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EXTRA VIEW



Comparative interactomics provides evidence for functional specialization of the nuclear pore complex

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ABSTRACT

The core architecture of the eukaryotic cell was established well over one billion years ago, and is largely retained in all extant lineages. However, eukaryotic cells also possess lineage-specific features, frequently keyed to specific functional requirements. One quintessential core eukaryotic structure is the nuclear pore complex (NPC), responsible for regulating exchange of macromolecules between the nucleus and cytoplasm as well as acting as a nuclear organizational hub. NPC architecture has been best documented in one eukaryotic supergroup, the Opisthokonts (e.g. *Saccharomyces cerevisiae* and *Homo sapiens*), which although compositionally similar, have significant variations in certain NPC subcomplex structures. The variation of NPC structure across other taxa in the eukaryotic kingdom however, remains poorly understood. We explored trypanosomes, highly divergent organisms, and mapped and assigned their NPC proteins to specific substructures to reveal their NPC architecture. We showed that the NPC central structural scaffold is conserved, likely across all eukaryotes, but more peripheral elements can exhibit very significant lineage-specific losses, duplications or other alterations in their components. Amazingly, trypanosomes lack the major components of the mRNA export platform that are asymmetrically localized within yeast and vertebrate NPCs. Concomitant with this, the trypanosome NPC is ALMOST completely symmetric with the nuclear basket being the only major source of asymmetry. We suggest these features point toward a stepwise evolution of the NPC in which a coating scaffold first stabilized the pore after which selective gating emerged and expanded, leading to the addition of peripheral remodeling machineries on the nucleoplasmic and cytoplasmic sides of the pore.

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In the beginning.....

The origin of the eukaryotic cell (eukaryogenesis) is a major evolutionary transition in the history of life,¹ accompanied by the emergence of extensive intracellular compartmentalization. The most pronounced compartment is the nucleus, surrounded by a double-membrane nuclear envelope (NE) that is contiguous with the endoplasmic reticulum. The most prominent macromolecular assembly, present in all NEs, are nuclear pore complexes (NPCs - 8-fold symmetric cylindrical multiprotein structures (~50 MDa in yeast) that mediate macromolecular exchange between the nucleus and the cytoplasm (Fig. 1). Since the NPC is uniquely and ubiquitously a eukaryotic feature, we

can facilitate a reconstruction of evolutionary history, identify potential adaptive mechanisms, and look for the imprints that the transition from the first to the last eukaryote common ancestor (FECA to LECA) left at the structural and molecular levels through a detailed study and comparison of the NPC's components from key organisms across the eukaryotic lineage.

Ultrastructural studies hint at a high level of morphological NPC conservation across eukarya. However, morphological similarity, especially at comparatively low resolution, cannot define molecular structure or function that requires a significantly greater level of dissection. Until recently, detailed

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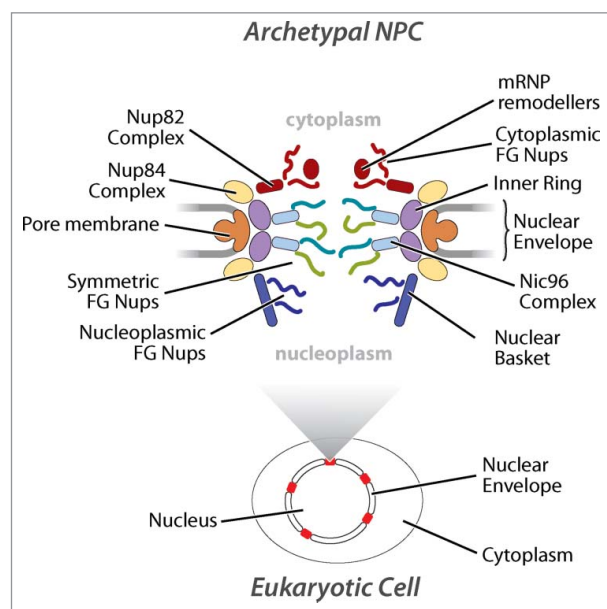


Figure 1. Schematic of opisthokont NPCs. A schematic of textbook NPCs highlighting each distinct nuclear pore subcomplex. The approximate location messenger ribonucleoprotein (mRNP) remodeling factors required for mRNA export out of the NPC is also shown.

compositional, structural and functional information of the NPC was only available for *Saccharomyces cerevisiae* (Sc) and *Homo sapiens* (Hs), both members of the Opisthokont supergroup, and thus relatively, closely related.²⁻⁴ In the last 7 years, NPC components have been well cataloged in 2 further supergroups, Excavata (*Trypanosoma brucei* (Tb)) by us,^{4,5} and Archaeplastida (*Arabidopsis thaliana* (At)),^{6,7} resulting in remarkable initial insights into the structure, evolution and species-specific adaptations of the NPC.

