Minireview

Protein import into the nucleus

Gabriel Schlenstedt*

Medizinische Biochemie, Universität des Saarlandes, D-66421 Homburg, Germany

Received 17 May 1996

Abstract The transport of proteins from the cytoplasm into the nucleus is a multistep process. The nuclear localization sequence (NLS) of a transport substrate associates with the heterodimeric NLS-receptor which binds to a subset of proteins of the nuclear pore complex (NPC). Translocation through the NPC is energy-dependent and requires the small GTPase Ran. Proteins that interact with Ran in either the GDP-bound or the GTP-bound state coordinate transfer through the NPC. Lastly, the NLS-receptor/substrate complex and Ran reach the nuclear side of the NPC where the complex disassembles.

Key words: Nuclear transport; Nuclear localization sequence; NLS-receptor; Ran; Nuclear pore complex

1. Introduction

The nuclear pore complex (NPC) is the site of all transport of macromolecules between the cytoplasm and the nucleoplasm. This review will focus on new findings on protein import into the nucleus. Export of proteins and import/export of RNA have been reviewed recently [1–4]. Molecules of up to ~50 kDa may pass the NPC by diffusion. However, even small proteins require nuclear localization sequences (NLS) for efficient targeting [5]. A typical NLS contains two clusters of basic amino acids separated by about 10 residues (e.g. the bipartite NLS of nucleoplasmin) or only one short basic stretch of amino acids (e.g. the SV40 large T antigen NLS) [6]. Active uptake of proteins into the nucleus is a rapid, specific, and evolutionarily conserved process. Experimentally, two steps can be distinguished: NLS-dependent but energy-independent binding of substrate to the cytoplasmic side of the NPC, followed by its energy-dependent transport through the NPC [7,8].

The development of an in vitro system that mimics the properties of import in vivo has allowed the characterization of a number of proteins required for transport. Solubilization of the plasma membrane of mammalian cells by digitonin-treatment releases soluble proteins from the cytoplasm. However, the nuclear envelope (NE) and other organellar membranes remain intact. Addition of a cytosol preparation is required for nuclear uptake of a fluorescent import substrate [9]. Subsequent subfractionation of cytosol has identified two separable activities; one fraction stimulates binding at the NPC, while the second fraction is required for energy-dependent translocation [10]. Four proteins have been identified which can substitute for the cytosol requirement: the two subunits of the NLS-receptor (binding activity), the small GTPase Ran, and p10 (transport activity). Alternative biochemical and genetic approaches resulted in the identification of these and additional important proteins. The relevant factors will be discussed in the following sections.

2. NLS-receptor (NR)

The NLS-receptor (NR) consists of two subunits, NRα and NRβ. The NR is also termed importin, karyopherin, and pore-targeting complex [11–13]. The 54/56 kDa 'NLS-receptor' and p97 described by Adam and coworkers represent the bovine NR [14–16]. The molecular weights in different species are 54–60 kDa for NRα and 90–97 kDa for NRβ. NRα was purified from *Xenopus* egg extracts as an essential protein for NLS-dependent binding of a fluorescent transport substrate to the NE and subsequent import into HeLa cell nuclei [11]. NRα is found in a complex with NRβ [17,12,13,18,19]. Both subunits are required for binding and transport [17,20]. NRα from yeast, also termed Srp1p or Kap60p [18,21], is most likely identical to the previously characterized NLS-binding protein NBP70 [22,23]. While there is only one yeast gene encoding NRα, higher eukaryotes contain a group of similar genes, which suggests differences in substrate specificity [11,20,24–28].

NRα binds directly to nuclear localization sequences [14,22,17,20,26,29,30]. NRβ was found to have weak [17] or no NLS-binding activity by itself [31,32] but it cooperates with NRα in NLS-recognition and binding of import substrate to the NE [16,17,32]. A large domain of so-called arm repeats [33] within NRα is responsible for NLS-binding [19,25]. NRα has eight repeats [11,34] whereas NRβ has a number of less well defined repeats. The NR heterodimer was observed to bind import substrates in cytosolic extracts [12,13,17]. The NR/substrate complex docks via NRβ to the NPC [20,35] and is translocated through the NPC by an energy-dependent and Ran-dependent mechanism. The amino-terminal ~50 amino acids of NRα are necessary and sufficient for NRβ binding. A fusion protein consisting of this peptide and a reporter was imported into the nucleus independently of NRα. Unlike NRα, this fusion protein was not exported from the nucleus [19,36]. Thus translocation through the NPC is mediated only by NRβ. Mammalian NRα was observed both in the cytoplasm and in the nucleus [19,30,36]. Yeast NRα was localized to the nucleus, or the NE, or the cytoplasm [21,22,37–39]. NRβ is located predominantly at the NE, also in the cytoplasm but not in the nucleoplasm [16,20,39]. Immunoelectron microscopy revealed that NRβ is located on both sides of the NPC [35]. According to the localization data, NRα and NRβ do not form a heterodimer within the nucleus and the heterodimer dissociates at the nuclear side of the NPC. It is not known whether dissociation of the NR heterodimer is accompanied by release of

*Corresponding author. Fax: (49)(6841) 166288.

