The Nuclear Pore Complex and Nuclear Transport

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The nuclear pore complex (NPC) is an essential gateway between the cell nucleus and the cytoplasm. The NPC is formed by multiple copies of ~30 different proteins called nucleoporins, which can be divided into scaffold, membrane-anchored and barrier components. Thousands of phenylalanine-glycine (FG) repeats, found in barrier nucleoporins, interact to form the selective permeability barrier of the NPC channel. Shuttling nuclear transport receptors are able to interact with these FG repeats and mediate the passage of large macromolecular cargoes through the barrier. Combinations of shuttling receptors, their adaptors and localisation signals in cargo molecules define a wide array of nuclear import and export pathways. Recent research has pointed to some dynamic features in NPC components, as well as a number of nucleoporin-related human diseases which are characterised by highly cell-type-specific phenotypes.

Introduction

The NPC is a massive supramolecular protein assembly which spans the double membranes of the nuclear envelope and forms an essential gateway between the nucleus and the cytoplasm. NPCs are composed of multiple copies of ~30 different proteins called nucleoporins. These proteins are organised into distinct subcomplexes, which assemble together into the intricate structure of the mature NPC, embedded in the nuclear membranes. The overall structure of the NPC is conserved from unicellular eukaryotes to humans and is characterised by an eightfold rotational symmetry around a central cylindrical channel. Ions and small molecules are able to passively diffuse through the aqueous NPC channel, while most macromolecules depend on selective and active transport mechanisms to cross this barrier. Macromolecular trafficking events are brought about by shuttling nuclear transport receptors (NTRs), which recognise a multitude of different cargoes and mediate their passage through the NPC. **See also: The Cell Nucleus**; **Nuclear–Cytoplasmic Transport**; **Nuclear Envelope: Organization and Dynamics**; **Nuclear Membranes**

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NPC Structure

The nucleus is separated from the cytoplasmic environment by the nuclear envelope, which is composed of two separate lipid bilayer membranes. These inner and outer nuclear membranes converge into highly curved pore-membrane domains which form the sites of integration of NPCs (Gerace and Burke, 1988; Hetzer and Wente, 2009). The structure of the NPC is dominated by a massive central framework, also called the scaffold, which is embedded into the curved pore membrane through specific 'anchors' formed by integral membrane proteins (**Figure 1**). At the core of this framework is a spoke-ring assembly surrounding the central channel and displaying an apparent eightfold rotational symmetry. The spoke-ring assembly is flanked by two additional rings of similar dimensions: the cytoplasmic ring and the nuclear ring. Eight free-ended proteinaceous filaments protrude from the cytoplasmic ring and eight similar filaments terminating in a distal ring form the distinctive nuclear basket structure on the nuclear facade of the NPC (Beck *et al*., 2004; Fahrenkrog *et al*., 2004).

In addition to the massive central framework, the NPC also contains flexible and dynamic components. These, together with its enormous size and complexity prevent the isolation of the entire structure in intact form from cells. Instead, structural studies have relied on the visualisation of intact NPCs embedded in membranes and on averaging techniques combining the three-dimensional (3D) data acquired from hundreds or thousands of individual NPCs. High-resolution images of intact NPCs have been obtained by scanning and transmission electron microscopy, as well as cryo-electron tomography depicting the entire structure up to a resolution of ~6.5 nm (Beck *et al*., 2007; Hinshaw *et al*., 1992; Maimon and Medalia, 2010). Direct surface