Copy and paste: the NPC's scaffold arose through ancient duplications of a progenitor coating complex

NPCs are comprised of multiples of ~30 different proteins termed nucleoporins (Nups) that are classified into 3 major subtypes; pore membrane (Poms), core scaffold, and phenylalanine-glycine (FG)-repeat containing Nups (FG-Nups) (Fig. 1).³⁻¹¹ Poms and the core scaffold form the major structural framework while FG-Nups establish the permeability barrier of the NPC and facilitate nucleocytoplasmic transport through interactions with soluble transport factors (karyopherins), with directionality dependent on a Ran GTP/GDP gradient.¹²

There is extremely low sequence similarity between excavate and opisthokont Nups.⁴ Despite this,

trypanosome Nups share a remarkable architectural and domain organization with opisthokonts and plants, highlighting that structural considerations such as detailed fold arrangements are key to the function of these proteins (Table 1).^{4,5} The core scaffold of the NPC comprises the inner and outer rings that are composed almost exclusively of proteins comprised of β -propellers, α -solenoids, or an N-terminal β -propeller and C-terminal α -solenoid (β - α).^{13,14} These characteristics are shared with major classes of membrane interacting proteins, such as vesicle coat proteins (COPI, COPII, clathrin and tethering complexes) and intraflagellar complexes, suggestive of a common ancestry between the endomembrane trafficking system and the NPC, a theory known as the “protocoatomer hypothesis”.^{8,13,14} Moreover, the overall architecture of the NPC's core scaffold appears to consist of multiple kinds of structural modules that nevertheless resemble each other, both in composition and in arrangement of fold and domain types.^{8,15,16} This supports the idea that the elaborate architecture of the NPC arose through repeated duplication events from a simple progenitor coating complex.^{8,17,18}


Variations on a theme: using the same building blocks to assemble different NPC structures

At the heart of the NPC there appears to be a remarkably conserved core subcomplex. This is the inner ring complex, almost identical in protein composition and arrangement between all eukaryotes studied, including trypanosomes.^{5,7,8,16,19,20} This structure must already have been present in LECA, with relatively few post-LECA innovations in any organism. Surely, this forms a keystone to hold the NPC together.




However, the modular scaffold architecture of the NPC – a scaffold of similar building blocks – can open a simple path to the evolutionary elaboration and diversification of the NPC, through altering the number or design of each building block. And indeed, there is a compositional flexibility in the outer ring complex, which revolves around the presence or absence of a subset of proteins in different organisms (Table 1). Most variable is the complement of β -propeller proteins, as only Sec 13 is present in all characterized versions of this complex. Sec 13 is also present in COPII complexes, further underscoring the evolutionary relationship between the NPC and coating complexes.^{13,14} Nonetheless, what appear to be minor species-specific

Table 1. Protein folds and the components of the NPC in trypanosomes, plants and opisthokonts. (A) The NPC is principally comprised of proteins with the fold types highlighted. α -solenoids are stacked pairs of α (α) helices, composed of anti parallel repeating α subunits that in turn form a kind of "superhelix";⁸⁷ β -propeller proteins typically have between 4 to 8 blade-shaped β (β) sheets arranged in a toroidal fashion;⁸⁸ RRM is RNA recognition motif, TM is *trans* membrane domain, coiled coils are 2 or more α helices that wind around each other to form larger helical bundles and FG-repeats are phenylalanine glycine repeats typically found in the intrinsically disordered protein domains of Nups that interact with transport factors in the nucleus. (B) A catalog of trypanosome Nups compared with those in other well-studied taxa. Orthologs of individual TbNups are based on interactome mapping and immunolocalization of NPC subcomplexes in the trypanosome NPC. Trypanosomes lack pore membrane Nups and cytoplasmic or nuclear biased Nups. Furthermore, several trypanosome FG-Nups and the nuclear basket Nups are not orthologous to those in opisthokonts and plants. Trypanosome nuclear basket Nups are half the size of those in other eukaryotes studied to date and appear to have arisen independently through evolution.^{38,39} The RNA-recognition motif (RRM) containing TbNup65 has a *trans*-membrane (TM) domain instead of an amphipathic lipid-sensing (ALPS) motif^{30,31} that is found in the equivalent opisthokont and plant orthologs, and maybe the way trypanosomes compensate for a lack of pore membrane (POMs) Nups. TbNup158* lacks the catalytic residues required to undergo autoproteolysis that generates 2 individual proteins such as yeast Nup145N/Nup145C and vertebrate Nup98/Nup96^{4,5}, and as such remains a single N-terminal FG-domain/C-terminal α -solenoid FG-Nup.^{4,5} Nup358# is a metazoan specific multi-domain protein that contains FG-Nups, Ran binding domains, multiple Zinc finger domains, an E3-SUMO ligase domain and a cylophilin domain.⁸⁹