PII S0014-5793(96)00583-2

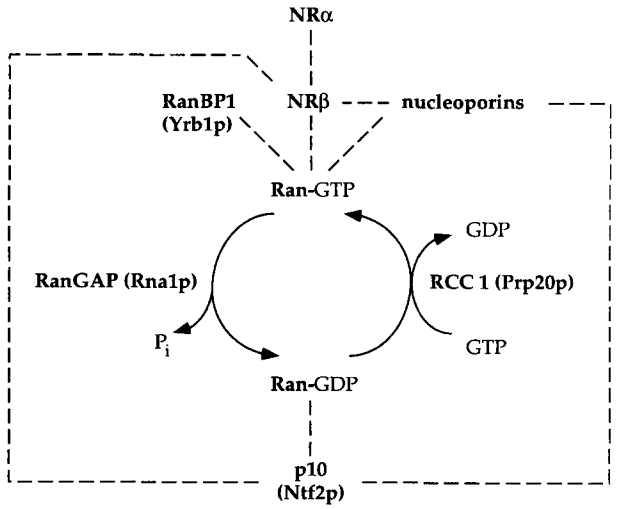

Fig. 1. The GTPase cycle of Ran (Gsp1p) and protein interactions. The cycle is regulated by the GDP/GTP exchange factor RCC1 (Prp20p) and the GTPase activating protein RanGAP (Rna1p). Other factors that regulate the guanine nucleotide bound state may exist. Dotted lines indicate direct interactions between two proteins. NRβ (Kap95p/Rsl1p) binds either to NRα (Srp1p/Kap60p) or to Ran-GTP. Names of *S. cerevisiae* homologues are in parentheses.

the import substrate from NRα. By one model, release could be achieved by NRα modification, e.g. dephosphorylation. This idea is supported by the observation that only phosphorylated NRα bound NLSs in a blot binding assay [40] and by the partial purification of a NRα kinase from yeast which was activated by import substrates [29].

Interestingly, it was recently observed that the GTP form of Ran bound to NRβ and caused dissociation of the NR heterodimer in vitro [32]. One interpretation is that this event represents import termination and that NRβ returns to the cytoplasm complexed to Ran-GTP. Homologues of NRβ which were found in a database search [17] might play a role in the export of NRα, or they might be alternative partners of NRα during import. It is not known whether the NR subunits are involved in export of proteins or RNA. It is unlikely that they play a role in mRNA export. Temperature-sensitive yeast strains which are mutated in NRα (which exhibit pleiotropic defects like cell cycle arrest) [23,34] and NRβ showed defects in protein import but not in mRNA export [23,39].

3. Nuclear pore complex (NPC)

The NPC [41–43] consists of about 100 different proteins [44], also termed nucleoporins. Although the overall architecture of the NPC is known in detail, information about the function and localization within pore substructures of individual nucleoporins is limited. Mutations in several nucleoporins lead to defects in protein import and/or RNA export. A number of nucleoporins contain more or less extended characteristic domains of repeats. The core of the repeats consists of degenerate FXFG or GLFG peptide motifs (amino acid single letter code). In many cases only FG is present as a motif. Among the 22 known yeast nucleoporins 12 contain FG repeats. Mammalian FG repeats containing nucleoporins are located at both sides and the central part of the NPC. The exact function of the repeats remains unclear. However, there is accumulating evidence that they serve as multiple binding sites for transport factors. The import factor p10 was observed to interact with FXFG repeats of Nup36p [45] and was purified by its ability to bind to the FXFG nucleoporin p62 [46]. Similarly, incubation of immobilized GLFG repeats of Nup98 with a cytosolic subfraction led to depletion of the binding activity [47]; NRβ was identified as one protein bound to these repeats [12]. Binding of the NLS-receptor to some but not all FG nucleoporins has also been reported by others [20,32,45,48–50] and required only the repeat domains [32,45,49,50].

