The nuclear pore complex: from molecular architecture to functional dynamics

Daniel Stoffler*, Birthe Fahrenkrog† and Ueli Aebi‡

Toward dissecting the molecular composition and architecture of the nuclear pore complex (NPC), over the past 18 months novel nucleoporins and NPC subcomplexes were identified and characterized. The three-dimensional structure of isolated yeast NPCs was determined by electron cryomicroscopy. New specimen preparation and labeling protocols localized a number of nucleoporins and NPC subcomplexes within the three-dimensional architecture of the yeast NPC. Structural changes of native NPCs mediated by physiological effectors such as calcium or ATP were monitored by time-lapse atomic force microscopy, thus revealing a first glimpse of the NPC's functional dynamics.

Addresses
Biozentrum, ME Müller Institute for Structural Biology, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland
*e-mail: stoffler@ubaclu.unibas.ch
† e-mail: fahrenkrog@ubaclu.unibas.ch
‡ e-mail: aebi@ubaclu.unibas.ch

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Abbreviations

AFM	atomic force microscopy
EM	electron microscopy
FESEM	field emission scanning EM
GFP	green fluorescent protein
IEM	immuno-electronmicroscopy
MDa	megaDalton
NE	nuclear envelope
NPC	nuclear pore complex
Nup	nucleoporin
SPB	spindle pole body
TEM	transmission electron microscopy
WGA	wheat germ agglutinin

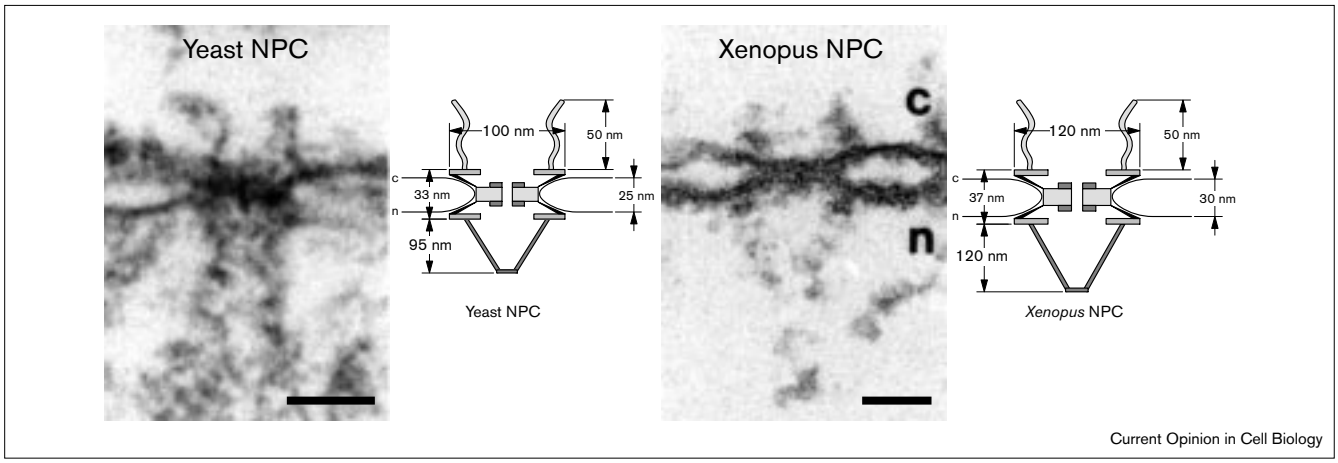
Introduction

The vertebrate nuclear pore complex (NPC) exhibits a tripartite architecture (perpendicular to the plane of the nuclear envelope [NE; see Figure 1 for schematic representation]) with a total mass of ~125 MDa (reviewed in [1]). Its ~55 MDa central framework is a ring-like assembly built of eight multidomain spokes each consisting of two roughly identical halves (see Figure 2) so that its asymmetric unit (i.e. one half-spoke) represents one sixteenth of its mass or roughly the size of a ribosome. This central framework is sandwiched between a ~32 MDa cytoplasmic ring and a ~21 MDa nuclear ring (see Figure 1). From the cytoplasmic ring eight short, kinky fibrils emanate, whereas the nuclear ring anchors a basket (or fishtrap), assembled from eight thin ~50 nm long filaments joined distally by a 30–50 nm diameter ring. The ring-like, central framework embraces the central pore of the NPC, which acts as a gated channel. The central pore is often plugged with a distinct particle of highly variable appearance — called the central plug or transporter — whose molecular architecture and functional significance remain to be established. In this review, we summarize recent insights into the subunit composition, molecular architecture and functional dynamics of vertebrate, invertebrate and yeast NPCs.

A closer look at nuclear pore complex conservation

The overall three-dimensional architecture of the NPC is evolutionarily conserved from yeast to higher eukaryotes

Figure 1



Cross-sections through the NE of yeast and *Xenopus* oocyte nuclei (c, cytoplasm; n, nucleus), and schematic comparison of yeast and *Xenopus* oocyte NPCs [2••]. Scale bars, 100 nm.

(Figure 1). Although the linear dimensions of the yeast NPC were determined to be 15% smaller than those of *Xenopus* oocyte NPC [2••], both NPCs revealed cytoplasmic fibrils and a nuclear basket. The recently solved three-dimensional structure of isolated yeast NPCs by electron cryomicroscopy revealed a surprisingly flat ~822 symmetric spoke complex, embracing a cylindrical plug, with no clear indication of a cytoplasmic or nuclear ring being attached to it [3••](Figure 2). The mass of the yeast NPCs used for the three-dimensional reconstruction was determined to be ~60 MDa, a value consistent with their smaller linear dimensions. Comparison of the overall dimensions of the yeast and vertebrate three-dimensional reconstructions (Figure 2), however, reveals that the yeast NPC must be significantly more compact than the vertebrate NPC, otherwise its mass would only amount to ~30 MDa. Although this work is a promising start, it is only the first word on the three-dimensional architecture of yeast NPCs.

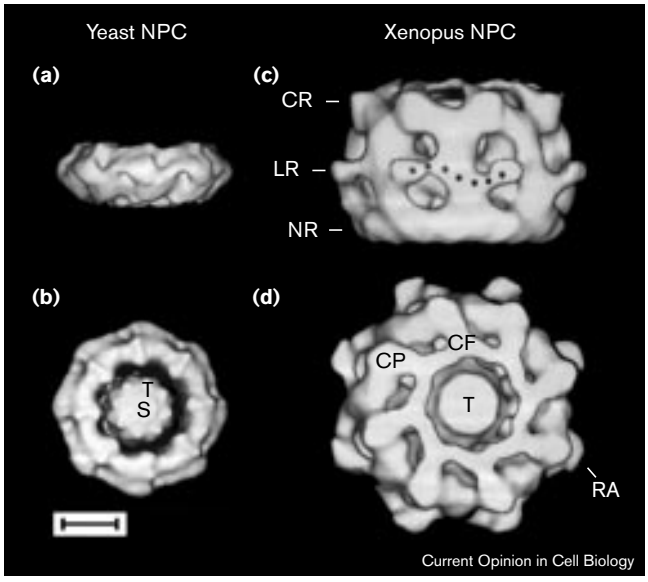
To further exploit the conservation of NPC architecture across species, chicken oocytes were investigated [4]. Although their overall structure is remarkably similar to that of amphibian NPCs, two differences were evident: first, the chicken cytoplasmic coaxial ring subunits were smaller; and second, in the chicken NPC a second ring residing on top of the nuclear basket ring was identified, which is possibly involved in 'funneling' export particles into the NPC. In the context of investigating the dynamic behaviour of NPC structure during Balbiani ring particle translocation in salivary gland cells of the insect *Chironomus thummi* [5•], it was found that in most respects, *Chironomus* NPCs were similar to amphibian NPCs, thus suggesting a strong evolutionary conservation of NPC architecture between invertebrates and vertebrates.

