Cleave to Leave

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in transactivation, one possibility is that these protein domains—and not a ligand—regulate the receptor's function. Finally, there is the possibility that upon synthesis of the HNF4 protein, a different fatty acid can be introduced into the protein. This is not a completely farfetched idea, as an increasing number of both endogenous as well as exogenous compounds have been found to regulate the expression of the HNF4α gene (Sladek and Seidel, 2001). However, even if it can be shown that a ligand can be exchanged under some physiological, or therapeutic, condition, it must also be shown that different ligands have the ability to impart different activities on the protein. At this point, it is not clear how that would happen, since the AF-2 does not contact the ligand. Although, the *Structure* paper notes that the bacterially expressed HNF4γ bound a fatty acid rarely found in *E. coli* (C17:1), suggesting that there might at least be a selection process for the ligand/cofactor.

Finally, the findings of Wisely et al. (2002) may alter our thinking about the evolution of nuclear receptors. It was previously proposed that HNF4 and two other highly conserved family members were ancestral nuclear receptors that lacked ligands and that the ability to bind ligands arose independently more than once during evolution (Escriva et al., 2000). Now, however, one must consider the possibility that the original nuclear receptor did bind a lipophilic compound, but its role was as an integral component of the protein structure, not as an interchangeable element that induced allosteric and functional changes, as do the more modern ligands. The binding of a nonexchangeable cofactor makes it easier to understand conceptually why the LBD has been conserved during evolution and how the first true ligands arose, i.e., as a variation on a preexisting theme as opposed to a completely new characteristic. In any case, in this post-genomic era it is humbling to consider the possibility that perhaps at least part of what makes us different from other organisms is not our genes or the proteins they encode but rather the ligands they bind.

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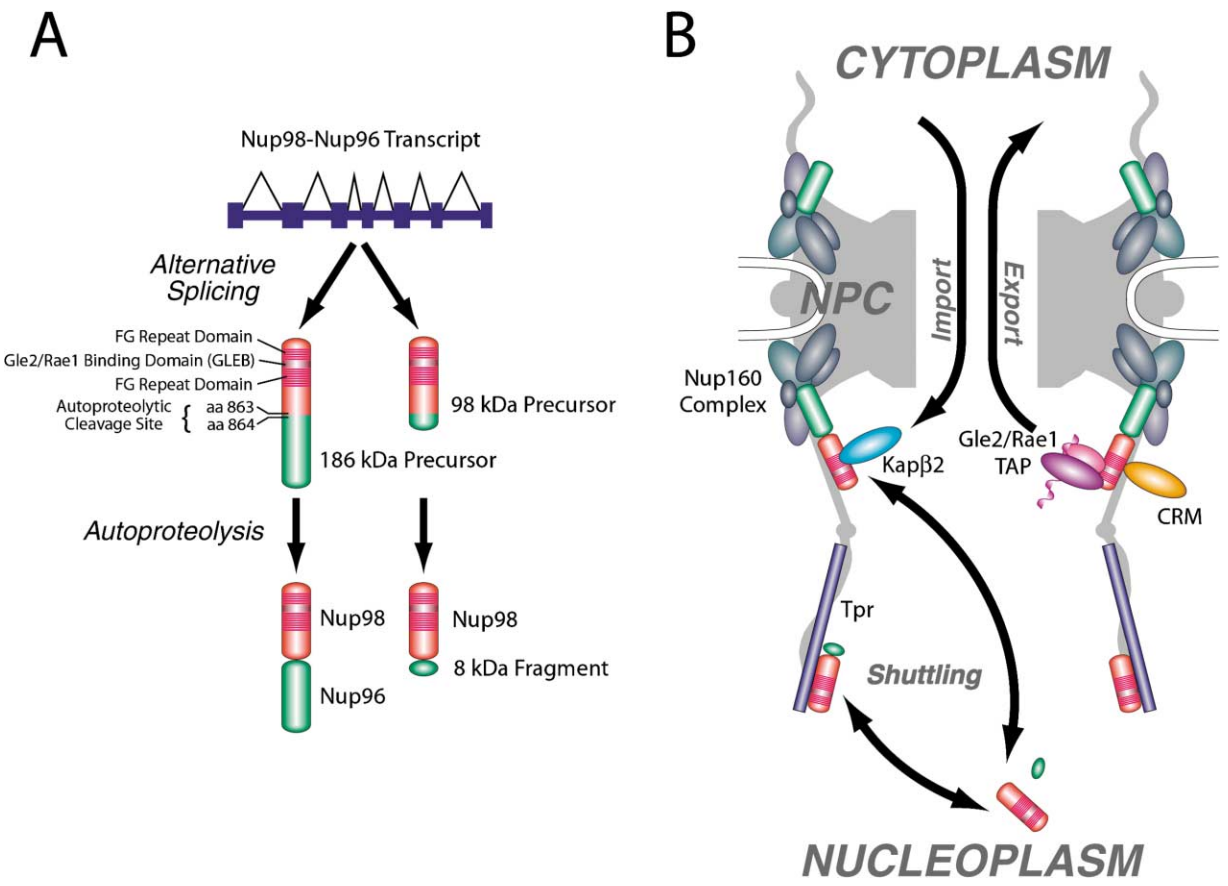
Cleave to Leave: Structural Insights into the Dynamic Organization of the Nuclear Pore Complex

