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Nuclear pore complexes: dynamics in unexpected places

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In vivo studies on the dynamics of the nuclear pore complex (NPC) in yeast suggested that NPCs are highly mobile in the nuclear envelope. However, new evidence indicates that in mammalian cells NPCs are stably attached to a flexible lamina framework, but a peripheral component can exchange rapidly with an intranuclear pool.

Over 25 years ago, when the composition and properties of the nuclear pore complex (NPC)* were still enigmatic, Aaronson and Blobel (1974) carried out pioneering biochemical fractionation studies on nuclei that framed subsequent thinking on how NPCs are organized in the nuclear envelope (NE). They made the striking observation that treatment of isolated rat liver nuclei with a nonionic detergent left NPCs intact and still attached to the membrane-denuded nuclear surface. This suggested that the NPC itself is a stable supramolecular assembly and is not dependent on membrane phospholipids for its structural integrity. The basis for attachment of NPCs to the nuclear surface was clarified when nonionic detergent extraction of isolated NEs revealed that the detergent-resistant NPCs were linked at their nucleoplasmic side to an ~15-nm thick fibrous structure (Aaronson and Blobel, 1975). This appeared to correspond to the nuclear lamina, a structure underlying the NE that previously had been visualized only in a few specialized cell types. The subsequent characterization of the major lamina polypeptides, termed nuclear lamins (Gerace et al., 1978), led to the realization that the nuclear lamina is ubiquitous in higher eukaryotes. The biochemical fractionation studies, together with the findings that lamins are members of the intermediate filament protein superfamily and form a polymeric array at the NE, gave rise to the model that the lamina is a scaffolding structure of the NE and serves to anchor NPCs (Fig. 1) and to provide an attachment site at the NE for chromatin (for review see Gerace and Burke, 1988; Gruenbaum et al., 2000). The properties of NPCs that were suggested by biochemical fractionation studies in the 1970s now have been given strong physiological validation by the results of Daigle et al. (2001, this issue), who have examined the dynamics of NPCs in living cells by using NPC proteins (nucleoporins) tagged with green fluorescent protein (GFP). They determined that a structural core of the NPC forms an extremely stable supramolecular assembly during interphase, and moreover that NPCs undergo little independent movement within the NE, probably by virtue of their attachment to the lamina. Their examination of Nup153, a nucleoporin localized at the nucleoplasmic periphery of the NPC, yielded the surprising result that Nup153 underwent rapid dynamic exchange between an NPC-attached and an intranuclear pool. This unexpected finding provides a new perspective on mechanisms of nuclear transport.

Stability of NPCs and their immobilization in the NE

NPCs span the double membrane of the NE and provide passageways for signal-mediated transport between the nucleus and cytoplasm (for review see Gorlich and Kutay, 1999; Nakielny and Dreyfuss, 1999). Most well-characterized trafficking of protein and RNA through the NPC involves the binding of signal-bearing macromolecules to nuclear transport receptors, which are translocated through the NPC by interaction with specific nucleoporins. The NPC has a mass of ~125 MDa in vertebrates, and is estimated to contain up to ~50 or more different polypeptides. Structural studies have shown that the framework of the NPC is a central ring–spoke assembly with eightfold rotational symmetry, from which 50–100-nm long fibrils extend into the nucleoplasm and cytoplasm (Fig. 1). A substantial number of nucleoporins have been molecularly characterized and localized to discrete NPC regions (for review see Stoffler et al., 1999).

Daigle et al. (2001) chose two nucleoporins for their analysis of NPC dynamics (Fig. 1). One of these, POM121, is an integral membrane protein that is localized to the ring spoke area and has been presumed to provide a membrane anchor for the NPC. The second nucleoporin is Nup153, a peripheral membrane protein that is localized to the nucleoplasmic fibrils and serves as a binding site for both import and export receptors (see Stoffler et al., 1999). By expressing POM121 and Nup153 tagged with multiple copies of GFP in cultured mammalian cells, it was possible to obtain a fluorescent signal of sufficient sensitivity to visualize individual NPCs and small clusters of NPCs as discrete foci on the nuclear surface.

The mobility and stability of integration of these GFP-tagged proteins in the NPC were examined by FRAP. Strikingly, in nondividing cultured cells expressing POM121-GFP

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*Abbreviations used in this paper: AL, annulate lamellae; GFP, green fluorescent protein; NE, nuclear envelope; NPC, nuclear pore complex.