A



B

	Excavates	Opisthokonts	Plants		
					
Secondary structure	<i>T. brucei</i>	<i>S. cerevisiae</i>	<i>H. sapiens</i>	<i>A. thaliana</i>	
α -solenoid	TbNup225	Nup192	Nup205	Nup205	Inner ring
	TbNup181	Nup188	Nup188	Nup188	
	TbNup96	Nic96	Nup93	Nup93	
	TbNup158*	Nup145C	Nup96	Nup96	
Outer ring	TbNup89	Nup85	Nup75	Nup75	
	TbNup82	Nup84	Nup107	Nup107	
	TbNup144	Nup170	Nup155	Nup155	
β -propeller α -solenoid (β - α)	TbNup119	Nup157	Nup155	Nup155	Inner ring
	TbNup152	Nup120	Nup160	Nup160	
	TbNup132	Nup133	Nup133	Nup133	
β -propeller	TbSec13	Sec13	Sec13	Sec13	Outer ring
	-	Seh1	Seh1	Seh1	
	TbNup41	-	Nup43	Nup43	
	TbNup48	-	Nup37	-	
β -propeller, coiled coil	TbNup76	Nup82	Nup88	Nup88	Cytoplasmic (opisthokonts), Symmetric (Trypanosomes)
	-	-	-	-	
RRM, <i>Trans</i> -membrane*	TbNup65*	Nup53/59	Nup35	Nup35	Inner ring
<i>Trans</i> -membrane	-	Pom152	Gp210	Gp210	Pore membrane ring
	-	NDC1	NDC1	NDC1	
	-	Pom34	-	-	
Coiled Coils	TbNup110	-	Pom121	-	Nuclear basket
	TbNup92	-	-	-	
	-	Mlp1	TPR	NUA	
FG-Nups, Coiled coils	TbNup62	Nsp1	Nup62	Nup62	Inner ring/central channel
	TbNup53a	Nup57	Nup54	Nup54	
	TbNup53b	Nup49	Nup58/45	Nup58	
	TbNup158*	Nup145N	Nup98	Nup98	
FG-Nups	TbNup149	-	-	-	Outer ring (Nuclear bias in yeast)
	TbNup140	-	-	-	Symmetric - Nup76 complex
	TbNup98	-	-	-	Symmetric - multi-complex FG-Nups
	TbNup75	-	-	-	
	TbNup64	-	-	-	Outer ring (Cytoplasmic)
	-	Nup116	-	-	
	-	Nup100	-	-	Cytoplasmic - Nup82/88 complex
	-	-	Nup358#	-	
	-	Nup159	Nup214	Nup214	
	-	Nup42	Nlp1	CG1	
-	Nup60	Nup153	Nup153	Nuclear bias	
-	Nup1	-	Nup1		
-	Nup2	Nup50	Nup50		

variations of the outer ring complex in opisthokonts have revealed an interesting architectural divergence between yeast and metazoa. The most compelling example is the metazoan outer ring, which despite near identical compositional and architectural similarity with yeast, comprises 2 reticulated rings,^{11,21} in contrast to a single ring in yeast.^{8,15,22} Thus, architectural and functional similarities cannot be assumed simply from a similar list of components. The presence or absence of a “few small β -propeller proteins” may disguise previously unsuspected and significant species-specific elaborations of this outer ring in other eukaryotic lineages. Indeed, the major difference we discovered through interactomics between trypanosomes and other well studied taxa is the presence of 3 β - α Nups in the outer ring complex, as opposed to just 2 in opisthokonts and plants (Table 1). This difference in composition may represent a completely unique outer ring structure in trypanosome NPCs (TbNPC). Discerning the stoichiometry, arrangement and morphology of TbNPC components is thus clearly essentially to fully understand the architecture of the trypanosome outer ring, and hence understand its function.

