Nuclear pores and nuclear assembly

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between the nucleus and cytoplasm occurs through large macromolecular structures, the nuclear pores. Quantitative scanning transmission electron microscopy has estimated the mass of a nuclear pore to be 60 million Daltons in yeast and 120 million Daltons in vertebrates. The past two years were noteworthy in that they saw: 1) the purification of both the yeast and vertebrate nuclear pores, 2) the initial description of routes through the pore for specific transport receptors, 3) glimpses of intranuclear organization imposed by the nuclear pores and envelope and 4) the revelation of new and pivotal roles for the small GTPase Ran not only in nuclear import but in spindle assembly and nuclear membrane fusion.

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Current Opinion in Cell Biology 2001, **13**:363–375

0955-0674/01/$ – see front matter

Abbreviations

BAPTA	1,2-bis (o-aminophenoxy)ethane-N,N,N′,N′-tetraaceticacid
ER	endoplasmic reticulum
FG	phenylalanine-glycine
FXFG	phenylalanine-X-phenylalanine-glycine
GFP	green fluorescent protein
GLFG	glycine-leucine-phenylalanine-glycine
LAP	lamin-associated protein
LBR	potential lamin B receptor
NES	nuclear export sequence
NLS	nuclear localization sequence
Nup	nucleoporin
POM	integral membrane protein of the nuclear pore
Ran-GAP	Ran GTPase activating protein
Ran-GEF	Ran GTP exchange factor
SUMO-1	small ubiquitin-like modifier 1
WGA	wheat germ agglutinin

Introduction

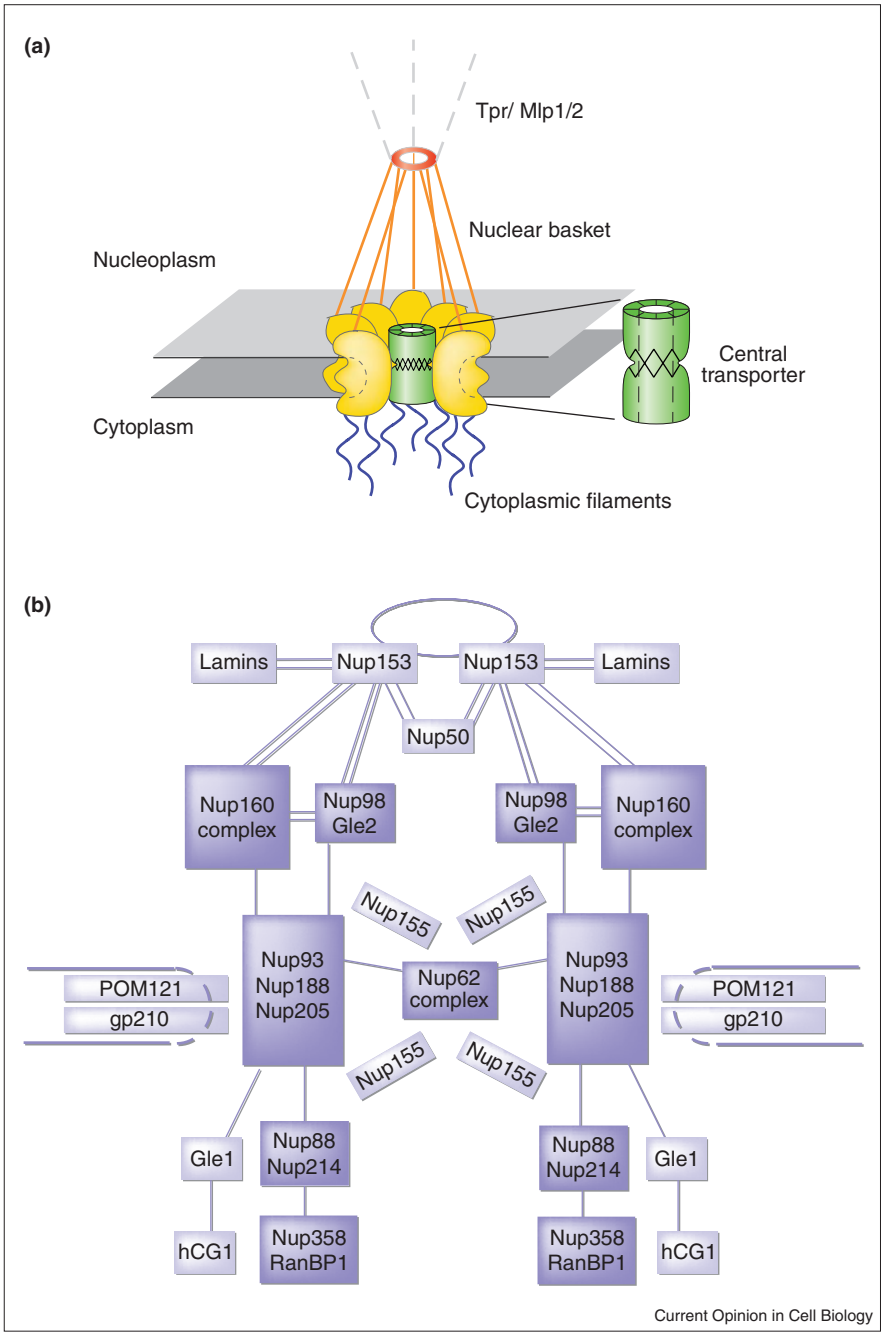
Nuclear pores, cellular megaliths 30 times the size of a ribosome, are the gates for all traffic between the nucleus and the cytoplasm. The nuclear pore consists of two integral membrane proteins and a large cast of nucleoporins recruited from the cytoplasm. Together these are assembled at points of fusion between the inner and outer nuclear membranes. The nuclear pore structure consists of a scaffold of eight spokes, a central transporter, eight cytoplasmic filaments and eight nuclear filaments intersecting at a distal ring, termed the nuclear basket (Figure 1a).

Nuclear import uses soluble receptors that bind and import cargo proteins containing nuclear localization signals or NLSs. The most-studied import receptor is composed of two subunits, importin α and importin β (or karyopherin α and β). Importin α binds to the NLS cargo, whereas importin β binds to nucleoporins. The resulting import complex, composed of importin α, importin β and the NLS cargo, is ~300–1000-fold smaller than the nuclear pore. It is hypothesized to bind sequentially to different nucleoporins as it traverses the large nuclear pore; during this time the import complex is neither distorted nor unfolded. Actual translocation through the pore occurs by an unknown mechanism and requires no obligatory nucleotide hydrolysis (see below). Once an import complex reaches the nuclear side of the pore, the small GTPase, Ran-GTP, binds to importin β, disassembling the complex and completing import. The directionality of import is imparted by two factors: one, the nuclear pore proteins unique to each face of the pore and two, a Ran-GTP/Ran-GDP gradient that is maintained across the nuclear envelope. Specifically, Ran-GTP is found only in the nucleus as a result of the exclusive localization of Ran's exchange factor, Ran-GEF or RCC1, on the chromosomes. In contrast, Ran-GDP is found in the cytoplasm, as a result of the localization of the Ran-GAP on the cytoplasmic filaments of the pore and in the cytoplasm ([1,2]; see also E Conti, E Izaurralde, pp 310–319 of this issue). Energy input is required to set up and maintain this Ran gradient, imparting an indirect energy requirement for nuclear import and export.

In reality, a family of importin β-related receptors is used for the import or export of diverse cellular cargoes. However, Ran-GTP does not disassemble export complexes, but instead stabilizes them. Export complexes consist of an export receptor such as the NES (nuclear export sequence) receptor exportin1/Crm, an NES-containing cargo and Ran-GTP itself. NES–protein export complexes, which are much smaller than the nuclear pore, are thought to bind to various nucleoporins as they transit to the cytoplasm. Other cargoes, however, such as large mRNAs coated with multiple different transport proteins ([3]; see also E Conti, E Izarraulde, pp 310–319 of this issue), thread their way carefully through the pore.

The questions that drive the field include: what are the proteins of the pore — in yeast and in the larger vertebrate pore? How does one define a pore protein? Is a pore protein or nucleoporin a static component dedicated only to pore business or can it transit on and off the pore. Can a pore protein fill other functional roles in the cell? Nucleoporins fitting each of these definitions have been found [4,5,6••]. Why are yeast pores largely symmetrical on both nuclear and cytoplasmic sides, whereas vertebrate pores contain many asymmetrically located proteins? Does this represent a true difference accumulated over the billion years separating yeast and vertebrates or a reflection of our incomplete knowledge? What is the actual translocation mechanism of nuclear import and export? New models are emerging. This review focuses on the structure of the pore, potential

