear protein kinase and activated by a corresponding phosphatase [404]. The kinase and phosphatase are stimulated by EGF or insulin, respectively, causing an opposite effect on the overall transport-rate of mRNA [406].

Phosphorylation of several proteins, such as nopp140 and nucleolin seems to activate rRNA transport [91,291], while for the retrograde (cytoplasm → nucleus) transport of U nRNA-s the cooperation of protein and RNA nuclear localization signals is required [144].

Some questions of further research: How are the intranuclear elements of mRNA, rRNA and tRNA transport regulated? What is the mechanism of the NTPase / p110 complex-mediated regulation of mRNA transport?

5.1.4. Protein kinases and phosphoprotein phosphatases

The previous sections gave numerous examples of the involvement of nuclear protein kinases and phosphatases in the regulation of transport processes. The key importance of these enzymes in regulation of membrane-related nuclear events and their partial association with nuclear membranes prompted us to include them briefly in the present review. A -rather incomplete- list of nuclear protein kinases and phosphatases is given in Table 5. Many of these enzymes, such as DNA-dependent protein kinase are residing in the cell nucleus, while others, e.g. MAP kinase, protein kinase A and some isoforms of protein kinase C, are translocated to the nucleus after various stimuli. There is a recurring debate on the nuclear localization of receptor tyrosine kinases, such as the EGF, or insulin receptor tyrosine kinase [162,197,232,350,358], however, the accumulating evidences encouraged us to denote them as — partially — nuclear kinases. Participation of a given kinase or phosphatase in Table 5 does not mean at all, that it is exclusively nuclear. In fact, many of them associate the nucleus only transiently, others are predominantly cytoplasmic and only a portion (subpopulation) is found in the nucleus. Relatively few data are available on the correct subnuclear localization of the enzymes. We have noted, where a possible association with the nuclear double mem-

Table 5
Nuclear protein kinases and phosphatases

Kinase or phosphatase	References
A. Nuclear Ser / Thr kinases	
calmodulin-dependent protein kinase	[392]
cdc2 kinase	[17,34,374]
casein kinase I	[454]
casein kinase II (*)	[3,133,241,262]
DNA-dependent protein kinase	[59,212,256]
myosin light chain kinase (*)	[419]
protein kinase A (*)	[332]
protein kinase C (*,m)	[164,340]
raf (*,m)	[261]
42/44 kDa MAP kinases (ERKs) (*)	[66,163,396,450]
pp90rsk S6 kinase (*)	[66]
Ndr kinase	[302]
B. Nuclear tyrosine kinases	
insulin receptor (*,d)	[18,232]
EGF receptor (*)	[197,220]
FGF receptor (*)	[364]
p185neu	[489]
c-src (*)	[508]
rak (src-homologue)	[53]
fgr	[461]
c-abl	[455]
wee1	[185]
p94fer, p51ferT (*,m)	[178,184]
C. Nuclear Ser / Thr-phosphatases	
protein phosphatase 1	[213]
protein phosphatase 2A	[244]
calcineurin (PP2B)	[36]
PP4	[41]
PP5	[65]
D. Nuclear phosphotyrosine-phosphatases	
cdc25 (d)	[299]
PAC-1 (d)	[385]
T cell PTPase	[125,368]
dPTP61F	[289]
PEST-enriched phosphatase	[136]

(*) = translocates to the nucleus after stimulus.
(m) = known to be associated with the nuclear envelope.
(d) = protein kinase/phosphatase with double (Ser-Thr/Tyr) specificity.

brane (envelope) is suspected. Again, this does not mean that all the enzyme is membrane-bound, and the absence of the sign shows mostly the unavailability of the corresponding data, than the evidence of exclusive intranuclear localization. Besides those in the list, several other nuclear protein kinases and phosphatases have been identified, such as the nuclear lamina-associated protein kinase or lamin B-receptor kinase [102,420], whose identity is not known or not certain. Though the intracellular localization of members of the 'traditional' MAP-kinase cascade has been mostly established, very few data are available on the possible nuclear translocation of protein kinases belonging to the recently discovered Janus kinases or stress-kinase cascade [227,246].

