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The nuclear pore complex core scaffold and permeability barrier: variations of a common theme

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Abstract

The study of the nuclear pore complex (NPC) is a fascinating endeavor, as it not only implies uncovering the 'engineering marvel' of its architecture and function, but also provides a key window into a significant evolutionary event: the origin of the eukaryotic cell. The combined efforts of many groups in the field, with the help of novel methodologies and new model organisms, are facilitating a much deeper understanding of this complex assembly. Here we cover recent advances on the characterization of the structure of the NPC scaffold and of the biophysical mechanisms that define the permeability barrier. We identify common architectural and functional principles between those two NPC compartments, expanding the previous protocoatomer hypothesis to suggest possible evolutionary origins for the FG nucleoporins and the NPC permeability barrier.

Introduction: the nuclear pore complex and nuclear transport

The hallmark organelle of the eukaryotic cell is the nucleus, a compartment delimited by a double membrane termed the nuclear envelope (NE). The nucleus confers the primary compartmentalization within the eukaryotic cell, segregating the DNA, and associated processes in the nucleoplasm, from the cytoplasm; however, compartmentalization comes with a cost, as it requires systems that ensure proper communication and exchange of macromolecules between different cellular compartments. In the case of the nucleus, the nuclear pore complex (NPC) ensures efficient trafficking between nucleus and cytoplasm. The NPC is a massive protein assembly, of 50–100 MDa depending on the organism, that fenestrates the NE and forms a channel of approximately 40 nm wide; it is an eightfold symmetrical assembly, formed by more than 500 copies of 30 different proteins called nucleoporins (nups) [1] that have been shown to be arranged in conserved, biochemically stable subcomplexes acting as the NPC's building blocks [2–4]. These building blocks coat the NE membrane with a highly modular symmetrical scaffold, formed by eight protomers called spokes, connecting radially to form several concentric rings [2,3]: the outer ring—formed by a head-to-tail arrangement of a Y-shaped complex [5–7]; the inner ring; and the membrane ring. Attached to these rings are two asymmetrically localized modules, the cytoplasmic Nup82 complex and the nuclear basket. From the scaffold, a special type of nups, called FG nups for their high content in phenylalanine–glycine repeats, project their

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intrinsically disordered FG repeat containing domains into the central channel [3] (Figure 1). An estimated ~6 MDa worth of FG disordered domains fill the NPC central channel to form the permeability barrier that confers selectivity and specificity to the NPC. Such a 'cloud' of FG domains prevents passive diffusion of most macromolecules through the NPC, while at the same time provides docking sites for specific transport factors (TFs), mainly belonging to the karyopherin family of TFs [8]; after binding to their cargo, TFs transiently interact with the FG regions and rapidly transit through the central channel in a matter of milliseconds [9].

Most components of the NPC core scaffold have been shown to be structurally and evolutionarily related to vesicle coating complexes [10–13], which led to the proposal of the protocoatomer hypothesis, that suggests a common evolutionary origin for NPCs and coated vesicles in an early membrane-curving module (the proto-coatomer) [10]. However, tracing the evolutionary origin of other parts of the NPC, including the permeability barrier, have been more challenging than that of the scaffold. Here, we give an overview of the recent advances in our understanding of: (i) the detailed architecture of the NPC scaffold; and (ii) the biophysical characteristics of intrinsically disordered FG domains and their molecular behaviors. Finally, we integrate this new knowledge into a hypothesis for the evolutionary origin of the FG nups/karyopherins and the NPC permeability barrier.

