Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Towards understanding nuclear pore complex architecture and dynamics in the age of integrative structural analysis

Ed Hurt[1] and Martin Beck[2]

Determining the functional architecture of the nuclear pore complex, that remains only partially understood, requires bridging across different length scales. Recent technological advances in quantitative and cross-linking mass spectrometry, super-resolution fluorescence microscopy and electron microscopy have enormously accelerated the integration of different types of data into coherent structural models. Moreover, high-resolution structural analysis of nucleoporins and their *in vitro* reconstitution into complexes is now facilitated by the use of thermostable orthologs. In this review we highlight how the application of such technologies has led to novel insights into nuclear pore architecture and to a paradigm shift. Today nuclear pores are not anymore seen as static facilitators of nucleocytoplasmic transport but ensembles of multiple overlaying functional states that are involved in various cellular processes.

Addresses
[1] Biochemistry Center of Heidelberg University, INF328, D-69120 Heidelberg, Germany
[2] European Molecular Biology Laboratory, Structural and Computational Biology Unit, Meyerhofstrasse 1, D-69117 Heidelberg, Germany

Corresponding authors: Hurt, Ed (ed.hurt@bzh.uni-heidelberg.de) and Beck, Martin (martin.beck@embl.de)

Current Opinion in Cell Biology 2015, **34**:31–38

This review comes from a themed issue on **Cell nucleus**

Edited by **Karsten Weis** and **Katherine L Wilson**

http://dx.doi.org/10.1016/j.ceb.2015.04.009

Introduction

The huge nuclear pore complexes (NPCs), which mediate nucleocytoplasmic exchange, exhibit a conserved overall structure consisting of sandwiched cytoplasmic, inner (spoke) and nuclear rings that form a central channel, through which the actual transport processes occurs. NPCs are assembled from a relatively small number of proteins (~30 nucleoporins), which are organized into complexes that are present in multiple copies in each NPC. Conserved modules include the (i) Nup82–Nup159–Nsp1 complex (in vertebrates vNup88–Nup214–Nup62) that is located at the cytoplasmic ring and plays a role in terminating mRNA export, (ii) the Nsp1–Nup49–Nup57 complex (vNup62–Nup45/Nup54–Nup58) that is located within the central channel and broadly involved in import and export reactions; (iii) the heptameric Y-complex also called Nup84 complex (vNup107–160), is the presently best characterized module and located within the cytoplasmic and nuclear rings. At last, (iv) the Nic96 complex, with its large structural nucleoporins Nup192 (vNup205) and Nup188, Nup157/Nup170 (vNup155), as well as Nic96 (vNup93) and Nup53/59 (vNup35) is located within the inner ring. Several other Nups that bind more peripherally to the NPC and are crucial for its transport function, are discussed elsewhere (for review see e.g. [1]). To determine how these various modules interact with each other in the context of the fused inner and outer nuclear membranes (INM, ONM) to form the massive NPC imposes a formidable challenge. Here we review scientific progress towards this goal in the last three years.

The intricate nature and compositional complexity of the NPC and the functional redundancy of its components, necessitates the application of integrated structural approaches that bridge across different length scales from atomic resolution and the subcomplex level up to the overall architectural organization. To generate such multi-scale structures, various data sets are combined using structural modeling approaches. The most popular experimental structure determination techniques for integration are X-ray crystallography to obtain static high-resolution information of suitable units, nuclear magnetic resonance (NMR) to obtain dynamic high-resolution information of small units, electron microscopy (EM) to obtain partly dynamic moderate to high-resolution information of large units, small angle X-ray scattering (SAXS) to obtain dynamic low resolution shape information and cross-linking mass spectrometry to obtain sparse, spatial restraints at moderate resolution — but of course various other techniques exist that are not mentioned here (for review see e.g. [2]). As a consequence, there is a high need for integrative structures to include metrics and assessments to describe the accuracy, precision and reliability of the final results and also how to deposit them into public databases. In some cases, for example, the fitting of X-ray structures into cryo-EM maps such quality measures have been well defined, however, due to the manifold possibilities of a rapidly developing field such criteria are still subject to debate in some other cases. Before the review period, several concepts of the NPC architecture — derived by very different means — had been put forward [3–5]. In principle the integrative structural biology community would define such architectural arrangements as a 'structure' [6] if they are experimentally derived, as for example, in case of the work of Alber *et al.* [3], while there are referred to as 'model' if derived by more conceptual means. This terminology is however not yet widely used in the field of nucleocytoplasmic transport, possibly due to the enormous scale of the experimental problem and the inherent difficulty to validate the resulting integrative structures. In any case, further work is required to reach a comprehensive understanding of nuclear pore architecture at high resolution. The key question in this field was and still is to unravel the structural principles, which allow the various NPC modules to become organized into the octagonal structural NPC framework with an estimated mass of 50–110 MDa. During the review period, impressive progress towards this goal has been made by the integration of multiple sources of experimental data, such as cross-linking mass spectrometry with crystallographic and electron microscopic data obtained of both isolated subcomplexes and fully assembled nuclear pores. Here, we discuss these findings with a focus on the two major scaffolding modules, namely the Y-complex and inner ring complex.