The mechanism of both the nuclear translocation and the retention in the nucleus is seldom determined. Protein kinase C may bind to its nuclear receptors (reviewed in [44]) and/or may associate with nuclear membrane structures having an increased diacylglycerol content. Protein kinase C isoenzymes may use a cryptic nuclear localization signal to promote their translocation [340]. The kinase activity of MAP kinase is not required for its translocation to the nucleus, in contrast to its serum-induced transport to the plasma membrane which is not observed with a kinase-deficient mutant [163]. Heat stable inhibitors of protein kinase A induce the release (export) of its catalytic subunit from the nucleus [471].

Nuclear kinases and phosphatases are in an intensive cross-talk, displaying an elaborate network of cross-regulation. The interactions of PAC1 tyrosine phosphatase with MAP kinase [463], or protein kinase A and casein kinase II with protein phosphatase 1 [456] are some of the first experimental evidences for these intranuclear signalling processes. For other useful information the reader is referred to recent reviews on the subject [208,461].

Some questions of further research: What is the mechanism of nuclear targeting of protein kinases and phosphatases? What types of interactions occur between them? How are they associated with nuclear membranes or lipid-structures? What is the subnuclear compartmentalization of the various enzymes and how does this influence the phosphorylation-related intranuclear signalling?

5.2. Cell cycle

Nuclear transport undergoes a general activation or inactivation during various phases of the cell cycle. Quiescent, confluent cells display seven times slower nuclear protein transport, than their dividing counterparts and the pore number is known to approximately double during S phase [126,138]. Nuclear lipids are significantly reduced in S phase [273], the decrease is especially pronounced in phosphatidylinositol (PI) levels, which further supports the involvement of PI-s in DNA synthesis [497]. The mechanism of interphase nuclear pore assembly/disassembly and PI fluctuation remains to be elucidated.

Nuclear transport plays an important role in the regulation of the cell cycle. A- and B-type cyclins translocate to the nucleus in S and M phases of the cell cycle, respectively [356]. The intranuclear cyclin dependent kinase(cdk)/cyclin complex phosphorylates all types of proteins helping the cell to replicate its DNA and preparing it to enter mitosis. Degradation of mitotic cyclins is mediated by a ubiquitin-dependent pathway. The ubiquitin-activating enzyme, E1 translocates to the cell nucleus in G2 phase [168]. As another example of cell cycle-dependent protein translocation to the nucleus, protein phosphatase 1 (PP1) becomes nuclear in G2 phase allowing the cell to prepare for the exit from mitosis [208]. Nuclear translocation of PP1 parallels a decrease of the nuclear transport/retention of its inhibitor, PI-2 [39]. The elucidation of cell cycle regulation by changes in the nuclear transport of other cyclins, cyclin-dependent kinases, their activators and inhibitors awaits further investigation. Recent observations of Wetzler et al. [474] indicate that another serine/threonine kinase, the BCR protein product also shows cell-cycle dependent changes in its subcellular localization.

Blow and Laskey [30] hypothesized that a licensing factor is necessary for the beginning of DNA replication, which does not have an access to the cell nucleus during interphase but associates with the chromosomes at mitosis and 'licences' the cell for one round of DNA-replication. Recent findings gave some initial evidences to validate this model by identifying Cdc46, Cdc47 and Mcm3 proteins as a family which enters the nucleus at the end of mitosis, necessary for DNA replication and leaves the nucleus after entry into *S*-phase [29,82,93].