A detailed understanding of the fine structure of the nuclear pore complex has remained elusive. Now, studies on a small protein domain have shed light on the dynamic organization of this massive assembly.

In the new age of proteomics, determining the interactions between proteins in large, dynamic supramolecular assemblies is a major goal. In a cell, the number of interacting proteins is dauntingly large, with interactions changing depending on the subcellular localization of the partners or the state of the cell. Furthermore, varying affinities and expression levels often make detection of functional complexes a formidable challenge.

The nuclear pore complex (NPC), weighing in at $\sim$100 MDa, is an example of such a large and dynamic supramolecular assembly. Located in the nuclear envelope, NPCs are the gatekeepers between the nucleus and cytoplasm. NPCs consist of approximately 30 different proteins, termed Nups. These multimerize to form an octagonal tube, from which fibers extend into both the cytoplasm and the nucleus (see Figure, panel B). Some soluble transport factors such as GTP-bound Ran power the process, and others such as karyopherins act as carriers, facilitating transport of their cargoes by interacting with particular sequence motifs in specific Nups. To understand its role as a transport machine, recent "holistic" approaches have studied the entire NPC or reconstituted significant constituent subcomplexes. In this issue of *Molecular Cell*, Hodel et al. (2002) have taken an alternative approach, showing how one can obtain important information about a large and dynamic complex like the NPC by instead looking in detail at key structural domains found in certain of its components.

The approach Hodel et al. have taken includes an



Halve the Nup, Double the Roles?

(A) Alternative splicing and autoproteolysis steps leading to the production of Nup98, Nup96, and an 8 kDa fragment (see text for details). (B) Nup96 is part of the Nup160 complex and appears to be bound to Nup98 on the nuclear side of the NPC. The protein import and export factors Kapβ2 and CRM, respectively, bind the FG repeat domain of Nup98, as does the mRNA export factor TAP. Gle2/Rae1 also binds via a dedicated binding site. Nup98 can also shuttle off Nup96 onto Tpr and into the nuclear interior.

elegant quantitative GFP-based in vivo folding assay to screen for domains of proteins that form independent folding units amenable to expression in *E. coli*. The screen is based on the observation that, in a fusion protein, a C-terminal GFP will form a functional fluorophore only if the N-terminal protein can be expressed in a correctly folded, soluble form (Sachdev and Chirgwin, 1998; Waldo et al., 1999). The target of their studies is the interface between Nup98 and Nup96. These are among the most intriguing nucleoporins, with a distinctive biogenesis. Both Nup98 and Nup96 are encoded by the same gene, which produces several alternatively spliced mRNAs (see Figure, panel A). The largest of these mRNAs codes for a 186 kDa precursor, and the smallest codes for a 98 kDa precursor. Both precursors are then cleaved by autoproteolysis at a shared sequence, the former generating Nup98 and Nup96, the latter forming the same Nup98 plus an 8 kDa polypeptide. Nup145N and Nup145C, yeast homologs of Nup98 and Nup96, respectively, are also generated by autoproteolysis of a precursor (Nup145), indicating the process is conserved (Teixeira et al., 1997; Emtage et al., 1997; Fontoura et al., 1999; Rosenblum and Blobel, 1999).