An extremely well conserved component of the outer ring complex with interesting properties is TbNup158 (HsNup98–96 or ScNup145N/C; see Table 1). It is easily identifiable as it encoded in most eukaryotic genomes as a single protein comprised of a large N-terminal FG-Nup domain and a large C-terminal α -solenoid separated by a β -sandwich that contains within it catalytic residues that lead to its autoproteolytic cleavage into 2 separate proteins.²³ However, although the 2 proteins still associate with one another in the NPC, it is generally believed that cleavage into 2 different polypeptides allows the FG-Nup domain of this protein to be dynamic and have a role in gene regulation, while the α -solenoid portion remains a stable structural member of the outer ring complex.^{24,25} The tripeptide catalytic residues in the trypanosome ortholog (TbNup158) have altered during evolution to prevent cleavage, resulting in the protein being incorporated into the TbNPC outer ring as a single protein, possibly to eliminate this dynamism and negate a role in gene regulation which is unusual in trypanosomes (see below and Fig. 2). Once again, apparently minor deviations in the composition of the trypanosome NPC can lead to significant alterations in structure and presumably,

function. This is not limited to trypanosomes. The same catalytic activity is lacking in another flagellated excavate *Giardia lamblia*,⁴ and autoproteolytic cleavage has been shown to be dispensable for cell growth in fission yeast.²⁶ Additionally, the Apicomplexan parasite *Plasmodium falciparum* has an unusual fusion of a portion of the α solenoid domain of this protein (ScNup145C) with PfSec13.²⁷ Thus, trypanosomes do not represent the exception – instead, they are part of the rule that the outer ring complex (comprising one-third of the NPC’s mass) can apparently vary considerably in form and function between different organisms.

TM and ALPS: Multiple ways to tether a leviathan

Opisthokonts also have *trans*-membrane (TM) proteins that interact with core scaffold Nups to anchor the NPC to the pore.^{3,9} Although not well conserved,²⁸ opisthokonts and plants all have orthologs of Gp210 (Pom152 in yeast, Gp210 in plants and humans) and Ndc1 (Table 1), pointing to ancient and possibly pre-LECA origins for both proteins, as plants - like trypanosomes - diverged early from the eukaryotic lineage. Remarkably, we found that both proteins are absent from the trypanosome NPC, suggestive of secondary loss. Instead, we found that trypanosomes rely on the ortholog of the RNA-recognition motif (RRM) containing HsNup35/ScNup53 to anchor the NPC.⁵ Nup35 is crucial for NPC biogenesis in opisthokonts.²⁹ Furthermore, it connects components of the inner ring in opisthokonts with the pore membrane where it interacts *via* an amphipathic lipid-packing sensor (ALPS) motif,³⁰⁻³² and is a key nucleator for *in vitro* assembly of an inner ring complex, raising the possibility that it is key *in vivo* for directing assembly of the inner ring, and so whole NPC, from the pore membrane inwards.³³ By contrast, TbNup65 (Table 1), the trypanosome ortholog of Nup35, instead interacts with the pore membrane through a canonical *trans*-membrane (TM) domain. Somehow, ALPS and TM domains can be interchanged in evolution, and there is thus plasticity in the exact mechanism used for anchoring the NPC to the pore membrane. Indeed, all Poms in the *Aspergillus nidulans* NPC can be deleted without compromising the viability of the cells, in the presence of an intact outer ring complex.³⁴ Thus, additional anchoring in trypanosomes may rely heavily on potential