One of the most striking events of the cell cycle in higher eukaryotes (except some insects and yeast) is that in mitotic cells, where the nucleus disassembles and after mitosis re-forms again. At the initiation of mitosis phosphorylation of several proteins (those of the nuclear pore complex, lamin B, its receptor and of numerous other proteins of the nuclear membrane) by the p34cdc2 kinase/cyclin complex and by at least one more heretofore unidentified protein kinase triggers the stepwise disassembly of the nuclear pores and lamina [83,137,138,301]. Demethylation of lamin B may also participate in the process [147]. Nuclear pore complexes may disassemble first, followed by the disassembly of the nuclear lamina and by vesicularization of the nuclear membrane. During disassembly some areas of the nuclear membrane become free of nuclear pore complexes before others [159]. It is likely, that vesicles are constantly budding from, and fusing with the nuclear membrane during interphase [187]. At mitosis vesicle budding may be accelerated via increased coat-recruiting of shedding vesicles of the nuclear membrane [32].

Most of our knowledge about nuclear reassembly is gathered by using the in vitro system of *Xenopus oocytes* [8,301]. Chromosomes (and possibly parts of the chromo-

some periphery [192]) act as templates for the assembly. At the beginning of the process immature nuclear pores may form around, and within the chromatin mass [413]. Subsequently, membrane vesicles bind to chromatin sites spaced 100 kb apart, and fuse via GTP- and Ca-sensitive steps [327,435,436]. At this intermediate stage, the resulting single membrane surrounds the individual chromosomes and it is covered by the outer membrane only at the nuclear periphery [413]. Next the association of the lamin B receptor/lamin B complex occurs, and the slow polymerization of the nuclear lamina completes the process, which may last even in G1 phase [290]. During this later phase the inner nuclear membrane withdraws to the nuclear periphery, and the pre-pores mature to fully competent nuclear pore complexes [413] by acquiring their cytoplasmic peripheral structures [50]. Assembly is accompanied by dephosphorylation of lamins and several other nuclear proteins [8]. After completion of the mitotic process, the original integrity of the cell nucleus and the cytoplasm is reestablished again, since cytoplasmic large fluorescent dextranes are excluded from newly formed interphase nuclei [438].

Some questions of further research: How are the various subpopulations of nuclear vesicles established in early mitosis? How and to what extent does the single inner nuclear membrane withdraw from the inside of the forming nucleus? How is the original integrity of the cell nucleus and the cytoplasm preserved?

5.3. Cellular stress

In this section we will give a survey of changes in nuclear transport and lipid metabolism after various forms of cellular stress, such as heat shock, osmotic shock, oxidative shock, UV illumination etc.

After *heat-shock* more than 50 newly synthesized proteins appear in the cell nucleus [253,373]. We have summarized some of their major constituents in Table 2 of Section 4.1. In spite of the extensive list, which includes almost all major members of heat-shock proteins (molecular chaperones), we know surprisingly little on the function of heat shock proteins in the protection of cell nucleus after heat stress. As one of the exceptions Ciavarra et al. [72] proposed a role of the 70 kDa heat shock protein (hsp70) in the protection of topoisomerase I. Heat shock induces profound changes in nuclear morphology, lipid metabolism and protein phosphorylation. Cleavage of polar-head lipids increases, concomitantly with nuclear diacylglycerol level and a subsequent translocation and activation of protein kinase C [379]. MAP kinase is activated by heat-shock [116]. Though the nuclear translocation of this kinase accompanies its activation by other stimuli [66,396], a direct evidence of the nuclear localization of heat-shock-activated MAP kinase is missing.

Lowering the temperature induces the synthesis of a distinct set of proteins, called *cold-shock* proteins, which are different from those induced by heat-shock. Initial studies of the phenomenon were mostly done in *E. coli* so cold-shock-induced changes in nuclear transport have not been investigated in detail. One of the yeast cold-shock proteins is the nucleolin homologue NSR1, which plays an important role in rRNA biogenesis. This suggests, that cold-shock may influence both RNA transport and nucleocytoplasmic shuttling [222].