Figure 1



The nuclear pore. **(a)** An artist's rendition of the nuclear pore is shown. Pores are assembled such that the inner and outer nuclear membranes fuse [7]. Both yeast and vertebrate pores have eight large spokes that form the major scaffold of the pore. This scaffold is ~1200 Å in diameter by 800 Å in height in vertebrates, and 960 Å in diameter by 350–380 Å high in yeast [7]. The spokes surround a central transporter (~350–420 Å in diameter by 625 Å in height in vertebrates, and 350–360 by 300 Å in yeast). Eight filaments of ~500 Å extend into the cytoplasm. On the nuclear side of the pore, eight long filaments (~1200 Å in vertebrates, 950 Å in yeast) connect at their distal end to a small ring [31]. This structure is termed the nuclear pore basket. Pore-associated filaments (dotted lines) extend from the basket of the pore into the nucleus and contain the proteins MLP1/2 in yeast and Tpr in vertebrates [66]. The yeast pore has a similar overall structure but is smaller (see above) [7]. The spokes of the yeast pore are abbreviated and have fewer domains, whereas the central transporter may contain one chamber rather than two [7]. A hypothetical FG nucleoporin meshwork [53••] is drawn across the equator of the central transporter in the figure to potentially fit with vertebrate experimental results [49,53••]. **(b)** A hypothetical model of the vertebrate nuclear pore is presented incorporating the known pore subcomplexes (boxes). The data from a number of immunolocalization and biochemical studies has been collated in the model (see also Table 2). The Nup62 subcomplex contains Nup62, Nup58, Nup54 and Nup45 (Table 2), whereas the Nup160 complex minimally contains Nup160, Nup133, Nup107, Nup96, sec13 and possibly a vertebrate homologue of ySeh1p (Table 2; [30] S Vasu, D Forbes, unpublished data). Double lines indicate experimentally demonstrated connections between subcomplexes; single lines indicate hypothetical connections. Confirmed interactions between Nup153 and Nup50 [48•], Nup153 and the Nup160 complex (S Vasu, D Forbes, unpublished data), and Nup62 and Nup93 [65] are shown. Not shown is a second subcomplex between Nup62 and Nup214. It is important to note that although certain subcomplexes are drawn on different filaments in the figure, it is very likely that all basket subcomplexes are aligned one after another on a basket filament and all cytoplasmic complexes are aligned linearly on a cytoplasmic filament. Placement of the Nup93–Nup188–Nup205 complex and Nup155 near the center of the pore is hypothetical. Arguments for doing so are based partly on vertebrate and partly on yeast experimental findings (see text). The reader is referred to [6••] for a recent working model of the yeast pore. In both yeast and vertebrates, each subcomplex is predicted to be present in at least eight copies per pore and possibly up to 56 copies per pore.

mechanisms of import, nuclear assembly and newly discovered roles for the transport factor Ran-GTP.

Structure and components of the yeast nuclear pore

A seemingly comprehensive description of the components of the yeast nuclear pore was published in 2000 ([6••]; Table 1). This study was provocative both in the way it significantly moved the field forward and in the way it pinpointed controversial areas that await more definitive answers. Purified *Saccharomyces cerevisiae* nuclear pores [7] were subjected to two dimensional gel analysis. Mass spectroscopy sequence analysis was performed on over 400 individual protein spots. Using a definition of a pore

Table 1

Yeast nucleoporins.

			Putative vertebrate homologues			
Yeast Nup	Location in yeast	FG repeats	Strong	Weak	Very distant	YPD and recent vert refs
yNup1	Nuclear	FG			FGs	YPD
yNup60	Nuclear	FN				YPD; [6••]
yNup2	Nuclear	FG			FGs	YPD
yNup133	Both				**vNup133**	YPD; Vasu *et al.*
yNup120	Both				**vNup160**	YPD; Vasu *et al.*
yNup85	Both				?	YPD
ySec13	Both		vSec13			YPD; [29•]; Vasu *et al.*
ySeh1	Both		vSeh1??			YPD; [29•]
yNup84	Both			vNup107		YPD
yNup145-C	Both			**vNup96**		YPD; [29•,42•]
yNup145-N	Bias to nucleus	GLFG		vNup98		YPD
yNsp1	Both	FG	vNup62			YPD
yNup49	Both	GLFG			vNup58?	YPD
yNup57	Near center	FG			vNup54	YPD
yNic96	Both		vNup93			YPD
yNup188	Both			**vNup188**		YPD; [33]
yNup192	Both			vNup205		YPD, [62]
yNup157	Both			vNup155		YPD
yNup53	Both	FG - 4			?	YPD
yNup59	Near center	FG - 6			?	YPD
yNup170	Near center			vNup155		YPD
yPOM152	Intgr memb				none	YPD
yPOM34	Intgr memb				none	YPD; [6••]
yNdc1	Intgr memb				?	YPD
yCdc31	Intgr memb				?	YPD; [6••]
yNup100	Bias to Cytoplasmic side	GLFG		vNup98		YPD
yGle1	Bias to Cytoplasmic side		vGle1			YPD
yNup42 (Rip)	Cytoplasmic	FG		hCG1		YPD; [101]
yGle2 (Rae1)	Both		vGle2			YPD
yNup116	Bias to cytoplasmic side	GLFG		vNup98		YPD
yNup82	Cytoplasmic				vNup88?	YPD
yNup159	Cytoplasmic	FG			FGs	YPD

Yeast (Table 1) and vertebrate (Table 2) nucleoporins are listed. The yeast nucleoporins are grouped on the vertical axis by their presence in subcomplexes of the pore. (The yeast integral membrane proteins are grouped together only for convenience. They have not been demonstrated to associate with one another.) Where a Nup exists in more than one complex, only the most abundant is shown. An effort is made to place complexes in an order that indicates potential neighboring complexes. New nucleoporins discovered in 1999–2001 are marked in bold. Parentheses mark an alternative name for a nucleoporin. Location indicates nuclear side of the pore (Nuclear), both sides (Both) or other locations within the pore. For each yeast nucleoporin (Table 1), 'Strong' homology indicates a case where a sequence is highly related for a significant portion of its length. 'Weak' indicates a case where homology is seen in a portion of the protein sequence, although even this region is not highly conserved. 'Very distant' homology denotes genes that take multiple iterations with Psi-Blast to reveal even small regions of homology. '?' or 'None' indicates cases where no homologues can or have been found. This is generally the case for the POMs and the cytoplasmic filament and basket FG nucleoporins, except that the latter contain common FG repeats, denoted 'FGs'. Vertebrate Nup98 and its yeast relatives Nup100, Nup116, and Nup145 are related in blocks in different parts of their sequences [2]. YPD indicates that the reader is referred to http://www.proteome.com/databases/YPD/reports/NSP1.html, where the protein sequences, comprehensive descriptions, experimental findings, and links to the original references can be found for each yeast Nup, simply by inserting the nucleoporin gene name (i.e., 'NUP157') in the place of 'NSP1' in the above web address. It should be noted that genes referred to as 'Related genes' in the Yeast Proteome Database often are related only by possession of common FG repeats. Original references to previously described vertebrate nucleoporins can be found in the Tables in [2,31] or by searching for a specific Nup at http://www.ncbi.nlm.nih.gov/PubMed/. For the newly discovered Nups (bold), references are as noted in the last column. 'Vasu *et al.*' indicates S Vasu, D Forbes, unpublished data.

protein as one found exclusively in the purified pore preparation and not in other cellular fractions, 30 yeast nuclear pore proteins were defined. Extensive genetic and biochemical studies in a number of laboratories had previously identified 27 of these as yeast nucleoporins (Table 1). Three new nucleoporins were revealed in the study,

Table 2

Vertebrate nucleoporins.

Nucleoporin	Location in vertebrates	FGs?	Yeast homologue?	References
vNup153	Nuclear basket ring	FG	FGs	[2,31]
vNup50	Nuclear basket	FG	?	[46•,47]
vNup160	Nuclear side		Very distant	Vasu *et al.*
vNup133	Nuclear side		Very distant	Vasu *et al.*
vNup107	Nuclear side		Weak	[2,31]
vNup96	Nuclear side		Weak	[29,42•]
sec13			Strong	[29•]
vNup98	Nuclear basket	GLFG	3 Related	[2,31]
vGle2 (Rae1)			Strong	[2,31,39]
vNup155	Both sides		Weak	[2,31]
vNup188	Spokes?/nuclear		Weak	[2,31,33]
vNup205	Spokes?/nuclear		Weak	[2,31,63]
vNup93	Spokes?/nuclear		Strong	[2,31,63]
vNup62	Central transporter?	FG	Strong	[2,31]
vNup58	Central transporter?	FG	Very distant	[2,31]
vNup54	Central transporter?	FG	Very distant	[2,31]
vNup45	Central transporter?	FG	?	[2,31]
vPOM121	Integral membrane	FG	None	[2,31]
v gp210	Integral membrane		None	[2,31]
vNup88 (84)	Cytoplasmic filaments		Very distant	[2,31]
vNup214 (CAN)	Cytoplasmic filaments	FG	FGs	
vGle1	Cytoplasmic filaments and ?		Strong	[2,31]
vNup358 (RanBP2)	Cytoplasmic filaments	FG	FGs	[2,31]
RanBP1	Cytoplasmic filaments		Strong	[2,31]
hCG1	Cytoplasmic filaments	FG	Weak	[2,31,101]

See legend for Table 1.

Nup60p, POM34p and cdc31p (bold type, Table 1). Nup60 localizes to the yeast nuclear pore basket. POM34p joins POM152p as one of two integral membrane proteins (POMs) exclusive to the yeast nuclear pore. Cdc31p and the previously discovered Ndc1p prove to be integral membrane proteins but with a dual purpose. These proteins are present in both yeast nuclear pores and spindle pole bodies [6••,8]. A soluble protein with a dual function, sec13p, originally identified as playing a role in secretion, was recently discovered to also be a component of a pore subcomplex required for RNA export (Table 1; [9,10•]). Each yeast Nup is present in 8 to 56 copies per pore [6••]. The derived total mass matches the predicted mass of the yeast pore (60 million Daltons) fairly well [7]. From these and other studies, we now have a good grasp of what may be the complete components of the yeast nuclear pore (Table 1). However, this number may expand if additional dual-purpose nucleoporins or even loosely associated nucleoporins are discovered. For example, Nup2 was not included by the Rout definition [6••], but several groups find Nup2 to be a nucleoporin with specific functions in the pore (see below).

