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Versatility at the nuclear pore complex: lessons learned from the nucleoporin Nup153

Received: 27 May 2005 / Revised: 8 July 2005 / Accepted: 10 July 2005
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Abstract The vertebrate pore protein Nup153 plays pivotal roles in nuclear pore function. In addition to being important to pore architecture, Nup153 is a key participant in both import and export. The scope of Nup153 function also extends beyond the canonical view of the pore as a trafficking gateway. During the transition into mitosis, Nup153 directs proteins involved in membrane remodeling to the nuclear envelope. As cells exit mitosis, Nup153 is recruited to the chromosomal surface, where nuclear pores are formed anew in a complicated process still under much experimental scrutiny. In addition, Nup153 is targeted for protease cleavage during apoptosis and in response to certain viral infections, providing molecular insight into pore reconfiguration during cell response. Overall, the versatile nature of Nup153 underscores an emerging view of the nuclear pore at the nexus of many key cellular processes.

Nuclear pore complex overview

Exchange of molecules between the nucleus and cytoplasm takes place through nuclear pore complexes (NPCs), which bridge the two membrane bilayers of the nuclear envelope (NE). NPCs are abundant, with 3,000–4,000 in a proliferating somatic cell (Gorlich and Kutay 1999), and each NPC is capable of orchestrating hundreds of transport events per minute (Gorlich and Mattaj 1996). This large volume of selective traffic results in unique environments within the nucleus and cytoplasm, while simultaneously allowing constant communication between these compartments. The vertebrate NPC has an estimated mass of

125 MDa (Reichelt et al. 1990), yet despite the immense size of this cellular machine, it is formed by only ~30 different nucleoporins (Nups), with multiple copies of each protein arranged in eightfold rotational symmetry (Cronshaw et al. 2002). The central domain of the pore complex, including the trafficking channel, is sandwiched between cytoplasmic and nuclear coaxial rings (see Fig. 1). Two distinct types of peripheral extensions are anchored to each ring. Flexible filaments fan out into the cytoplasm (Fahrenkrog and Aebi 2003; Suntharalingam and Wentz 2003; Weis 2003). On the nuclear face of the pore, fibers emanating from the nuclear coaxial ring are tethered distally to a second ring, forming an arrangement referred to as a fishtrap (Ris 1997) or, now more commonly, as the nuclear basket. During each cell division, higher eukaryotes go through an open mitosis characterized by dissolution of the nuclear envelope. In a coordinate fashion, the NPC is also disassembled. Notably, some Nups play important roles during or after mitotic remodeling of the pore itself (Joseph et al. 2004; Liu et al. 2003; Salina et al. 2003).

Nup153: identification and organization

Nup153, named for its predicted mass as is the convention for most nucleoporins, is essential (Galy et al. 2003; Harborth et al. 2001). Through both directed (Dimaano et al. 2001; McMorrow et al. 1994; Shah et al. 1998; Sukegawa and Blobel 1993) and genomic approaches, Nup153 has now been sequenced in multiple organisms; human Nup153 coordinates will be used in this review. Of note, there is no nucleoporin in yeast that shares the same overall domain composition as Nup153, although certain Nups in *Saccharomyces cerevisiae* (Nup1, Nup60) and *Saccharomyces pombe* (Nup124) share some specific functional and sequence features (Cronshaw et al. 2002; Hase and Cordes 2003; Varadarajan et al. 2005).

Nup153 is a serine/threonine-enriched (27%) glycoprotein. Like several other nucleoporins, Nup153 has a region containing many copies of a degenerate pentapeptide

Communicated by E.A. Nigg

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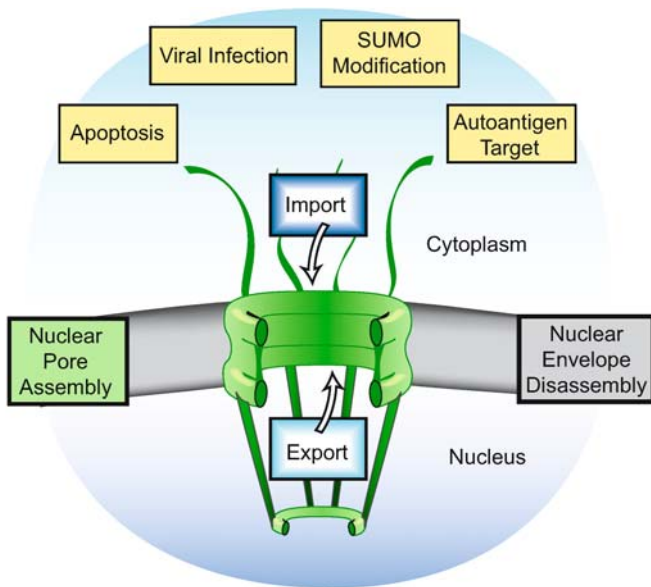


Fig. 1 Nup153 is at the nexus of many key cellular processes. Following mitosis, Nup153 is involved in the stepwise recruitment of nuclear pore proteins to the chromosomal surface, which leads to assembly of the macromolecular pore complex and the nuclear envelope itself. Poised at this gateway between the nucleus and cytoplasm, Nup153 plays a key role in facilitating movement of specific molecules between these main compartments. When the cell cycle progresses to mitosis, Nup153 recruits factors required for nuclear envelope breakdown. Nup153 exerts an influence on the status of posttranslational modification by SUMO due to an interaction between Nup153 and a SUMO-specific protease. Nup153 is a target of the caspase pathway during apoptosis and is involved in pathways of cellular dysregulation, including autoimmunity and viral infection

repeat, xFxFG [xxxFG, xFxFx, xFxFG (Denning et al. 2003); where x is any amino acid, F is phenylalanine, and G is glycine]. In the case of Nup153, this region is at the carboxy end and contains ~30 motifs (Fig. 2a). Nup153 is phosphorylated in interphase and hyperphosphorylated in mitosis (Bodoor et al. 1999; Favreau et al. 1996; Walther et al. 2003b). Nup153 is also modified with O-linked N-acetylglucosamine (Meier et al. 1995; Radu et al. 1995). In the context of pore proteins, the significance of this modification remains poorly understood; however, one general proposal is that glycosylation works in opposition with phosphorylation (Hart 1997).