After cleavage, the Nup98 and Nup96 products make different lifestyle choices (see Figure, panel B). Nup96 is the steady type—it is found in the Nup160 subcomplex (homologous to the Y-shaped Nup84 complex in yeast), which forms a structural core of the NPC on both the nuclear and cytoplasmic side of the NPC (Belgareh et al., 2001; Lutzmann et al., 2002). On the other hand, Nup98 is an itinerant worker. It has been shown to shuttle between its position at the NPC on the nuclear filaments to several locations in the nuclear interior where it may also associate with the putative nuclear filament protein, Tpr (Fontoura et al., 2001; Griffis et al., 2002). By virtue of bearing characteristic Phe-Gly repeat motifs, Nup98 belongs to the family of "FG Nups." The FG repeat domain serves as a docking site for transport factor/cargo complexes as they transverse the NPC (reviewed in Vasu and Forbes, 2001). Consistent with this, Nup98 is implicated in nuclear import and is crucial for nuclear export. Furthermore, Nup98 contains a binding site for Rae1/Gle2, another mRNA export factor (see Figure, panel B). Clearly, the dynamic interaction interface between Nup98 and Nup96 is a particularly interesting one to study, because Nup98 must be both attached to the

NPC with enough strength to act as a firm base for the docking of transport factors while at the same time being sufficiently free to allow Nup98 to move around for its other nuclear functions.

Hodel et al. (2002) have solved the three-dimensional structure of the Nup98-Nup96 autoproteolytic interface by crystallizing three different protein fragments: a Nup98 fragment terminated at the autoproteolytic cleavage site and Nup98-Nup96 fragments in pre- and postcleavage forms. The structures, solved at ~3 Å resolution, reveal a new protein fold and give structural insight into the mechanism of cleavage and association of the two resulting proteins. The autoproteolytic domain of Nup98 adopts a half-open, β sandwich-like fold anchored by a large β sheet that expands across the entire length of a domain. The atomic details of the proteolytic active site in both pre- and postcleavage forms confirm the intein-like mechanism proposed by Rosenblum and Blobel (1999) but also introduce new players in the catalytic event. The cleavage has remarkably little effect on the overall structure of the autoproteolytic domain except in the residues immediately adjacent to the cleavage site, suggesting that, after cleavage, both the Nup96 and 8 kDa fragments remain bound to Nup98 in a conformation similar to that observed before cleavage. Therefore, the authors suggest that the structure showing Nup98-Nup96 interaction after the cleavage should also represent the three-dimensional structure of a pore-docking event.

Why the biogenesis of Nup98 and Nup96 has evolved as such still remains largely speculative. Probably, the ancestors of Nup98 and Nup96 were fused together as a single polypeptide, just like their 186 kDa precursor (see Figure, panel A). Only upon acquiring the ability to perform autoproteolytic cleavage was the Nup98 ancestor able to take on additional roles as a mobile protein. In fact, it is also a mystery why Nup98 should need to shuttle on and off the NPC at all. Interestingly, Nup98 is not the only "mobile" Nup—for example, Nup50 (and its yeast counterpart, Nup2) also appears to shuttle between the NPC and nuclear interior (Guan et al., 2000; Dilworth et al., 2001). A growing idea is that this shuttling helps chaperone transport cargoes from deep in the nuclear interior to the NPC. Because Nup98 binds RNA export factors, it may associate with transcripts near their site of production within the nucleus. This would help target them to the NPC, binding to Nup96 and passing their associated transcript out into the cytoplasm. The concept is supported by recent data showing a positive link between transcription and Nup98 shuttling activity (Griffis et al., 2002). Presumably, the binding site for the RNA export factor Gle2/Rae1 in Nup98 would help it to chaperone RNAs. Curiously, though, the Gle2/Rae1 binding site is not found in Nup145N, the similarly nucleoplasmic yeast homolog of Nup98, but only in Nup116. Both Nup116 and Nup100 are homologs of Nup98, but are not autoproteolytically cleaved and localize mainly (though not exclusively) to the cytoplasmic face of the NPC. Thus, how the Gle2/Rae1 binding domain functions on different sides of the NPC in different organisms is still mysterious. Remarkably, the surface residues of the pore-docking site are highly conserved in the three yeast Nup98 homologs (Nup145N, Nup100, and Nup116). The authors speculate that Nup98 and its yeast homologs might have another, yet unidentified, evolutionarily conserved interaction partner at the NPC. It also seems likely that as all three share the docking site interface, all should interact with Nup145C (the Nup96 homolog): Nup145N on the nuclear-sided copies of Nup145C, and Nup116 with Nup100 on the cytoplasmically sided copies.

This work represents an important milestone, showing how high-throughput approaches rapidly led to the first high-resolution structure of a complete domain in the NPC. We are lucky indeed that the methods used here represent another in an increasing array of powerful new tools available to the biologist. Surely the time is not too distant when not only the NPC but many other dynamic supramolecular assemblies will be understood in unprecedented detail.

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