Using electron microscopy (EM) and Protein A-tagging, an undertaking was made to map individual nucleoporins within the yeast pore [6••]. Surprisingly all but five yeast nucleoporins were found to be symmetrically located on both sides of the pore, despite the fact that the two faces of the yeast pore differ markedly in structure (Figure 1a; [7,11]). Previous sequence analysis by many groups had revealed that a third of the yeast nucleoporins contain phenylalanine–glycine or FG repeats (Table 1), either as FG, FXFG or GLFG repeats (collectively referred to as FG repeats). Different FG nucleoporins are major sites of interaction for specific transport factors (extensively reviewed in [2]). Rout *et al.* [6••] found that the FG-containing Nups were localized throughout the yeast pore. Unfortunately, when the Nups were examined by EM they did not localize over the bulk of the protein scaffold, as the antibodies cannot access the tightly packed interior of the pore structure without simultaneously destroying it. Thus, the map of the yeast nucleoporins [6••] coincides only with the exposed edges of the nuclear pore. A different structural order may lie beneath the surface than we at present suspect. For example, using pre-embedding immunogold electron microscopy, Aebi and colleagues [11] find that yeast Nsp1p is a member of three different subcomplexes mapping to three separate sections of the yeast pore. Nic96 and Nup53 also map to three locations within the yeast pore [12].

Only a small group of yeast nucleoporins are localized to one side of the pore or the other [6••,13,14]. Nup60p, Nup1p and Nup2p are found exclusively on the nuclear basket of the yeast pore, and the GLFG-containing protein Nup145Np is biased to that side [6••,13,14]. Such 'sidedness' of the pore, whether conferred by a few proteins, as in yeast, or by a greater number of proteins, as in vertebrates (Tables 1 and 2), imparts a directionality to transport in an unexpected way. Instead of import complexes having the highest affinity for the cytoplasmic side of the pore, they appear to show a graded affinity with the strongest binding to proteins of the nuclear pore basket [15–17]. Specifically, yeast basket nucleoporins Nup1p and Nup2p bind incoming importin–karyopherin-α import complexes [13,14], and vertebrate Nup153 on the terminal ring of the pore basket binds importin-α–β–NLS import complexes at high levels [16,17].

During the early stages of export, basket nucleoporins also interact with recycling import factors and export receptors. For example, the yeast basket protein Nup2p binds to recycling importin α, whereas Nup1p binds to the export factor Msn5 [13,14,18•]. However, in the late stages of export, transport factors and export receptors interact with nucleoporins on the cytoplasmic filament side of the pore, both in yeast and vertebrates. Yeast Nup159p, Nup82p and Nup42p are found exclusively on the cytoplasmic face of the yeast pore [6••], and Nup116p and Nup100p are biased to that side [6••,19–21]. Nup159p interacts with RNA helicase DBP5p and other factors involved in the last step(s) of mRNA export ([22,23]; see also references in the review by E Conti, E Izaurralde, pp 310–319 of this isue), whereas yeast Nup116p and Nup100p interact with the transport factor Mex67 [19]. In vertebrates, Nup214 on the cytoplasmic filaments binds to NES/Crm1 export complexes in what is presumed to be the export complex's last binding site on the way out of the pore (see, for example, [24–26]). Here RanBP1 (Ran-binding protein 1) causes hydrolysis of RanGTP, disassembling the export complex and releasing the receptor from the cytoplasmic filament nucleoporins [25]. Thus, both yeast and vertebrate nuclear pores fit a paradigm where the nuclear basket seems to contain strong binding sites for many (but perhaps not all) import complexes as they move into the nucleus, whereas the cytoplasmic filaments contain strong binding sites for export complexes as they move toward the cytoplasm.

All of the binding-site nucleoporins mentioned above contain FG repeats. The interaction of such repeats with transport factors is more fully discussed in [2] and Conti and Izaurralde (pp 310–319 of this issue). Recently, an impressive fluorescence resonance energy transfer (FRET) study, using tagged nucleoporin and receptor protein pairs in living yeast cells, identified numerous contacts that two specific transport receptors make as they pass through the pore [18•]. In addition, a novel FXFG nucleoporin SpNup124p (observed to date only in *Schizosaccharomyce pombe*) is required for the nuclear import of the *S. pombe* retrotransposon Tf1 [27].

Structure and components of the vertebrate nuclear pore

Approximately 23 vertebrate nuclear pore proteins have been identified using biochemical association, immunological means, functional assays or through interaction with known nucleoporins (Table 2). From mass estimates and purification studies, it is estimated that 30–50 different proteins are present [2,28,29•,30••,31]. However, from purification studies of the vertebrate pore a subset of this number are likely to be transport factors [30••], further reducing the expected number of different vertebrate nucleoporins. These pore proteins are found in a set of approximately 12 subcomplexes into which the pore disassembles at mitosis and from which it is reassembled at the end of telophase (Figure 1b; Table 2; e.g., see [32–34]). New pores are also assembled from pore subcomplexes during S phase, when the pore number is known to double in vertebrates.

The nuclear basket of the vertebrate pore contains Nup153, Nup98/Gle2, Nup50 and a new Nup160 subcomplex containing Nup160–Nup133–Nup96–Nup107 and accompanying proteins (Table 2; Figure 1b). Potential members of the central scaffold of the pore are the Nup93–Nup205–Nup188 complex and Nup155 (see below). The Nup62–Nup58–Nup54–Nup45 subcomplex maps in or near to the central transporter region. To date, the cytoplasmic filaments are known to contain Nup214, Nup88, Nup358, RanGAP and RanBP1 [2]. A specific ubiquitin-like SUMO-1 modification targets RanGAP to Nup358 on the cytoplasmic filaments [35]. The integral membrane proteins gp210, with a large lumenal domain and a short cytoplasmic domain, and POM121, with a small lumenal domain but a large FG-containing cytoplasmic domain, complete the list of known vertebrate pore proteins and are thought to anchor the pore within the membrane. As in yeast, each subcomplex might be expected to be present in 8 to 56 copies per pore, giving 500–1000 total proteins per pore.

Many of the latest findings on the vertebrate pore have focused on the pore basket. Nup153, at the distal ring of the basket, appears to play a role in initial nuclear membrane and/or lamina assembly, prior to pore formation [34]. In the pore, Nup153 is important both for terminal steps of import and for RNA export [5,16,17,36,37]. Some evidence indicates that Nup153 shuttles between the nucleus and the cytoplasm [4]; other evidence indicates a more stationary role [5]. Nup98 is present in the pore basket [2,31] and is involved in RNA export [38]. Nup98 contains a unique binding site for the transport factor Rae1/Gle2 [39] and binds other transport factors, such as TAP and Crm, on its amino-terminal GLFG repeats [40,41]. The unique carboxyl terminus of Nup98 is the binding site for the newly discovered Nup160 complex of nucleoporins, a complex that includes the novel vertebrate nucleoporins Nup160 and Nup133 (S Vasu, D Forbes, unpublished data), as well as Nup96, Nup107 and sec13 [30•,43].

Nup98 is unique among vertebrate pore proteins in that it is derived from a protein precursor containing two distinct nucleoporins. An autoproteolytic activity contained within the Nup98 sequence cleaves the precursor into Nup98 and Nup96, the latter being a newly discovered vertebrate nucleoporin that maps to the basket of the pore [29•,42•]. This cleavage has been conserved through evolution: yeast Nup145p is cleaved into two nucleoporins related to vertebrate Nup98 and Nup96 (Nup145-Np, Nup145-Cp; see [43] and references therein). Interestingly, the GLFG region of Nup98 is an important target in vesicular stomatitis virus (VSV) viral infection. Specifically, VSV matrix protein binds to Nup98 [44••] and shuts off host mRNA export [44••] and nuclear protein import [45•].

Two additional vertebrate nucleoporins emerged last year, Nup50 [46•] and Nup188 (see below; [33]). Nup50 is an FG repeat-containing nucleoporin localized to the pore basket and is involved in Crm-mediated NES-export but not NLS import [46•]. Biochemical experiments argue that Nup50 is an early binding site for outgoing NES export complexes [46•]. It interacts with its neighbor, Nup153, and with the cell cycle inhibitor p27(Kip1) [47]. However, Nup50 null fibroblasts do not show major cellular defects [47], perhaps indicating that this receptor binding nucleoporin is functionally redundant. Certainly functional redundancy between the FG nucleoporins has been observed in yeast [2]. On a separate note, it is interesting that immunofluorescence of Nup50, Nup98, Nup96 and Nup153 all show an intranuclear stain in addition to a nuclear pore stain [4,5,29•,46•]. Whether this is due to these nucleoporins performing a shuttling role in transport or because they play additional roles within the nucleus is not yet known.

Comparison between yeast and vertebrate pore proteins

Drawing direct analogies between the proteins of yeast and vertebrate pores is not easy. A few nucleoporins are reasonably well conserved: these are yNic96/vNup93, yNsp1/vNup62, yNup157/170–vNup155 and interspecies pairs of the small proteins Gle1, Gle2 and sec13 ('Strong homology', Table 1). The yeast GLFG nucleoporins Nup145Np, Nup116p and Nup100p all resemble blocks of the single mammalian GLFG nucleoporin, Nup98. Six other yeast nucleoporins have weak to moderately conserved versions in vertebrates ('Weak homolgy'; Table 1). Five yeast Nups have extremely distant vertebrate homologues where relatedness is difficult to discern ('Very distant homology'; Table 1; D Forbes, unpublished data). Other yeast nucleoporins have no sequence relatives but most probably have functional orthologs (for example, yeast POM152p and POM34p). Nup153, Nup214 and Nup358, vertebrate nucleoporins central to transport, have no yeast sequence homologues. Nup153 and Nup358 both contain zinc finger domains, which are not observed in yeast nucleoporins. However, there are FG nucleoporins in the same general regions of the yeast pore (yeast Nup1p, Nup2p, Nup60p and Nup159p), and these are probably functional orthologs of vertebrate Nup153, Nup214 and Nup358.