Little is known on the consequences of other environmental stresses on nuclear transport and metabolism. NF-kappa-B is known to translocate to the cell nucleus after oxidative stress [403]. In hypoxia, a specific hypoxia-inducible transcription factor (HIF-1), fos, jun and other proto-oncogenes translocate to the cell nucleus [409,466]. UV illumination causes the translocation of NF-kappa-B to the nucleus and the activation of several protein kinases, including c-src, raf, and a family of stress-activated c-jun kinases [103,104,246]. UV-induced nuclear translocation of these cytoplasmic kinases has not been addressed yet. Psychosocial (emotional or overcrowd) stress is known to elevate blood cortisol [309] and nerve growth factor levels [10]. These agents are known to induce profound changes in nuclear transport processes.

Some questions of further research: How do nuclear heat shock proteins protect the cell nucleus from the consequences of heat-stress? What other protein kinases and phosphatases are translocated to or activated in the cell nucleus besides protein kinase C after heat-shock? What are the consequences of various cellular stresses on nuclear transport mechanisms?

6. Nuclear transport and membrane changes in disease

6.1. Cancer

Cancer can be regarded as a set of deleterious changes of the signalling system that controls cell proliferation and differentiation. Transformed cells may acquire *gain-of-function mutations*, such as the example of those protein kinases/transcription factors, which have escaped from their normal control, being constitutively active. Transformed cells may also possess *loss-of-function mutations*, where an otherwise functioning inhibitor has been switched off. In some cases, the proper activity appears at a wrong place, or can not reach its regular location. As a profound example of these latter changes, nuclear transport of proteins may be promoted or hindered in cancerous cells. In the following we will give some examples of these malignant changes.

p53 induces the transcription of numerous genes, among others p21, an inhibitor of cyclin-dependent kinase-(cdk)-2/cyclin E and /cyclin A complexes, which cause cellular arrest in G_1 or apoptosis. Mutations of p53 are widespread in many types of cancer [179]. In breast cancer, however, another defect may also occur: wild type p53 is excluded

from the cell nucleus, thus it can not induce the inhibitors preventing abnormal cell growth [308]. The c-abl nuclear tyrosine kinase, which negatively effects cell growth, is excluded from the nucleus in its transforming p160gag/v-abl form [455]. c-abl is also redirected by its fusion with the BCR product in Philadelphia leukemias [474].

In contrast, v-fos evades the translocation control of c-fos, which is sequestered by a cytoplasmic inhibitor in the absence of serum stimulation and becomes completely nuclear [388]. A similar change may occur at the NF-kappa-B homologues, v-rel [154,176], or at v-cbl [27], where a C-terminal truncation allows the proteins to enter the nucleus constitutively possibly contributing to their oncogenic potential. Gene fusions may also direct proteins unwantedly to the cell nucleus as it is the case with alk tyrosine kinase/numatrin fusion product which sequesters the cytoplasmic kinase to the nucleolus and participates in the induction of many non-Hodgkin's lymphomas [318] or with Tpr, a possible component of the cytoplasmic fibrils of the nuclear pore complex which re-directs several protooncogens, such as the trk, met and raf via gene fusion [50]. Timing is also important: c-jun has a constant translocation rate to the cell nucleus throughout the whole cell cycle, while a single amino acid mutation in the nuclear localization signal of v-jun renders the translocation of the latter protein cell cycle dependent [68].

Since the molecular approach of carcinogenesis-related research concentrated mostly on the identification of the key players and on their interactions, the investigation of cancer-related changes in their nuclear transport is lagging behind. Further research will probably yield numerous examples, where the incorrect intracellular localization of proteins contributes to their oncogenic activity.

Several types of cancer can be induced or promoted by certain DNA viruses, such as the SV40, human papilloma virus, adenovirus, etc. The involvement of nuclear transport mechanisms in viral infections will be summarized in the next section.

Some questions of further research: Is there any change in the nuclear translocation of oncogenic protein kinases, such as v-raf? What are the other examples of cancer-related changes in nuclear transport of transcription factors? How is the nuclear translocation of various cdk-s, cyclins and their complexes affected in cancer?