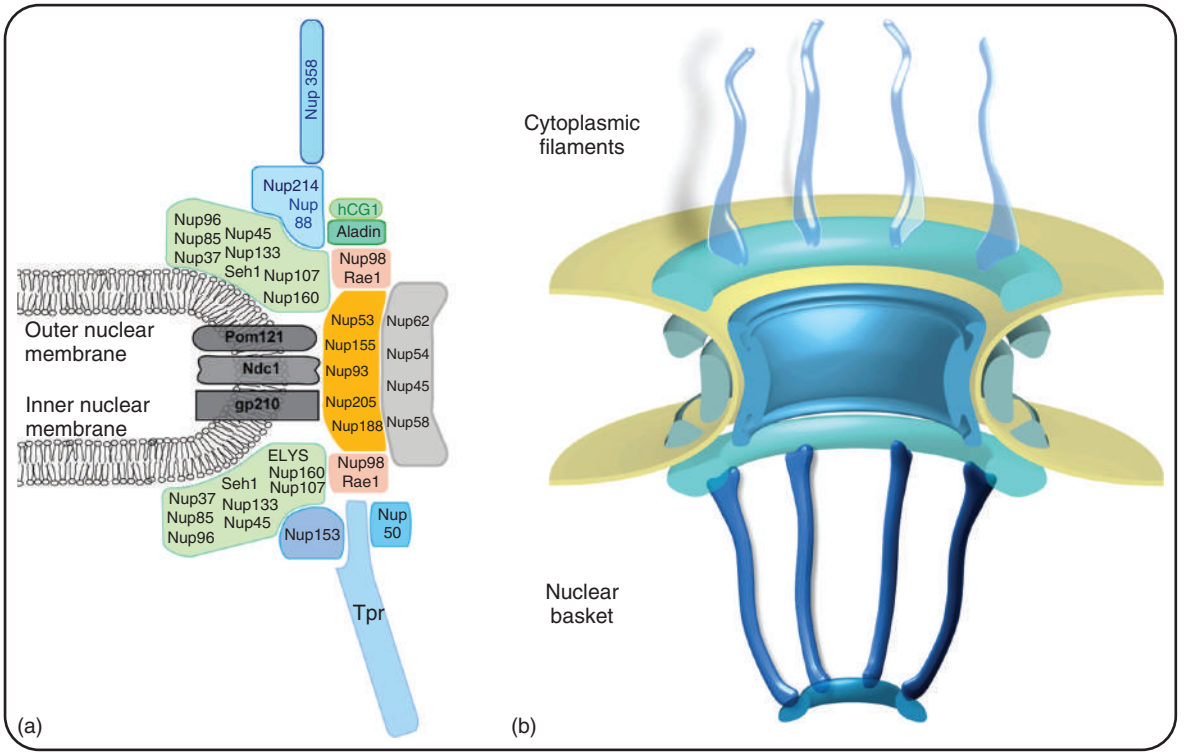

Figure 1 (a, b) Structure and composition of the nuclear pore complex. A schematic cross section of the NPC is shown on the right, emphasising the major structural modules (in different shades of blue) and the rotational symmetry of the structure. Membranes are shown in yellow. The whole structure is tightly embedded within the curved pore-membrane domain connecting the inner and outer nuclear membranes. On the left: a diagram showing the division of vertebrate nucleoporins into major subcomplexes and their approximate positions within the structure. Note that the exact contact sites among subcomplexes and other features of this supramolecular assembly are still being investigated and debated.

imaging of exposed mammalian cell nuclei by scanning electron microscopy shows large fields of interspersed NPCs embedded in the nuclear envelope (**Figure 2**). Some of these NPCs exhibit an apparently open channel and even partial glimpses of the internal nuclear basket structure (Fichtman *et al*., 2014). Other NPCs contain large particles of varying dimension within the central pore channel. Such particles have long been thought to represent large cargo caught in transit through the NPC but have not yet been characterised by molecular means (Beck *et al*., 2004; Jarnik and Aebi, 1991). **See also: Nuclear Pores: Methods for Preparation**

Atomic resolution structures of individual nucleoporins and combinations of these proteins approaching full subcomplexes have been obtained over the past decade by X-ray crystallography. These crystallographic studies have provided detailed information on domain folds and many critical protein interfaces in the main scaffold structure (Brohawn *et al*., 2009; Hoelz *et al*., 2011). It is estimated that the complete supramolecular structure of the NPC contains 500–1000 individual polypeptide chains. A major challenge in current research is to determine how multiple copies of the different nucleoporin subcomplexes are arranged within the 3D structure and how they interact with each other. A recent study aimed at these questions has used an integrated approach based on cryo-electron tomography, transmission electron microscopy of isolated subcomplexes and cross-linking mass spectrometry (Bui *et al*., 2013). This has led to a new model describing the arrangement of scaffold subcomplexes within reticulated rings on the nuclear and cytoplasmic sides of the central framework (see **Figure 3**).