Interestingly, even though the pores of yeast and vertebrates contain such divergent proteins, the subcomplexes into which the pores disassemble have in many cases been maintained throughout evolution (Tables 1 and 2). In addition, extremely distantly related yeast/vertebrate protein pairs are often of identical size, as if the greater puzzle into which the piece fits allows no other size. Exceptions to this size rule are ScNup120 and the very distantly related vNup160, which contains an extra 33 kDa (S Vasu, D Forbes, unpublished data; Table 1). At present, it would not be surprising to us to find that the vertebrate pore contains a similar number of proteins in its orchestra as the yeast pore. What is confusing is why the instruments have diverged so much (Tables 1 and 2), why their placement in the orchestra appears to have changed, and why the vertebrate pore structure appears so much larger ([7]; see legend to Figure 1a).

Potential models for translocation

In scanning and transmission EM, the central transporter of the pore appears to be a distinct central entity [7,48]. Small gold particles pass directly through a central 90 Å channel in the 1200 Å structure of the pore [49•]. Larger NES- or NLS-tagged gold particles and mRNP particles also translocate through this channel, somehow inducing its effective diameter to expand up to ≥260 Å [49,50]. This visualization of a single central passageway is consistent with biochemical data from a novel *in vitro* system that assays passive diffusion, nuclear import, and nuclear export through single nuclear pores using optical recording [51,52]. One recent model for how the central transporter functions is based on the fact that yeast FG nucleoporins are found throughout the pore and that FG nucleoporins have affinity for transport receptors. The 'Brownian ratchet' model proposes that the transporter is composed of many waving FG filaments that have little structure or defined conformation [6••]. Import or export complexes could potentially be able to enter a channel of such filaments because of their affinity for the FG repeats in the channel nucleoporins. Other proteins not escorted by a transport receptor would be deflected by the postulated waving filaments.

More recently, a second theoretical model has been proposed, termed the selective phase model [53••]. In this model, the FG repeats of the channel nucleoporins are proposed to biochemically interact to form a meshwork across the central plane of the pore (Figure 1a). The meshwork would act as a permeability barrier impervious to non-nuclear proteins. In contrast, transport receptors with their cargo would become 'soluble' in the meshwork, owing to low affinity interactions with the channel FG nucleoporins, and emerge on the opposite side of the pore [53••]. The selective phase model was proposed to explain the extremely rapid nuclear entry of empty transportin receptors (~1000 per pore per second) and other import factors.

Which nucleoporins could conceivably form a channel of FG filaments or an FG meshwork permeability barrier? Structurally, vertebrate nuclear pores have 10 FG nucleoporins identified to date: Nup153, Nup98 and Nup50

located on the basket, a complex of Nup62–Nup58–Nup54–Nup45 located in or near the central transporter and Nup214 and Nup358 on the cytoplasmic filaments (Table 2). (The integral membrane protein POM121 also contains FG repeats.) The FG nucleoporins found in the basket and the cytoplasmic filaments are seemingly too distant from the pore's point of selectivity (and probably too widely spaced) to play a role in excluding inappropriate protein traffic. In reality, this has been experimentally demonstrated by gold particle injections [49]. The observed point of selectivity for the pore coincides with the exact equator of the central transporter, hundreds of angstroms internal from the major portions of the basket and cytoplasmic filaments (see figures in [49]). Thus, the Nup62–Nup58–Nup54–Nup45 complex would seem to be the major source of FG repeats in the central transporter region. As reconstituted nuclei lacking the Nup62 complex are defective for import [54], the Nup62 complex may well play the pivotal role in such FG models. With reference to this, Nup62 shows a very low but detectable affinity for importin-α–β–NLS cargo complexes compared to that seen for Nup153 and Nup358 [16], consistent with the central FG Nups of the pore being sites of low affinity interaction, as proposed in the selective phase model. The related yeast NSP1p complex is also quite important for nuclear transport [55]. Although proof for the two FG models lies ahead, they have already stimulated lively discussion.

Assembly of the nuclear pore

The great majority of pore proteins are synthesized as cytoplasmic proteins that then assemble at points of fusion between the nuclear membranes. In yeast, which has a closed mitosis, the nuclear envelope does not break down and the assembly of new pores occurs throughout the cell cycle [56]. In vertebrates, the number of nuclear pores doubles at S phase. These pores are disassembled at mitosis and must reassemble within the nuclear membrane during telophase, with nuclear membrane assembly preceding nuclear pore assembly [57].

The vertebrate nuclear pore possesses only two known integral membrane proteins, gp210 and POM121, whereas there four are known in yeast, POM152p, POM34p, ndc1p and cdc31p (Tables 1 and 2). One or more in each species could initiate fusion between the outer and inner nuclear membranes to assemble a new nuclear pore. Immunofluorescence on vertebrate cells, examining the order in which different proteins return to the nuclear envelope at the end of mitosis [58•,59], found that the integral membrane protein POM121 is one of the earliest associating proteins [58•]. This suggests that POM121 might be the protein required for the membrane fusion step of pore assembly [58•]. gp210 associates with the pore much later, perhaps downplaying a role for gp210 in fusion. However, it may be that a subpopulation of gp210 initiates fusion between the two nuclear membranes, but the bulk of gp210 accumulates at the pore at a later time — to provide stability for the pore, an anchoring function for other nucleoporins, or for a role not yet understood. In yeast it is not known which POM initiates fusion and which play ancillary roles.

Once the membrane fusion step of pore assembly has occurred, the major scaffold of the pore must be built within the resulting cavity. High resolution scanning EM reveals a number of potential intermediates in pore formation that can be ordered logically (if not yet experimentally) as dimples, then holes, within which a 'star-ring' of eight spokes appears, followed by the addition of higher order pore structures [60]. The assembly of nuclear pores can be disrupted by the chemical inhibitor BAPTA, by GTPγS or by the lectin WGA at early intermediate steps [57,60]. To date, the pore proteins that form the spokes of the major scaffold of the pore are not known. However, in yeast immunoEM suggests that Nup 57p, Nup170p, Nup188p and Nup192p lie nearest to the central plane of the pore [6••].

Insight into central elements of the pore can be derived from determining the effects on pore assembly after the experimental removal of particular pore porteins. Yeast Nup188p is an abundant protein that interacts genetically with Nic96p, Nup170p, Nup192p and POM152p [61,62]. Together these are the most abundant proteins of the yeast pore. Disruption of yeast Nic96 or Nup192 genes by temperature-sensitive mutation drastically reduces the number of pores assembled per nucleus [56], suggesting that yeast Nic96p and Nup192p play a role in pore assembly and/or in forming the major structures of the pore. In vertebrates, there are three proteins related to yeast Nic96p, Nup188p and Nup192p; they also exist in a complex of vertebrate Nup93, Nup188 and Nup205 (Figure 1b; [33,63]). Immunodepletion of the vertebrate complex from *Xenopus* nuclear reconstitution extracts, followed by nuclear assembly, results in nuclei containing fewer pores, again arguing that these proteins play a role in pore assembly [63]. Lastly, yeast mutants deleted for Nup188 or Nup170 have been analyzed functionally and show higher rates of passive diffusion [64], consistent with the proteins playing a role in establishing the resting diameter of the central transporter.

Interactions between pore proteins and chromatin or lamina proteins may well play a role in nuclear pore assembly. However, neither is necessary for pore assembly itself. Structures identical to nuclear pores can form in double membrane stacks, termed annulate lamellae, either in *Xenopus* egg extracts or in the cytoplasm of somatic cells (see [30••,65] and references therein). So abundant are the annulate lamellae formed *in vitro* that they have been used as the source for purification of the first intact vertebrate nuclear pores [30••]. Many of the same nucleoporins can be visualized in a preparation of pore proteins derived from selective solubilization of rat nuclear envelopes [29•]. Knowledge of the full complement of vertebrate pore proteins is perhaps not far off.

Pore-associated filaments and higher order function within the nucleus

Attached to the nuclear pores are filaments leading into the nucleus (dotted lines, Figure 1a). Major constituents of these filaments are the myosin-like proteins MLP1/2p in yeast and Tpr in vertebrates ([66,67••] and references therein). In MLP1/2 null mutants, telomeres are no longer localized to the nuclear periphery, double-strand DNA break repair is impaired, and telomeric gene silencing is reduced [67••]. All these normally take place at the nuclear periphery. Deletion of the yeast nucleoporin gene Nup145 gives the same defects as an MLP1/2 null strain [67••]. These result imply that Nup145p participates in tethering the MLP filaments to the pore and that MLP tethering is a prerequisite for a number of distinct nuclear functions.

Nuclear assembly and disassembly

Animals from *Drosophila* to man undergo open mitosis. Chromosomes condense, the nuclear lamina disassembles and the nuclear membranes either partially (flies) or completely (vertebrates) disappear. The 120 million Dalton nuclear pore is disassembled into subcomplexes of up to a million Dalton (Figure 1b). Pore breakdown is initiated by direct phosphorylation of a subset of nucleoporins by mitotic cdc2/cyclin B kinase [2,31,32,68–72]. Examination of mitotic nuclear pore subcomplexes has been used to reveal nearest neighbor interactions within the pore ([24,29•,32,33,69,73]; S Vasu, D Forbes, unpublished data), as well as to reveal strong interactions of Nups with import and export factors [2,16,24].

EM had first suggested that metazoan nuclear membranes become vesiculated at mitosis. Certainly inner nuclear membrane proteins and POMs are present in small vesicles in the extracts of mitotic cells (see below and [74] with references therein). Recently, however, an elegant study in living cells using a GFP-tagged version of the inner nuclear membrane protein LBR (a potential lamin B receptor) demonstrated that the inner and outer nuclear membranes do not vesiculate at mitosis *in vivo*. Instead, in this study [75•] and in a confocal immunoEM study [76•], the nuclear membranes were seen to retract into the endoplasmic reticulum (ER) at mitosis [75•,76•,77]. It is surmised that inner nuclear membrane proteins that normally bind to chromatin are phosphorylated at mitosis and release their chromatin partners, allowing the freed nuclear membrane sheets to retract into the ER to which the outer nuclear membrane is connected. As mitosis ends, the inner nuclear membrane proteins become dephosphorylated, rebind to their chromatin or lamin partners and once again draw the nuclear membranes around the chromosomes. The membrane proteins involved in this process probably include a number of recently discovered inner nuclear membrane proteins, such as emerin, LAPs (lamin-associated proteins), different lamin isoforms, otefin, LBR, nurim and MAN1. These proteins and their role in nuclear assembly and human disease have been the subject of recent reviews to which the reader is referred for an in depth discussion of this increasingly interesting area [77–80].