The N-terminal region (1–610) of Nup153 is unique and contains both a pore targeting interface as well as an RNA binding domain (250–400; see Fig. 2a). Localization of HA-tagged Nup153 fragments in BHK cells revealed two overlapping segments that are involved in Nup153 localization (Bastos et al. 1996; Enarson et al. 1998). One segment, the nuclear envelope targeting cassette (NETC) comprising residues 2–144, contains a predicted amphipathic helix and directs Nup153 into proximity with the inner membrane of the NE. Another segment, the nuclear pore associating region (NPAR) mapping to residues 39–339, is sufficient to direct incorporation into NPCs (Enarson et al. 1998).

The zinc finger region (650–880) contains four C₂–C₂ type zinc fingers that are most similar to zinc fingers found in Nup358 (Wu et al. 1995; Yokoyama et al. 1995) and, more generally, to the Npl4 zinc finger (NZF) family (Meyer et al. 2002; Wang et al. 2003). Although this region of Nup153 has been shown to associate with DNA by blot overlay analysis (Sukegawa and Blobel 1993), the characteristics of DNA binding have not been formally defined. Additional partnerships with this and other domains of Nup153 are highlighted in Fig. 2b, more comprehensively summarized in Table 1, and discussed further below.

Nup153 localization: clues to pore architecture

Early studies of Nup153 localization by immuno-gold electron microscopy revealed that this protein resides primarily on the nuclear basket of the NPC (Sukegawa and Blobel 1993). However, pinpointing a precise location for Nup153 was not trivial, and further studies revealed a more complicated localization. Overall, the data are consistent with the notion that different epitopes of Nup153 are exposed at different regions of the nuclear pore basket (Fahrenkrog et al. 2002; Krull et al. 2004; Pante et al. 1994, 2000; Walther et al. 2001). In particular, the zinc finger region is most accessible at the distal ring of the pore basket, whereas the N-terminal region is predominantly detected closer to the membrane, presumably on the proximal ring of the basket. This could be explained either by Nup153 adopting an extended struc-

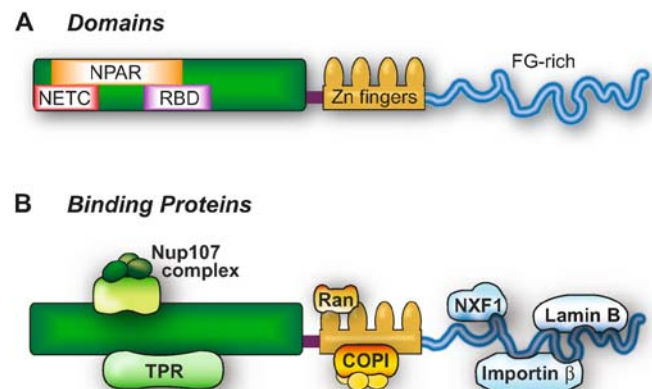


Fig. 2 Nup153 organization. **a** The domain architecture of Nup153 is depicted. The N-terminal region of Nup153 contains the nuclear envelope targeting cassette (NETC; 2–144), which is required for targeting Nup153 into proximity with the inner nuclear envelope, the nuclear pore associating region (NPAR; 39–339), which directs Nup153 incorporation into the nuclear pore complex, and an RNA binding domain (RBD; 250–400). The central zinc finger region (650–880) is comprised of four C₂–C₂ type zinc fingers. And the C-terminal region (881–1475) contains FG-rich motifs that are typical of many pore proteins and thought to be natively unfolded. **b** Protein partners of Nup153. A subset of protein partners are illustrated, each docked in the vicinity of the region they associate with. A comprehensive list of Nup153 partners can be found in Table 1. Nup153 likely does not serve as a scaffold for all partners simultaneously (as depicted), but the spatial and temporal control of partnerships has not yet been experimentally addressed

Table 1 Nup153 partnerships

	Protein transport	
	Importin- β	(Shah et al. 1998; Moroianu et al. 1995)
	RanGTPase	(Nakielny et al. 1999; Saitoh et al. 1996)
	Importin- α 2	(Moroianu et al. 1997)
	Kap β 3/RanBP5	(Yaseen and Blobel 1997)
	Transportin-1	(Shah and Forbes 1998; Nakielny et al. 1999)
	Crm1/Exportin-1	(Nakielny et al. 1999)
	Importin-7/RanBP7	(Walther et al. 2003a)
	Nup50/Npap60	(Smitherman et al. 2000)
	NTF2	(Cushman et al. 2004)
	RNA transport	
	NXF1/TAP	(Bachi et al. 2000)
	Exportin-t	(Kuersten et al. 2002)
	Exportin-5	(Brownawell and Macara 2002)
	eIF5A	(Hofmann et al. 2001)
	NPC/lamina	
	Nup160 complex	(Vasu et al. 2001; Walther et al. 2003a)
	Tpr	(Hase and Cordes 2003)
	Lamin B	(Smythe et al. 2000)
	Membrane remodeling	
	COPI	(Liu et al. 2003)
	Transcription factors	
	Smad2	(Xu et al. 2002)
	Stat1	(Marg et al. 2004)
	PU.1	(Zhong et al. 2005)
	Cellular regulation	
	Caspase-3 (inferred)	(Buendia et al. 1999; Ferrando-May et al. 2001)
	SENP2 (SUMO protease)	(Hang and Dasso 2002; Zhang et al. 2002)
	Nucleic acid	
	DNA	(Sukegawa and Blobel 1993)
	RNA	(Ullman et al. 1999; Dimaano et al. 2001; Ball et al. 2004)