Nucleoporins

Nucleoporins, which constitute the individual protein building blocks of the NPC, can be classified into three broad groups: scaffold nucleoporins, membrane-anchored nucleoporins and peripheral (also known as barrier) nucleoporins (Cronshaw *et al*., 2002; Rout *et al*., 2000; Schwartz, 2005). Although the primary sequence conservation among nucleoporins from different organisms is low, they are considered to be generally conserved at the structural level and this has been proven time and again by crystallographic analysis. Individual nucleoporins have been estimated to occur in multiples of eight copies per NPC, because of the rotational symmetry of the overall structure. Scaffold nucleoporins, in particular, appear to function as modular building blocks largely composed of α-helical domains, β-propellers or a tandem combination of these protein folds (Brohawn *et al*., 2009; Hoelz *et al*., 2011).

The largest scaffold module is the Nup107-160 subcomplex (also known as the Y-complex), with seven universally conserved

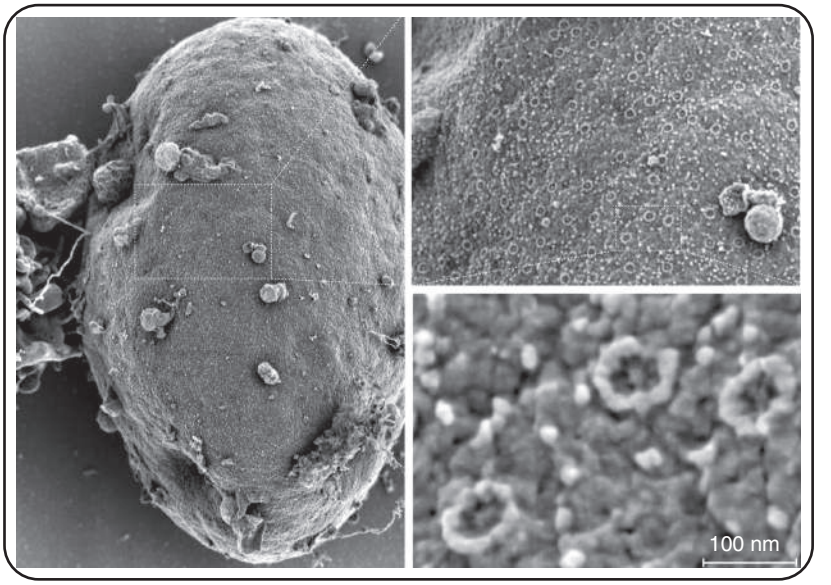

Figure 2 Direct surface imaging of NPCs in a human cell nucleus. Primary fibroblasts were subjected to hypotonic treatment in order to expose their nuclei and obtain high-resolution images by scanning electron microscopy. See Fichtman *et al.* (2014) for further details of this procedure. Three successive magnifications are shown; starting with a whole intact nucleus and finally focusing on an area containing three individual NPCs. Smaller particles protruding from the outer nuclear membrane are thought to be ribosomes. The sample was coated with a thin layer of iridium in order to allow direct surface imaging in a scanning electron microscope. Partially collapsed cytoplasmic filaments are observed on the cytoplasmic facade of NPCs and some details of internal structures are seen through open pore channels.

members from yeast to man and three additional proteins present only in vertebrate cells (Belgareh *et al*., 2001; Harel *et al*., 2003). One of these additional members is the large DNA-binding protein ELYS, which serves as an essential adaptor for seeding chromatin with initiation sites for post-mitotic NPC assembly in vertebrates (Rasala *et al*., 2006; Rotem *et al*., 2009). As shown in **Figure 3**, the latest 3D model of the human NPC shows the scaffold structure resolved to ~3.2 nm and positions 16 copies of the Y-complex within each of the cytoplasmic and nuclear rings (Bui *et al*., 2013).