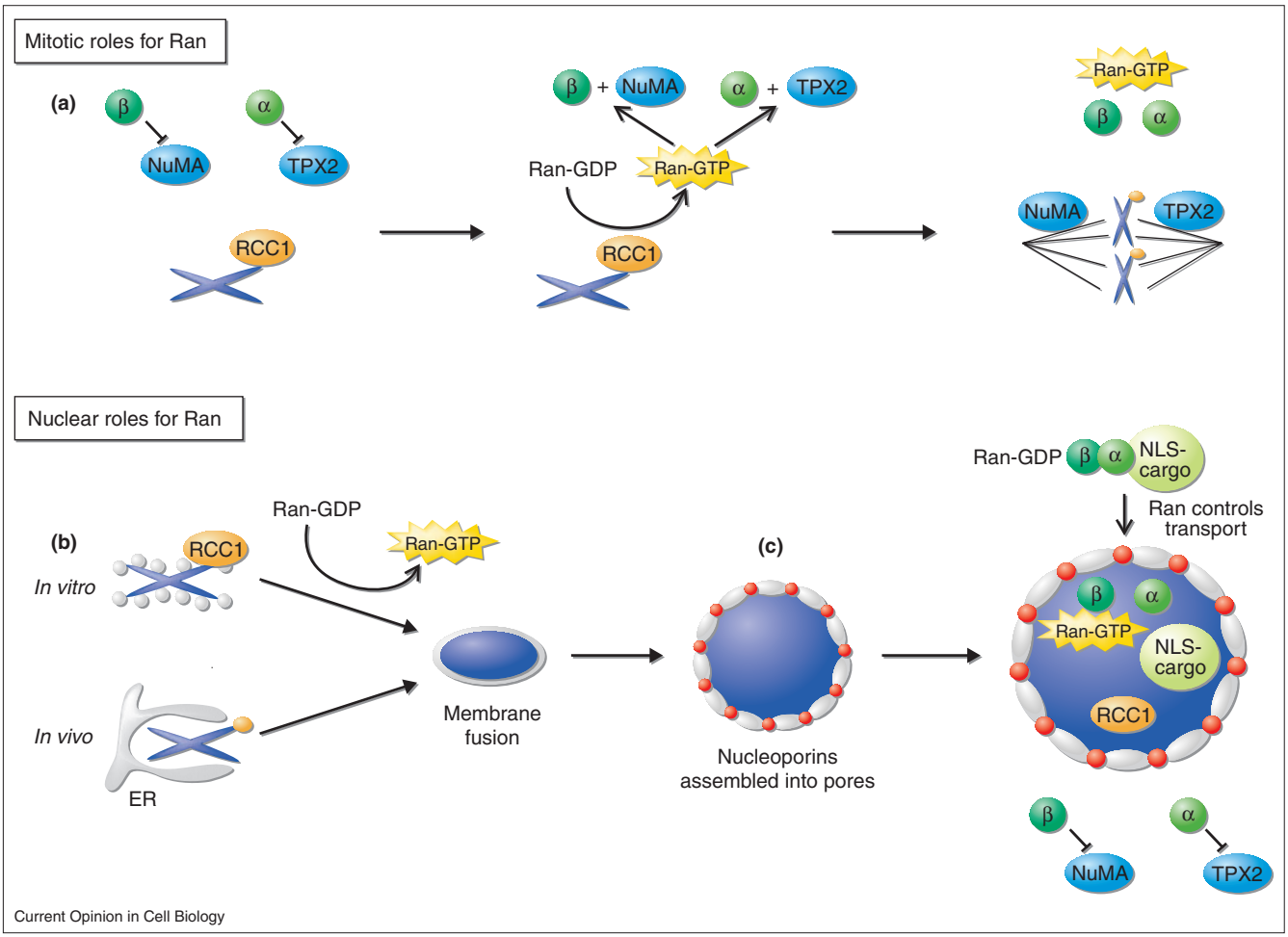
In vitro assays and new roles for Ran

Disassembly of the nucleus at mitosis necessitates the reassembly of all nuclear structures at the end of each cell cycle. With a structure as large and complex as the eukaryotic nucleus, defining the steps required to carry out its assembly were at first considered beyond the scope of experimentation. It was with surprise that scientists found that nuclear assembly could occur *in vitro* with little help from the experimentalist. Lysates of *Xenopus* eggs, which contain sufficient components to form 4000 embryonic nuclei, can spontaneously assemble nuclear structures around exogenously added DNA or chromatin [81–83]. The resulting nuclei contain double nuclear membranes, nuclear lamina and nuclear pores. They are also functional for nuclear import, DNA replication, and mitotic breakdown [68,77–83]. The *Xenopus* system has been used to ask a number of questions of nuclear assembly. (Only the most recent studies or those relevant to pore assembly or Ran action will be discussed.) Extracts of *Drosophila* and sea urchin eggs can also carry out nuclear assembly with added DNA [71,84]. Lysates of mammalian mitotic cells will initiate a similar course of nuclear assembly around their mitotic chromosomes, albeit with a lesser enthusiasm, probably due to the fact that they do not contain the vast stores of nuclear assembly components found in eggs [85]. The finding that long linear DNA from any source, bacteriophage or mammalian, could be assembled into nuclear structures indicated that specific eukaryotic sequences such as centromeres or telomeres are not required for the major structural steps in nuclear envelope assembly [82].

For *in vitro* nuclear assembly membrane vesicles containing inner nuclear membrane proteins in two distinct vesicle populations bind to a chromatin substrate, then fuse side to side to form a double nuclear membrane ([68,77,84,86] and references therein). (This is not inconsistent with *in vivo* work, as the lysis of cells fragments the mitotic ER network into vesicles.) Nuclear pores are then assembled within the double membrane. The extreme robustness of nuclear envelope formation is highlighted by the finding that a normal nuclear envelope with functional nuclear pores can be assembled in *Xenopus* extracts even around DNA-coated magnetic beads [87] and other unusual templates (see below).

In nuclear transport, the small GTPase Ran plays a pivotal role, both in disassembling nuclear import complexes and as a component of nuclear export complexes. Ran acts as the molecular switch whose GTP/GDP state controls these complexes [1,2]. Indeed, an artificial reversal of the RanGTP/GDP gradient between nucleus and cytoplasm is capable of, in some sense, running transport backwards [88]. In consequence, Ran, which was originally implicated in many seemingly unrelated cellular processes from cell cycle control and chromosome structure to nuclear transport, was

Figure 2



Multiple roles for Ran-GTP: a protein for all seasons. **(a)** When a metazoan cell enters mitosis, Ran-GTP is thought to be produced by RCC1 on the mitotic chromosomes. Ran-GTP is postulated to locally disrupt complexes that contain importin β and NuMA, and importin α and TPX2, freeing NuMA and TPX2. NuMA and TPX2 then stimulate microtubule and spindle assembly around the mitotic chromosomes. **(b)** When the cell cycle proceeds to telophase, RCC1 on the mitotic chromosomes continues to generate locally high concentrations of Ran-GTP. Ran-GTP promotes membrane vesicle fusion around the chromosomes (or related templates) in an unknown manner, forming a double nuclear membrane [99••,100••]. **(c)** Once nuclear pores are assembled in the nuclear membranes, Ran-GTP controls nuclear import and export [1–3]. Ran-GTP, now only in the nucleus owing to the exclusive nuclear localization of RCC1, causes the disassembly of incoming import complexes. An importin-α–β–NLS import complex is shown. Ran-GTP is also an obligatory component of nuclear export complexes (not shown). The sequestration of RCC1 in the nucleus assures that Ran-GTP is not present in the cytoplasm in significant amounts during interphase. The large concentration of cytoplasmic importin α and β can thus keep NuMA and TPX2 in an inhibited state and prevent spindle formation in interphase.

reconsidered in 1993 and thought to owe its role in all of these to a central role in nuclear transport [89].

In 1999, however, a number of groups made the startling observation that RanGTP added to mitotic cytosol from cytostatic factor (CSF)-treated *Xenopus* egg extracts induces microtubule growth from centrioles and, if chromosomes are present, induces the formation of spindles [90–95]. As the Ran exchange factor RCC1 is found tightly associated with chromosomes, these groups speculated that high local levels of Ran-GTP, generated by the RCC1 on the chromosomes, provided the conditions necessary to initiate spindle formation (Figure 2). Spindle microtubules would form only at mitosis, when chromosomes and their RCC1 became mixed with the cytoplasm.

Very recent studies suggest that there is a direct connection between Ran's role in nuclear transport and in microtubule assembly. These studies found that importin α and β actually bind to and inhibit proteins required to promote microtubule and spindle organization [96••–98••]. For example, importin α inhibits TPX2, a protein that aids in organizing spindles through targeting a required kinesin-like motor (Xklp2) to the microtubules [97••]. Importin β inhibits NuMA, a protein that organizes the minus ends of microtubules into spindle poles [96••,98••]. When RanGTP

is produced in the cytoplasm, as it would be in the vicinity of RCC1-rich mitotic chromosomes, importin α and β are removed from TPX2 and NuMA (Figure 2), allowing spindles to form around the mitotic chromosomes.