Nup153 has been shown to interact with nucleic acids and several proteins. For simplicity, these interactions are listed and grouped within functional categories related to transport, nuclear envelope assembly and disassembly, and cellular regulation

ture or by there being two populations of Nup153, positioned at different sites with distinct regions exposed (Fahrenkrog and Aebi 2003; Fahrenkrog et al. 2002; see Fig. 3).

Immuno-gold labeled antibodies to the C-terminal FG-rich region of Nup153 decorate both proximal and distal regions of the nuclear pore basket and, surprisingly, map even to cytoplasmic features of the pore (Fahrenkrog et al. 2002). To circumvent potential issues with antibody specificity within this repetitive region, epitope-tagged versions of Nup153 were examined. In contrast to an N-terminal epitope tag, which was detected predominantly at the nuclear coaxial ring, the C-terminal epitope tag was detected in a spread distribution within the basket and, again, on the cytoplasmic side of the pore (Fahrenkrog et al. 2002). These results suggest that the C-terminal region is flexible and is not folded into a structure that is constrained to a fixed point within the pore (see Fig. 3). Consistent with this, biophysical measurements of several FG-rich domains have led to the proposal that these regions are natively unfolded and exist in equilibrium between many conformations (Denning et al. 2003).

Overall, these localization studies point toward a distinctive arrangement of Nup153 at the nuclear pore, which is discussed further below. The results also underscore the

importance of domain-specific antibodies when determining the placement of a relatively large protein within a macromolecular structure such as the NPC. This has proven to be true for other pore proteins as well, including TPR (Frosst et al. 2002; Krull et al. 2004) and Nup214 (Paulillo et al. 2005). More surprises about nuclear pore organization may arise as other nucleoporins are re-examined in a similar manner.

Nup153 in real-time: reconsidering the nature of the nuclear pore

The vertebrate NPC demonstrates little lateral mobility within the NE (Daigle et al. 2001) and, for many years, was considered to be a highly stable structure itself. However, the advent of techniques that allow real-time assessment of protein localization has drastically altered this view of the NPC. Fluorescence recovery after photobleaching (FRAP) analysis of GFP-tagged Nup153 at the pore has revealed that Nup153 is a highly mobile nucleoporin (Daigle et al. 2001) that is exchanging with a soluble population in the nucleoplasm over a time frame of a few minutes. In a recent survey of many nucleoporins (using a related technique, iFRAP), several were found to have some degree of

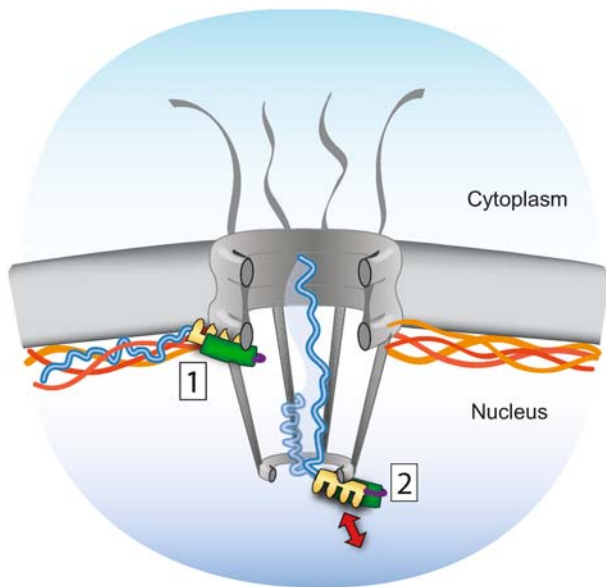


Fig. 3 A working model for Nup153 localization. Information about Nup153 localization collected to date indicates that its distribution is multifaceted. This particular rendition is intended to illustrate a few key points and how they can be integrated, but there are certainly other possible models as well. Nup153 is a mobile nucleoporin, exchanging on and off the pore as indicated by the *red arrow*. Domain-specific antibodies indicate that the zinc finger domain is exposed at the distal ring of the pore basket, whereas the N-terminal domain is accessible to antibody only in proximity to the nuclear membrane. One possibility is that this domain exposure reflects two populations of Nup153, each positioned such that distinct domains are masked. The C-terminal FG-rich domain of Nup153 is detected in a wider distribution at the pore, perhaps because this domain itself exists in various conformations. Observations that Nup153 aids in anchoring the pore and interacts with lamin suggest that a subset of Nup153 is dedicated to a more structural role, here depicted as a *population 1*

mobility, although Nup153 was one of the most highly dynamic (Rabut et al. 2004a,b). Kinetic analysis further reveals that Nup153 exchanges at the pore with two different rates, which are both very rapid but separable (Griffis et al. 2004; Rabut et al. 2004a). There is also evidence of an immobile population of Nup153, discussed further below. It is worth noting that Nup153 does not appear to be in exchange with a cytoplasmic population (other than newly synthesized Nup153 en route to the pore) (Daigle et al. 2001).