A second large module of the NPC scaffold contains five proteins (in vertebrates: Nup53, Nup93, Nup155, Nup188 and Nup205) and is closely associated with the pore membrane (**Figure 1a**). Membrane-anchored nucleoporins play a critical role in embedding the whole structure into the highly curved pore-membrane domain but are only poorly conserved between lower and higher eukaryotes. Only NDC1, a protein with multiple membrane spanning segments and large C-terminal domain on the pore side, is conserved from yeast to mammals. POM121, a vertebrate-specific membrane nucleoporin, plays a role in different aspects of nuclear envelope expansion and NPC assembly (Antonin *et al*., 2005; Shaulov *et al*., 2011; Stavru *et al*., 2006). Both of these membrane-anchored nucleoporins are involved in specific interactions with members of the scaffold subcomplexes.

The third group of proteins, comprising about one-third of NPC components in all eukaryotes, are the peripheral or barrier nucleoporins. The defining characteristic of these proteins is the presence of one or more domains of multiple phenylalanine-glycine (FG) repeats, which are sometimes divided into subtypes (such as simple FG, FXFG and GLFG repeats). These hydrophobic FG repeats are interspersed with short stretches of hydrophilic amino acids and play an essential role in forming the selective permeability barrier of the NPC (Hurt, 1988; Radu *et al*., 1995; Rout *et al*., 2000). The barrier nucleoporins line the entire passage route through the NPC with thousands of FG repeats protruding into the central channel area. The importance of the FG repeats was recognised in the mid-1990s, in parallel with the identification of the shuttling nuclear transport receptors and related transport factors (see the following sections). Despite this, the molecular mechanisms that control the translocation of receptor–cargo complexes through the NPC barrier have been a matter of intense debate. Although the division into scaffold, membrane-anchored and barrier components seems to be clear cut, there are some exceptional nucleoporins that fit more than one category. Thus, the membrane-anchored POM121 contains an extensive FG repeat domain close to its C-terminus, which appears to integrate into the barrier along with the repeats of other proteins. Nup214 and Nup358 are considered to be barrier nucleoporins and also form distinct architectural elements in the cytoplasmic ring and filaments, respectively. These two proteins are more stably associated with the NPC than other FG repeat-containing nucleoporins and therefore resemble scaffold components in this feature.

The Selective Permeability Barrier

As mentioned in the Introduction, ions and small molecules are able to passively diffuse through the aqueous central channel

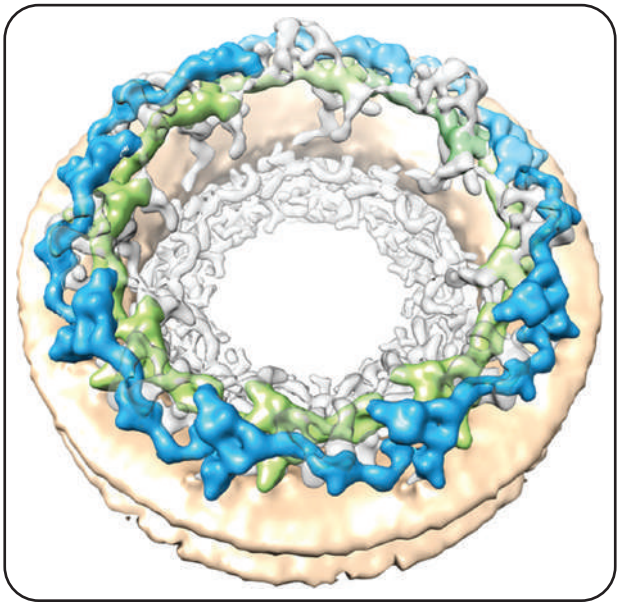

Figure 3 The Y-complex within the NPC scaffold. A three-dimensional model showing the position of 16 copies of the human Nup107–160 subcomplex within the cytoplasmic ring of the NPC. The tomographic scaffold structure (top view, from the cytoplasmic side) shows general protein densities in grey, membranes in yellow and pairs of inner and outer copies of the Nup107–160 subcomplex in green and blue, respectively. The elongated Y-shaped structure of a single subcomplex unit can be identified at the 6 o'clock position. Sixteen additional copies are similarly arranged within the nuclear ring, on the other side of the scaffold. Image was kindly provided by Martin Beck. See (Bui *et al.*, 2013) for further details.