6.2. Viral, retroviral infection, AIDS

Most DNA viruses, class V viruses and retroviruses enter the cell nucleus for the replication of their genetic material. After a partial assembly subviral particles leave the nucleus interfering with the export of cellular RNA-s. In the following section we will describe some examples of both phases of viral-nuclear interactions.

Virus particles *enter* the nucleus *via* the nuclear pore complexes. This process is usually aided by the nuclear localization signal of one or several of their capsid proteins [138]. Before or during transport several proteins may dissociate from the virus, such as the M1 matrix protein of influenza virus [277]. From the pore complex the nef protein of the HIV type 1 virus is transported to the nucleoplasm along distinct, curvilinear tracks [321]. In case of the rev and tat proteins of the HIV virus and the rex protein of HTLV-1 virus, the nucleolar localization is achieved by an R-Q-R-like subsignal adjacent or overlapping with the nuclear localization signal of these proteins [144].

The *export* of HIV-1 mRNA from the nucleus is promoted by the virally encoded rev protein, which interacts directly with the translocation apparatus competing with the export of host mRNA-s and specifically promoting the export of viral RNA [353]. Influenza viral RNA-s exit the nucleus via the bound viral matrix protein M1 [276]. However, RNA export is specific, since the template influenza RNA-s, which are synthesized early after infection, remain in the nucleus to direct virion RNA synthesis [412]. Adenovirus mRNA-export is helped by the heterodimer of the E1B and E4 proteins [242].

Essential products of the viral genom have to be transported back to the cell nucleus. Nuclear transport of adenovirus DNA polymerase is facilitated by the 80 kDa adenovirus preterminal protein, which may promote their concerted arrival to the cell nucleus [507]. The 'HIV-exporter' rev may utilize the shuttling numatrin for its retrotransport back to the nucleus [124].

Viral proteins and particles interfere with the life of the host cells in many ways. Influenza virus NS1 protein inhibits pre-mRNA splicing and blocks mRNA export of the host cell [139]. Viral protein export may hinder the nuclear export of host-proteins. However, as a notable exception, the HIV protease cleaves the 105 kDa NF-kappa-B precursor, which activates the transcription factor by promoting its translocation to the cell nucleus [380]. The human T-cell leukemia virus type 1 encoded protein, Tax also induces the nuclear translocation of NF-kappa-B [319].

Anti-viral activity of cellular proteins may also depend on their intracellular localization. Members of the Mx GTPase family interfering with various steps of viral transport processes exhibit anti-viral activities against different viruses depending on their cytoplasmic or nuclear localization [219].

In contrast to influenza and other viruses which bud from the plasma membrane of the host, nucleocapsids of herpes virus and Newcastle disease virus acquire their lipids from the inner leaflet of the nuclear envelope. In these latter cases, viral assembly occurs in the nucleoplasm and the enveloped viral particle free in the perinuclear space [95,274]. Glycoproteins of Sindbis and Vesicular Somatitis viruses may also be detected in the *inner* nuclear membrane [24,448], which probably reflects their diffusion via the pore membrane region of the nuclear envelope.

Some questions of further research: How does the pore complex accommodate the 'giant' viral particles? Does 'viral-gene-gating' operate? How big is the asymmetry in viral transcription and export around the cell nucleus? What determines if a virus buds from the plasma membrane or from the nuclear envelope?

6.3. Autoimmune diseases, diabetes

Most of nuclear proteins are confined inside the cell, and never meet the immune system under normal conditions. However, a subpopulation of the 'traditional' nuclear proteins, such as nucleolin [223,235,408] and the pre-mRNA-binding hnRNP U protein [223] appears on the surface of various cell types. In case of cell-damage or abnormal protein targeting, other nuclear proteins may appear in the cell surface. These, usually 'unavailable' nuclear proteins, or the malfunctioning of the inhibitory 'circuits' of the immunological homunculus [74] may induce nuclear autoantibodies, and an autoimmune disease may develop. Numerous autoantibodies have been described against nuclear proteins, such as lamin A [288], lamin B [71,172], fibrillarin [336], a 180 kDa nucleoporin [479] and many other unidentified nucleoplasmic or nucleolar proteins [265,492].