Interestingly, Nup153 dynamics are dependent on active transcription (Griffis et al. 2004), suggesting a link between RNA cargo formation and the status of ongoing pore reconfiguration. Analysis of the mobility of Nup153 fragments demonstrated the N-terminal residues 1–339, essentially the minimal pore targeting domain, are sufficient to link mobility to RNA pol I transcription. However, additional residues between 339 and 610 are required for RNA pol II dependence (Griffis et al. 2004). The dependence on transcription has not been examined systematically for all mobile Nups, but it had been previously found to be the case for Nup98 (Griffis et al. 2002). A deletion analysis of Nup98 also uncoupled requirements for pol I and pol II dependence, although there is no clear

similarity between the regions mapped within Nup98 and those mapped within Nup153.

With the relevant regions now identified, it should be possible to decipher the molecular links between nucleoporin mobility and transcription. Intriguingly, loss of sensitivity to pol II inhibition corresponds to truncation of the RNA binding domain within Nup153 (see below for further information on this domain). This suggests that direct contact with newly synthesized pol II transcripts may contribute to dynamic exchange of Nup153, but this has not been directly demonstrated. Nonetheless, Nup153 mobility opens up the possibility that this pore protein, and other mobile nucleoporins, associate with RNA cargo—whether directly, indirectly, or both—at an early step in RNA biogenesis to facilitate recruitment to the NPC.

Nup153 and the interface between cargo-receptor complexes and the NPC

Although small molecules (<~50 kDa or 9 nm in diameter) can move through the pore by simple diffusion, the transport of larger molecules and even some small molecules through the NPC is regulated (Breeuwer and Goldfarb 1990; Zasloff 1983). Transport receptors, in many cases, play a critical role in facilitating movement of cargo through the pore via contact with pore proteins. To date, there are three classes of nuclear transport receptors (Weis 2003). One class, the importin- β family, includes both importins and exportins and is distinguished by a shared binding motif for the small GTPase Ran (Gorlich et al. 1997). Importin- β itself often works in conjunction with a family of adaptors termed importin- α (or karyopherin- α) proteins (Goldfarb et al. 2004).

Interaction between importin- β (also referred to as karyopherin- β 1) and Nup153 in particular was first demonstrated by blot overlay analysis (Moroianu et al. 1995) and by coimmunoprecipitation from *Xenopus* egg extracts (Shah et al. 1998). When an importin- β binding fragment of Nup153, comprising a portion of the FG repeat region, was tested for its ability to inhibit transport mediated by importin- α/β or transportin-1 (karyopherin- β 2), only importin- β -mediated import was affected (Shah and Forbes 1998). Interestingly, a different fragment of Nup153, derived from the juncture of the N-terminal region and zinc finger domain, did interfere with transportin-1-mediated import, suggesting that transport pathways converge at Nup153 but utilize unique interfaces (Shah and Forbes 1998).

Interactions have been found between Nup153 and other importin- β related proteins: Kap β 3/RanBP5 (Yaseen and Blobel 1997), importin-7/RanBP7 (Walther et al. 2003a) exportin-5 (Brownawell and Macara 2002), exportin-t (Kuersten et al. 2002), and exportin-1 (Nakielny et al. 1999). In addition, Nup153 interfaces with other classes of transport receptors: NXF1/Tap, the mRNA export receptor (Bachi et al. 2000), Imp α 2 (Moroianu et al. 1997), and NTF2, the import receptor for Ran (Cushman et al. 2004). Overall, these data suggest that Nup153 is at the intersec-

tion for many paths of transport through the pore. Other pore proteins clearly participate in these same pathways; however, distinct subsets of Nups seem to play prominent roles in the transport of distinct classes of cargo (Rout and Aitchison 2000). One must also consider (not in a mutually exclusive way) that interactions with transport receptors are involved in other roles of Nup153. This is illustrated by the role of importin- β in NPC assembly (Harel and Forbes 2004, and see below) but may extend to other transport receptors and other circumstances that are not yet appreciated.

Although interactions between transport receptors and nucleoporins typically bridge cargo to the NPC, in some cases, transport cargoes directly contact nuclear pore proteins and, in turn, traverse the pore independently of canonical transport receptors. Three transcription factors, Stat1, Smad2, and PU.1, are proposed to fall into this category and, further, have been shown to be capable of direct interaction with Nup153 (Marg et al. 2004; Xu et al. 2002; Zhong et al. 2005). These results suggest Nup153 participates in cellular response to TGF β , interferon, and cues that direct hematopoietic cell differentiation. A growing appreciation of connections between pore proteins and specific chromosomal regions (Casolari et al. 2004; Galy et al. 2000), as well as the specific observation that there is a nucleoplasmic population of Nup153 in dynamic exchange with a pore-associated population (Daigle et al. 2001; Griffis et al. 2004), raises the possibility that interaction with Nup153 reflects a functional connection beyond transport itself. Although speculative, such interactions may serve to target Nup153 to a subset of enhancer regions, and this proximity may influence the role of Nup153 with respect to export of the mRNA being synthesized or with respect to transcription regulation itself.

Movement through the NPC

The mechanism of cargo–receptor translocation through the central channel of the pore is still a subject of debate. Models to explain selectivity in transit through the pore include an entropic barrier to entry, a protein–protein meshwork that creates a hydrophobic phase within the pore, and an affinity gradient of docking sites (for review, see Bayliss et al. 2000; Rabut and Ellenberg 2001; Rout et al. 2003). Regardless of the exact mechanism, or combination of mechanisms, by which cargo–receptor complexes traverse the nuclear pore, there is consensus that the small GTPase Ran plays a prominent role in many transport paths. Ran appears to modulate both cargo–receptor interactions and receptor–nucleoporin interactions. Generally, RanGTP destabilizes importin–cargo complexes and some nucleoporin–receptor interactions, whereas RanGTP facilitates formation of exportin–cargo complexes (Weis 2003). Ran is predominantly associated with GTP in the nucleus (Bischoff et al. 2002; Kalab et al. 2002) and thus serves as a critical switch for transport

initiation and termination of appropriate receptor–cargo complexes.