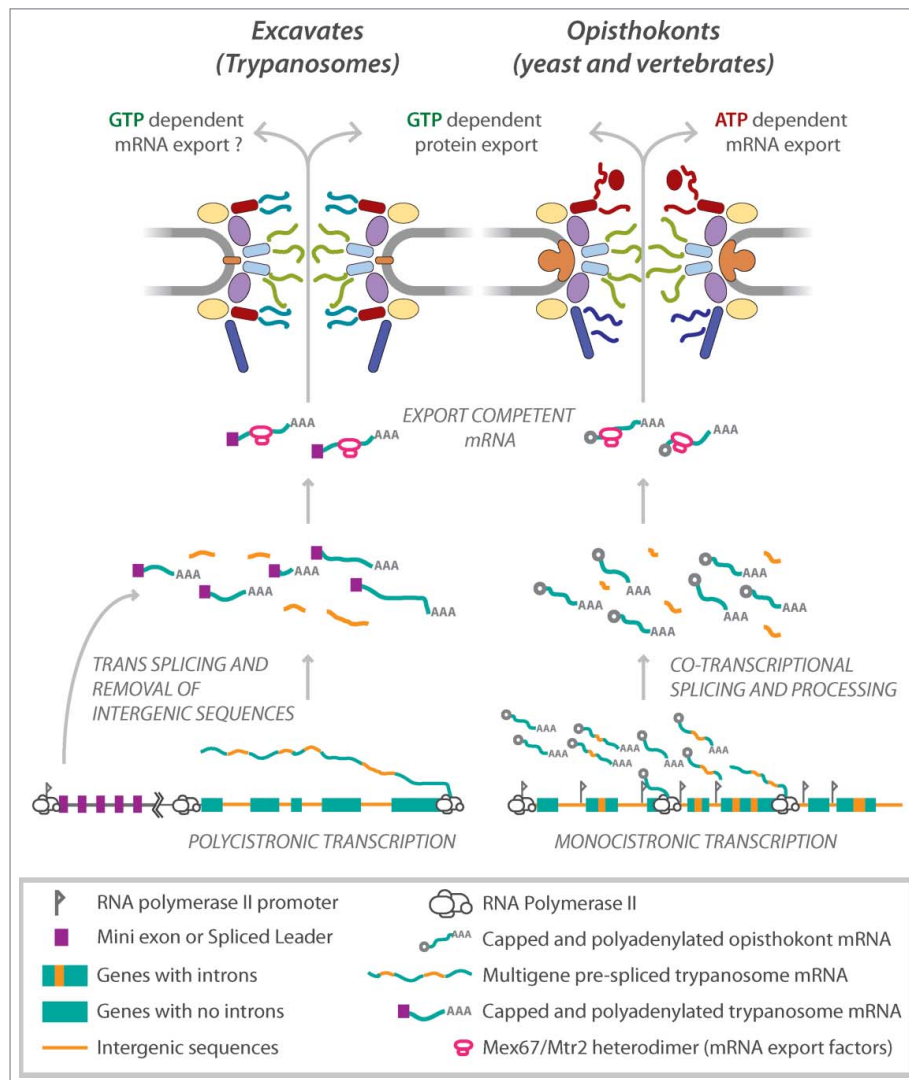


Figure 2. (A) comparison between Excavate and Opisthokont NPCs and transport through them. Trypanosomes (Excavates) have a symmetric NPC with the exception of the nuclear basket unlike in opisthokonts (yeast and humans). Furthermore, trypanosomes lack the cytoplasmic mRNA platform including mRNP (ribonucleoproteins) remodeling factors such as the ATP-dependent DEAD box helicase Dbp5 and Gle1 that are crucial for mRNA export in opisthokonts.^{15,41} Instead, mRNA export in trypanosomes appears to rely on the RanGDP/GTP gradient similar to protein export, as opposed to ATP in opisthokonts.⁵ This may be related to the unusual mechanisms trypanosomes use for controlling gene expression. Trypanosome protein-coding genes are intronless and each gene lacks an individual polymerase II promoter.⁵⁸ Thus, trypanosome genes (green boxes) are transcribed into long multigene (polycistronic) transcripts that are resolved into single mRNAs by *trans*-splicing of a mini exon, also known as the spliced leader sequence (purple box) at the 5' end of each gene, splicing out of intergenic (orange lines) sequences and polyadenylated (AAA). Processed and export competent mRNA are exported through the NPC by the Mex67/Mtr2 heterodimer (pink ovals). Protein coding genes in opisthokonts (and most other eukaryotes) have introns (orange boxes) and individual promoters (flags) and are transcribed as singly (monocistronic). Transcribed mRNAs are co-transcriptionally 5'-capped (gray circles), and introns spliced (orange lines) before export by Mex67/Mtr2 in conjunction with the actions of the ATP dependent helicase Dbp5.

ALPS motifs that have been identified on the β -propepters of several outer ring β - α Nups in opisthokonts³² or be provided by the lamin analog NUP-1 that copurifies with members of the trypanosome outer ring complex.^{5,35} Unfortunately, little is known about how the NPC assembles outside of opisthokonts, so the role of these motifs in NPC assembly or function remains obscure.

Through the looking glass: FG-Nups are symmetrically distributed within the TbNPC

FG-Nups are proteins carrying significant intrinsically disordered domains that contain multiple repeats of degenerate phenylalanine-glycine (FG) motifs. These domains collectively fill the central channel of the NPC, and facilitate nucleocytoplasmic transport

through interactions with soluble cargo-carrying transport factors.¹² Approximately one third of FG-Nups in opisthokonts exhibit a biased localization within the NPC, being present either on the cytoplasmic side or the nucleoplasmic side of the pore.^{9,36} This asymmetry is required for aspects of NPC function, especially mRNA export, which relies on the cytoplasmically biased Nup82 complex for messenger ribonucleoproteins (mRNPs) remodeling before release into the cytoplasm for translation by ribosomes.^{15,37}