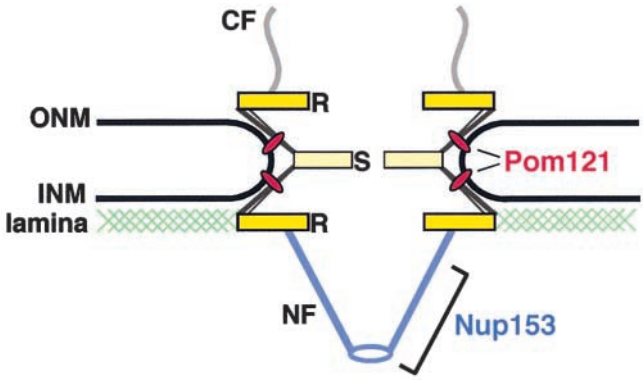

Figure 1. **Model of the nuclear pore complex.** The NPC occurs in regions of the NE where the outer nuclear membrane (ONM) and inner nuclear membrane (INM) are continuous. The framework of the NPC consists of eight central spokes (S) that are connected to flanking rings (R), from which cytoplasmic (CF) and nuclear (NF) fibrils emanate. The nuclear fibrils often are joined at their distal end to form a basket-like structure, as shown. POM121 is an integral membrane protein localized to the ring–spoke region of the NPC, whereas Nup153 is a peripheral membrane protein found in the nuclear fibrils. The NPC is attached at its nucleoplasmic side to the lamina.

fusions, the GFP fluorescence in a bleached zone of the NE recovered very slowly, with a $t_{1/2}$ of ~20 h. Similarly, in growing cells complete recovery of the bleached zone was seen only after the next mitosis, when the NPC was reversibly disassembled. This indicates that any individual molecule of POM121 remains integrated in the NPC for a very long time, implying that a structural core of the NPC remains stably assembled over a similarly long period.

Using POM121 as a marker for the stable core of the NPC, the authors investigated the mobility of individual NPCs in the plane of the NE by tracking a group of NPC foci localized in one zone of the nucleus. They observed that the foci showed no independent movement relative to each other, but rather moved in synchronous waves that likely reflected large scale movement of the nuclear surface. To simultaneously visualize the movement of lamins and NPC, they coexpressed POM121–yellow fluorescent protein and lamin B1–GFP fusions. Consistent with previous studies (Broers et al., 1999; Moir et al., 2000), lamin B1–GFP showed very slow recovery after photobleaching, demonstrating its low mobility and indicating that lamins form a highly stable protein assembly in interphase. This allowed the authors to demarcate specific regions of the lamina by bleaching the GFP in a grid pattern on the nuclear surface. They then could track the movement of individual NPCs, as well as regions of the lamina itself, with respect to this grid. They found that the lamina and NPCs behaved as if they were part of the same elastic network, which underwent periodic deformations and then relaxed back to its previous form. This provides a compelling argument for a role of the nuclear lamina and its associated structures in providing a flexible yet stable supporting scaffolding for NPCs, and for the NE as a whole.

The finding that NPCs are stably anchored in the NE of mammalian cells contrasts with observations made in *Saccharomyces cerevisiae*, in which an examination of cells containing GFP-tagged nucleoporins indicated that NPCs are diffusionally mobile in the plane of the NE at the time of nuclear fusion or karyogamy (Belgareh and Doye, 1997; Bucci and Wente, 1997). This diffusional mobility is consistent with the absence of nuclear lamins in budding yeast. Nonetheless, it remains possible that some as-yet-undescribed functional counterparts of lamins are present in yeast and simply are disassembled during karyogamy when the NE needs to undergo major restructuring.

The mechanism of attachment of NPCs to the lamina in higher eukaryotes remains an important, unresolved question. Nup153 is associated with lamin B3 in *Xenopus* egg extracts, and the addition of a dominant negative mutant of lamin B3 to a cell-free nuclear assembly assay leads to sequestration of lamin B3 in intranuclear aggregates and selectively blocks appearance of Nup153 in the NPC (Smythe et al., 2000). This suggests that a Nup153–lamin interaction may have a role in anchoring NPCs to the lamina. However, since Nup153 assembles in the NE before lamin B, this interaction clearly is not required for NPC assembly. This is consistent with the finding that lamins are not present in annulate lamellae (see below).

Induction of annulate lamellae by overexpressed nucleoporins

Annulate lamellae (AL), which usually are found in the cytoplasm, are tightly stacked layers of double membranes perforated with a high density of pore complexes that are ultrastructurally very similar to NPCs (for review see Kessel, 1992). Of the nucleoporins that have been examined, most are present in AL, including POM121 and Nup153. Although AL usually are present only at low frequency, if at all, in somatic cells, they are abundant in oocytes and embryonic cells. In cultured cells, the overexpression of either POM121 or Nup153, but not of certain other nucleoporins, induces a significant proliferation of cytoplasmic AL (Imreh and Hallberg, 2000; Daigle et al., 2001). Overexpression of Nup153 also induces intranuclear arrays of closely stacked double membranes lacking pore complexes (Bastos et al., 1996), similar to a phenotype obtained by overexpression of the nucleoporin Nup53p in yeast (Marelli et al., 2001). The cytoplasmic AL induction by overexpression of POM121 and Nup153 may be due to an upregulation of the levels of other nucleoporins, although elevated levels of POM121 and Nup153 could also promote the formation of pore complexes from a hypothetical pool of unassembled nucleoporins. These findings raise the intriguing question of how the formation of pore complexes is triggered. Clearly this must be a concerted process involving fusion between two membranes to form a pore, coupled with assembly of the different architectural subunits that comprise a pore complex. Since AL assembly induced by the overexpression of POM121 and Nup153 occurs without a simultaneous increase in NPC assembly in the NE (Daigle et al., 2001), an equally interesting question is how NPC density in the NE is regulated. Perhaps this is linked to some property of the lamina, which needs to be locally interrupted in regions of the NE underlying forming NPCs.