The topology of Nup153 at the pore suggests, however, that the order of events may not be as straightforward as previously envisioned because sites of receptor–nucleoporin interaction may not be discretely situated at distinct architectural elements of the pore. Specifically, the large spread in distribution found for the C-terminal region of Nup153 leads to the notion that this flexible domain can protrude to the cytoplasmic face of the pore (Fahrenkrog et al. 2002). Consistent with this, a monoclonal antibody (SA1) against an epitope within the C-terminal FG-rich region of Nup153 can “piggyback” efficiently from the cytoplasm to the nuclear face of the pore even though Nup153 is not a traditional shuttling protein (Nakielny et al. 1999). Thus, although Nup153 is thought to be the site of termination for importin- β -mediated import, an import complex may encounter Nup153 early during passage through the pore. This leads to additional implications about the potential role of the FG region as a guide for transport as it extends and retracts from its site of anchoring. Although this influences the notion of how cargo–receptor complexes may come to the terminal site of pore transit, it does not alter the view that Ran plays a key role once the Nup–receptor–cargo is in the environment rich in RanGTP. Indeed, perhaps such flexibility in nucleoporin topology accounts for the ability to reverse the directionality of transport by reversing the orientation of the RanGTP gradient (Nachury and Weis 1999) and for the observation that nuclear pores can function with a highly restricted number of FG repeat domains (Strawn et al. 2004; Zeitler and Weis 2004). The precedent set by Nup153, in terms of domain extension within the pore, may similarly apply to other FG-rich nucleoporins; evidence for this has been obtained in recent experiments (Paulillo et al. 2005). Further, a relationship between the transport status of the NPC and the arrangement of FG-rich regions was observed, suggesting an important functional connection.

Transport through the NPC could prove to involve more than simply getting from one compartment to another. Posttranslational modification by the ubiquitin-like peptide SUMO, which modulates protein activity, may be dynamically regulated at the NPC (Melchior et al. 2003). Specifically, Nup153 has been found to interact in an *in vitro* assay with SENP2, a SUMO protease (Hang and Dasso 2002; Zhang et al. 2002). Moreover, the region of SENP2 that confers the ability to associate with Nup153 is required for the localization of SENP2 on the nuclear side of the pore (Hang and Dasso 2002). Although the FG-rich region of Nup153 is known to bind SENP2, this is thought to be indirect (M. Matunis, personal communication). This is consistent with a precedent set in yeast in which SUMO proteases are tethered indirectly to nucleoporins via interactions with transport receptors (Panse et al. 2003). Interestingly, two other proteins involved in ligating SUMO to its targets are found at the pore as well: Ubc9, a SUMO E2-conjugating enzyme (Zhang et al. 2002) and the nuclear

pore protein Nup358, a SUMO E3-ligase (Pichler et al. 2002). The NPC localization of this machinery has fueled the hypothesis that, in some cases, transport is coupled to or hinges on the regulation of SUMO modification (Melchior et al. 2003). Given the mobility of Nup153, it is again worth considering that this nucleoporin may additionally serve to deliver SENP2 to specific nucleoplasmic sites. There is clearly much that remains to be learned about the SUMO–NPC connection and, further, whether the machinery for other modes of posttranslational modification similarly takes advantage of the NPC as a scaffold.

Nup153 is an RNA binding protein with sequence specificity

To date, Nup153 is unique among the vertebrate pore proteins in that an ability to directly bind RNA has been mapped to a discrete region (250–400) within this protein (Dimaano et al. 2001). RNA binding ability is conserved in *Drosophila*, *Xenopus*, and human Nup153, underscoring its importance (Dimaano et al. 2001). In a survey of individual RNAs, the RNA binding domain (RBD) of Nup153 was found to preferentially bind single-stranded RNA (Ball et al. 2004). This result indicates that the class of RNA contacted by Nup153 is mRNA. Consistent with this, several mRNAs tested were found to be recognized by the RBD in vitro. Indeed, this global affinity for mRNA led to the conclusion that the RBD has a very general preference for single-stranded RNA (Ball et al. 2004). However, when a particular RNA was analyzed in more detail for the determinants of binding, Nup153 was found to bind with different affinities to various fragments of the RNA (Ball et al. unpublished data). The exact nature of the motif recognized by Nup153 remains to be fully characterized, but appears to be loose enough that it is present at least once within many mRNAs.

Although Nup153 plays a role in mRNA export (Bastos et al. 1996; Ullman et al. 1999), it is not known how the RNA binding domain contributes to this role. It is tempting to speculate that recognition of a general motif found in single-stranded RNA [within the “mRNA identity element” (Erkman et al. 2005; Masuyama et al. 2004; Ohno et al. 2002; Rodrigues et al. 2001; Ullman 2002)] by Nup153 plays a role in directing RNA into the mRNA export route, but further investigation is needed. In any event, a role for the Nup153 RBD in mRNA export would not be in isolation. mRNA is packaged with many proteins, ultimately leading to the recruitment of the export receptor, NXF1 (for review, see Cullen 2003; Dimaano and Ullman 2004; Stutz and Izaurralde 2003). In this light, Nup153 is poised to recognize two key determinants of mRNA: a single-stranded stretch and NXF1, which can interact with the C-terminal region of Nup153 (Bachi et al. 2000). Previous reports of a direct interaction between mRNA and both Nup62 (Dargemont et al. 1995) and the pore-associated protein Rae1/Gle2 (Kraemer and Blobel 1997) suggest that other pore components may participate simi-