Critical-point dried amphibian NEs investigated by field emission scanning EM (FESEM) revealed hollow cables of 50 nm in diameter consisting of eight 6 nm thick filaments that emanated from the distal rings of the nuclear baskets and reached deeply into the nucleus [6]. Similarly, NPC-attached, p270/Tpr-containing intranuclear filaments forming bundles, which projected as much as 350 nm into the nuclear interior, were identified after *Xenopus* oocytes were chemically fixed and centrifuged [7]. The molecular composition and functional significance of these hollow cables or intranuclear bundles, however, remain to be determined.

Identification and functional characterization of nucleoporins and nuclear pore complex subcomplexes

On the basis of its molecular mass of ~125 MDa and the high degree of symmetry of its central framework (i.e. one-eight-fold and two quasi-two-fold axes of symmetry; see Introduction and Figure 2), it has been assumed that the vertebrate NPC is composed of multiple copies (i.e. 8 or 16) of ~100 different proteins, called nucleoporins (Nups;

Figure 2



A comparison between the three-dimensional structures of yeast and vertebrate NPCs. **(a)** Side view of the yeast NPC showing the marked difference in height relative to the *Xenopus* NPC and the lack of thin rings on both sides. **(b)** The yeast NPC as viewed from the putative cytoplasmic surface. The transporter is marked (T) and putative cargo is marked (S). **(c)** Side view of the *Xenopus* NPC reveals the cytoplasmic (CR) and nuclear (NR) thin rings that are integral parts of the spoke complex. A lumenal ring (LR) is formed by the lumenal spoke domain and the adjacent radial arms (black dots). **(d)** The *Xenopus* NPC as viewed from the cytoplasmic surface. The transporter (T) is partly obscured by a ring of collapsed cytoplasmic filaments (CF) that emanate from the cytoplasmic particles (CP). The radial arms are labeled (RA). Scale bar, 30 nm. This figure has been adapted from [3••].

reviewed in [1]). Similarly, the ~60 MDa yeast NPC is assumed to be composed of 30–50 different nucleoporins. To date, ~30 yeast (Table 1) and ~20 vertebrate (Table 2) nucleoporins have been identified and characterized. These nucleoporins exhibit epitopes predominantly at the cytoplasmic or the nuclear periphery of the NPC, both in vertebrates and yeast (Figure 3).

A common feature of many nucleoporins is the presence of repeating FXFG, GLFG, and FG sequence motifs (in the single letter code for amino acids, where X is any amino acid). These repeat motifs are not required for targeting the corresponding nucleoporins to the NPC and, in most cases, they are not essential for viability. A number of recent investigations, however, have suggested a functional role of the FG repeats in nucleocytoplasmic transport. *In vivo* assays involving *Xenopus* egg extracts, isolated rat liver nuclei, or lysates from yeast nuclei, have revealed that Nup153, Tpr, Nup159p, Nup116p, Nup100p, Nsp1p, and the newly identified Nup53p interact with transport factors (i.e. importin-α importin-βs or RanGTP) [8,9••,10,11•,12,13•]. Moreover, blot overlays and solution binding assays, immunoprecipitation, and microinjection of transport factors into cultured cell