Evolving view of the NPC structure

The structural characterization of the NPC has been an especially challenging endeavor, due to the sheer size of the assembly, its intrinsic flexibility, the dynamic nature of some of its components, and the fact that ~1/3 of its mass is predicted to be disordered. Nevertheless, recent groundbreaking advances in the structural analysis of the NPC are revealing exciting new details about this beautifully complex assembly. One of these breakthroughs came thanks to technical advances in cryo electron microscopy [14], that allowed Martin Beck and colleagues to generate a first 30 Å resolution cryo-electron tomography map of the human NPC [15]. The resolution of this map, and that of further refinements achieving resolutions around the 20 Å mark [16••,17••,18••], along with bio-chemical reconstitutions [19,20••, 21,22••] and cross-linking and mass spectrometry data [15,17••], allowed the fitting of available crystal structures [23•,4,25•,26–31] into new hybrid models covering a substantial portion of the NPC symmetric core [17••,18••,22••]. These analyses showed that the outer rings of the vertebrate NPC are formed by a reticulated arrangement of two concentric rings of 8 Y-complexes (32 copies total). The monomeric complexes run in a head-to-tail fashion and seem to connect both through direct contacts and through bridges formed by other nups, like Nup358 [17••,22••]; in yeast, though, the arrangement is simpler [3]. The Y-complexes contact the NE membrane directly through membrane interacting motifs located in the beta-propeller tips of Nup133 and Nup120–Nup160 [32,33]. Similar membrane binding motifs are used to contact the NE by other nups, like vNup155 [18••], yNup60, yNup1 [34•] and Nup53 [35,36], suggesting the presence of a network of contacts across the surface of the NPC facing the pore membrane that probably helps stabilize and shape the NPC; an important future goal will be to fully map these contacts to understand how the NPC is anchored in the NE. The outer and inner rings of the vertebrate NPC are connected through vertically arranged copies of vNup155. The core of the inner ring is proposed to be formed

by vNup93, vNup155, vNup205 or vNup188, and the trimeric vNup62 complex [23•,25•]. In each spoke, they appear organized in four compositionally similar modules, including one copy of each protein. The modules are arranged along an oblique two-fold rotational symmetry axis dividing the spoke into a nuclear and a cytoplasmic half. On each half, the two modules run parallel in an approximate head-to-tail fashion, and the trimeric coiled-coil bundles of vNup62–vNup54–vNup45 project laterally into the central channel, placing their associated FG domains roughly around the equator of the NPC. It would also seem that a number of these features are similar in the yeast NPC [3]. However, we should keep in mind that there's certainly no one single NPC architecture, and that, although many architectural principles and components seem quite conserved, evolution has shaped NPCs in different ways; indeed, it has been shown that even between different human tissues, NPCs seem to be compositionally heterogeneous [15]. Moreover, recent studies in evolutionarily distant eukaryotes are portraying a fascinating and unexpected diversity in the NPC architecture. Work on *Trypanosomes* are indicating a seemingly alternative, symmetric configuration for the mRNA export platform in the NPC [37], and it has also been shown that in the ciliate *Tetrahymena,* two nuclei with different and specific NPC compositions (and thus most certainly alternative architectures) coexist within the same cell [38•,39,40].

In the case of the NPC asymmetric modules, their molecular architecture is changing our classical view of the NPC organization, as exemplified by the integrative structural analysis of the NPC subcomplex thought to form the peripheral cytoplasmic filaments: the Nup82 complex [41••,42••]. The detailed study of this tetrameric complex showed that it has a roughly P-like shape [41••,42••]. This has recently been shown to arise from the asymmetric assembly of two compositionally identical subunits formed by hetero-trimeric coiled-coils [42••] that position all the large FG regions associated to the complex (those of Nup159 and Nsp1) emanating as a plume from one of the extremes of the complex [42••]. Unexpectedly, these studies show that the Nup82 complex does not form any kind of filament emanating towards the cytoplasm, but instead it connects to the Nup85–Seh1 arm of the Nup84 complex and projects towards the NPC central channel, where it also serves as a scaffold for the organization of the mRNP remodeling machinery [42••]. This arrangement positions the Nup82 complex associated FG regions facing the central channel, and not away from it as has been traditionally considered [43].