larly in an interface with mRNA cargo. Like many other RNA binding proteins, the Nup153 RBD exhibits affinity for single-stranded DNA as well (J.R. Ball and K.S. Ullman, unpublished results). This warrants further study in order to assess whether there is an intersection between the diverse functions of Nup153 and sites of single-stranded DNA, such as telomeres, promoters, and origins of DNA replication. Finally, recent results illustrating an unexpected role for RNA association in the mitotic function of Rae1/Gle2 (Blower et al. 2005) bring to light new possibilities by which interaction with RNA can contribute to the role of nucleoporins and pore-associated proteins.

Nuclear pore assembly: fitting Nup153 into a sequence of steps

After the separation of chromosomes at mitosis, formation of new nuclei immediately commences. Nucleoporins, nuclear lamina proteins, and integral membrane proteins must be reorganized in association with newly recruited membranes to reestablish nuclear and cytoplasmic environments (Marshall and Wilson 1997; Mattaj 2004). Localization studies reveal that Nup153 is recruited to the chromatids early in this stepwise process, at late anaphase to early telophase (Bodoor et al. 1999; Haraguchi et al. 2000).

Investigation of the requirements for nucleoporin assembly into NPCs has also revealed clues about key interactions during NPC assembly. For example, genomic knockout of Nup98 demonstrated that this nucleoporin is not required for Nup153 incorporation into the pore (Wu et al. 2001). Similarly, depletion of TPR, Nup205, and Nup93 by RNAi demonstrated that Nup153 does not require these Nups for assembly into the NPC (Hase and Cordes 2003). In contrast, depletion of nucleoporins in the Nup107–160 complex decreased incorporation of Nup153 into pores (Boehmer et al. 2003; Krull et al. 2004; Walther et al. 2003a). This pore subunit has an essential role in overall pore formation (Harel and Forbes 2004; Walther et al. 2003a); however, a direct role in recruiting Nup153 is supported by biochemical data. Indeed, the Nup107 complex binds to a sequence (210–338; Vasu et al. 2001; Walther et al. 2003a) within the pore-targeting region of Nup153 (NPAR; Enarson et al. 1998).

RNAi depletion of Nup153 inhibits incorporation of Tpr and Nup50 into the pore (Hase and Cordes 2003; Krull et al. 2004). Consistent with this, both Tpr and Nup50 are partners of Nup153 (Hase and Cordes 2003; Smitherman et al. 2000). When Nup153 is prevented from incorporating into the pores of nuclei reconstituted in vitro, there is some discrepancy in the impact this has on other nucleoporins (Smythe et al. 2000; Walther et al. 2001). This may relate to the extent to which Nup153 incorporation is prevented or the mode of inhibition. These two studies do converge, however, on the concept that Nup153 provides a link between the pore and the nuclear lamina that is critical for pore anchoring.

The order of nucleoporin recruitment as well as the interdependence of nucleoporin targeting has laid an important groundwork for understanding how NPCs are assembled. However, this begs the question of how pore assembly initiates at the right time and place to begin with. Recent results have pointed toward an elegant mechanism that guides deposition of the nucleoporins at the chromosome surface. Specifically, the interaction between importin- β and Nup153 (as well as other Nups) is modulated by Ran; RanGTP disrupts importin- β association with the nucleoporins that it chaperones (Walther et al. 2003b). Since RanGTP is locally concentrated near the chromosomal surface, this small GTPase is hypothesized to work in cooperation with importin- β to target pore protein delivery and to spatially restrict interactions between Nups (Harel and Forbes 2004).

Reconciling a structural role for Nup153 with its dynamic properties

How is it possible for a mobile nucleoporin to tether Tpr, a stable component of the nuclear basket structure? In one scenario, only a subset of the eight copies of Nup153 would have to be at the pore at any one time to fulfill its anchoring role. A second model to consider is that Nup153 delivers Tpr to the pore but does not remain stably engaged once Tpr is incorporated via other interactions. Finally, at least two distinct populations of Nup153 may exist at the pore, one in dynamic association and one stably integrated (see Fig. 3). As mentioned, there is in fact evidence for an immobile population: in FRAP analyses, the GFP-Nup153 fluorescence signal is incompletely recovered, indicating that a subset of Nup153 molecules are not exchanging (Daigle et al. 2001; Griffis et al. 2004). However, the extent of this immobile population varies in different studies, leaving it unclearly defined at this point. The number of GFP tags on Nup153 may influence its ability to incorporate stably into the pore and contribute to variability in the amount of immobile Nup153 detected.

The presence of both mobile and immobile populations of Nup153 might appear to be in conflict with estimates of only eight Nup153 molecules at the pore (Cronshaw et al. 2002)—at least the presumption is that these would be symmetrically arranged and tethered. However, Nup153 in dynamic exchange at the pore may have been lost during biochemical isolation of the NPC, enriching for the population of Nup153 putatively dedicated to a structural role at the pore. Independently targeted populations of Nup153 with different dynamic properties are consistent with the observed exposure of different epitopes at different regions within the pore basket (Fahrenkrog et al. 2002), i.e., a population of Nup153 in dynamic association at the distal ring of the basket may be situated such that the zinc finger domain is exposed to antibody, whereas this domain could be buried in a more stable population integrated into the nuclear coaxial ring (Fig. 3). Finally, the presence of an immobile population of Nup153 at the pore is also consistent with its proposed role in anchoring the

pore to the nuclear lamina (Smythe et al. 2000; Walther et al. 2001). Overall, although the nature of Nup153 localization is not yet fully elucidated, the apparent paradox of a structural role and transient pore association is not inherently in conflict.