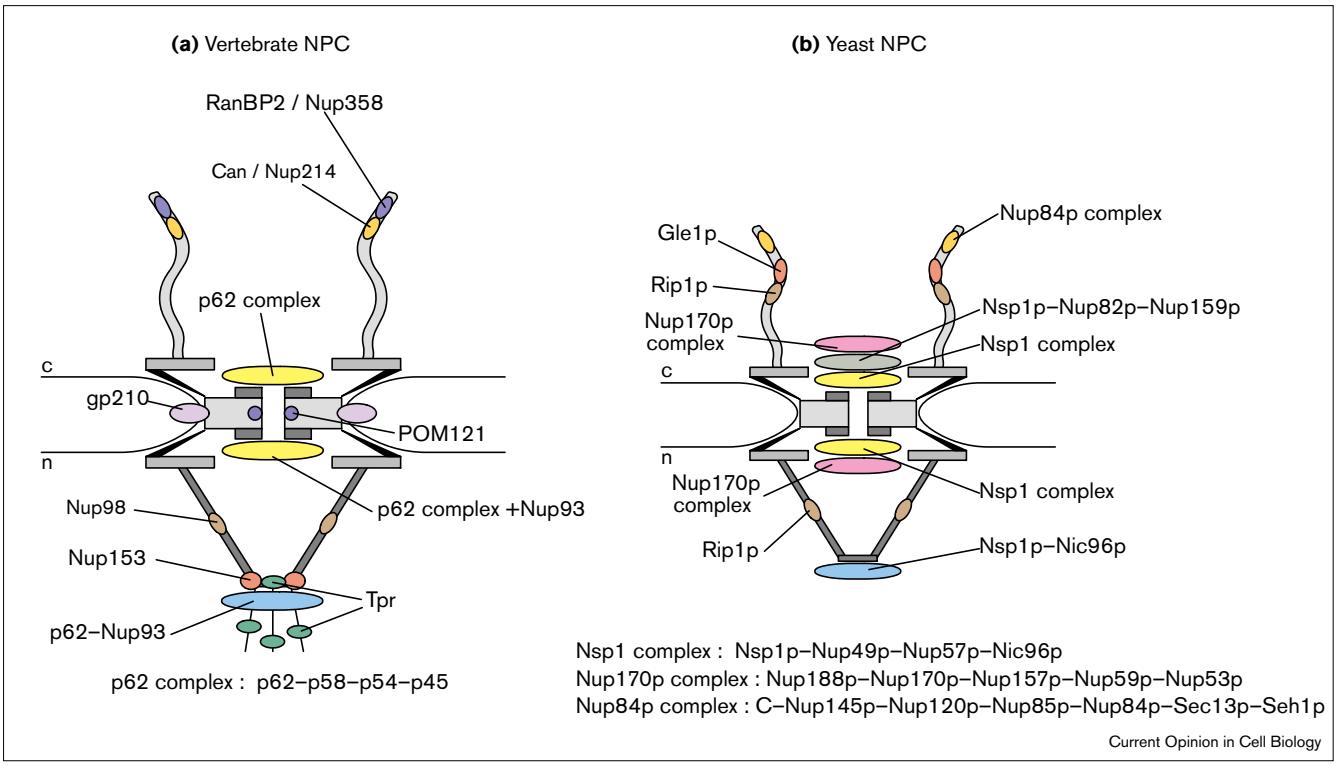
Table 1

***Saccharomyces cerevisiae* nucleoporins.**

Name*	Putative homologue(s)	Motifs†	Location	Properties and function	References
Snl1p (18 kDa)	–	Transmembrane	NE and ER	Stabilizing role in NPC structure and function	[53]
Yrb2p/Nup36p	h RanBP3	FXFG, Ran binding	Unknown	Ran binding protein	[29]
Sec13p (32 kDa)	–	WD	Cytoplasmic fibrils[a]	Part of Nup84p complex; vesicular transport from ER to Golgi	[29](a)
Seh1p (39 kDa)	–	WD	Cytoplasmic fibrils[a]	Part of Nup84p complex; vesicular transport from ER to Golgi	[29](a)
Gle2p (40 kDa)	*Sp* Rae1p	–	Unknown	Role in mRNA export	[29]
Rip1p (42 kDa)	h Rip1/Rab	FG	Cytoplasmic fibrils; nuclear basket; nucleus[b]	essential for export of heat shock RNA	[29](b)
Nup49p	r p58/p45 *Sp* Nup49	GLFG Coiled coil	Cytoplasmic and nuclear periphery of the central gated channel	Role in protein import and RNA export	[2••,29]
Nup53p	*X* MP44	FG Coiled coil	Cytoplasmic and nuclear face of the NPC core	Role in import of ribosomal proteins; phosphorylated during mitosis	[13•]
Nup57p	r p54 *Sp* Nup57	GLFG Coiled coil	Cytoplasmic and nuclear periphery of the central gated channel	Role in protein import and RNA export	[2••,29]
Nup59p	*X* MP44	FG Coiled coil	Cytoplasmic and nuclear face of the NPC core		[13•]
Gle1p (62 kDa)	hGdel	NES	Cytoplasmic fibrils; cytoplasm[b]	Role in mRNA export	[23,54](b)
Npl4p (64 kDa)		Degenerated Repeat motifs: GSXS, GSSX, GSXF, GFXS	Unknown	Role in protein import, RNA export and biogenesis	[29]
Ndc1p (74 kDa)	*Sp* Cut11p	Transmembrane	NPC and SPB	Required for proper SPB duplication; NPC assembly?	[35•,36]
Nup2p (78 kDa)	–	FXFG, coiled coil Ran binding	Unknown		[29]
Nup82p	–	Coiled coil	Cytoplasmic periphery of the central gated channel	Docking site for Nsp1p–Nup159p complex; role in RNA export	[2••,29,50•,51•]
Nup84p	r Nup107	–	Cytoplasmic fibrils[a]	Role in RNA export	[29]
Nsp1p (86 kDa)	r, h, *X* p62	FXFG Coiled coil	Cytoplasmic and nuclear periphery of the central gated channel; nuclear basket	C-terminal domain essential; in complexes with Nup49p, Nup57p, Nup82p, Nic96p, Nup159p; role in protein import	[2••,12,29,51•](a)
Nic96p	r, h, *X* Nup93 *Sp* Npp106	Coiled coil	Cytoplasmic and nuclear periphery of central gated channel; nuclear basket	Anchors Nsp1p–Nup49p–Nup57p into the NPC; N-terminal domain essential; role in NPC assembly; role in mRNA export	[2••,28•,29,55]
Nup100p	r, h, *X* Nup98	GLFG	Unknown		[11•,14•,29]
Nup116p	r, h, *X* Nup98	GLFG	Unknown	C-terminus necessary for targeting and association with the NPC; role in mRNA export; recycling of Kap95p	[11•,14•,29]
Nup1 (113 kDa)	C-terminus of Nup153	FXFG	Unknown	Role in nucleocytoplasmic transport and NPC morphology	[17•,29]
Nup120p	–	–	Cytoplasmic fibrils[a]	Role in mRNA export	[29](a)
Nup 133p	–	–	Unknown	Role in mRNA export, and NPC morphology	[29]
Nup145p	r, h, *X* Nup98	GLFG	C-terminus at the cytoplasmic fibrils[a]; N-terminus unknown	*In vivo* cleavage; C-terminal domain is part of Nup84p complex and essential for mRNA export and NPC morphology	[29,56•,57,58](a)
Pom152p	–	Transmembrane	Not determined	Anchors the NPC into the NE	[29]
Nup157p	r Nup155 *D* Nup154	–	Cytoplasmic and nuclear face of the NPC core	NPC core protein	[13•,29]
Nup159p	–	FG Coiled coil	Cytoplasmic periphery of the central gated channel	In complex with Nsp1p–Nup82p; C-terminus essential; N-terminus involved in mRNA export	[29,50•,51•,59]
Nup170p	r Nup155	–	Cytoplasmic and nuclear face of the NPC core	NPC core protein	[13•,29]
Nup188p	–	–	Cytoplasmic and nuclear face of the NPC core	NPC core protein	[29]
Nup192p	r, h p205	–	Unknown		[29]

C-terminus, carboxyl terminus; D, *Drosophila*; h, human; N-terminus, amino terminus; r, rat; *Sc*, *S. cerevisiae*; *Sp*, *S. pombe*; X, Xenopus. For other nomenclature see legend to Table 2.

Figure 3



Schematic diagram summarizing the immunolocalization of nucleoporin epitopes within the three-dimensional architecture of **(a)** the vertebrate NPC and **(b)** the yeast NPC. (a) In vertebrates, CAN/Nup214 and RanBP2 exhibit epitopes at the cytoplasmic fibrils [1]. The p62 complex, consisting of p62, p58, p54, and p45, exhibits epitopes at the cytoplasmic and the nuclear periphery of the central gated channel [1]; additionally, p62 exhibits an epitope at the nuclear basket (see [2••]). Nup93 epitopes are located at the nuclear periphery of the central gated channel and at the nuclear basket [28•]. Nup153, Nup98 and Tpr exhibit epitopes at the nuclear basket, the latter also at the intranuclear filaments [1,18•,19•,20]. The transmembrane proteins gp210 and POM121 are predicted to have epitopes in the lumen of the NE and on the NPC proper, respectively [1]. (b) In yeast, the nucleoporins of the Nup84p complex (i.e. C-Nup145p, Nup120p, Nup85p, Nup84p, Sec13p, and Seh1p) display epitopes at the cytoplasmic fibrils (S Siniossoglou, personal communication). Gle1p and Rip1p epitopes reside at the cytoplasmic fibrils, Rip1p additionally at the nuclear basket (B Fahrenkrog, F Stutz, unpublished data). The epitopes of the Nsp1p complex (Nsp1p–Nup49p–Nup57p–Nic96p) are located at the cytoplasmic and the nuclear periphery of the central gated channel [2••]. Moreover, Nsp1p and Nic96p show epitopes at the distal ring of the nuclear basket [2••]. Epitopes of the Nsp1p–Nup82p–Nup159p complex are displayed at the cytoplasmic periphery of the central gated channel [2••,50•,51•]. The Nup170p complex (i.e. Nup188p, Nup170p, Nup157p, Nup59p, and Nup53p), exhibit epitopes at the cytoplasmic and nuclear face of the NPC core, with no clear assignment to distinct substructures of the NPC [13•]. As all these localization studies have been performed with antibodies directed against epitopes of nucleoporins or tags fused to nucleoporins, however, we are only at the beginning of understanding the complete three-dimensional molecular architecture of the NPC.

lines demonstrated that Nup153, Nup98, CAN/Nup214, and Nup116p interact with members of the importin-β family as well as other transport factors (e.g. importin-α and Gle2p), most probably via their FG repeats [14•,15•,16,17•]. For example, Nup153, located at the distal end of the nuclear basket (Figure 3), might function as a termination site for nuclear protein import [9••] or as a site for importin α recycling [17•]; moreover, Tpr, of yet unknown function in nucleocytoplasmic transport [7,18•], might also participate in the recycling of importin α and β, or in mRNA export [19•].