An important paradigm shift in our understanding of the architecture of the NPC came from deciphering the structural role of non-FG unstructured domains in nups. For many years, the function of these domains was not well understood, if not even considered an ‘inconvenient’ feature that needed to be removed to obtain constructs suitable for structural analyses. This view has radically changed thanks to groundbreaking studies by Ed Hurt's group. They showed that the thermophilic fungus *Chaetomium thermophilum* (ct) nups [19] ctNup53, ctNic96, ctNup145N and their *Saccahromyces cerevisae* homologous nups (yNup53, yNic96 and yNup145N, yNup116 and yNup100 respectively) contain conserved arrays of short linear motifs (SLiMs [44]) in their intrinsically disordered regions (IDRs) that mediate interactions with multiple inner ring components [19,20••] (Figure 1). These SLiM-containing IDRs are thus extended and inter-connect the inner ring modules through multiple, relatively weak interactions [17••,19,20••,22••]. The identification of these flexible linkers bridging inner ring subcomplexes and components has two significant implications:

(i) it has been suggested that the disordered linkers provide flexibility to the inner ring and a straightforward mechanism for NPC disassembly [20••,22••], as it has been shown that phosphorylation of the SLiM containing region of hNup98 is required for NPC disassembly during mitosis [45]; (ii) the SLiM-containing IDRs have been shown to connect mainly to the alpha-helical solenoids of inner ring components (Nup170, Nup192, Nic96 [19,20••, 22••]), indicating that the FG regions contiguous to some SLiM linkers would emanate from central positions in the NPC. We still do not understand how these flexible linkers contribute collectively to the structure of the NPC; hence, an important future endeavor for the field would be to map these linkers within the structure of the NPC as well as accurately define the emanating points for all their associated FG regions. But the precise organization and position of the FG nups in the NPC are only a piece in the NPC functional and evolutionary puzzle. To assemble the rest of the pieces we require the characterization of the FG nups biophysical properties, their interaction with TFs and their behavior within the permeability barrier (Figure 2).

Nuclear transport: fast and loose

Until recently, most studies on the mechanism of the nuclear transport focused on the macroscopic morphology and behavior of FG Nups, mainly centering on their ‘cohesive’ properties. Although the term ‘cohesive’ has been used in the field rather elusively, most proposed models generally fall between the two models with contrasting degrees of cohesion between FG Nups; the ‘hydrogel/selective phase’ model advocating hydrogel structures with static cohesion among FG Nups [46–49], and the ‘polymer brush/virtual gating model’ emphasizing the dynamism of FG Nups [1,50]. The hydrogel/selective phase model is based on the propensity of FG Nups to form macroscopic hydrogels *in vitro*, which are characterized by very slow-moving, amyloid like interactions [51]. A recent study on Nup98 from various species focused on ‘phase-separation’ properties of FG Nups, observing formation of ‘FG particles’ *in vitro* [46]. The authors maintain emphasis on the importance of FG–FG cohesion as in the hydrogel studies [47–49], but it is not clear how such macroscopic structures, on the scale of micrometers, relate to nanoscopic NPCs. In support of the selective phase model, Zahn *et al*. interpreted that an FG layer on a surface, as quantified by quartz crystal microbalance with dissipation analysis, is significantly more compact than a theoretical non-cohesive polymer brush, suggesting a ‘pore-filling cohesive meshwork’ or ‘condensed polymer brush’ resulting from FG–FG cohesion in a nano-confined NPC milieux [52]. However, future analyses should address the existence of such NPC hydrogel structures or cohesive meshworks *in vivo*.