Nup153 provides mechanistic insight into nuclear disassembly

During mitosis/meiosis in higher eukaryotes, the nuclear envelope disperses (Burke and Ellenberg 2002; Rabut et al. 2004b). Nuclear pore complexes are also dismantled, with some Nups relocating throughout the cytoplasm, as is thought to be the case for Nup153, and others additionally targeting to kinetochores (Belgareh et al. 2001; Joseph et al. 2004). Nucleoporins are released from the pore in a stepwise manner. Nup98 and Nup50 are among the first nucleoporins released, followed by a wave of Nup153 dispersal (Hase and Cordes 2003; Lenart et al. 2003). Tpr, Nup96, and a second Nup153 pool are then released, followed by Nup107 (and presumably its associated pore subcomplex; Loiodice et al. 2004). Along the lines of the previous section, the stepwise release of Nup153 could reflect the release (or decrease in association) of a mobile population, followed later by the exit of a second population more integral to pore structure. Although phosphorylation of several lamina and NPC components is likely important in releasing NE constituents, the contribution of additional factors in nuclear envelope breakdown was evident after investigating the role of Nup153.

Specifically, a recombinant protein encompassing the zinc finger region of Nup153 was found to dominantly interfere with nuclear disassembly recapitulated in *Xenopus* egg extract. Identification of proteins associating with the zinc finger region revealed members of the COPI coatomer complex (Liu et al. 2003). This complex had previously been characterized within the realm of secretory trafficking, in particular for its role in forming vesicles at the Golgi apparatus (Bonifacino and Glick 2004). Probing for a component of the COPI complex, however, revealed that β -COP (and presumably the entire complex) is recruited to the nuclear rim during early prophase. When the zinc finger region of Nup153 was included in the assembly/disassembly reaction, β -COP recruitment to the NE decreased, supporting the notion that Nup153 aides in coordinating the mitotic function of COPI at the NE (Liu et al. 2003). Moreover, antibodies to both Nup153 and to COPI were found to disrupt progression of nuclear envelope breakdown.

These results provide a mechanistic model for the rapid dispersal of nuclear membrane at mitosis. Specifically, the zinc finger module within Nup153 may serve to locally concentrate COPI, facilitating its recruitment to the nuclear membrane. Once juxtaposed at the membrane, COPI may promote membrane dispersal via vesicle formation. This stride forward in understanding players involved in nuclear envelope remodeling has brought with it many new questions. For instance, does COPI facilitate NE

breakdown globally in cell division or is the role more critical in rapidly dividing cells, such as the early embryo? Does the canonical COPI complex or a specialized subset of coatomer proteins participate in nuclear membrane remodeling?

Recent results suggest that Nup358, which contains a related zinc finger region, works in coordination with Nup153 during nuclear envelope breakdown (A. Prunuske, J. Liu, K.S. Ullman, unpublished results). A major question which remains to be addressed is what controls the temporal regulation of COPI recruitment to zinc finger domains. Early remodeling events at the pore and/or mitotic phosphorylation might play key roles in restricting this event to the right time and place. Given the central role for the small GTPase Ran in many interphase and mitotic events, it is also intriguing that this protein is a partner of the zinc finger region (Nakielnny et al. 1999).

Nup153 and pore remodeling during apoptosis

Apoptosis, or programmed cell death, results in several morphological changes to the cell. In addition to cytoskeletal alterations and plasma membrane blebbing, the nucleus undergoes remarkable remodeling during apoptosis. Chromatin condenses, and ultimately, nuclei themselves fragment (Strasser et al. 2000). Underlying the hallmark changes in morphology and cell function is a cascade of proteases, termed caspases. By targeting specific proteins for cleavage, caspases undermine normal elements of cell structure (Strasser et al. 2000). In turn, identification of caspase targets aids in deciphering how apoptosis is executed.

The dramatic changes at the nuclear periphery—including nuclear pore clustering (Buendia et al. 1999; Falcieri et al. 1994)—as well as the importance of nucleocytoplasmic trafficking during apoptosis (Yasuhara et al. 1997), led to investigation of NPC components. Nup153 was of particular interest due to its proposed role in anchoring the pore to the nuclear lamina. Indeed, following induction of apoptosis in tissue culture cells, Nup153 was cleaved in a caspase-3-dependent manner and was absent from pores found in clusters (Buendia et al. 1999; Ferrando-May et al. 2001). The site of cleavage was mapped to residues 36–391 (Ferrando-May et al. 2001) and likely corresponds to the putative caspase-3 site positioned at amino acid 343 (Buendia et al. 1999).

In addition to Nup153, several other nucleoporins are targeted for disruption during apoptosis (Buendia et al. 1999; Ferrando-May et al. 2001; Kihlmark et al. 2001). This attack on nuclear pore components appears to take place in a sequential order, eventually disabling structural and transport roles of the pore (Kihlmark et al. 2004). Cleavage of Nup153 and Lamin B (Kihlmark et al. 2001) corresponds to pore clustering and, in collaboration with cytoplasmic alterations (Croft et al. 2005), may contribute to disruption of nuclear morphology.

Nup153 has also been found to be targeted for degradation following oxidative stress, although it is not yet

clear if this is dependent on caspase cleavage (Kodiha et al. 2004). While further studies are needed to integrate information, it is now well established that modulation of Nup153 and other Nups occurs during various adaptive responses (also see below). This aspect of pore remodeling clearly could be serving as a central functional switch; however, more investigation into the causal relationship between individual nucleoporin cleavage and pore function is needed.