By IEM and colocalization using confocal immunofluorescence microscopy, Tpr epitopes were localized to the nuclear basket and the intranuclear filaments [7,9••,19•,20]. Nup116p and/or Nup100p, both of unknown NPC location, might also be involved in the recycling of importin β [11•]. In contrast to the previously described *in vitro* interactions between FG repeat nucleoporins and transport factors, the *in vivo* interactions appear highly specific, indicating that the FG repeat nucleoporins do indeed control transport pathways in and out of the nucleus. In addition, the FG motif can interact directly with viral proteins — for example the HIV proteins Vpr and Rev [21,22] — thereby regulating their nuclear import and export. In a pathogenic process involving chromosomal translocation associated with human acute myeloid leukemia, the FG repeats of Nup98 and CAN/Nup214 are incorporated into chimeric proteins [23–25] which then act as oncogenic transcription factors [25].

Table 2

Vertebrate nucleoporins.

Name*	Putative homologue(s)	Motifs†	Location	Properties and function	References
p45	*Sc* Nup49p	FG Coiled coil	Cytoplasmic and nuclear periphery of the central gated channel	Generated by alternative splicing of p58; role in nuclear protein import	[1,60]
p54	*Sc* Nup57p	FG, PA Coiled coil	Cytoplasmic and nuclear periphery of the central gated channel	Role in nuclear protein import	[1,60]
p58	*Sc* Nup49p	FG, PA Coiled coil	Cytoplasmic and nuclear periphery of the central gated channel	Role in nuclear protein import	[1,60]
p62	*Sc* Nsp1p *Hydra vulgaris* p62	FXFG Coiled coil	Cytoplasmic and nuclear periphery of the central gated channel; nuclear basket	In complex with p45, p54 and p58; role in nuclear protein import	[1,61]
Nup88	r Nup84	Coiled coil	Cytoplasmic face of the NPC	C-terminal domain contains CAN/Nup214 binding site	[15•,62•]
Nup93	*Sc* Nic96p *Sp* Npp106	Coiled coil	Nuclear periphery of the central gated channel; nuclear basket	role in NPC assembly; in complex with p205	[28•]
Nup98	*Sc* Nup100p, Nup116p, and Nup145p	FXFG, GLFG, FG	Nuclear basket and nucleus	Role in export of snRNAs, 5S RNA, rRNA, and mRNA, but not tRNA; role in import and export of HIV proteins; role in AMLs	[1,23–25,63]
Nup107		Leucine zipper	Unknown		[1]
Pom121	–	FXFG, Transmembrane	NPC core	Anchors NPC to the NE; N-terminal domain required for nuclear targeting; N-terminal and transmembrane domain required for NPC targeting	[1,64]
Nup153	*Sc* Nup1p	FXFG 4 Zn fingers	Nuclear basket	Termination site for nuclear protein import; N-terminus contains targeting and assembly information	[1,9••,52•]
Nup155	*D* Nup154 *Sc* Nup157p, and Nup170p	–	Cytoplasmic and nuclear face of the NPC		[1,65]
gp210	–	Transmembrane	Lumen of the NE	Anchors NPC to the NE; related to autoimmune diseases	[1]
CAN/Nup214	–	FXFG, FG Leucine zipper	Cytoplasmic fibrils	Role in nuclear protein import, mRNA export and cell cycle; involved in AMLs	[1,15•,32,62•]
Tpr (265 kDa)	–	Coiled coil	Nuclear basket and intranuclear filaments	C-terminus essential for nuclear import; N-terminus required for NPC association; possible role in mRNA export or recycling of transport factors; appears in oncogenic fusions with the oncogenes met, trk, and raf	[1,7,18•,19•,20]
RanBP2/ Nup358	–	Ran binding FXFG, FG 8 Zn fingers	Cytoplasmic fibrils	Nucleocytoplasmic transport	[1]

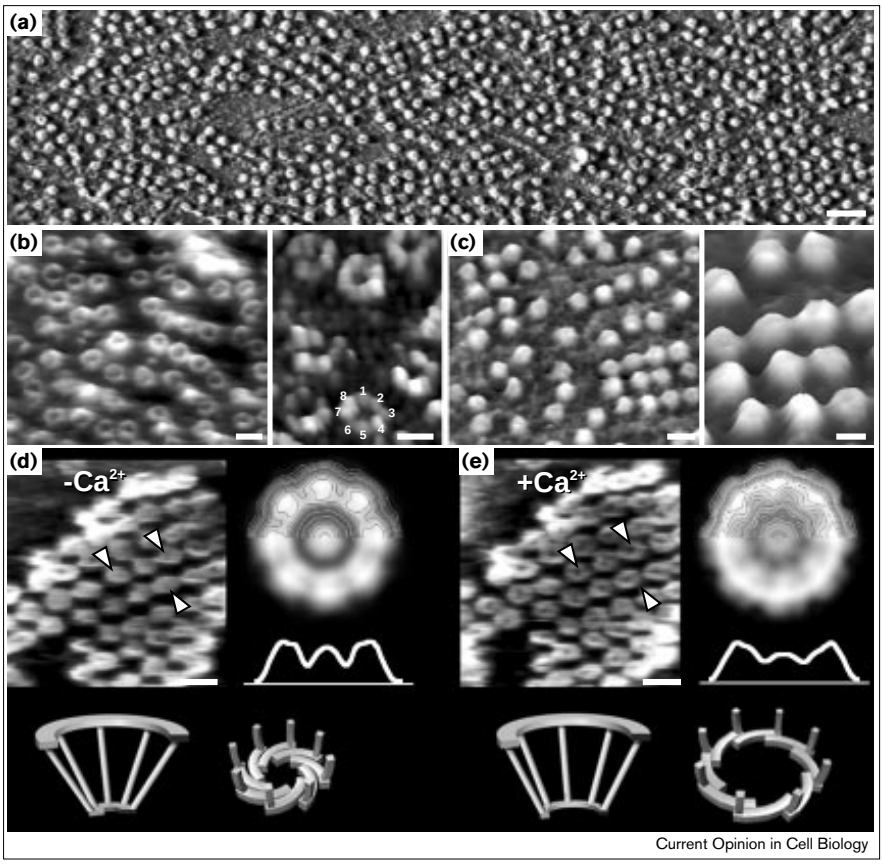
AML, acute myeloid leukemia; Gle, GLFG lethal; Nic, nucleoporin interacting component; NPC core, designation originally by the authors, most likely the central framework; Npl, nuclear protein localization; Nsp, nucleoskeletal-like protein; Nup, nucleoporin; Pom, pore membrane protein; Rip, Rev interacting protein; Seh, Sec13 homologue; Snl, suppressor of Nup116-C lethal; SPB, spindle pole body; Yrb, yeast ran binding.*Numerical assigment reflects either the predicted molecular mass (in kDa) or the genetic identification. †FXFG, GLFG, FG, GSXS, GSSX, GFXS, PA, and WD, repeat motifs represented by single-letter code for amino acids; NES, nuclear export sequence; coiled coil, predicted parallel two-stranded α-helical structure made of heptad repeats. (a) S Siniossoglou, E Hurt, personal communication. (b) B Fahrenkrog and F Stutz, unpublished data.