In contrast, most recent studies on FG Nups seem to highlight their mobile properties; fluorescence anisotropy measurements *in vivo* indicated that FG regions are unstructured and mobile with some position-dependent nematic organization [53], while atomic force microscopy characterization of whole NPCs on *Xenopus* nuclear envelope demonstrated the mobile and dynamic nature of the NPC milieux *in situ* [54]. A theoretical study also took into account the flexibility of FG Nups as one of the basic properties, along with their interaction with TFs and with each other, that could explain previous experimental observations [55], especially those of Kap-centric models [56–58]. Therefore, it appears to us that a body of evidence is emerging to support a more dynamic and fluid nature for the

NPC permeability barrier, as opposed to a static, very slow-moving, amyloid-like structure; however, the basic physicochemical characterization of FG Nup behaviors is still needed to fully explain the mechanism of nuclear transport. Thus, we would now like to focus our attention on recent studies that examined the nuclear transport system at the atomic/molecular level, which provided such basic molecular details of FG Nups behavior and their interactions with TFs.

Nuclear magnetic resonance (NMR) studies heralded an atomic-scale characterization of the permeability barrier, experimentally elucidating the dynamics of individual FG Nup molecules and providing us with basic physico-chemical characteristics of these intrinsically disordered proteins. First, FG Nups were demonstrated to be highly mobile and remain disordered in various conditions [59••,60••]; in various buffers, in the presence of TFs and nonspecific proteins [59••,60••], and in cellular milieux [59••]. Furthermore, interactions with TFs are localized to FG motif residues, while spacer residues are minimally involved and remain highly dynamic, permit-ting extremely fast on-off events [59••,60••]. *F*ull atom simulations with NTF2 corroborated those NMR findings, and additionally showed that FG motifs interacting with NTF2 can transition between weakly- and strongly-interacting states by ‘sliding’ on the surface of NTF2 [61]. Combined, those experimental and computational results indicate that FG Nups are ‘springs’ with high configurational entropy, preferring a dynamic state (*i.e.,* disordered) to a static one (*e.g.,* collapsed, folded). Thus, an interaction involving a FG Nup could induce physical constraint on the movement of the FG chain, which could result in a loss of configurational entropy of the FG Nup. TFs may possess a mechanism to overcome such a potential energetic cost, possibly through enthalpic gain, as suggested by the virtual gating models [1,50].

Therefore, most of the newly characterized molecular details underscore the ‘fast and loose’ character of the NPC transport system: (1) FG Nups are intrinsically disordered with fast dynamics, (2) interaction with TFs is virtually limited to FG motifs, (3) per-FG affinity is very low with fast on-off rates, and (4) FG–TF interactions are ‘fuzzy’ (and thus should not be treated like conventional interactions between folded proteins) [62]. These characteristics at the nanoscopic level are consistent with measured rapid transport rates *in vivo* [63–67]. Individual FG motifs interact very weakly (at millimolar affinities) with each site on a TF [59••,60••]; however, the existence of multiple interaction sites on both sides increases discriminating power of FG–TF interactions against nonspecific macromolecules, likely providing the specificity to the nuclear transport without compromising the rapid diffusion of TFs. It remains to be seen how high avidity (*i.e.,* strong overall affinity induced by multiple bindings) is prevented despite the seemingly multivalent FG–TF interaction.

Evolutionary origin of the FG nups and the NPC permeability barrier

In the previous sections we discussed the structural and functional characteristics of the main compartments of the NPC: the core scaffold and the permeability barrier. Although they appear to be fundamentally distinct entities, we think that the accumulating evidence suggests otherwise, because both compartments seemingly share a common overall architectural theme: the presence of IDRs containing arrays of SLiMs that establish multiple, relatively weak interactions with alpha-helical solenoid proteins. In the core scaffold, the