Stoichiometric measurements of the nuclear pore complex

Since 500–1000 building blocks (nucleoporins) are required to assemble a single NPC, one indispensible necessity for understanding its architecture is to determine the exact stoichiometry of all the involved players. Already more than a decade ago, protein stoichiometries of the yeast [7] and rat [8] nuclear pore components have been analyzed by quantitative Western blotting and gel band densitometry, respectively. More recently, the stoichiometry of the human NPC was determined using targeted proteomics with absolutely quantified, heavy labeled peptides as intrinsic standards [9], which is a highly accurate method. Although the results of the three proteomic studies were somewhat diverse, possibly due to differences across species, they consistently suggested iso-stoichiometric abundance of almost all members of the Y-complex in both yeast and mammals, which is in agreement with biochemical data obtained in isolation (see below). By contrast, an elevated abundance of Nsp1 (vNup62) and Nic96 (vNup93) as compared to related members of the Nsp1-Nup49-Nup57 and inner ring complex was observed. This finding might be explained by the multiple distinct roles of these two interacting proteins in NPC architecture and highlights the importance of determining the stoichiometry of isolated complexes in addition to fully assembled NPCs [10•,11].

In order to use the determined nucleoporin stoichiometries for structural modeling, these values yet have to be calibrated to copy numbers per NPC. This has initially been done by setting the lowest observed abundance level to 8 copies per NPC, which corresponds to 1 copy per asymmetric unit [3,8]. The first study that experimentally determined absolute abundances relied on quantification of fluorescence intensity of GFP-tagged Nups overexpressed in rat cells as compared to an intrinsic standard, although only in a limited number of cases the endogenous versus tagged protein ratio has been determined [12]. This challenge is easily resolved in yeast, where homologous recombination is straightforward to implement [13]. In human cells, fluorophore counting and image quantification was used as two independent, absolutely quantitative and consistent measurements, both strongly suggesting that 32 copies of vNup107 complex occur per NPC [14•]. Because 16 copies of the yeast Y-complex per NPC were previously proposed [3,8], this finding has led to some debate whether the nuclear pore oligomeric state is conserved across different species [3,15••,16••,17]. It has been argued that certain Nups that are absent in bakers or fission yeast (Nup43 and Nup37) are positioned into the scaffold such that they modulate higher order interactions in vertebrates [15••,16••].

Biochemical and structural analysis of the inner ring complex suggests relationship with nuclear transport receptors

During the review period the set of available high-resolution structures has been further complemented [18–22]. To structurally analyze the inner ring complex (Figure 1) turned out extremely challenging. Until relatively recently, the conserved large structural scaffold Nups 192 (vNup205) and 188 were difficult to handle as full-length proteins and purification from whole cell lysates (e.g. yeast) or recombinantly from *Escherichia coli* did not provide the necessary yields for successful structural analyses. During the past 3 years there was a breakthrough in the structural analysis of these Nups that was enabled by exploiting the suitable protein properties of eukaryotic thermophiles. The purification of larger amounts of *Chaetomium thermophilum* Nup192 and Nup188 allowed biochemical reconstitutions with other interacting partners, and to obtain the first EM structures of these large α-solenoid like, S-shaped Nups [23••]. Three different labs now succeeded to solve structures of the N-terminal and C-terminal fragments of Nup188 from *Myceliophthora thermophile* [24••], and the N-terminal Nup192 domains from *Chaetomium thermophilum* [25••] and *Saccharomyces cerevisiae* [26••]. These large Nups are built up by many stacked α-helical repeats, with either a 'heat' or 'arm' repeat organization, which in overall form a right-handed superhelical ring. Notably, specific inserts in the α-helical solenoid part, like the SH3 fold insertion in the case of Nup188 or a long disordered loop between the