During mitosis, vertebrate NPCs reversibly disassemble and reassemble. Hence, structural intermediates such as dimples, pores, star-rings, and thin rings, which were identified during NPC reassembly ([26•]; reviewed in [27]), may eventually provide important insights into the morphology and chemical composition of distinct NPC subcomplexes and how these integrate into the mature NPC architecture.

Elucidating the molecular architecture of the nuclear pore complex

To understand the functional role of a particular nucleoporin in molecular detail it is necessary not only to know how it interacts with transport factors and other nucleoporins (see Tables 1 and 2) but also to map its location within the three-dimensional architecture of the NPC. As

Figure 4



(a) Low-magnification overview of a native NE prepared from a *Xenopus* oocyte nucleus and spread on a carbon-coated Parlodion film supported by a copper grid with its cytoplasmic face adsorbed. While kept in buffer, the nuclear surface topography of the NE has been imaged by AFM in tapping mode. Arrays of NPCs forming patches could be observed that did not look different from EM data at this magnification. Scale bar, 500 nm. Higher-magnification views recorded in contact mode of corresponding AFM images revealed a distinct morphology for **(b)** the cytoplasmic and **(c)** the nuclear face of *Xenopus* oocyte NEs prepared and imaged as above. The inset in (b) depicts a high-magnification view of the cytoplasmic face so that the eight-fold rotational symmetry of individual NPCs is becoming resolved. (c) On the nuclear face note the 'remnants' of the nuclear lamina depicted in areas devoid of NPCs. The inset in (c) reveals a high-magnification view of the nuclear face. Both the structures of (b) the cytoplasmic fibrils and (c) the nuclear baskets are apparently too flexible to be resolved at higher detail. Figures (b) and (c) were tilted to 80° using the scanner image processing software to improve the three-dimensional appearance of the NPCs. Scale bars, 200 nm; 100 nm for insets. **(d,e)** Visualization of the reversible calcium-mediated closing ([d] $-Ca^{2+}$) and opening ([e] +100 μM Ca^{2+}) of the nuclear baskets (the distal rings) by time-lapse AFM of the type of specimen displayed in Figure 1a and c. The same specimen area has been imaged in the two distinct conformational states with three corresponding NPCs being marked by arrowheads. As the AFM is a 'surface' sensor and hence forms images of the surface topography of a particle rather than its internal structure, the effect of calcium on the entire NPC was also examined by energy-filtered transmission electron microscopy of completely unfixed/unstained NEs embedded in thick (250 nm) amorphous ice by so-called 'zero-loss' imaging. Single particle averages of 100 NPCs – displayed both as grey-level/± contours representations and radial mass density profiles – each in the presence and absence of calcium, revealed significant structural rearrangements within the entire NPC. In the two models, the distal ring might act as an iris-like diaphragm, as proposed in [1], opening upon addition of micromolar amounts of calcium and closing upon removal of calcium. In support of this possibility, Nup153, a nucleoporin being a constituent of the distal ring and consisting of three distinct domains [52•], was shown to play a role in protein import [9••,10] as well as in mRNA export [17•]. It is conceivable that Nup153, which forms an octameric complex, represents part of a central framework or scaffold of the distal ring. Hence it will be important in the future to dissect the exact role of Nup153 in nucleocytoplasmic transport.

yet, high cross-reactivity of anti-nucleoporin antibodies and limited EM sample preparation protocols have constrained these studies to 12 vertebrate nucleoporins (Figure 3; reviewed in [1]; [28•]).

A new EM sample preparation protocol applied to yeast strains expressing protein A tagged nucleoporins has now enabled the precise localization of a number of nucleoporins to distinct structural components of the yeast NPC (Figure 3; [2••]). Accordingly, Nsp1p and its interacting proteins Nup49p, Nup57p, Nup82p, and Nic96p reside at the cytoplasmic and nuclear periphery of the central gated channel, as well as at the distal ring of the nuclear basket. Although consistent with the known role of these complexes in either nuclear protein import or RNA export (reviewed in [29]), these locations also raise questions. For example, Nic96p anchors the Nsp1p–Nup57p–Nup49p complex, which may occur on either side at the periphery of the central gated channel, to the central framework of the NPC (reviewed in [29,30•]). The function of Nic96p in the newly identified Nsp1p–Nic96p complex at the nuclear basket, as well as the function of the complex itself remain elusive, however [2••]. The putative vertebrate homologue of Nsp1p, p62, has similarly been immunolocalized to the same three distinct NPC sites (reviewed in [1,2••]). In contrast, Nup93, the putative vertebrate homologue of Nic96p, resides only on the nucleoplasmic face of the NPC (Figure 3; [28•]). These differing locations and the limited sequence homologies between several vertebrate nucleoporins and their putative yeast homologues suggest that they may not necessarily represent functional homologues.

Stationary versus mobile nucleoporins

The multiple locations of some nucleoporins, for example, Nsp1p or p62 (Figure 3), indicate that the NPC is not a static but a rather dynamic structure. In this context, Nsp1p may represent a 'mobile' nucleoporin traversing the NPC in complex with cargo. In contrast, 'stationary' nucleoporins (e.g. Nup49p), form part of the structural backbone of the NPC [2••]. Indeed, Nup153 (a component of the nuclear basket; Figure 3) appears to be the first bona fide mobile nucleoporin shuttling between the nuclear and the cytoplasmic face of the NPC in NRK cells, evidently accompanying export cargos toward the cytoplasm [31•]. The location of a particular nucleoporin might not only vary as a function of transport but it might also depend on its expression level [32]. For example, in HeLa cells, overexpressed CAN/Nup214 not only localizes to the cytoplasmic fibrils but also to the nuclear baskets suggesting that multiple binding sites for this nucleoporin exist [32]. Mating assays combined with green fluorescent protein (GFP)-tagged nucleoporins demonstrated that even fully assembled NPCs can evidently cross the NE [33•,34]. The spindle pole body (SPB) too appears to move within the NE and shares at least one protein with the yeast NPC: Ndc1p in *Saccharomyces cerevisiae* [35•] and Cut11p in *Schizosaccharomyces pombe* [36]. This observation, together with the finding that mutations in the divergent actin gene *ACT2* cause defects in NPC structure and nuclear protein import [37], suggests that NPC assembly is coupled to the biogenesis of other cell organelles or compartments, such as the SPB and the cytoskeleton.