AL have been proposed to represent storage depots for excess nucleoporins, which then could be used to form NPCs on demand (see Kessel, 1992). Consistent with this, Daigle et al. (2001) found that a substantial fraction of POM121 (≤40%) in the induced AL was diffusionally mobile, in contrast to the virtually immobile POM121 in the NE. In principle, if the pore complexes of AL were relatively unstable, their components could disassemble quite rapidly and be mobilized for the assembly of new NPCs in the NE, which occurs throughout interphase in growing cells (for review see Maul, 1977). NPCs are globally disassembled during mitosis by a process that is correlated with the hyperphosphorylation of nucleoporins (Macaulay et al., 1995; Favreau et al., 1996), which may promote NPC disassembly. Analogously, the pore complexes of AL could be destabilized during interphase by a spatially restricted posttranslational modification such as phosphorylation. Alternatively, pore complexes of AL could lack some key stabilizing proteins found in interphase NPCs, and thus could be intrinsically more dynamic.

Exceptionally rapid exchange of Nup153 at the NPC

In examining the FRAP characteristics of a Nup153–GFP fusion, Daigle et al. (2001) found that in sharp contrast to the very low mobility of NPC-associated POM121-GFP, Nup153–GFP repopulated a bleached zone of the NE very rapidly, with a $t_{1/2}$ of ~15 s. Nup153 that was tagged at either its NH_2 or COOH terminus showed a similarly rapid recovery rate, arguing that the high mobility of Nup153 was not an artifact of being tagged with GFP. Since an intranuclear pool of Nup153 is present in nontransfected as well as transfected cells, these data imply that Nup153 undergoes rapid exchange between intranuclear (nonassembled) and NPC-associated pools to give rise to the short FRAP times observed. An apparent cytoplasmic pool of Nup153–GFP also was seen in the transfected cells, but this pool was not in rapid equilibrium with the nuclear pool, since all of the cytoplasmic fluorescence could be depleted by repeated bleaching of a defined region of the cytoplasm without significant loss in intensity of the nuclear pool. Thus, although NPC-associated and intranuclear Nup153 appear to undergo rapid exchange, these results argue that Nup153 does not shuttle between the nucleus and cytoplasm as proposed previously (Nakielny et al., 1999).

The description of Nup153 dynamics at the NPC raises several important questions. Is the association of Nup153 with the NPC regulated, or does it occur constitutively as an simple on/off binding reaction? Does Nup153 associate with only a single site at the NPC? Finally, what is the function of the rapid exchange between intranuclear and NPC-bound Nup153?

If this exchange is relevant to nucleocytoplasmic transport, several potential roles can be envisaged. Since Nup153 is suggested to be an initial binding site for export receptor complexes at the NPC (see Stoffler et al., 1999), an excess of unassembled Nup153 inside the nucleus might be expected to act as a competitive inhibitor for the binding of export complexes to the NPC. The finding that the intranuclear pool of Nup153 rapidly exchanges with the NPC can resolve this apparent conundrum, since export complexes bound to intranuclear Nup153 would be efficiently conducted to the NPC by this mechanism. Moreover, the nuclear dynamics of Nup153 could reflect a mechanism to chaperone nuclear export complexes from the nuclear interior to the NPC. Nuclear export complexes are formed by the cooperative binding of Ran-GTP and cargo to export receptors of the karyopherin family (see Nakielny and Dreyfuss, 1999). Previous biochemical studies have indicated that export receptors in complex with Ran and cargo bind with higher affinity to nucleoporins than do unliganded export receptors (Askjaer et al., 1999; Kehlenbach et al., 1999). If the stability of the export complex were further enhanced by binding to a nucleoporin such as Nup153, this would provide a means to help commit export complexes to the export pathway shortly after their formation in the nucleoplasm. In the future it will be important to determine which other nucleoporins have dynamic properties similar to those described for Nup153, and in particular to examine nucleoporins associated with the cytoplasmic fibrils of the NPC. Whatever the functions of reversible interactions of nucleoporins with the NE, the findings of Daigle et al. (2001) have further blurred the line between the mobile and stationary components of the nuclear transport machinery.

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