A third role for Ran came unexpectedly in the area of nuclear membrane fusion. RanGTP added to interphase *Xenopus* extracts has been found to promote membrane vesicle fusion around chromatin, creating a nuclear envelope [99••]. Moreover, a bead containing only coupled Ran (and no DNA) can initiate the formation of encircling double nuclear membranes with functional nuclear pores, when the Ran-bead is placed in interphase *Xenopus* extracts [100••]. The Ran-bead may attract RCC1 and then other chromatin proteins, in the end resembling a protein-coated DNA template for envelope formation. Addition of exogenous RCC1 to *Xenopus* egg extracts accelerates membrane fusion into nuclear envelopes, whereas addition of a defective Ran mutant or immunodepletion of RCC1 or Ran, blocks membrane fusion [99••,100••]. The nuclear membrane fusion events promoted by the Ran system require the generation of RanGTP by RCC1, as well as subsequent GTP hydrolysis by Ran [99••,100••]. It is not yet known what protein(s) RanGTP is acting upon to promote nuclear membrane fusion in these studies. However, Ran's cellular repertoire has now expanded to include roles in nuclear import, microtubule assembly, spindle organization, and nuclear membrane fusion. Can other controlling roles for Ran be far behind?

Conclusions

Studies during the past two years bring us closer to a full understanding of the constituents of the very large nuclear pore. If all nucleoporins are not yet known, it is likely that their neighbours are and that a close scrutiny will soon reveal the full ensemble of nuclear pore proteins. The making and testing of models for pore function will probably prove both stimulating and contentious in the coming year as the pace of discovery quickens. In related arenas, the unexpected breadth of Ran's functions suggests that we have yet to fully understand the scope of Ran within the cell. Is Ran a 'universal regulator' or does it only appears to be? Work with the MLP1/2 pore-associated filaments also gives us a glimpse into a potentially much more ordered nuclear environment. Success with *in vitro* nuclear assembly systems suggests that this higher order intranuclear organization may also yield to *in vitro* analysis.

Acknowledgements

The authors thank Rene Chan for preparation of the figures. They apologize for an inability to cite excellent papers relevant to the topics discussed due to space constraints. A number of authors/areas were instead cited in the companion review by Conti and Izaurralde (pp310–319 of this issue).

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

1. Gorlich D, Kutay U: **Transport between the cell nucleus and the cytoplasm.** *Annu Rev Cell Dev Biol* 1999, **15**:607-660.

2. Ryan KJ, Wente SR: **The nuclear pore complex: a protein machine bridging the nucleus and cytoplasm.** *Curr Opin Cell Biol* 2000, **12**:361-371.

3. Tang H, Wong-Staal F: **Specific interaction between RNA helicase A and Tap, two cellular proteins that bind to the constitutive transport element of type D retrovirus.** *J Biol Chem* 2000, **275**:32694-32700.

4. Nakielny S, Shaikh S, Burke B, Dreyfuss G: **Nup153 is an M9-containing mobile nucleoporin with a novel Ran-binding domain.** *EMBO J* 1999, **18**:1982-1995.

5. Zolotukhin AS, Felber BK: **Nucleoporins Nup98 and Nup214 participate in nuclear export of human immunodeficiency virus type 1 Rev.** *J Virol* 1999, **73**:120-127.

6. •• Rout MP, Aitchison JD, Suprapto A, Hjertaas K, Zhao Y, Chait BT: **The yeast nuclear pore complex. Composition, architecture, and transport mechanism.** *J Cell Biol* 2000, **148**:635-652.
Proteins from the purified yeast pore were subjected to mass spectroscopy sequencing. Three new Nups were identified, the stoichiometry of each yeast Nup was determined and electron microscopic examination of individually tagged Nups was performed to give a postulated localization within the yeast pore. A 'Brownian ratchet' model was proposed to explain nuclear translocation.

7. Yang Q, Rout MP, Akey CW: **Three-dimensional architecture of the isolated yeast nuclear pore complex: functional and evolutionary implications.** *Mol Cell* 1998, **1**:223-234.

8. Chial HJ, Rout MP, Giddings TH, Winey M: ***Saccharomyces cerevisiae* Ndc1p is a shared component of nuclear pore complexes and spindle pole bodies.** *J Cell Biol* 1998, **143**:1789-1800.

9. Siniossoglou S, Wimmer C, Rieger M, Doye V, Tekotte H, Weise C, Emig S, Segref A, Hurt EC: **A novel complex of nucleoporins, which includes Sec13p and a Sec13p homolog, is essential for normal nuclear pores.** *Cell* 1996, **84**:265-275.

10. • Siniossoglou S, Lutzmann M, Santos-Rosa H, Leonard K, Mueller S, Aebi U, Hurt E: **Structure and assembly of the Nup84p complex.** *J Cell Biol* 2000, **149**:41-54.
A detailed study of the Nup84 subcomplex of the yeast nuclear pore. This subcomplex, which is required for RNA export, contains Nup84, Nup85, Nup120, Nup145C, sec13, seh1 and has a Y-shaped structure. Mutants of sec13 disrupt pore morphology and are synthetically lethal with a Nup84 deletion, demonstrating that sec13 plays a significant role in the nuclear pore.

11. Fahrenkrog B, Hurt EC, Aebi U, Pante N. **Molecular architecture of the yeast nuclear pore complex: localization of Nsp1p subcomplexes.** *J Cell Biol* 1998, **143**:577-588.

12. Fahrenkrog B, Hubner W, Mandinova A, Pante N, Keller W, Aebi U: **The yeast nucleoporin Nup53p specifically interacts with nic96p and is directly involved in nuclear protein import.** *Mol Biol Cell* 2000, **11**:3885-3896.

13. Solsbacher J, Maurer P, Vogel F, Schlenstedt G: **Nup2p, a yeast nucleoporin, functions in bidirectional transport of importin alpha.** *Mol Cell Biol* 2000, **20**:8468-8479.

14. Hood JK, Casolari JM, Silver PA: **Nup2p is located on the nuclear side of the nuclear pore complex and coordinates Srp1p/importin-alpha export.** *J Cell Sci* 2000, **113**:1471-1480.

15. Rexach M, Blobel G: **Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins.** *Cell* 1995, **83**:683-692.

16. Shah S, Tugendreich S, Forbes D: **Major binding sites for the nuclear import receptor are the internal nucleoporin Nup153 and the adjacent nuclear filament protein Tpr.** *J Cell Biol* 1998, **141**:31-49.

17. Shah S, Forbes DJ: **Separate nuclear import pathways converge on the nucleoporin Nup153 and can be dissected with dominant-negative inhibitors.** *Curr Biol* 1998, **8**:1376-1386.

18. •• Damelin M, Silver PA: **Mapping interactions between nuclear transport factors in living cells reveals pathways through the nuclear pore complex.** *Mol Cell* 2000, **5**:133-140.
A novel approach is described that uses FRET analysis of interactions between fluorescently tagged nucleoporins and receptors to test for interactions within the living cell. The import receptor Pse1 interacts with many of the same Nups as the export receptor Msn5, but each also has distinct Nup interactions.

19. Strawn LA, Shen T, Wente SR: **The GLFG regions of Nup116p and Nup100p serve as binding sites for both Kap95p and Mex67p at the nuclear pore complex.** *J Biol Chem* 2000, **276**:6445-6452.

20. Ho AK, Shen TX, Ryan KJ, Kiseleva E, Levy MA, Allen TD, Wente SR: **Assembly and preferential localization of Nup116p on the cytoplasmic face of the nuclear pore complex by interaction with Nup82p.** *Mol Cell Biol* 2000, **20**:5736-5748.

21. Bailer SM, Balduf C, Katahira J, Podtelejnikov A, Rollenhagen C, Mann M, Pante N, Hurt E: **Nup116p associates with the Nup82p-Nsp1p-Nup159p nucleoporin complex.** *J Biol Chem* 2000, **275**:23540-23548

22. Hurwitz ME, Strambio-de-Castillia C, Blobel G: **Two yeast nuclear pore complex proteins involved in mRNA export form a cytoplasmically oriented subcomplex.** *Proc Natl Acad Sci USA* 1998, **95**:11241-11245.

23. Hodge CA, Colot HV, Stafford P, Cole CN: **Rat8p/Dbp5p is a shuttling transport factor that interacts with Rat7p/Nup159p and Gle1p and suppresses the mRNA export defect of xpo1-1 cells.** *EMBO J* 1999, **18**:5778-5788.

24. Fornerod M, van Deursen J, van Baal S, Reynolds A, Davis D, Murti KG, Fransen J, Grosveld G: **The human homologue of yeast CRM1 is in a dynamic subcomplex with CAN/Nup214 and a novel nuclear pore component Nup88.** *EMBO J* 1997, **16**:807-816.

25. Kehlenbach RH, Dickmanns A, Kehlenbach A, Guan T, Gerace L: **A role for RanBP1 in the release of CRM1 from the nuclear pore complex in a terminal step of nuclear export.** *J Cell Biol* 1999, **145**:645-657.

26. Black BE, Holaska JM, Levesque L, Ossareh-Nazari B, Gwizdek C, Dargemont C, Paschal BM: **NXT1 is necessary for the terminal step of Crm1-mediated nuclear export.** *J Cell Biol* 2001, **152**:141-155.

27. Dang VD, Levin HL: **Nuclear import of the retrotransposon Tf1 is governed by a nuclear localization signal that possesses a unique requirement for the FXFG nuclear pore factor Nup124p.** *Mol Cell Biol* 2000, **20**:7798-7812.

28. Reichelt R, Holzenburg A, Buhle EL, Jarnik M, Engel A, Aebi U: **Correlation between structure and mass distribution of the nuclear pore complex and of distinct pore complex components.** *J Cell Biol* 1990, **110**:883-894.