Identifying functional states of the nuclear pore complex structure

Distinct changes of NPC substructure, including some observed during NE disassembly [38] or NPC reassembly [26•], have been correlated with nucleocytoplasmic transport. For example, it was suggested that p10, a nuclear import factor, might regulate nucleocytoplasmic transport by modulating the functional size of the gated channel within the NPC during oogenesis [39•]. Moreover, ATP evidently affected NPC size and shape: atomic force microscopy (AFM) revealed a transient change in NPC height and diameter following the addition of ATP [40•]. Similarly, AFM studies indicated that depletion of calcium from the lumen of the endoplasmic reticulum or the NE, which is known to inhibit passive diffusion through the NPC, might cause a switch in NPC conformation ([41]; reviewed in [42]).

Employing time-lapse AFM of native *Xenopus* oocyte NEs in buffer solution, the repeated opening and closing of the nuclear baskets in response to adding and removing micromolar amounts of calcium was monitored [43••], an event most likely involving the basket's distal ring acting as a calcium-sensitive iris-like diaphragm (Figure 4). In contrast, the cytoplasmic NPC topography appeared rather insensitive to calcium, in particular, those NPCs being plugged in the absence of calcium remained plugged upon addition of calcium and vice versa.

The three-dimensional localization of nucleoplasmin and wheat germ agglutinin (WGA) within the NPC [44•], and diffusion studies of colloidal-gold coated polyethylene glycol particles [45] suggested a barrier or gate for nuclear import of cargo residing in the central pore. Three-dimensional reconstruction of ice-embedded *Xenopus* NPCs yielded a transporter occupying the central pore [46], which was also revealed in the three-dimentional reconstruction of yeast NPCs [3••]. A model of its substructure was proposed based on transmission EM (TEM) and FESEM data of *Chironomus* NEs consisting of two central cylinders and two globular assemblies undergoing conformational variations during Balbiani ring particle translocation [5•]. Depending on the isolation and/or specimen preparation procedures employed, however, both the abundance and appearance of the central 'transporter' are highly variable (reviewed in [1]). Hence, to what extent the central plug or transporter is a stationary component of the NPC or whether it represents, at least in part, cargo caught in transit, remains elusive.

Conclusions and perspectives

Identification and functional characterization of nucleoporins is moving quickly and so the molecular composition of the yeast NPC should soon be completely known. Despite much recent progress made toward a better understanding of the three-dimensional architecture of the NPC, the identity, molecular composition or functional significance of some of its components have remained controversial — for example, the central plug and the intranuclear bundles or hollow cables. As the NPC is the major gateway for passive diffusion of ions and small molecules and active transport of proteins, RNAs, and RNP particles in and out of the nucleus (reviewed in [47–49]; see also Adam this issue pp 402–406), identification and characterization of distinct structural states of the NPC will be a prerequisite to directly correlate its structure with function. To achieve this, time-lapse AFM of native NPCs combined with energy filtering TEM of unfixed/unstained samples embedded in thick ice holds great promise (see Figure 4; and discussed in [43••]).

Despite all this progress, determination of the complete three-dimensional molecular architecture of the NPC, hand in hand with a more rational understanding of its functional dynamics will readily consume another 5–10 years, to say the least!

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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A new protocol for preparing yeast cells for electron microscopy was developed which yielded structurally well-preserved yeast NPCs. A direct comparison of yeast and *Xenopus* NPCs revealed that the NPC structure is evolutionarily conserved, although yeast NPCs are 15% smaller in their linear dimensions. With this preparation protocol – and yeast strains expressing nucleoporins tagged with protein A – Nsp1p and its interacting partners Nup49p, Nup57p, Nup82p, and Nic96p were localized by IEM. Accordingly, Nsp1p resides in three distinct subcomplexes that are located at the entry and exit of the central gated channel and at the terminal ring of the nuclear basket.

3. •• Yang Q, Rout MP, Akey C: **Three-dimensional architecture of isolated yeast nuclear pore complex: functional and evolutionary implications.** *Mol Cell* 1998, **1**:223-234.
A three-dimensional map of the yeast NPC from frozen-hydrated specimens was calculated, thereby providing a direct comparison with the vertebrate NPC. Overall, the smaller yeast NPC is comprised of an octagonal inner spoke ring that is anchored within the NE by a novel membrane-interacting ring. In addition, a cylindrical transporter is located centrally within the spokes and exhibits a variable radial expansion in projection that may reflect gating. The inner spoke ring, a transmembrane spoke domain, and the transporter are conserved between yeast and vertebrates; hence, they are required to form a functional NPC. Significant alterations in NPC architecture have arisen during evolution, however, that may be correlated with differences in nuclear transport regulation or mitotic behavior.

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5. • Kiseleva E, Goldberg MW, Allen TD, Akey CW: **Active nuclear pore complexes in *Chironomous*: visualization of transporter configurations related to mRNP export.** *J Cell Sci* 1998, **111**:223-236.
The dynamics of NPC structure in salivary gland nuclei from *Chironomus* during Balbiani ring (BR) particle translocation was investigated by high resolution scanning and transmission electron microscopy revealing evidence of rearrangement of the transporter related to mRNP export. The transporter is an integral part of the NPC and is composed of a central short double cylinder that is retained within the inner spoke ring and two peripheral globular assemblies, which are tethered to the cytoplasmic and nucleoplasmic coaxial rings by eight conserved internal ring filaments. Distinct stages of BR particle nuclear export through the individual NPC components were directly visualized and placed in a linear transport sequence. The BR particle first binds to the NPC basket, which forms an expanded distal basket ring. Furthermore, analysis of the individual NPC components revealed a strong evolutionary conservation of NPC structure between vertebrates and invertebrates.

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9. •• Shah S, Tugendreich S, Forbes D: **Major binding sites for the nuclear import receptor are the integral nucleoporin Nup153 and the adjacent nuclear filament protein Tpr.** *J Cell Biol* 1998, **141**:31-49.
Immunoprecipitation experiments revealed that the nucleoporin Nup153 and the pore-associated filament protein Tpr each form stable subcomplexes with importin α and β in *Xenopus* egg extracts. Nup153 can bind to a complete import complex containing importin α, β, and an NLS substrate, consistent with an involvement of this nucleoporin in a terminal step of nuclear import. Importin β binds directly to Nup153 and can do so at multiple sites in the Nup153 FXFG repeat region *in vitro*. Tpr, which has no FXFG repeats, binds to importin β and to importin α/β heterodimers, but only to those that do not carry an NLS substrate. The GTP analogue GMP–PNP is able to disassemble importin β complexes with either Nup153 or Tpr. Importantly, analysis of extracts of isolated nuclei indicates that complexes between importin β and Nup153 or Tpr exist in assembled NPCs. Thus, Nup153 and Tpr are major physiological binding sites for importin β.

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It was determined that recycling of Kap95p requires a nuclear export signal (NES). A region containing the NES in Kap95p was sufficient to mediate active nuclear export in a microinjection assay. Mutation of the NES in Kap95p resulted in a temperature sensitive import mutant, and immunofluorescence microscopy experiments showed that the mutated Kap95p was not recycled but instead localized in the nucleus and at the NE. The NES mutation abolished Kap95p interaction with the GLFG repeat regions from the nucleoporins Nup116p and Nup100p. *In vivo* interaction was demonstrated by isolation of Kap95p from yeast nuclear lysates in either protein-A-tagged Nup116p or protein-A-tagged Nup100p complexes, supporting a model in which a step in the recycling of Kap95p is mediated by interaction of an NES with GLFG regions.