Mammalian RanBP2/Nup358 was the first nucleoporin that was shown to bind to Ran. It is located at the cytoplasmic side of the NPC and has 4 Ran binding domains (see below). It binds only the GTP-form of Ran [51,52]. The two yeast nucleoporins Nup2p [53] and Nup36p [45] also contain one Ran binding domain. The Ran binding domain of Nup2p interacted with yeast Ran as shown by two hybrid analysis [54,55]. Interestingly, all three of these Ran binding nucleoporins also contain FXFG repeats and were observed to interact with the NR [32,45,48,52]. Assuming that they concomitantly bind Ran-GTP and the NR/substrate complex and that GTP hydrolysis by Ran initiates translocation through the NPC, these nucleoporins could be the entry sites for translocation. Other evidence for the interconnection of FXFG nucleoporins, the NR, and Ran comes from yeast genetics, through genetic interactions between Nup1p and Nup2p [53], Nup1p and NRα [48], Nup2p and NRα (J. Loeb, unpublished), Rna1p (the Ran GTPase activating protein) and Nup1p [56], as well as Rna1p and NRβ [39].

4. Ran

Ran (Ras-related nuclear protein), also termed TC4, belongs to the family of Ras-like GTPases. However, it differs from other members of the family in that it lacks a carboxy-terminal membrane attachment, is very abundant, and is located mainly in the nucleus. Interactions with other proteins depend on the state of the nucleotide bound to Ran (Fig. 1) which is regulated by the GTPase activating protein (GAP) and the GDP/GTP exchange factor (GEF). Temperature-sensitive GAP and GEF mutants as well as Ran mutants that are defective in GTP-binding (GDP-form) or in GTP-hydrolysis (GTP-form) have been studied in different organisms. Ran and its regulators have been proposed to function in numerous nuclear events, e.g. maintenance of the nuclear structure, cell cycle control, DNA replication, transcription, RNA processing and export. A primary function of Ran in nuclear import is not universally accepted [57,58].

Ran was purified from cytosol as the major activity required for energy-dependent translocation of import substrate into permeabilized cells [59]. GTP hydrolysis by Ran is required for import [59–63] but it is not known whether other GTPases or ATPases are also involved. Ran is depleted from digitonin-treated cells [62] but accumulated at the NE and the nuclear interior during import in vitro [20,35,62]. Uptake of an import substrate is blocked in the presence of non-hydrolyzable GTP analogues. Under these conditions, Ran accumulated at the cytoplasmic side of the NPC. By immunoelectron microscopy, it was observed to be located at the same peripheral region of the NPC where substrates accumulated after ATP depletion [62]. This region, which corresponds to the