With respect to Nup153, one mechanistic question that arises is that of how proteolytic cleavage relates to Nup153 mobility. Indeed, apoptosis is experimentally induced in some cases by extended treatment with the transcription inhibitor actinomycin D, also known to arrest Nup153 mobility. It is interesting to note that the putative Nup153 caspase-3 cleavage site is within the RNA binding domain. Possibly, the lack of RNA cargo, under conditions of transcriptional inhibition (or the general block to mRNA export that occurs during apoptosis), leaves Nup153 vulnerable to caspase cleavage. More information about the dynamics of Nup153 under other conditions that lead to apoptosis is needed in order to understand whether there is a link between arresting the dynamic exchange of Nup153 at the pore and the ability of caspases to target this nucleoporin.

Cellular dysregulation and the nuclear pore

In order for viruses to successfully propagate, they must elude protective cellular mechanisms, while simultaneously utilizing cellular machinery for transcription, replication, translation, and other fundamental aspects of their life cycle. In many cases, viruses interface with nucleocytoplasmic transport machinery and the nuclear pore complex itself. This can occur during the course of nuclear entry (Cullen 2001; Whittaker et al. 2000) or in promoting the export of specific viral transcripts from the nucleus (Cullen 2003; Harris and Hope 2000; Zolotukhin and Felber 1999). In other cases, viral proteins target cellular transport machinery in order to alter the trafficking patterns of host proteins (for example, see Faria et al. 2005; Gustin 2003; Petersen et al. 2000; von Kobbe et al. 2000). Another interesting example of global alterations at the NPC has been found during infection with picornaviruses such as poliovirus and rhinovirus. The replication of these viruses takes place entirely in the cytoplasm. Thus, although viral proteins and RNA do not need to traverse the pore, the relocation of certain resident nuclear proteins may enhance viral RNA synthesis. Altering pore function in this case may be advantageous both to facilitate the viral life cycle and, additionally, to prevent a program of antiviral gene expression.

During poliovirus or rhinovirus infection, nuclear import is selectively inhibited in a manner that is dependent on translation but not transcription (Gustin and Sarnow 2001, 2002). Proteolytic cleavage of specific nucleoporins, including Nup153, has been shown to occur following infection with these two viruses and is believed to be the molecular basis for alterations observed in NPC function.

These cleavage events do not result in complete abrogation of pore function: the classical NLS and M9 NLS import pathways are inhibited (Gustin and Sarnow 2001, 2002), whereas the glucocorticoid receptor appears able to shuttle in a hormone-modulated manner (Gustin and Sarnow 2001). While certain nuclear proteins retain their selective intracellular distribution (Gustin and Sarnow 2001), there is evidence for alterations in passive diffusion following infection (Belov et al. 2000, 2004). The influence on any particular transport cargo may depend on whether retention mechanisms work in cooperation with active transport. The alterations observed in nucleocytoplasmic division, as well as in the overall appearance of the pore detected at the level of electron microscopy (Belov et al. 2004), correspond to dramatic reductions in the level of Nup153, Nup98, and Nup62 (Gustin and Sarnow 2001, 2002; K. Gustin, personal communication).

Although picornaviruses have been shown to induce apoptosis in a caspase-dependent manner (Agol et al. 1998), nucleoporin turnover appears to be modulated by a distinct pathway in infected cells (Gustin and Sarnow 2001, 2002). Learning the identity of the protease(s) responsible for nucleoporin cleavage during viral infection will yield significant further insight into cellular dysregulation caused by viral infection.

Recently, a region of Nup153 (448–634) was found capable of substituting for a domain within *S. pombe* Nup124 that is essential for LTR-retrotransposon propagation (Varadarajan et al. 2005). Similarities between retrotranspon Tfl-Gag and HIV Vpr raise the intriguing possibility that Nup153 may play a role in Vpr-mediated pathogenesis, such as nuclear blebbing (de Noronha et al. 2001). Other forms of cellular dysregulation during disease, although mechanistically quite distinct, involve nuclear pore proteins as well. In this regard, Nup153 has been recognized as a target of auto-immunity in disease (Enarson et al. 2004). Nup153 has also been noted to colocalize with aggregates of Lamin A protein under experimental conditions (Bechert et al. 2003), underscoring the possibility that laminopathies may involve alterations in the interface between Nup153 and the nuclear lamina.

Concluding remarks

Overall, our understanding of Nup153 to date clearly illustrates that, although composed of relatively few building blocks, the NPC is far from being a simple machine. Its complexity lies in the myriad roles taken on by individual components, which indeed extend beyond the pore itself. Nup153 has proven to be a remarkable example of functional flexibility. This trend is true for other pore proteins as well, although further studies are needed before concluding whether all Nups similarly multitask or whether there are pore constituents dedicated to specific tasks. Delving deeper into the role of individual nucleoporins and integrating this information will continue to yield new insight into how nucleocytoplasmic compartmentalization is achieved at interphase, modulated in response to

specific signals, and remodeled during the cell cycle. Further study of Nup153 and other Nups will also be important in elucidating the role of pore proteins in disease. And, given the trend so far, new surprises in pore function are likely to be revealed as well.

Acknowledgements Michael Matunis, Birthe Fahrenkrog, and Kurt Gustin kindly shared data prior to publication. We appreciate critical feedback on the review from Amy Prunuske, Valerie Blanc, Birthe Fahrenkrog, and Kurt Gustin. We thank Diana Lim for expert help with illustrations. The authors were supported by a grant from the NIH (GM61275).

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