of the NPC. This reflects the sieve-like properties of the barrier and a size entry limit of around 30–40 kDa for globular proteins. Larger macromolecules carrying specific localisation signals depend on active transport mechanisms to cross the NPC barrier and accumulate in their destination compartment against a concentration gradient (Gorlich and Kutay, 1999; Mattaj and Englmeier, 1998). This bidirectional traffic includes import and export from the nucleus of single molecules and larger complexes, most often in their native, folded conformations. Some of the larger cargoes that are able to gain access through the NPC barrier are messenger ribonucleoprotein particles (mRNPs), ribosomal subunits and proteasome particles (Daneholt, 2001; Tschochner and Hurt, 2003).

Early attempts to solve the complex puzzle of selective entry into the nucleus led to the identification of the first nuclear localisation signals (NLSs) in the 1980s. This, in turn, initiated a search for an elusive 'NLS receptor', which was first thought to be a component of the NPC itself, in analogy to other eukaryotic translocation systems. The advent of an efficient *in vitro* import assay, in digitonin-permeabilised mammalian cells, focused the searchlight onto soluble components of the cytosol (Adam *et al.*, 1990). This led to the identification of NTRs or shuttling nuclear transport receptors and their accessory proteins (see the following text). The modern view of nuclear trafficking involves the combined action of three separate components: nuclear import and export signals in cargo molecules, the shuttling NTRs and the barrier nucleoporins of the NPC.

The actual translocation or gating mechanism of the large receptor–cargo complexes in their passage through the NPC have been hotly debated for the past two decades. NTRs confer special properties on their associated cargoes, enabling them to overcome the usual size limit of the NPC barrier. Receptor–cargo complexes with a diameter of up to 40 nm can transverse the barrier, without opening the way to other macromolecules, which do not bear the correct localisation signals (Pante and Kann, 2002; Weis, 2003). An important clue was the early realisation that all NTRs specifically interact with the FG repeats of barrier nucleoporins.
See also: Nuclear–Cytoplasmic Transport

Different mechanistic models have been proposed to explain the biophysical details of the FG repeat–NTR connection and the selectivity of the permeability barrier. The 'oily spaghetti', 'virtual gating' and 'polymer brush' models represented different variations on an entropic barrier formed by extended natively unfolded FG repeat domains of the NPC. NTRs were suggested to be able to move aside or cause the collapse of these extended FG filaments, thus gaining access through the crowded NPC channel (Lim *et al.*, 2007). By contrast, the 'selective phase' model suggests that the FG repeats form a physical barrier, or 3D meshwork, based on multivalent hydrophobic interactions among thousands of phenylalanine residues. The size of this meshwork determines the upper size limit for diffusion, while NTRs are able to compete for these hydrophobic interactions and locally disrupt the FG meshwork. Receptor–cargo complexes therefore effectively dissolve or partition into the hydrophobic phase of the NPC barrier (Frey and Gorlich, 2007). The 'selective phase' model has gained wider acceptance in recent years, as researchers have been able to form saturated hydrogels from genuine FG repeat domains and reproduce many of the properties of the NPC barrier *in vitro*. The cohesive FG interactions of one specific nucleoporin, Nup98 which is characterised by GLFG repeats, appear to be most critical for creating the selective permeability barrier in vertebrate cells (Hulsmann *et al.*, 2012).