There are significant differences between opisthokont and trypanosome FG-motif domain sequences, making it near impossible to identify orthologs of opisthokont FG-Nups in trypanosomes by *in silico* means alone. However, through affinity capture, we were able to identify orthologs of FG-Nups, based on the core scaffold structure with which they associate.⁵ We identified inner ring (central channel) FG-Nups, outer ring FG-Nups, a group that associates with both inner and outer ring complexes (multi-complex) and a third complex which we termed the TbNup76 complex, which consists of TbNup76 (an ortholog of ScNup82/HsNup88), and TbNup140 and TbNup149, the 2 largest FG-Nups in the TbNPC (see Table 1). One might have thought that, having identified the associated complex, we would then know where the FG-Nups were placed in the TbNPC. But this was not the case. Rather, we discovered *via* immuno-electron microscopy (iEM) localization that, with the exception of the nuclear basket,^{5,38,39} all Nup classes and sub-complexes were *equally* and *symmetrically* distributed between the nuclear and cytoplasmic faces of the NPC.⁵ Astonishingly, this suggests that the trypanosome NPC lacks a clear nucleoplasmic or cytoplasmic biased localization of FG-Nups, in complete contrast to opisthokonts.^{9,36} The symmetric arrangement in trypanosomes is consistent with the hypothesis that inherent NPC asymmetry is not necessary for basic nucleocytoplasmic transport.⁴⁰

A major source of Nup asymmetry in opisthokonts is the exclusively cytoplasmic ScNup82/HsNup88 sub-complex, tethering specialized FG-Nups that provide the interaction platform for factors critical for mRNA export.^{15,37} However, TbNup76, the presumed trypanosome ortholog of ScNup82, is located on both the cytoplasmic and nucleoplasmic faces of the trypanosome NPC.⁵ Herein lies another cautionary tale of making sure that one does not assume that, just

because proteins are orthologous, they function in the same way in organisms that are evolutionarily distant. After all, a bat's wing is orthologous to our hands! Furthermore, the FG-Nups that associate with TbNup76 do not bear any resemblance to the equivalent Nup82/Nup88 complex FG-Nups in opisthokonts and plants which importantly, contain N-terminal β -propeller domains required to mediate interactions with the ATP-dependent DEAD box RNA helicase Dbp5 and the RNA export mediator Gle1 with its cofactor IP₆ (inositol hexakisphosphate) to form a remodeling factory to process messenger ribonucleoproteins (mRNPs) before nuclear exit.⁴¹⁻⁴³ In fact, no single FG-Nup in the TbNPC contains a N-terminal β -propeller domain.

Rules of the road: 2 major FG-Nup flavors, 2 modes of transport?

Interestingly, despite the fact that FG-Nups are symmetrically distributed in the TbNPC, there still are different FG “flavors” (Supplementary Figure 4 in Obado et al, 2016). A perusal of the flavors used by trypanosomes compared with opisthokonts suggests that these flavor types are roughly preserved, even though their nucleocytoplasmic asymmetry is not. The more centrally localized trypanosome FG Nups are enriched predominantly in “GGFG” motifs; the multi-complex FG-Nups, which seem to be more peripheral on the NPC, are enriched instead in “FSFG,” “FG,” “SVFG” and “PAFG” repeats. In opisthokonts, there are also only a few major FG repeat flavors, the majority of which fall into the category of either “GLFG”-like or “FxFG”-like.^{44,45} We also know that there are 2 major kinds of transport factor: karyopherin-like, and non-karyopherin-like, with very different preferences for FG repeat flavors.^{46,47} Certainly, trypanosomes show us that FG flavor may not necessarily be all about nucleocytoplasmic positioning or imparting a directionality to nuclear transport. Perhaps instead, these 2 major FG repeat flavors delineate specific transport conduits for the trafficking pathways across the NPC mediated by the 2 kinds of transport factor?

Nip and tuck: Sculpting the NPC to reflect biology

So how does the trypanosome mRNA export machinery function in the absence of FG-Nup asymmetry? Unlike protein transport, mRNA (poly-adenylated RNA) export in opisthokonts is Ran-independent,

being powered in an ATP-dependent manner by Dbp5 and is mediated by non-karyopherin RNA export factors (the Mex67:Mtr2 heterodimer in yeast, termed TAP:p15 in humans),^{41,48-50} Fortunately, transport factors (karyopherin and non-karyopherin) generally are extremely well conserved across the eukaryotic kingdom, including even in trypanosomes, which have clear orthologs of the RNA export factors Mex67 and Mtr2.^{51,52}