IDRs of Nup98/Nup145n/Nup116/Nup100 interconnect the alpha-solenoids of inner ring nups [17••,20••,22••]; in the permeability barrier, the FG IDRs establish multiple transient interactions mainly with the alpha-solenoids of karyopherins [68]. From this point of view, the two compartments of the modern NPC mostly differ in their degree of order/disorder, determined by the stability of the interactions between their components: higher in the core scaffold and very low within the permeability barrier. Thus, based on this shared architectural theme, we suggest a hypothesis for the evolutionary origin of the permeability barrier, as a relatively simple modification of the ancestral IDRs-alpha solenoids connections present in the proto-NPC coating complex (Figure 3). We propose that the NPC originated from a protocoatomer formed by interconnected combinations of folded domains (beta-propellers, alpha-solenoids) and SLiM-containing IDRs. Originally, the NPC was probably a simple fenestration of the NE, stabilized by the proto-NPC coating complex, which allowed transit of macromolecules with low selectivity. Selective pressure to increase the compartmentalization led to fine-tuning of the proto-NPC components directly facing the central channel. We suggest that the FG regions evolved as a highly specialized subset of the SLiM-containing IDRs, where the overall number of SLiMs was increased to enhance specificity, and yet their individual interaction surface size reduced to 2–4 amino acids (FG, GLFG, FxFG) to ensure fast interactions. The alpha-solenoid proteins interacting with the proto-FG regions would have likely evolved in parallel to become more dynamically associated, but without losing specificity for those regions. Several pieces of evidence support our hypothesis: (i) IDRs containing SLiMs are common, probably extremely ancient, protein modules featuring in many cases the amino acids that characterize FG regions (Phe, Gly, and charged residues) [69]; (ii) The NPCs from all organisms analyzed so far seemingly contain Nups with a combination of SLiM-containing IDRs contiguous to FG regions (*e.g.,* yeast Nup116/Nup100/Nup145n or vertebrate Nup98) [20••,38•,70,71]; (iii) those proteins establish connections to the inner ring solenoids through their SLiMs [17••, 20••,22••]; (iv) an available crystal structure shows that Nup145n interacts with the alpha-solenoid of Nup170 through short patches of hydrophobic residues (Phe, Leu, Ile) that bind to small hydrophobic pockets in the convex face of the Nup170 alpha-solenoid [22••], highly reminiscent of the FG-karyopherin mode of interaction [72]; (v) other FG nups feature IDRs between their folded domain and the FG region [11]; (vi) several independent structural analyses agree on an evolutionary relationship between karyopherins and alpha-solenoid nups [26,27]; (vii) large alpha-solenoid inner ring nups (Nup188, Nup192) can be made to interact directly with FG regions *in vitro* [27,73,74], maybe revealing a signature of their ancestral interactions within the proto-NPC; (viii) alpha-helical solenoid transporters are the main mediators of transport pathways [68]; (ix) the NPC coating Y-shaped complex contains what could be considered as a ‘molecular fossil’ of the proto-NPC organization we are suggesting, Nup145/Nup98–96, a nup formed by a COPII-related alpha-helical solenoid connected through a two IDRs-SLiM regions to a small folded domain and an FG region. Although in opisthokonts the Nup145 protein self-cleaves into two independent polypeptides [75], the two halves still interact with each other [76] and in some organisms they never separate and form a single protein [70]. This hypothesis can be tested: higher resolution structures of the entire NPC should provide a much needed insight into how much of the NPC's structure is actually interwoven with such FG-Nup linked SLiMs and how dependent

on them it is for integrity; and complementary structural data on NPCs in numerous highly divergent organisms will speak to the generality of SLiMs and their evolutionary origins.

Concluding remarks

Although some of the NPC's secrets are being revealed at a remarkable speed, we still have a long way to go. Defining the detailed average architecture of the whole assembly in several model organisms and dissecting the molecular details of numerous FG nup–TF interactions seem achievable goals for the near future. For the long run, we would expect advances allowing the incorporation of the multiple states that the transport machinery could adopt for all the different TFs and their cargos, throughout the life of a cell, and between different cell types, into a comprehensive, dynamic and more faithful picture of the biological assembly and its function. In addition, we expect to see how our actual view of the nuclear transport machinery, currently limited to the opisthokonts, is challenged through the analysis of more divergent eukaryotes. This is already revealing some surprising differences [37•,38•,39,40] that would be absolutely essential to understand the structural diversity of the NPC and its evolutionary history.