Although some of the secrets of selective passage through the NPC channel have been revealed, one of the most intriguing open questions is how multiple bidirectional transport events are coordinated *in vivo*. 'Real-life' trafficking through the NPC involves thousands of different types of large and small cargoes, simultaneous import and export and tremendous rates of transport. We are still far from understanding how these simultaneous events are coordinated and how cells respond to changes in physiological conditions by modulating specific transport pathways.

Nuclear Transport Pathways

The identification of the first soluble transport factors in the early 1990s led to a golden age of discovery in the nuclear transport field, with a strong emphasis on the so-called soluble phase of transport. The original NLS receptor turned out to be a heterodimer consisting of importin α, which recognises cargo proteins bearing the classical NLS, and importin β, which mediates the passage through the NPC via interactions with FG

repeat nucleoporins (Gorlich and Kutay, 1999). This facilitated diffusion, or translocation process, is coupled to the input of metabolic energy through the action of a third critical soluble component: the small GTPase Ran. Additional shuttling transport receptors were soon discovered and importin β became the founding member of a large superfamily of NTRs, all sharing an N-terminal binding domain for Ran (Gorlich *et al*., 1997; Mattaj and Englmeier, 1998; Weis, 2003).

Members of the importin β superfamily of receptors, also known as karyopherins, can be classified into importins and exportins. In general, importins recognise various cargo molecules in the cytoplasm by binding to exposed NLSs, either directly or through a specialised adaptor (such as importin α). The receptor–(adaptor)–cargo complexes cross the FG repeat barrier of the NPC and encounter the GTP-bound form of Ran inside the nucleus. The binding of RanGTP triggers the release of the cargo inside its destination compartment and also the recycling of the NTRs back to the cytoplasm. The general rules for nuclear export seem to follow a reverse order of events: cargo molecules exposing a nuclear export signal (NES) are bound by specific NTRs (exportins) together with RanGTP. These tertiary (cargo–receptor–RanGTP) complexes cross the NPC barrier and GTP hydrolysis on Ran, which occurs on the cytoplasmic facade of the NPC, triggers disassembly and releases the cargo into the cytoplasm. Although these import and export pathways are classified as active transport mechanisms and require energy, it is important to note that GTP hydrolysis is not directly required for crossing the selective barrier of the NPC channel. Instead, Ran's GTPase cycle determines the directionality of transport and regulates the recycling of 'empty' receptors back to the compartment of origin, thus making them genuine shuttling receptors (Gorlich and Kutay, 1999; Weis, 2003). **See also: Nuclear–Cytoplasmic Transport**

The human genome contains seven different genes encoding distinct types of importin α adaptors. Importin β can form heterodimers with any of these proteins, or with additional adaptors such as snurportin and XRIPα. Importin β can also bind some types of cargo directly, without the need for an adaptor. Other members of this superfamily recognise additional types of localisation signals, creating a large combinatorial array of different import and export complexes that pass through the NPC channel. Specific combinations of localisation signals, adaptors and NTRs define distinct transport pathways and allow for hierarchical regulation at different levels of these processes (Gorlich and Kutay, 1999; Terry *et al*., 2007; Weis, 2003). Other, Ran-independent, NTRs have evolved separately from the importin β superfamily and are also able to mediate passage through the NPC via specific interactions with the FG repeats. The most prominent of these additional receptors is TAP-p15 (or Mex67-Mtr2 in yeast), a conserved heterodimer which mediates the export of mRNAs from the nucleus. This complex pathway is coupled to multiple steps of post-transcriptional processing and quality control inside the nucleus and involves mRNP remodelling during the passage through the NPC (Dimaano and Ullman, 2004; Hodge *et al*., 2011). **See also: mRNA: Intranuclear Transport**

NPC Dynamics and Disease Connections

The NPC was once viewed as a static structure and a rather passive player in nuclear transport, with the soluble NTRs playing the more active roles. Molecular dissection of the underlying mechanisms has shed light on the central role of barrier nucleoporins and their FG repeat domains in achieving selective permeability. The NPC is now seen as a more dynamic and flexible structure, capable of modulating specific transport pathways and responding to various cellular cues (Makhnevych *et al*., 2003; Tran and Wente, 2006). Scaffold nucleoporins indeed represent the more stable part of the structure and have recently been shown to be among the most long-lived proteins in rat brain, with measured protein lifetimes of at least 1 year (Savas *et al*., 2012). By contrast, peripheral nucleoporins exhibit fast turnover rates and some of them even dissociate from the NPC and shuttle back and forth to the nuclear interior, where they may be involved in the regulation of gene expression (Capelson *et al*., 2010; Kalverda *et al*., 2010).