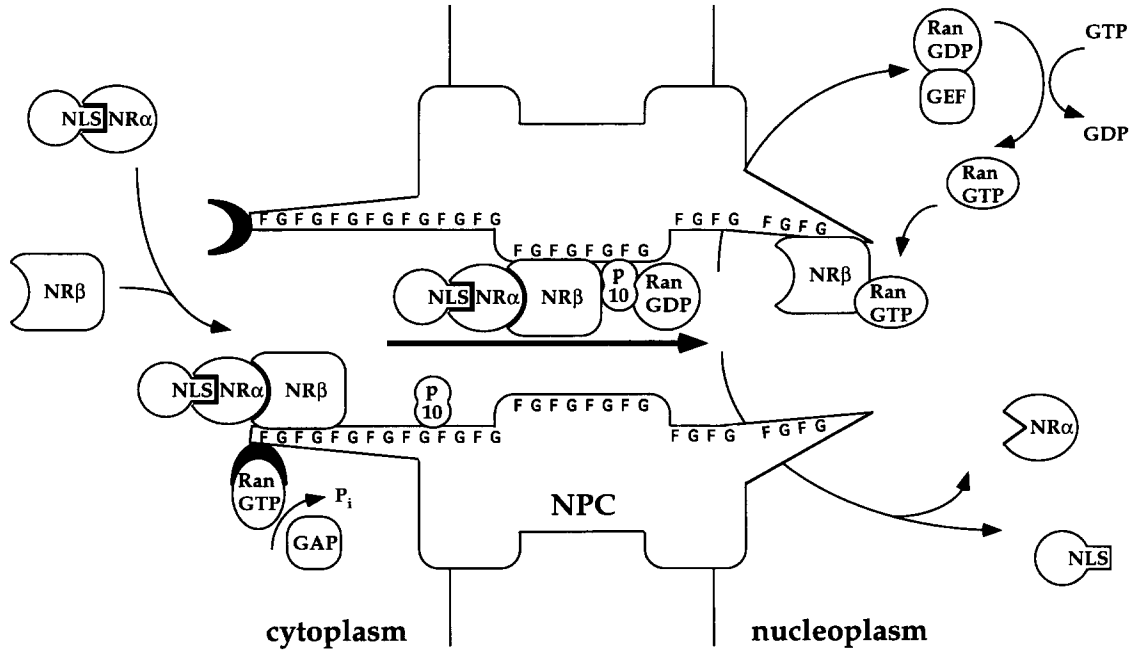

Fig. 2. Schematic model of protein import into the nucleus. The α-subunit of the NLS-receptor (NRα) associates with the nuclear localization sequence (NLS) of an import substrate. NRα can bind to pore-associated or cytoplasmic NRβ. The NR heterodimer binds via NRβ to FG repeats of a subset of nucleoporins. Translocation requires energy, Ran and p10. Ran-GTP binds to Ran binding domain (black) containing nucleoporins at the cytoplasmic side of the nuclear pore complex (NPC). Translocation is probably initiated by GTPase activating protein (GAP)-mediated GTP hydrolysis. The actual transfer mechanism is unknown. The NLS protein/NR/p10/Ran-GDP complex is a possible intermediate. The complex disassembles at the nuclear side of the NPC. This might be accomplished by Ran-GTP binding to NRβ. Then NRα releases the import substrate. Ran-GDP is converted to Ran-GTP by the GDP/GTP exchange factor (GEF). Alternative views about the role of Ran during transport are discussed in Section 7.

cytoplasmic fibrils that extend from the NPC, was proposed to be the initial binding site for import substrates [8]. The Ran-GTP binding nucleoporin RanBP2/Nup358 was also localized to this region [51] and was shown to be a major Ran binding component of rat liver nuclear envelopes [52,62].

Regulation by specific GAPs and GEFs is required for proper function of Ran. So far only one GAP and only one GEF have been identified, and they are located on opposite sides of the NE (Fig. 2). The nuclear protein RCC1 forms a complex with Ran-GDP and promotes guanine nucleotide exchange [64,65]. Like other Ras-like proteins, Ran has a low intrinsic GTPase activity. Hydrolysis of bound GTP is stimulated by a RanGAP [66] which was found to be a functional homologue of the cytoplasmic yeast protein Rna1p [63,67,68]. Mutants in *RCC1* and its budding yeast homologue, *PRP20*, display pleiotropic defects. They are blocked both in RNA export [69–72] and protein import [39,73]. Similar phenotypes were observed in *rna1* mutants [69]; they also exhibit defects in RNA export [74,75] and protein import [63]. Moreover, dominant-negative expression of a Ran mutant that is unable to hydrolyze GTP in yeast leads to a block in protein import and mRNA export [61]. These data together show that disturbance of the Ran GTPase cycle will cause defects in bi-directional nuclear transport in vivo. Recently, Rna1p was also shown to be involved in protein import in vitro. Cytosol prepared from the loss of function mutant *rna1-1* was unable to support import into competent nuclei of permeabilized yeast cells unless wild-type Rna1p was added [63].

It has been suggested that Ran shuttles between the cytoplasm and the nucleoplasm and that shuttling is coupled to the GTPase cycle of Ran and to nucleocytoplasmic transport. In this scenario, Ran-GTP and the import substrate/NR complex dock at the cytoplasmic side of the NPC, translocation is triggered by GAP-mediated GTP hydrolysis, and then Ran-GDP and the complex reach the nuclear side of the NPC, where the complex disassembles. Nuclear Ran-GDP is converted to Ran-GTP by the GEF (Fig. 2). If RNA export is a mirror image of protein import, a second set of a nuclear GAP and a cytoplasmic GEF have to be postulated but they have not been found yet [67,68].

Using recombinant yeast proteins, it was shown that addition of GTP-loaded Gsp1p (the essential yeast Ran), and not of Gsp1p-GDP, caused dissociation of a complex consisting of FXFG repeats of Nup1p, NRα, and NRβ, as well as of a complex consisting of import substrate, NRα, and NRβ [32]. As a result, a complex of NRβ and yeast Ran-GTP was formed in which the bound GTP was not accessible to Rna1p [32,76]. These observations have been interpreted as representing a dissociation step in repeated association/dissociation reactions during movement of an import substrate across the NPC [32], or to represent the terminal step of translocation [4].