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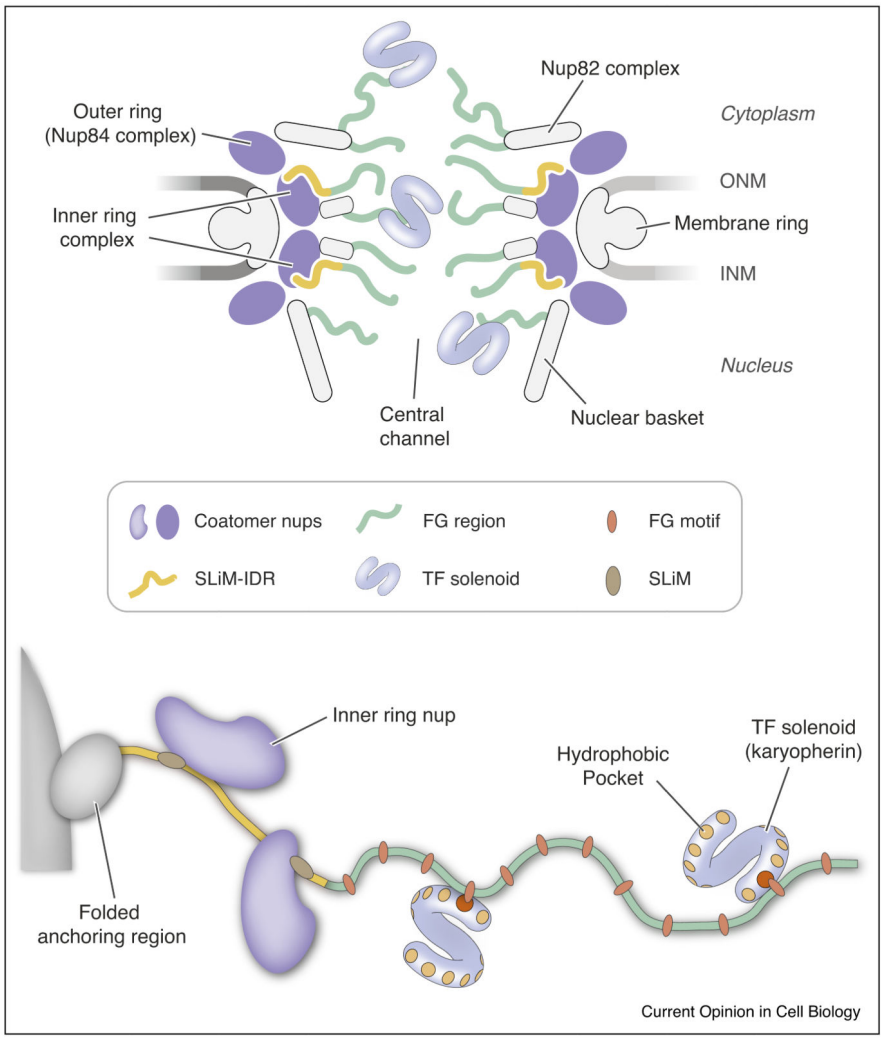

Figure 1.

The nuclear pore complex architecture.

Upper part, schematic illustrating the major features of the NPC organization. Lower part, schematic of linker nucleoporins yNup145N/yNup 100/yNup116/hNup98, showing their interaction with the NPC scaffold through their disordered connectors (based on Ref. [20••]) and interaction with transport factors through their FG repeat regions.