We tagged and affinity captured TbMex67 to identify the composition of any potential RNA processing and export factors in trypanosomes. As well as its partner TbMtr2,⁵¹ TbMex67 associates strongly with the TbNup76 complex, even though this is found on both sides of the NPC; in opisthokonts, Mex67 associates strongly with the Nup82/Nup88 complex, as one might expect.^{41,50} However, no potential orthologs of Dbp5 and Gle1 co-isolated with TbMex67, or even found in the genome.⁵ So, although at least some aspect of the function of this complex – binding to RNA export factors – seems to be conserved in trypanosomes, it does so in a very different spatial context and by a different mechanism that lacks the anticipated helicase processing factory. In addition, TbMex67 bound strongly to the GTPase Ran, to Ran binding protein 1 (RBP1) and to a GTPase activating protein (GAP). In opisthokonts, Ran, RBP1 and Ran-GAP work in synchrony to expedite the hydrolysis of RanGTP to RanGDP in the cytoplasm to facilitate the release of protein cargo from a RanGTP-karyopherin complex.⁵³⁻⁵⁵ Therefore, the co-isolation of Ran, RBP1 and the GAP with the non-karyopherin transporters Mex67-Mtr2 is extremely unusual and has not been previously observed in other organisms. This suggests that unlike in opisthokonts and plants, GTP and not ATP powers mRNA export in trypanosomes. Interestingly, non protein coding RNA in opisthokonts, such as tRNAs, small nuclear RNAs, micro RNAs and pre-ribosomal subunits, are exported out of the nucleus on a Ran gradient by karyopherins, similar to protein export (reviewed in^{47,56}). Additionally, a small subset of mRNA export is dependent on the karyopherin, Exportin 1 (Crm1).⁵⁷ Thus trypanosomes appear to have modified their NPC and the Ran-mediated export pathways, a system that already has enormous flexibility, expanding it to include all transport, including mRNA export. Why have they done this? We don't know; we have speculated it may be linked to their rather unusual mechanisms for controlling

gene expression. Trypanosome protein-coding genes are intron-less, lack individual RNA polymerase II promoters and are organized into large polycistronic transcription units (Fig. 2).^{58,59} Thus, the regulation of gene expression relies mainly on mRNA turnover and translation rates.^{60,61} Hence, perhaps gene expression is controlled at the point of nucleo-cytoplasmic export through differential export in a highly regulated manner. In addition, the exclusive *trans*-splicing of protein-coding mRNAs in trypanosomes (Fig. 2) may also relax requirements for extensive chaperoning during nuclear export. Moreover, these results indicate that the asymmetric localization of many FG Nups in opisthokont NPCs is at least in part strongly associated with the co-transcriptionally linked mRNA processing and export pathway, with the final stages of mRNA processing embedded in the NPC in opisthokonts, and asymmetrically disposed FG Nups providing the necessary docking sites. Thus, quality control occurs at the nuclear basket and nucleoplasmic FG-Nups and then the remodeling of mRNPs to facilitate export occurring at the cytoplasmic end of the NPC.^{15,62}

In any case, all this underscores the intimate link between the NPC and gene expression, as well as the incredible flexibility of the NPC, which although probably fully formed at LECA has the capacity to mold and adapt its structure to the demands of individual lineages by acting as an interaction platform for several nuclear processes while still maintaining its structural core and primary function as a transport hub.

What do we now know about the evolution of the NPC?

The origin of eukaryotes and the events surrounding the transition from prokaryotes to eukaryotes remain obscure despite several attempts at reconstructing the pathways involved. Trypanosomes diverged early in eukaryotic evolution and can provide important evolutionary insights into fundamental cell biologic processes shared by all eukaryotes. Indeed, several key features of molecular biology have first been identified in trypanosomes. Examples include antigenic variation, GPI-anchored proteins, RNA editing, polycistronic transcription and *trans*-splicing.⁶³⁻⁶⁷ Originally considered a “quirky” character of these parasites, these fundamental biologic processes have now been found to be common in other eukaryotes (including

RNA editing in mammals,^{68,69} polycistronic transcription and *trans*-splicing in the nematode *Caenorhabditis elegans*.^{70,71} Now, the trypanosome is performing this function again, providing insights into the structure and evolution of the NPC and mechanisms of nucleocytoplasmic transport.

The shared evolutionary relationship between the NPC core scaffold and the endomembrane system is well documented.¹⁸ Furthermore, the 2 large yeast inner ring α -solenoid ScNups188/192 (Table 1) share architectural features with karyopherins (also α -solenoid proteins), suggestive of a co-evolution of transport factors, structural components of the NPC, and the endomembrane trafficking system.^{72,73} Indeed, it is noteworthy that the assembly of vesicle coats and the translocation of proteins through membranes are controlled by Ras-like GTPases termed Rabs,⁷⁴ just as the translocation of cargo in the NPC is controlled by the Ras-like GTPase, Ran.