5. p10

Ran associates in *Xenopus* ovary extracts with a dimer of p10, also termed pp15 or Ntf2p. The p10 dimer further stimulates energy-dependent translocation in the presence of Ran [77]. Although its addition is not required to all batches of

permeabilized cells [35] it seems to be an essential import factor. The protein was also purified from HeLa cells by biochemical complementation of cytosol which was depleted of import activity by preincubation with recombinant nucleoporin p62 [46]. The yeast protein is essential and is located at the NE [45,78]. The human homologue is able to function in *S. cerevisiae*. Temperature-sensitive *ntf2* mutants exhibit defects in protein import but show no defect in mRNA export [78].

Recombinant p10 was observed to bind to several repeat-containing nucleoporins. It also bound to NRβ and Ran-GDP, but not to Ran-GTP. Assembly of the NR heterodimer on FXFG repeats of Nup36p yielded cooperative binding of Ran-GDP and p10 in vitro. Addition of Ran-GTP to this complex (which did not contain an import substrate) caused release of the NR heterodimer. Surprisingly, addition of GTP caused release of NRα [45]. These observations favor an extended model for translocation by repeated association/dissociation reactions. The Nup repeats/NRα/NRβ/Ran-GDP/p10 complex would form as an intermediate and stimulate a Prp20p-independent GDP/GTP exchange reaction in the microenvironment of the NPC. The formation of Ran-GTP would lead to dissociation and movement of the import substrate [45].

6. Other factors

Except NRα, none of the import factors purified from mammalian cytosol is predominantly located in the cytoplasm. The successful isolation of these proteins has relied on the fact that cytosol preparations contain nuclear and NPC-associated proteins and that digitonin treatment of mammalian cells extracts or inactivates transport factors [16,20,62]. Interestingly, the binding activity (i.e. the NLS-receptor) is not an absolutely required cytosolic factor in the yeast in vitro system [79]. Here, permeabilization is achieved not by digitonin treatment but by a freeze/thaw cycle. It seems possible that additional import factors can be purified by using more stringent permeabilization conditions.

Other factors like Hsp70 heat shock proteins [80,81] have been reported to play a role in protein import. Furthermore, several other Ran-GTP binding proteins have been characterized [82]. The 23 kDa protein RanBP1/Yrb1p binds Ran only in its GTP-form [54,55,83–86] and acts as a co-activator of the Ran GTPase [55,84]. It shares a domain of 120–150 amino acids, the 'Ran binding domain', with other proteins, some of which are nucleoporins (discussed above). The 70-residue core region of the Ran binding domain is highly conserved. Mutations therein disrupt the interaction with Ran [55,85]. A Ran mutant lacking the 6 carboxy-terminal amino acids was unable to bind to RanBP1 but still associated with NRβ. Binding of RanBP1 and NRβ to Ran-GTP do not seem to compete with each other [87]. The yeast protein, Yrb1p, is located in the cytoplasm but is concentrated in a region around the NE. Mutants in *YRB1* exhibit defects in protein import and mRNA export [55] and cause synthetic lethality with mutations in *NUP1* [88]. Cytosol prepared from *yrb1* mutants [55], or cytosol immunodepleted of Yrb1p (Schlenstedt and Silver, unpublished), as well as the carboxy-terminal Ran deletion mutant [89] still supported protein import in vitro. The function of RanBP1/Yrb1p remains unclear. It could play a role in export of Ran-GTP from the nucleus, in release of Ran-GTP from NRβ, in protection of cytoplasmic Ran-GTP from Rna1p, or in targeting of cytoplasmic Ran-GTP to the NPC.

7. Questions and mechanisms

It is obvious that more import factors will have to be identified and characterized before we understand at the molecular level how proteins move into the nucleus. We do not know whether Ran alone is responsible for the energy requirement of transport. It is unclear what ensures the directionality of translocation. Does a functional complex exist which consists of import substrate/NR and Ran? What causes disassembly of the complex in the nucleus? How do transport components return to the cytoplasm? Are they involved in export of protein or RNA? How is transport regulated overall?