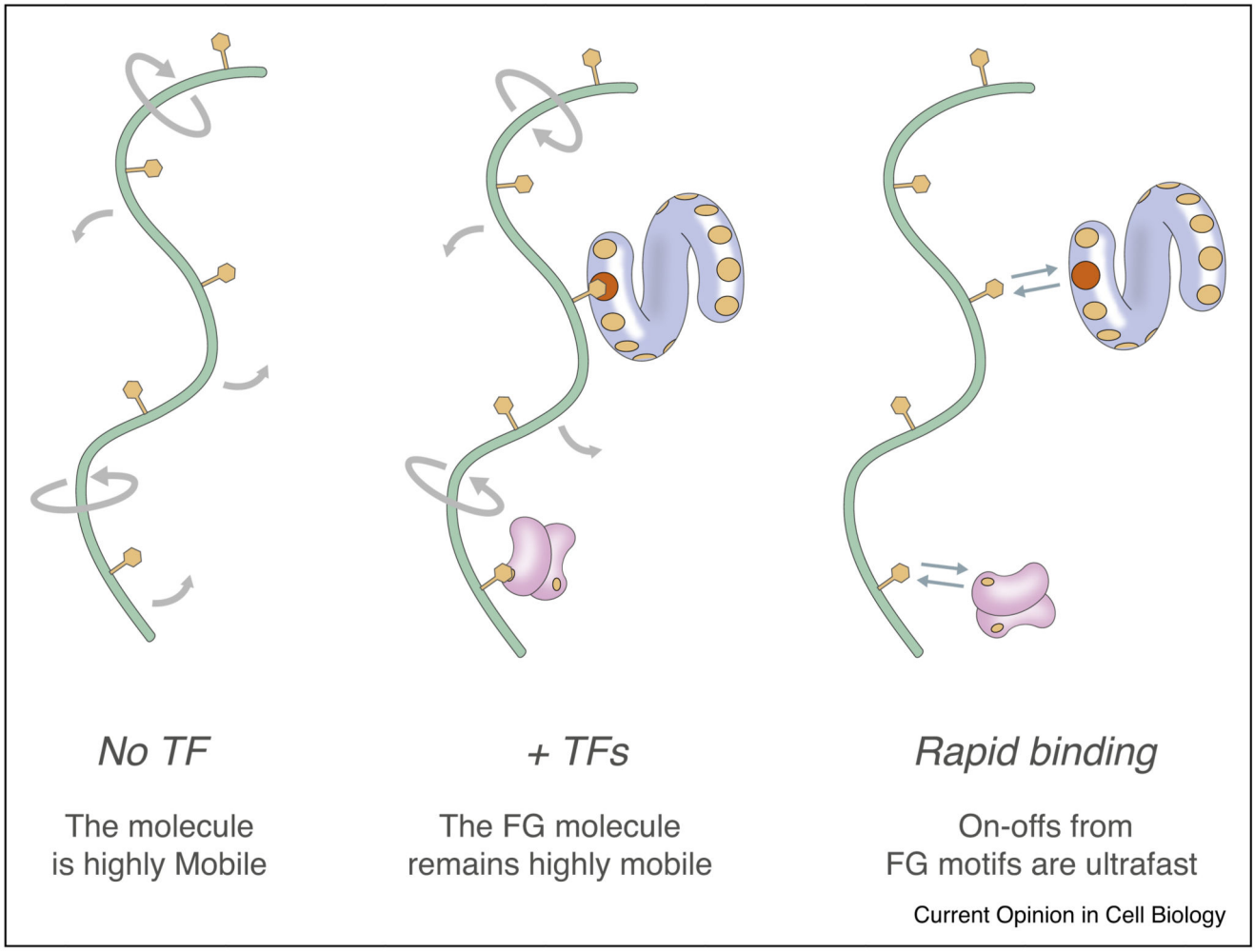

Figure 2.

'Fast and loose' nature of FG–TF interaction.

FG Nups are intrinsically disordered without any secondary structure. They are highly mobile as indicated by the gray arrows (left). Interactions with TFs are minimally localized to FG motifs while the spacer sequences remain mobile even in the presence of TFs (middle). Each FG motif has a low affinity for interaction sites on a TF, and thus, the per-site on-off events are rapid (right).