29. • Fontoura, BM, Blobel, G, and Matunis, MJ: **A conserved biogenesis pathway for nucleoporins: proteolytic processing of a 186-kilodalton precursor generates Nup98 and the novel nucleoporin, Nup96.** *J Cell Biol* 1999, **144**:1097-1112.
See annotation [42•].

30. •• Miller BR, Forbes DJ: **Purification of the vertebrate nuclear pore complex by biochemical criteria.** *Traffic* 2000, **1**:941-951.
Pores prepared by *in vitro* assembly of annulate lamellae were fractionated and purified ~500-fold to the theoretical limit. Known nucleoporins were shown to be present, whereas the bulk of unrelated nuclear, cytoplasmic and integral membrane proteins were removed. A subset of transport receptors copurified, but Ran and NTF2 did not.

31. Stoffler D, Fahrenkrog B, Aebi U: **The nuclear pore complex: from molecular architecture to functional dynamics.** *Curr Opin Cell Biol* 1999a, **11**:391-401.

32. Matsuoka Y, Takagi M, Ban T, Miyazaki M, Yamamoto T, Kondo Y, Yoneda Y: **Identification and characterization of nuclear pore subcomplexes in mitotic extract of human somatic cells.** *Biochem Biophys Res Commun* 1999, **254**:417-423.

33. Miller BR, Powers M, Park M, Fischer W, Forbes DJ: **Identification of a new vertebrate nucleoporin, Nup188, with the use of a novel organelle trap assay.** *Mol Biol Cell* 2000, **11**:3381-3396.

34. Smythe C, Jenkins HE, Hutchison CJ: **Incorporation of the nuclear pore basket protein Nup153 into nuclear pore structures is dependent upon lamina assembly: evidence from cell-free extracts of *Xenopus* eggs.** *EMBO J* 2000, **19**:3918-3931.

35. Melchior F: **Sumo nonclassical ubiquitin.** *Annu Rev Cell Dev Biol* 2000,**16**:591-626.

36. Bastos R, Lin A, Enarson M, Burke B: **Targeting and function in mRNA export of nuclear pore complex protein Nup153.** *J Cell Biol* 1996, **134**:1141-1156.

37. Ullman K, Shah S, Powers M, Forbes D: **The nucleoporin Nup153 plays a critical role in multiple types of nuclear export.** *Mol Biol Cell* 1999a, **10**:649-664.

38. Powers MA, Forbes DJ, Dahlberg JE, Lund E: **The vertebrate GLFG nucleoporin, Nup98, is an essential component of multiple RNA export pathways.** *J Cell Biol* 1997, **136**:241-250.

39. Pritchard CE, Fornerod M, Kasper LH, van Deursen JM: **RAE1 is a shuttling mRNA export factor that binds to a GLEBS-like NUP98 motif at the nuclear pore complex through multiple domains.** *J Cell Biol* 1999, **145**:237-254.

40. Fontoura BM, Blobel G, Yaseen NR: **The nucleoporin Nup98 is a site for GDP/GTP exchange on ran and termination of karyopherin beta 2-mediated nuclear import.** *J Biol Chem* 2000, **275**:31289-31296.

41. Bachi A, Braun IC, Rodrigues JP, Pante N, Ribbeck K, von Kobbe C, Kutay U, Wilm M, Gorlich D, Carmo-Fonseca M, Izaurralde E: **The C-terminal domain of TAP interacts with the nuclear pore complex and promotes export of specific CTE-bearing RNA substrates.** *RNA* 2000, **6**:136-158.

42. • Rosenblum JS, Blobel G: **Autoproteolysis in nucleoporin biogenesis.** *Proc Natl Acad Sci USA* 1999, **96**:11370-11375.
Papers [29•] and [42•] find that the nucleoporin Nup98 has an endoproteolytic activity and can cleave its protein precursor into two nucleoporins. Cleavage is necessary for incorporation of Nup96 into the nuclear pore. [29•] shows that Nup96 is in a complex containing the nucleoporin Nup107 and probably sec13. A potential homologue of yeast seh1p is noted.

43. Teixeira MT, Fabre E, Dujon B: **Self-catalyzed cleavage of the yeast nucleoporin Nup145p precursor.** *J Biol Chem.* 1999, **274**:32439-32444.

44. •• von Kobbe C, van Deursen JM, Rodrigues JP, Sitterlin D, Bachi A, Wu X, Wilm M, Carmo-Fonseca M, Izaurralde E: **Vesicular stomatitis virus matrix protein inhibits host cell gene expression by targeting the nucleoporin Nup98.** *Mol Cell* 2000, **6**:1243-1252.
See annotation [45•].

45. • Petersen JM, Her LS, Varvel V, Lund E, Dahlberg JE: **The matrix protein of vesicular stomatitis virus inhibits nucleocytoplasmic transport when It is in the nucleus and associated with nuclear pore complexes.** *Mol Cell Biol* 2000, **20**:8590-8601.
Papers [44•] and [45••] demonstrate the mechanism by which VSV virus impedes host gene expression. Specific inhibition of cellular mRNA export occurs through the binding of the VSV M protein to the GLFG domain of Nup98 [45••]. Inhibition of protein import is also seen [44•].

46. • Guan T, Kehlenbach RH, Schirmer EC, Kehlenbach A, Fan F, Clurman BE, Arnheim N, Gerace L: **Nup50, a nucleoplasmically oriented nucleoporin with a role in nuclear protein export.** *Mol Cell Biol* 2000, **20**:5619-5630.
A novel nucleoporin on the basket of the vertebrate nuclear pore was analyzed that plays a role in NES-mediated protein export.

47. Smitherman M, Lee K, Swanger J, Kapur R, Clurman BE: **Characterization and targeted disruption of murine Nup50, ap27(Kip1)-interacting component of the nuclear pore complex.** *Mol Cell Biol* 2000, **20**:5631-5642.

48. Kiseleva E, Goldberg MW, Allen TD, Akey CW: **Active nuclear pore complexes in Chironomus: visualization of transporter configurations related to mRNP export.** *J Cell Sci* 1998, **111**:223-236.

49. Feldherr CM, Akin D: **The location of the transport gate in the nuclear pore complex.** *J Cell Sci* 1997, **110**:3065-3070.

50. Kiseleva E, Goldberg MW, Daneholt B, Allen TD: **RNP export is mediated by structural reorganization of the nuclear pore basket.** *J Mol Biol* 1996, **260**:304-311.

51. Keminer O, Peters R: **Permeability of single nuclear pores.** *Biophys J* 1999, **77**:217-228.

52. Keminer O, Siebrasse JP, Zerf K, Peters R: **Optical recording of signal-mediated protein transport through single nuclear pore complexes.** *Proc Natl Acad Sci USA* 1999, **96**:11842-11847.

53. •• Ribbeck K, Gorlich D: **Kinetic analysis of translocation through nuclear pore complexes.** *EMBO J* 2001, in press.
The authors analyze the import of empty transportin receptors, transportin receptors carrying cargo, NTF2 and other proteins and determine that the rate of transport is unexpectedly high. They propose the selective phase model as a way to mechanistically explain the exceedingly high transport rates observed.

54. Finlay DR, Meier E, Bradley P, Horecka J, Forbes DJ: **A complex of nuclear pore proteins required for pore function.** *J Cell Biol* 1991, **114**:169-183.

55. Schlenstedt G, Hurt E, Doye V, Silver PA: **Reconstitution of nuclear protein transport with semi-intact yeast cells.** *J Cell Biol* 1993, **123**:785-798.

56. Gomez-Ospina N, Morgan G, Giddings TH Jr, Kosova B, Hurt E, Winey M: **Yeast nuclear pore complex assembly defects determined by nuclear envelope reconstruction.** *J Struct Biol* 2000, **132**:1-5.

57. Macaulay C, Forbes DJ: **Assembly of the nuclear pore: biochemically distinct steps revealed with NEM, GTP gamma S, and BAPTA.** *J Cell Biol* 1996, **132**:5-20.

58. • Bodoor K, Shaikh S, Salina D, Raharjo WH, Bastos R, Lohka M, Burke B: **Sequential recruitment of NPC proteins to the nuclear periphery at the end of mitosis**. *J Cell Sci* 1999, **112**:2253-2264.
The authors examine the order in which pore and nuclear envelope proteins associate with a reforming nucleus at telophase in living cells. The observed order is POM121, Nup62, Nup214 and gp210. Nup153 associates with chromosomes in a membrane-independent manner.

59. Haraguchi T, Koujin T, Hayakawa T, Kaneda T, Tsutsumi C, Imamoto N, Akazawa C, Sukegawa J, Yoneda Y, Hiraoka Y: **Live fluorescence imaging reveals early recruitment of emerin, LBR, RanBP2, and Nup153 to reforming functional nuclear envelopes.** *J Cell Sci* 2000, **113**:779-794.

60. Goldberg MW, Wiese C, Allen TD, Wilson KL: **Dimples, pores, star-rings, and thin rings on growing nuclear envelopes: evidence for structural intermediates in nuclear pore complex assembly.** *J Cell Sci* 1997, **110**:409-420.

61. Nehrbass U, Rout MP, Maguire S, Blobel G, Wozniak RW: **The yeast nucleoporin Nup188p interacts genetically and physically with the core structures of the nuclear pore complex.** *J Cell Biol* 1996, **133**:1153-1162.

62. Kosova B, Pante N, Rollenhagen C, Hurt E: **Nup192p is a conserved nucleoporin with a preferential location at the inner site of the nuclear membrane.** *J Biol Chem* 1999, **274**:22646-22651.

63. Grandi P, Dang T, Pane N, Shevchenko A, Mann M, Forbes D, Hurt E: **Nup93, a vertebrate homologue of yeast Nic96p, forms a complex with a novel 205-kDa protein and is required for correct nuclear pore assembly.** *Mol Biol Cell* 1997, **8**:2017-2038.