The symmetry of the TbNPC alludes to our previous theory that there may have been a stepwise acquisition of complexity in the NPC in the FECA to LECA transition in which an early non-specific pore comprised of coating proteins but lacking a gating function (Fig. 3).^{17,18} Later, the evolution of FG-Nups led to a more sophisticated gating system, that was further elaborated to include cytoplasmic and nucleoplasm biased FG-Nups and to include the elaborate mRNA export machinery in opisthokonts (Fig. 3).

Trypanosomes have no cytoplasmic or nucleoplasmic biased FG-Nups. However, they have retained a level of asymmetry through the addition of the nuclear basket, which interestingly appears to have a distinct evolutionary history to that of opisthokonts.^{38,39} In the opisthokonts and plants, the nuclear basket is composed of large (~200–250 kDa) coiled coil proteins, that associate with and coordinate several nuclear peripheral processes, thereby extending NPC functionality.^{36,62,75–78} Although the 2 nuclear basket proteins are much smaller (~100kDa) in trypanosomes, our study showed by iEM, that they extend an average of 36nm into the nucleoplasm from the center of the TbNPC.⁵ Additionally, they have retained similar functions to those in other eukarya, by creating heterochromatin free zones around nuclear pores that are evident by electron microscopy in all eukaryotic nuclei, as well as recapitulating interactions with the spindle organizer.^{4,38,79–81} Thus, in conjunction with divergent lamin-like proteins,^{35,82} and unconventional

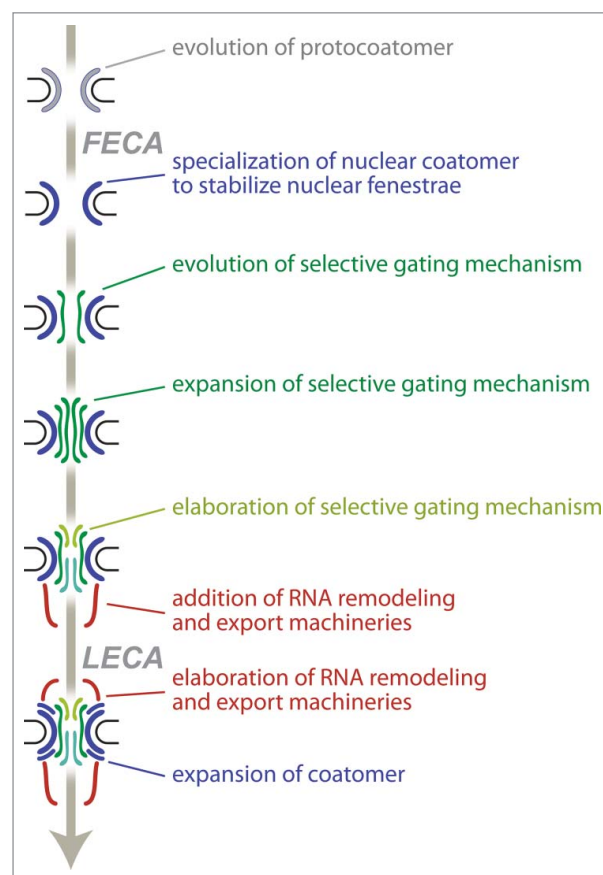


Figure 3. Evolution of the NPC. We propose a model in which the NPC evolved gating functions in a stepwise manner starting with a simple coat that acquired complexity through a series of duplications as observed in endomembrane trafficking.^{17,18} This then led to the evolution of FG-Nups and then further diversification into the current metazoan type NPC with a nuclear basket and cytoplasmic filaments.

kinetochores,⁸³ it seems that the trypanosome nucleus uses unique protein complexes in parallel with conserved core elements such as the spindle microtubules and outer kinetochore components, Ndc80/Nuf2 to facilitate trypanosome biology.^{84,85}

Did trypanosomes retain an ancient symmetric assembly or reconfigure their molecular biology through the loss of introns and individual gene promoters (Fig. 2) and thereby dispense with the need to utilize ATP as an energy source for separately powering mRNA export? Perhaps investigations of the nuclear transport machineries in other divergent eukaryotes will help answer this question. Indeed, a recent study has shown that mRNA export pathways in Apicomplexa also appears to be divergent⁸⁶; in addition, so far investigators have been unable to identify any Mex67 orthologs in *Toxoplasma gondii*, indicating that this organism may be even more divergent

than trypanosomes in its nucleocytoplasmic transport machineries.⁸⁶ In fact, very little is known about mRNA processing and export in most protists. Thus our study highlights and reinforces the need to sample and study a broad distribution of eukaryotic taxa to gain insight into evolutionary origins of function and mechanism at the nuclear envelope – as well as of course in many other cellular processes.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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