One controversial question at this point is: what is the actual translocation mechanism? There are different views about the exact series of interactions and events during import. They differ mainly in speculations about the role of Ran. (1) *Binding/release model:* The model of repeated association/dissociation reactions during movement of an import substrate across the NPC implies that the NR heterodimer and the import substrate are not translocated as a complex and that multiple GTPase cycles of Ran drive binding and release of the factors to the NPC. The function of Rna1p would be to convert any cytoplasmic Ran-GTP (which prevents dimerization of the NR) to the primary active Ran-GDP [32,45]. (2) *Walking model:* It has also been suggested that the NR heterodimer and the import substrate are translocated as a single complex and moves in discrete steps along an array of binding sites with increasing affinity. Multiple Ran GTPase cycles would provide the energy for this walking mechanism by regulating the affinity for alternate binding sites [4]. (3) *Sliding unit model:* Alternatively, a single Rna1p-triggered GTP hydrolysis event could lead to the assembly of a transport-competent unit (like a substrate/NR/p10/Ran-GDP complex). This unit would slide along the FG repeat coated inner surface of the NPC. The sliding movement could be driven by putative attached motor proteins. As the last step of import, Ran-GTP would bind to NRβ at the nuclear side of the NPC and dissociate the unit (Fig. 2).

Acknowledgements: I would like to thank Jonathan Loeb, Richard Zimmermann and Jens Solsbacher for helpful comments on the manuscript.

References

[1] Fabre, E. and Hurt, E.C. (1994) Curr. Opin. Cell Biol. 6, 335–342.
[2] Izaurralde, E. and Mattaj, I.W. (1995) Cell 81, 153–159.
[3] Gerace, L. (1995) Cell 82, 341–344.
[4] Görlich, D. and Mattaj, I.W. (1996) Science 271, 1513–1518.
[5] Breeuwer, M. and Goldfarb, D.S. (1990) Cell 60, 999–1008.
[6] Dingwall, C. and Laskey, R.A. (1991) Trends Biochem. Sci. 16, 178–181.
[7] Newmeyer, D.D. and Forbes, D.J. (1988) Cell 52, 641–653.
[8] Richardson, W.D., Mills, A.D., Dilworth, S.M., Laskey, R.A. and Dingwall, C. (1988) Cell 52, 655–664.
[9] Adam, S.A., Sterne-Marr, R. and Gerace, L. (1990) J. Cell Biol. 111, 807–816.
[10] Moore, M.S. and Blobel, G. (1992) Cell 69, 939–950.
[11] Görlich, D., Prehn, S., Laskey, R.A. and Hartmann, E. (1994) Cell 79, 767–778.