64. Shulga N, Mosammaparast N, Wozniak R, Goldfarb DS: **Yeast nucleoporins involved in passive nuclear envelope permeability.** *J Cell Biol* 2000, **149**:1027-1038.

65. Dabauvalle MC, Muller E, Ewald A, Kress W, Krohne G, Muller CR: **Distribution of emerin during the cell cycle.** *Eur J Cell Biol* 1999, **78**:749-756.

66. Paddy MR: **The Tpr protein: linking structure and function in the nuclear interior?** *Am J Hum Genet* 1998, **63**:305-310.

67. •• Galy V, Olivo-Marin JC, Scherthan H, Doye V, Rascalou N, Nehrbass U: **Nuclear pore complexes in the organization of silent telomeric chromatin.** *Nature* 2000, **403**:108-112.
Deletion of the pore-associated filament proteins MLP1/2 releases telomeres from the nuclear periphery and simultaneously inhibits double-strand DNA break repair and telomeric gene silencing, both of which normally occur at the nuclear periphery. A Nup145 deletion has the same phenotype, arguing that the organization of MLP1/2 filaments at the pore is required for proper functioning of an array of nuclear activities.

68. Wiese C, Goldberg MW, Allen TD, Wilson KL: **Nuclear envelope assembly in *Xenopus* extracts visualized by scanning EM reveals a transport-dependent 'envelope smoothing' event.** *J Cell Sci* 1997, **110**:1489-1502.

69. Macaulay C, Meier E, Forbes DJ: **Differential mitotic phosphorylation of proteins of the nuclear pore complex.** *J Biol Chem* 1995, **270**:254-262.

70. Favreau C, Worman HJ, Wozniak RW, Frappier T, Courvalin JC: **Cell cycle-dependent phosphorylation of nucleoporins and nuclear pore membrane protein Gp210.** *Biochemistry* 1996, **35**:8035-8044.

71. Collas P: **Nuclear envelope disassembly in mitotic extract requires functional nuclear pores and a nuclear lamina.** *J Cell Sci* 1998, **111**:1293-1303.

72. Ganeshan R, Parnaik VK: **Phosphorylation of NPA58, a rat nuclear pore-associated protein, correlates with its mitotic distribution.** *Exp Cell Res* 2000, **261**:199-208.

73. Askjaer P, Bachi A, Wilm M, Bischoff FR, Weeks DL, Ogniewski V, Ohno M, Niehrs C, Kjems J, Mattaj IW, Fornerod M: **RanGTP-regulated interactions of CRM1 with nucleoporins and a shuttling DEAD-box helicase.** *Mol Cell Biol* 1999, **19**:6276-6285.

74. Ewald A, Hofbauer S, Dabauvalle MC, Lourim D: **Preassembly of annulate lamellae in egg extracts inhibits nuclear pore complex formation, but not nuclear membrane assembly.** *Eur J Cell Biol* 1997, **73**:259-269.

75. • Ellenberg J, Siggia ED, Moreira JE, Smith CL, Presley JF, Worman HJ, Lippincott-Schwartz J: **Nuclear membrane dynamics and reassembly in living cells: targeting of an inner nuclear membrane protein in interphase and mitosis.** *J Cell Biol* 1997, **138**:1193-1206.
See annotation [76•].

76. • Yang L, Guan T, Gerace L: **Integral membrane proteins of the nuclear envelope are dispersed throughout the endoplasmic reticulum during mitosis.** *J Cell Biol* 1997, **137**:1199-1210.
Papers [75•] and [76•] detail the visualization of inner nuclear membrane proteins by GFP-tagging in living cells or by immunoEM, revealing that the metazoan nuclear envelope does not vesiculate at mitosis but retracts into the ER membrane system.

77. Collas I, Courvalin JC: **Sorting nuclear membrane proteins at mitosis.** *Trends Cell Biol* 2000, **10**:5-8.

78. Moir RD, Yoon M, Khuon S, Goldman RD: **Nuclear lamins A and B1: different pathways of assembly during nuclear envelope formation in living cells.** *J Cell Biol* 2000, **151**:1155-1168.

79. Cohen M, Gruenbaum Y, Lee KK, Wilson KL: **Transcriptional repression, apoptosis, human disease and the functional evolution of the nuclear lamina.** *Trends Biochem Sci* 2001, **26**:41-47.

80. Hutchison CJ, Alvarez-Reyes M, Vaughan OA: **Lamins in disease: why do ubiquitously expressed nuclear envelope proteins give rise to tissue-specific disease phenotypes?** *J Cell Sci* 2001, **114**:9-19.

81. Lohka MJ, Masui Y: **Formation *in vitro* of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components.** *Science* 1983, **220**:719-721.

82. Forbes DJ, Kirschner MW, Newport JW: **Spontaneous formation of nucleus-like structures around bacteriophage DNA microinjected into *Xenopus* eggs.** *Cell* 1983, **34**:13-23.

83. Newport J: **Nuclear reconstitution *in vitro*: stages of assembly around protein-free DNA.** *Cell* 1987, **48**:205-217.

84. Ulitzur N, Harel A, Goldberg M, Feinstein N, Gruenbaum Y: **Nuclear membrane vesicle targeting to chromatin in a *Drosophila* embryo cell-free system.** *Mol Biol Cell* 1997, **8**:1439-1448.

85. Burke B, Gerace L: **A cell free system to study reassembly of the nuclear envelope at the end of mitosis.** *Cell* 1986, **44**:639-652.

86. Sasagawa S, Yamamoto A, Ichimura T, Omata S, Horigome T: ***In vitro* nuclear assembly with affinity-purified nuclear envelope precursor vesicle fractions, PV1 and PV2.** *Eur J Cell Biol* 1999, **78**:593-600.

87. Heald R, Tournebize R, Blank T, Sandaltzopoulos R, Becker P, Hyman A, Karsenti E: **Self-organization of microtubules into bipolar spindles around artificial chromosomes in *Xenopus* egg extracts.** *Nature* 1996, **382**:420-425.

88. Nachury MV, Weis K: **The direction of transport through the nuclear pore can be inverted.** *Proc Natl Acad Sci USA* 1999, **96**:9622-9627.

89. Dasso M: **Running on Ran: Nuclear transport and the mitotic spindle.** *Cell* 2001, **104**:321-324.

90. Carazo-Salas RE, Guarguaglini G, Gruss OJ, Segref A, Karsenti E, Mattaj IW: **Generation of GTP-bound Ran by RCC1 is required for chromatin-induced mitotic spindle formation.** *Nature* 1999, **400**:178-181.

91. Ohba T, Nakamura M, Nishitani H, Nishimoto T: **Self-organization of microtubule asters I nduced in *Xenopus* egg extracts by GTP-bound Ran.** *Science* 1999, **284**:1356-1358.

92. Kalab P, Pu RT, Dasso M: **The ran GTPase regulates mitotic spindle assembly.** *Curr Biol* 1999, **9**:481-484.

93. Wilde A, Zheng Y: **Stimulation of microtubule aster formation and spindle assembly by the small GTPase Ran.** *Science* 1999, **284**:1359-1362.

94. Guarguaglini G, Renzi L, D'Ottavio F, Di Fiore B, Casenghi M, Cundari E, Lavia P: **Regulated Ran-binding protein 1 activity is required for organization and function of the mitotic spindle in mammalian cells *in vivo*.** *Cell Growth Differ* 2000, **11**:455-465.

95. Sazer S, Dasso M: **The ran decathlon: multiple roles of Ran.** *J Cell Sci* 2000, **113**:1111-1118.

96. •• Wiese C, Wilde A, Moore MS, Adam SA, Merdes A, Zheng Y: **Role of importin-beta in coupling Ran to downstream targets in microtubule assembly.** *Science* 2001, **291**:653-656.
See annotation [98••].

97. •• Gruss OJ, Carazo-Salas RE, Schatz CA, Guarguaglini G, Kast J, Wilm M, Le Bot N, Vernos I, Karsenti E, Mattaj IW: **Ran induces spindle assembly by reversing the inhibitory effect of importin alpha on TPX2 activity.** *Cell* 2001, **104**:83-93.
See annotation [98••].

98. •• Nachury MV, Maresca TJ, Salmon WC, Waterman-Storer CM, Heald R, Weis K: **Importin beta is a mitotic target of the small GTPase Ran in spindle assembly.** *Cell* 2001, **104**:95-106.
Papers [96••–98••] delineate the roles of importin α and β in binding to and inactivation of the microtubule-binding proteins NuMA and TPX2. Reversal of this binding by RanGTP allows microtubule assembly and spindle formation. These papers provide a link between Ran's role in nuclear transport and its role in microtubule dynamics.

99. •• Hetzer M, Bilbao-Cortes D, Walther TC, Gruss OJ, Mattaj IW: **GTP hydrolysis by Ran is required for nuclear envelope assembly.** *Mol Cell* 2000, **5**:1013-1024.
See annotation [100••].

100. •• Zhang C, Clarke PR: **Chromatin-independent nuclear envelope assembly induced by Ran GTPase in *Xenopus* egg extracts.** *Science* 2000, **288**:1429-1432.
Papers [99••,100••] reveal that Ran-GTP plays a role in nuclear membrane fusion and formation. This fusion requires both the actual production of Ran-GTP and the hydrolysis of GTP. Paper [99•] describes a novel fusion assay between two populations of fluorescently tagged membrane vesicles.

101. Strahm Y, Fahrenkrog B, Zenklusen D, Rychner E, Kantor J, Rosbach M, Stutz F: **The RNA export factor Gle1p is located on the cytoplasmic fibrils of the NPC and physically interacts with the FG-nucleoporin Rip1p, the DEAD-box protein Rat8p/Dbp5p and a new protein Ymr 255p.** *EMBO J* 1999, **18**:5761-5777.