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A specific karyopherin docking complex was identified within the yeast NPC that contains two novel, structurally related nucleoporins, Nup53p and Nup59p, and the NPC core protein Nup170p. The localization of Nup53p, Nup59p, and Nup170p within the NPC by IEM suggested that the Nup53p-containing complex is positioned on both the cytoplasmic and nucleoplasmic faces of the NPC core. Associated with the isolated complex is the nuclear transport factor Kap121p (Pse1p), mediated by its interaction with Nup53p. Kap121p can be released from Nup53p by the GTP bound form of the small GTPase Ran.

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Nup116p and Nup100p are highly related yeast GLFG nucleoporins, but only Nup116p is stoichiometrically bound to Gle2p, a previously identified mRNA export factor. A short Gle2p-binding sequence within Nup116p (GLEBS) is sufficient and necessary to anchor Gle2p at the NPCs. The GLEBS is evolutionarily conserved and found in rat and *Xenopus* Nup98 and an uncharacterized *Caenorhabditis elegans* open reading frame, but is absent from Nup100p. When the GLEBS is deleted from Nup116p, Gle2p dissociates from the NE and clusters of herniated nuclear pores form. When the GLEBS is inserted into Nup100p, Nup100p–GLEBS complements both the thermosensitive and NPC-herniated phenotype of *nup116*$^{-}$ cells, and Gle2p is retargeted concomitantly to the NPCs.

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CAN/Nup214 associates with two proteins of 88 and 112 kDa, which were cloned and characterized. The 88 kDa protein is a novel NPC component, named Nup88. The localization of Nup88 to the NPC is dependent on CAN binding. The 112 kDa protein is the human homologue of yeast CRM1, that shares a domain of significant similarity with importin β. Human CRM1 (hCRM1) localized to the NPC as well as to the nucleoplasm. Nuclear overexpression of the FG-repeat region of CAN, containing its hCRM1-interaction domain, resulted in depletion of hCRM1 from the NPC. In CAN$^{-/-}$ mouse embryos lacking CAN, hCRM1 remained in the NE. Therefore, hCRM1 might be a soluble nuclear transport factor that interacts with repeat-containing nucleoporins.

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An interaction between karyopherin α2 and the nucleoporin Nup153 was identified using binding assays and their interacting domains were mapped. An *in vitro* assay demonstrated that karyopherin α export was stimulated by

added GTPase Ran, required GTP hydrolysis, and was inhibited by WGA. RanGTP-mediated export of karyopherin α was inhibited by peptides representing the interacting domains of Nup153 and karyopherin α2; moreover, a cryptic fragment of karyopherin β1, termed β1*, inhibited import, but not export, of karyopherin α. Hence, karyopherin α import into and export from nuclei are asymmetric processes.

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Transfection studies of cultured mammalian cells identified a short region within the carboxy-terminal domain of Trp that is essential and sufficient to mediate nuclear import of Tpr and that can also confer nuclear accumulation of the soluble cytoplasmic protein pyruvate kinase. Tpr deletion mutants that contain this nuclear targeting segment but lack the amino-terminal domain appeared evenly dispersed throughout the nucleus without any noticeable association with the NPC. In contrast, the amino-terminal domain lacking the carboxy-terminal region remained located within the cytoplasm, forming aggregate-like structures not associated with the NE. When tagged with the short nuclear targeting segment of Trp or with the nuclear localization signal of the SV40 large T protein, the amino-terminal domain was imported into the nucleus where it then associated with the NPC.

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• **Functional analysis of Tpr: identification of nuclear pore complex association and nuclear localization domain and a role in mRNA export.** *J Cell Biol* 1998, **143**:1801-1812.

Full-length Tpr and several subdomains in mammalian cell lines were expressed and their effects on NPC function examined. An amino-terminal domain was identified to be sufficient for association with the nucleoplasmic aspect of the NPC. The acidic carboxyl terminus was efficiently transported into the nuclear interior mediated by a putative nuclear localization sequence. Overexpression of Tpr caused a dramatic accumulation of poly(A)$^+$ RNA within the nucleus but did not appear to affect nuclear import. Therefore, Tpr might be tethered to intranuclear filaments of the NPC by its coiled-coil domain leaving the acidic carboxyl terminus free to interact with soluble transport factors and mediate export of macromolecules from the nucleus.

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Field emission in-lens scanning EM was employed to examine newly-assembled, growing NEs in *Xenopus* egg extracts thereby revealing rare 'dimples' (outer membrane depressions, 5–35 nm diameter), more abundant holes (pores), pores containing one to eight triangular 'star-ring' subunits, and more complicated structures. Neither mature complexes – nor these novel structures – formed when WGA was added directly to the assembly reaction at high concentrations (~500 μg/ml) . At intermediate concentrations (50–100 μg/ml), WGA caused a dramatic, sugar-reversible accumulation of 'empty' pores and other structures; this effect correlated with the lectin-induced precipitation of a variable proportion of each major *Xenopus* WGA-binding nucleoporin. When 1 mM dibromo-BAPTA, a highly selective calcium chelating agent, was added immediately to the reconstitution assay, no pore-related structures formed. When dibromo-BAPTA was added to growing nuclei 40–45 minutes after initiating assembly, however, star-rings and other structures accumulated, suggesting that dibromo-BAPTA can inhibit multiple stages in pore complex assembly.

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A vertebrate nucleoporin, Nup93, was identified in both human and *Xenopus* that has proved to be an evolutionarily related homologue of the yeast nucleoporin Nic96p. Immunofluorescence and IEM localized vertebrate Nup93 at the nuclear basket and at or near the nuclear entry to the gated channel of the pore. Immunoprecipitation from both mammalian and *Xenopus* cell extracts indicated that a small fraction of Nup93 physically interacts with the nucleoporin p62, whereas a large fraction of Nup93 is present in *Xenopus* egg extracts in complex with a newly discovered 205 kDa protein. The putative human nucleoporin of 205 kDa has related sequence homologues in *Caenorhabditis elegans* and *Saccharomyces cerevisiae*. Nuclei depleted in Nup93 complex were clearly defective for correct NPC assembly.

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To identify factors that regulate NPC formation and dynamics, a novel fluorescence-based strategy was used. NPC proteins were tagged with GFP, and the hypothesis that NPC assembly mutants will have distinct GFP-NPC signals compared to wild-type cells was analyzed by fluorescence-activated cell sorting (FACS).

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A phage display system was employed to identify proteins that interact with transportin 1, the import receptor of shuttling hnRNP proteins, with an M9 nuclear localization signal (NLS). Accordingly, Nup153 harbors an M9 NLS and several import and export receptors interact with Nup153 and in a Ran•GTP-regulated fashion. Whereas Ran•GTP dissociates Nup153–import receptor complexes it is required for Nup153–export receptor complexes. Moreover, Nup153 shuttles between the nuclear and cytoplasmic face of the NPC and thus represents a mobile constituent of the NPC that evidently accompanies export cargo toward the cytoplasm.