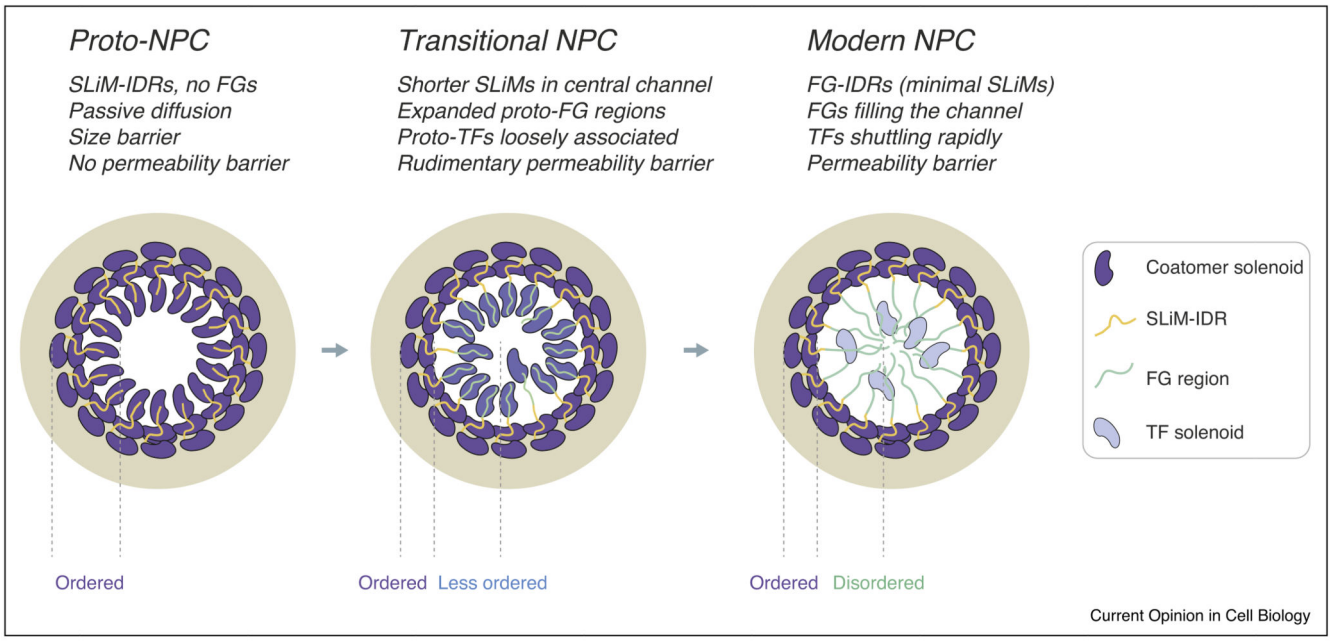

Figure 3.

Connector-to-FG hypothesis for the evolutionary origin of the NPC permeability barrier. Zenithal schematics of the NPC assembly through three evolutionary stages: (i) left, ancestral NPC, formed by protocoatomer modules that included inter-connected alpha-solenoids and intrinsically disordered connectors, forming a channel through the NE. The channel allowed communication between the nucleus and the cytoplasm through basic diffusion and with very limited selectivity; (ii) middle, transitional NPC, showing the evolution of the modules directly exposed to the central channel to increase the compartmentalization between nucleus and cytoplasm. FG regions evolved as highly specialized, short SLiMs in expanded IDRs. The proto-TF evolved in parallel to maintain loose connectivity and high specificity; (iii) right, modern NPC, FG regions covering the whole channel and establishing multiple, fast and specific interactions with shuttling TFs. A highly efficient permeability barrier forms the gating mechanism that regulates exchange between nuclear and cytoplasmic compartments