[12] Radu, A., Blobel, G. and Moore, M.S. (1995) Proc. Natl. Acad. Sci. USA 92, 1769–1773.
[13] Imamoto, N., Tachibana, T., Matsubae, M. and Yoneda, Y. (1995) J. Biol. Chem. 270, 8559–8565.
[14] Adam, S.A. and Gerace, L. (1991) Cell 66, 837–847.
[15] Adam, E.J.H. and Adam, S.A. (1994) J. Cell Biol. 125, 547–555.
[16] Chi, N.C., Adam, J.H.E. and Adam, S.A. (1995) J. Cell Biol. 130, 265–274.
[17] Görlich, D., Kostka, S., Kraft, R., Dingwall, C., Laskey, R.A., Hartmann, E. and Prehn, S. (1995) Curr. Biol. 5, 383–392.
[18] Enenkel, C., Blobel, G. and Rexach, M. (1995) J. Biol. Chem. 270, 16499–16502.
[19] Weis, K., Ryder, U. and Lammond, A.I. (1996) EMBO J. 15, 1818–1825.
[20] Moroianu, J., Hijikata, M., Blobel, G. and Radu, A. (1995) Proc. Natl. Acad. Sci. USA 92, 6532–6536.
[21] Yano, R., Oakes, M., Tabb, M.M. and Nomura, M. (1992) Mol. Cell. Biol. 12, 5640–5651.
[22] Stochaj, U., Osborne, M., Kurihara, T. and Silver, P. (1991) J. Cell Biol. 113, 1243–1254.
[23] Loeb, J.D.L., Schlenstedt, G., Pellman, D., Kornitzer, D., Silver, P.A. and Fink, G.R. (1995) Proc. Natl. Acad. Sci. USA 92, 7647–7651.
[24] Cuomo, C.A., Kirch, S.A., Gyuris, J., Brent, R. and Oettinger, M.A. (1994) Proc. Natl. Acad. Sci. USA 91, 6156–6160.
[25] Cortes, P., Ye, Z.-S. and Baltimore, D. (1994) Proc. Natl. Acad. Sci. USA 91, 7633–7637.
[26] Weis, K., Mattaj, I.W. and Lamond, A.I. (1995) Science 268, 1049–1053.
[27] Török, I., Strand, D., Schmitt, R., Tick, G., Török, T., Kiss, I. and Mechler, B.M. (1995) J. Cell Biol. 129, 1473–1489.
[28] Küssel, P. and Frasch, M. (1995) J. Cell Biol. 129, 1491–1507.
[29] Azuma, Y., Tabb, M.M., Vu, L. and Nomura, M. (1995) Proc. Natl. Acad. Sci. USA 92, 5159–5163.
[30] Imamoto, N., Shimamoto, T., Takao, T., Tachibana, T., Kose, S., Matsubae, M., Sekimoto, T., Shimonishi, Y. and Yoneda, Y. (1995) EMBO J. 14, 3617–3626.
[31] Moroianu, J., Blobel, G. and Radu, A. (1995) Proc. Natl. Acad. Sci. USA 92, 2008–2011.
[32] Rexach, M. and Blobel, G. (1995) Cell 83, 683–692.
[33] Peifer, M., Berg, S. and Reynolds, A.B. (1994) Cell 76, 789–791.
[34] Yano, R., Oakes, M.L., Tabb, M.M. and Normura, M. (1994) Proc. Natl. Acad. Sci. USA 91, 6880–6884.
[35] Görlich, D., Vogel, F., Mills, A.D., Hartmann, E. and Laskey, R.A. (1995) Nature 377, 246–248.
[36] Görlich, D., Henklein, P., Laskey, R.A. and Hartmann, E. (1996) EMBO J. 15, 1810–1817.
[37] Küssel, P. and Frasch, M. (1995) Mol. Gen. Genet. 248, 351–363.
[38] Aitchison, J.D., Blobel, G. and Rout, M.P. (1995) J. Cell Biol. 131, 1659–1675.
[39] Koepp, D.M., Wong, D.H., Corbett, A.H. and Silver, P.A. (1996) J. Cell Biol., in press.
[40] Stochaj, U. and Silver, P.A. (1992) J. Cell Biol. 117, 473–482.
[41] Hurt, E.C. (1993) FEBS Lett. 325, 76–80.
[42] Panté, N. and Aebi, U. (1993) J. Cell Biol. 122, 977–984.
[43] Rout, M.P. and Wente, S.R. (1994) Trends Cell Biol. 4, 357–365.
[44] Rout, M.P. and Blobel, G. (1993) J. Cell Biol. 123, 771–783.
[45] Nehrbass, U. and Blobel, G. (1996) Science 272, 120–122.
[46] Paschal, B.M. and Gerace, L. (1995) J. Cell Biol. 129, 925–937.
[47] Radu, A., Moore, M.S. and Blobel, G. (1995) Cell 81, 215–222.
[48] Belanger, K.D., Kenna, M.A., Wei, S. and Davis, L.I. (1994) J Cell Biol. 126, 619–630.
[49] Iovine, M.K., Watkins, J.L. and Wente, S.R. (1995) J. Cell Biol. 131, 1699–1713.
[50] Kraemer, D.M., Strambio-de-Castillia, C., Blobel, G. and Rout, M.P. (1995) J. Biol. Chem. 270, 19017–19021.
[51] Yokoyama, N., Hayashi, N., Seki, T., Panté, N., Ohba, T., Nishii, K., Kuma, K., Hayashida, T., Miyata, T., Aebi, U., Fukui, M. and Nishimoto, T. (1995) Nature 376, 184–188.
[52] Wu, J., Matunis, M.J., Kraemer, D., Blobel, G. and Coutavas, E. (1995) J. Biol. Chem. 270, 14209–14213.