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• **yeast.** *J Cell Biol* 1997, **136**:1185-1199.

To investigate the dynamics of NPCs, a live-cell assay in the yeast *Saccharomyces cerevisiae* was developed. The nucleoporin Nup49p was fused to GFP and expressed in *nup49* null haploid yeast cells. When the GFP–Nup49p donor cell was mated with a recipient cell harboring only unlabeled Nup49p, the nuclei fused as a consequence of the normal mating process. By monitoring the distribution of the GFP–Nup49p, fluorescent NPCs were observed to move and encircle the entire nucleus within 25 minutes after fusion. In a mutant strain, where nuclear fusion does not occur, GFP–Nup49p appearance in the recipient nucleus occurred at a very slow rate. GFP–Nup49p labeled NPCs, assembled at 23°C, moved into clusters when the cells were shifted to growth at 37°C, indicating that NPCs can move through the double nuclear membranes and can do so to form NPC clusters in mutant strains.

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It was demonstrated that Ndc1p is a membrane protein of the NE that localizes to both NPCs and spindle pole bodies (SPBs). Indirect immunofluorescence microscopy revealed that Ndc1p displays punctate, nuclear peripheral localization that colocalizes with a known NPC component, Nup49p, and also with a known SPB component, Spc42p. IEM showed that Ndc1p localizes to the regions of NPCs and SPBs that interact with the NE. A shared function of Ndc1p in the assembly of these organelles into the NE was proposed.

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Using nucleoplasmin-coated gold as a transport substrate, it was determined that the shift in synthesis from small to large RNAs during oogenesis is accompanied by an increase in both the rates of signal-mediated nuclear import and the functional size of NCPs. It was observed that, nuclear import in oocytes is limited by translocation factors rather than by cytoplasmic binding factors. Analysis of extracts prepared from early and late stage oocytes revealed that the transport factor p10 is more abundant in early stage cells. Indeed, microinjection of purified p10 into later stage oocytes reduced the nuclear import of large gold particles to the level observed in early stage cells. Evidently, p10 can modulate transport through the pores by regulating the functional size of the central transporter element.

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The effect of ATP on NPC conformation in isolated NEs from *Xenopus laevis* oocytes was investigated using AFM. All experiments were conducted in a saline solution mimicking the cytosol. ATP was added during the scanning procedure and the resultant conformational changes of the NPCs were directly monitored, revealing dramatic conformational changes of NPCs subsequent to the addition of ATP. The height of the pores protruding from the cytoplasmic surface of the NE visibly increased whereas the diameter of the pore opening decreased. The observed changes occurred within minutes, were transient and could represent a NPC 'contraction.'

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Understanding structural changes at the level of individual pores will be a prerequisite to eventually correlate the molecular architecture of the NPC with its distinct functional states during nucleocytoplasmic transport. Time-lapse AFM of native NPCs kept in buffer allowed the direct observation of calcium-mediated structural changes such as the opening (i.e., $+Ca^{2+}$) and closing (i.e., $-Ca^{2+}$) of individual nuclear baskets. Most probably, this structural change of the nuclear basket involves its distal ring, which may act as an iris-like diaphragm. To directly correlate distinct structural features with corresponding functional states and dynamic aspects, the putative role of the 'central plug' or 'transporter' was investigated revealing that in the absence of ATP cytoplasmic plugging/unplugging of the NPC is insensitive to calcium.

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The three-dimensional localization of nucleoplasmin and WGA at the NE of *Xenopus* oocytes was investigated by microinjecting protein coated gold colloids and examining their distribution using both stereo transmission EM and FEISEM. Binding of the WGA gold in the central region of the NPCs appeared to form a barrier, preventing the import of nucleoplasmin gold, and included central localization along radial 'tracks' which correspond to the internal filaments connecting the cytoplasmic ring and the central region of the NPC. It was suggested that these filaments might in some way be involved in opening and closing of the central channel of the NPC for transport. Transport of nucleoplasmin through the central region of the NPCs appeared to be in 'single file' regardless of the size of the colloidal gold, and distribution into the nucleoplasm appeared to be through the basket rings with no association of the nucleoplasmin gold with the basket filaments being observed.

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The yeast nucleoporin Nup82 was sublocalized to the cytoplasmic side of the NPC by IEM. *In vitro* binding assays demonstrated that Nup82 interacts with the C-terminal region of Nup159, a yeast nucleoporin that previously was also localized to the cytoplasmic side of the NPC. It was shown that overexpression of Rss1/Gle1 also partially rescued depletion of Nup82, as previously shown for Nup159p. Nup159 and Nup82 might form a cytoplasmically oriented subcomplex of the NPC that is likely associated with Rss1/Gle1, and this complex is essential for RNA export.

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It was demonstrated that Nup159p specifically interacts through its carboxy-terminal domain with both Nsp1p and Nup82p. A deletion within the carboxy-terminal domain of Nup82p prevents its interaction with Nsp1p, but does not affect the interaction between Nup159p and Nsp1p. Nup82p may act as a docking site for a Nup159p-Nsp1p complex. A previously observed poly(A)$^+$ RNA export defect in *nup82* mutant cells might be due to the loss from the NPCs of Nup159p.

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Nup153 is a large O-linked glycoprotein that is a component of the nuclear basket. The Nup153 molecule has a tripartite structure consisting of N- and C-terminal domains flanking a central zinc finger domain. All of the targeting and assembly information contained within Nup153 is contributed by the N-domain, and can target a cytosolic protein, pyruvate kinase, to the nucleoplasmic face of the NPC. The zinc finger and C-terminal domains appear to have no role in these targeting and assembly activities. Deletion analysis revealed that there are two distinct regions within the Nup153 N-domain that contain different targeting functions. One of these is directly involved in assembly into the NPC while a second overlapping region may target Nup153, as well as other reporter molecules, to the inner face of the NE.

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Nup145p was cleaved *in vivo* to yield two functionally distinct domains: a carboxy-terminal domain (C-Nup145p), which is located at the NPC and assembles into the Nup84p complex, and a GLFG-containing amino-terminal domain (N-Nup145p), which is not part of this complex. Whereas the essential C-Nup145p accomplished the functions required for efficient mRNA export and normal NPC distribution, N-Nup145p was not necessary for cell growth, but becomes essential in a *nup188* mutant background. Generating a free amino-domain is a prerequisite for complementation of this peculiar synthetic lethal mutant. These data suggested that amino- and carboxyl-domains of Nup145p perform independent functions, and that the *in vivo* cleavage observed is of functional importance.

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Immunoprecipitation studies revealed a novel nonglycosylated nucleoporin, Nup84, that is tightly associated with CAN/Nup214, and was found to be exposed on the cytoplasmic face of the NPC. Nup84 contains neither the GLFG nor the XFXFG repeats, but secondary structure predictions suggested that Nup84 contains a coiled-coil carboxy-terminal domain. Mutagenesis and expression studies indicated that the putative coiled-coil domain is required for association with the cytoplasmic face of the NPC, whereas the amino-terminal region of Nup84 contains the site of interaction with CAN/Nup214. Nup84 may function in the attachment of CAN/Nup214 to the central framework of the NPC, and could play a central role in the organization of the interface between the pore complex and the cytoplasm.

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