Type-I (insulin-dependent) diabetes is an autoimmune disease where the self-destructing immune response is directed towards the glutamate decarboxylase of insulin-producing pancreatic β cells [14]. Type-II (non-insulin dependent) diabetes occurs, when elements of the insulin-related intracellular signalling are not functioning properly. Insulin deficiency and high blood glucose levels change many aspects of cellular metabolism. However, almost no studies investigated changes in nuclear lipid metabolism or transport in diabetes.

Some questions of further research: What is the role of nuclear proteins on the cell surface? How do they get there? How do nuclear lipid metabolism and transport change in diabetes?

6.4. Aging, neurodegeneration, Alzheimer disease

Aging impairs the transcriptional activity of several transcription factors, such as the c-jun/c-fos complexes (AP-1) and Sp1 [11,475]. In other cases a transcriptional repressor, such as the p50/p50 dimer of NF-kappa-B of androgen receptor expression may be amplified during the aging process [437]. These changes may be mediated by various mechanisms involving protein kinases, phosphatases, chaperone-like activators and changes in nuclear transport. Indeed, Malviya and co-workers reported a significant decrease in nuclear protein kinase C activation in senescent rats [384]. Besides the above possibilities age-induced oxidation of the normally reductive nuclear environment [22] may also contribute to transcriptional inactivation [442].

Few studies were done to elucidate nuclear changes in *Alzheimer disease*, which can be considered a pathological form of aging. Aluminium, which may have a role in the development of the disease, accumulates in neuronal nuclei of Alzheimer patients [268]. On the other hand, nuclear creatinine phosphokinase content is reported to decrease in affected neurons [5]. As a pathological hallmark of the disease, amyloid fibrils accumulate many times near and on the nuclear membrane [278]. The reason and consequences of this juxtanuclear localization are not known.

Some questions of further research: What are the mechanisms inducing age-dependent attenuation of transcriptional activity? How do nuclear transport and lipid metabolism change in Alzheimer disease?

7. Conclusions and perspectives

All research evokes more questions than it answers. We have listed some of the nuclear transport-related new questions at the end of each previous section. Now, as a summary, first we would like to emphasize the far-reaching consequences of the gene-gating hypothesis of Blobel [28]. The cell nucleus is highly organized and this structural complexity allows and mandates an 'organized tour' of maturing mRNA instead of its free diffusion in the nucleus. As a -simplified- consequence, each nuclear pore may have its 'fingerprint', releasing only a certain set of gene-transcripts. This involves and generates a tremendous asymmetry both inside the cell nucleus and in the cytoplasm and predicts the existence of highly organized membrane domains both in the nuclear envelope and in the surrounding endoplasmic reticulum. With the advent of new techniques, such as confocal microscopy, high resolution electron microscopy and atomic force microscopy [157,159,335] our hopes are more and more justified to gain exciting and relevant informations on this complexity.

The higher the organization, the more its rigidity. The high complexity of the nucleus may hinder its efficient functioning. A type of 'Parkinson's Law' may be valid to the cell nucleus as well. However, the nucleus is in a constant change which needs high flexibility and continuous restructuring. Nuclear and -traditionally- cytoplasmic molecular chaperones play a so far rather poorly studied, but essential role in maintaining the adaptation and required speed of nuclear processes.

In spite of the large (but 'plugged') nuclear pores, the myriads of transport processes, the direct connection of the nuclear envelope with the endoplasmic reticulum, the cell nucleus is rather isolated from the cytoplasm. The nuclear environment has a specific response to most stimuli. Investigation of nuclear signalling, the specialties of nuclear phosphatidylinositol cycle, the nuclear zinc metabolism, protein phosphorylation and intranuclear signals will clearly result great surprises in the near future.

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