[53] Loeb, J.D.J., Davis, L.I. and Fink, G.R. (1993) Mol. Biol. Cell 4, 209–222.
[54] Dingwall, C., Kandels-Lewis, S. and Séraphin, B. (1995) Proc. Natl. Acad. Sci. USA 92, 7525–7529.
[55] Schlenstedt, G., Wong, D.H., Koepp, D.M. and Silver, P.A. (1995) EMBO J. 14, 5367–5378.
[56] Bogerd, A.M., Hoffman, J.A., Amberg, D.C., Fink, G.R. and Davis, L.I. (1994) J. Cell Biol. 127, 319–332.
[57] Rush, M.G., Drivas, G. and D'Eustachio, P. (1996) Bioessays 18, 103–112.
[58] Sazer, S. (1996) Trends Cell Biol. 6, 81–85.
[59] Moore, M.S. and Blobel, G. (1993) Nature 365, 661–663.
[60] Melchior, F., Paschal, B., Evans, J. and Gerace, L. (1993) J. Cell Biol. 123, 1649–1659.
[61] Schlenstedt, G., Saavedra, C., Loeb, J.D.J., Cole, C.N. and Silver, P.A. (1995) Proc. Natl. Acad. Sci. USA 92, 225–229.
[62] Melchior, F., Guan, T., Yokoyama, N., Nishimoto, T. and Gerace, L. (1995) J. Cell Biol. 131, 571–581.
[63] Corbett, A.H., Koepp, D.M., Schlenstedt, G., Lee, M.S., Hopper, A.K. and Silver, P.A. (1995) J. Cell Biol. 130, 1017–1026.
[64] Bischoff, F.R. and Ponstingl, H. (1991) Proc. Natl. Acad. Sci. USA 88, 10830–10834.
[65] Bischoff, F.R. and Ponstingl, H. (1991) Nature 354, 80–82.
[66] Bischoff, F.R., Klebe, C., Kretschmer, J., Wittinghofer, A. and Ponstingl, H. (1994) Proc. Natl. Acad. Sci. USA 91, 2587–2591.
[67] Bischoff, F.R., Krebber, H., Kempf, T., Hermes, I. and Ponstingl, H. (1995) Proc. Natl. Acad. Sci. USA 92, 1749–1753.
[68] Becker, J., Melchior, F., Gerke, V., Bischoff, F.R., Ponstingl, H. and Wittinghofer, A. (1995) J. Biol. Chem. 270, 11860–11865.
[69] Forrester, W., Stutz, F., Rosbash, M. and Wickens, M. (1992) Genes Dev. 6, 1914–1926.
[70] Kadowaki, T., Zhao, Y. and Tartakoff, A. (1992) Proc. Natl. Acad. Sci. USA 89, 2312–2316.
[71] Amberg, D.C., Fleischmann, M., Stagljar, I., Cole, C.N. and Aebi, M. (1993) EMBO J. 12, 233–241.
[72] Cheng, Y., Dahlberg, J.E. and Lund, E. (1995) Science 267, 1807–1810.
[73] Tachibana, T., Imamoto, N., Seino, H., Nishimoto, T. and Yoneda, Y. (1994) J. Biol. Chem. 269, 24542–24545.
[74] Hopper, A.K., Banks, F. and Evangelidis, V. (1978) Cell 19, 211–219.
[75] Amberg, D.C., Goldstein, A.L. and Cole, C.N. (1992) Genes Dev. 6, 1173–1189.
[76] Floer, M. and Blobel, G. (1996) J. Biol. Chem. 271, 5313–5316.
[77] Moore, M.S. and Blobel, G. (1994) Proc. Natl. Acad. Sci. USA 91, 10212–10216.
[78] Corbett, A.H. and Silver, P.A. (1996) J. Biol. Chem. 271, in press.
[79] Schlenstedt, G., Hurt, E., Doye, V. and Silver, P.A. (1993) J. Cell Biol. 123, 785–798.
[80] Imamoto, N., Matsuoka, Y., Kurihara, T., Kohno, K., Miyagi, M., Sakiyama, F., Okada, Y., Tsunasawa, S. and Yoneda, Y. (1992) J. Cell Biol. 119, 1047–1061.
[81] Shi, Y. and Thomas, J.O. (1992) Mol. Cell. Biol. 12, 2186–2192.
[82] Lounsbury, K.M., Beddow, A.L. and Macara, I.G. (1994) J. Biol. Chem. 269, 11285–11290.
[83] Coutavas, E., Ren, M., Oppenheim, J.D., D'Eustachio, P. and Rush, M.G. (1993) Nature 366, 585–587.
[84] Bischoff, F.R., Krebber, H., Smirnova, E., Dong, W. and Ponstingl, H. (1995) EMBO J. 14, 705–715.
[85] Beddow, A.L., Richards, S.A., Orem, N.R. and Macara, I.G. (1995) Proc. Natl. Acad. Sci. USA 92, 3328–3332.
[86] Ouspenski, I.I., Mueller, U.W., Matynia, A., Sazer, S., Elledge, S.J. and Brinkley, B.R. (1995) J. Biol. Chem. 270, 1975–1978.
[87] Lounsbury, K.M., Richards, S.A., Perlhungher, R.R. and Macara, I.G. (1996) J. Biol. Chem. 271, 2357–2360.
[88] Kenna, M.A., Petranka, J.G., Reilly, J.L. and Davis, L.I. (1996) Mol. Cell. Biol. 16, 2025–2036.
[89] Ren, M., Villamarin, A., Shih, A., Coutavas, E., Moore, M.S., LoCurico, M., Clarke, V., Oppenheim, J.D., D'Eustachio, P. and Rush, M.G. (1995) Mol. Cell. Biol. 15, 2117–2124.