The currently accepted view in the field is that NPC structure and function are conserved from unicellular eukaryotes to mammalian cells. Very little data is available, however, about subtle differences among individual NPCs in the same nucleus or in nuclei from different cells and tissues. Recent studies have reported variations in subunit stoichiometry of the human NPC in different cell culture lines and even microheterogeneity of specific barrier nucleoporins between different NPCs in the same nucleus (Kinoshita *et al*., 2012; Ori *et al*., 2013). There is also evidence for cell-type-specific expression of the membrane-anchored gp210 (now renamed Nup210), which has been suggested to play a causal role in myogenic and neuronal differentiation in mammals (D'Angelo *et al*., 2012). It is still unclear whether these observations reflect exceptions to the general rule or inherent architectural variations and cell-type-specific functions of individual nucleoporins.

Only a handful of inherited human diseases have been linked to genetic changes in nucleoporins to date (Basel-Vanagaite *et al*., 2006; Cronshaw and Matunis, 2003; Zhang *et al*., 2008). This has been suggested to reflect the central role of the NPC in cellular physiology and the prediction that mutations leading to functional defects in the NPC would be embryonic lethal. A different rationalisation relies on functional redundancy among closely related nucleoporins, which may compensate for the loss of a specific component of the large supramolecular structure. In addition to the rare inherited diseases, several nucleoporins have been found to be targets for repeated chromosomal translocations that lead to haematopoietic malignancies (Gough *et al*., 2011). All of the known NPC-related diseases exhibit complex and cell-type-specific phenotypes, which are still poorly understood at the mechanistic level.

Different recessive mutations in the nucleoporin Aladin cause triple A syndrome, which combines adrenal insufficiency with oesophageal achalasia and alacrima (a failure to produce tears). These mutations disrupt the targeting of the protein to the NPC, but no structural abnormalities or defects in specific transport functions have been identified in this case (Cronshaw and Matunis, 2003). A point mutation in Nup155 causes an autosomal

recessive form of atrial fibrillation and early sudden cardiac death. On the basis of transient transfection of reporter constructs in mammalian cells, it was suggested that the mutant form of Nup155 shows defective targeting to the NPC, consistent with a-loss-of function mechanism. A mouse Nup155 knockout model is embryonic lethal, while heterozygous mice show an atrial fibrillation phenotype, supporting a conserved functional role for the protein in cardiovascular physiology (Zhang *et al*., 2008). A single-point mutation in human Nup62 causes a severe neurological disorder: infantile bilateral striatal necrosis, with damage focused primarily in the basal ganglia of the brain. Again, this autosomal recessive phenotype hints at a possible cell-type-specific role of the protein, but no changes in the protein, its targeting to the NPC or specific transport functions have been identified in patient cells or in a transfected mutant protein assay (Basel-Vanagaite *et al*., 2006). **See also: Genetics of Diseases of the Nuclear Envelope**; **Lamins: Organisation, Dynamics and Functions**

Our currently limited knowledge about nucleoporin-related diseases and their outcomes poses an interesting conceptual dilemma: are the damaged tissues and organs affected because of highly cell-type-specific roles of the mutated proteins? Do these distinct phenotypes reflect redundancy among nucleoporins, such that most functions in most cells can be compensated for by other NPC building blocks? Alternatively, could the affected tissues or cells represent the hardest hit targets, while more subtle changes would also be expected to exist in other cell types? These and other open questions, mentioned earlier, are driving current research in the field.

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