Biochemical Society Transactions (2021) **49** 1601–1619
https://doi.org/10.1042/BST20200570

Review Article

Evolution and diversification of the nuclear pore complex

Alexandr A. Makarov[1,*], Norma E. Padilla-Mejia[1,*] and Mark C. Field[1,2]

[1]School of Life Sciences, University of Dundee, Dundee DD1 5EH, U.K.; [2]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, 37005 České Budějovice, Czech Republic

Correspondence: Mark C. Field (mfield@mac.com)

The nuclear pore complex (NPC) is responsible for transport between the cytoplasm and nucleoplasm and one of the more intricate structures of eukaryotic cells. Typically composed of over 300 polypeptides, the NPC shares evolutionary origins with endo-membrane and intraflagellar transport system complexes. The modern NPC was fully established by the time of the last eukaryotic common ancestor and, hence, prior to eukaryote diversification. Despite the complexity, the NPC structure is surprisingly flexible with considerable variation between lineages. Here, we review diversification of the NPC in major taxa in view of recent advances in genomic and structural characterisation of plant, protist and nucleomorph NPCs and discuss the implications for NPC evolution. Furthermore, we highlight these changes in the context of mRNA export and consider how this process may have influenced NPC diversity. We reveal the NPC as a platform for continual evolution and adaptation.

Introduction

The nuclear pore complex (NPC), responsible for bidirectional transport of proteins and RNA between the cytosol and nucleus, is an octagonally symmetric structure consisting of multiple co-axial rings, each built of eight spokes (Figure 1). The inner (IR) and outer cytoplasmic and nuclear rings (CR and NR) form the core scaffold, anchored to membrane ring (MR) *trans*-membrane domain proteins and housing the bulk of FG-nucleoporins (Nups) that maintain the NPC permeability barrier. The nuclear basket (NB) and cytoplasmic filaments (CFs) are attached to the core scaffold and contribute towards both protein and mRNA transport [1].

The protocoatomer hypothesis [2] was proposed in recognition of common architectures between core proteins of multiple complexes within eukaryotic cells, including the NPC. Protocoatomers are membrane-deforming proteins consisting of β-propellers and α-solenoids, and while the primary structure between family members is frequently poorly retained, the secondary structure is considerably better conserved. Inferred from this is that an archetypal membrane coating complex evolved in the earliest eukaryotes (Figure 1A), supported by the presence of β-propeller and α-solenoid-encoding genes within the closest known prokaryotic ancestors of eukaryotes, the archaea. The model proposes that through paralogous duplication, extensive type I and II coat families arose: Type I coats feature an N-terminal β-propeller followed by a continuous α-solenoid, while type II coats bear a perversion within the α-solenoid [3,4]. NPCs contain proteins of both type I and II architectures, suggesting evolution followed the establishment of the major coat types. In *Saccharomyces cerevisiae* two clearly paralogous columns parallel to the NPC axis form each IR spoke and each spoke is, in turn, duplicated vertically. Similarly, the spokes in the CR and NR are built of two columns each. The column building block is an amalgam of type I and II coat proteins, and this subcomplex, via paralogous expansions, possibly populated the NPC architecture in the LECA (Figure 1B).

*A.A.M. and N.E.P.-M. made equal contributions to this work.

Received: 26 April 2021
Revised: 18 June 2021
Accepted: 21 June 2021

Version of Record published: 20 July 2021

Biochemical Society Transactions (2021) **49** 1601–1619
https://doi.org/10.1042/BST20200570

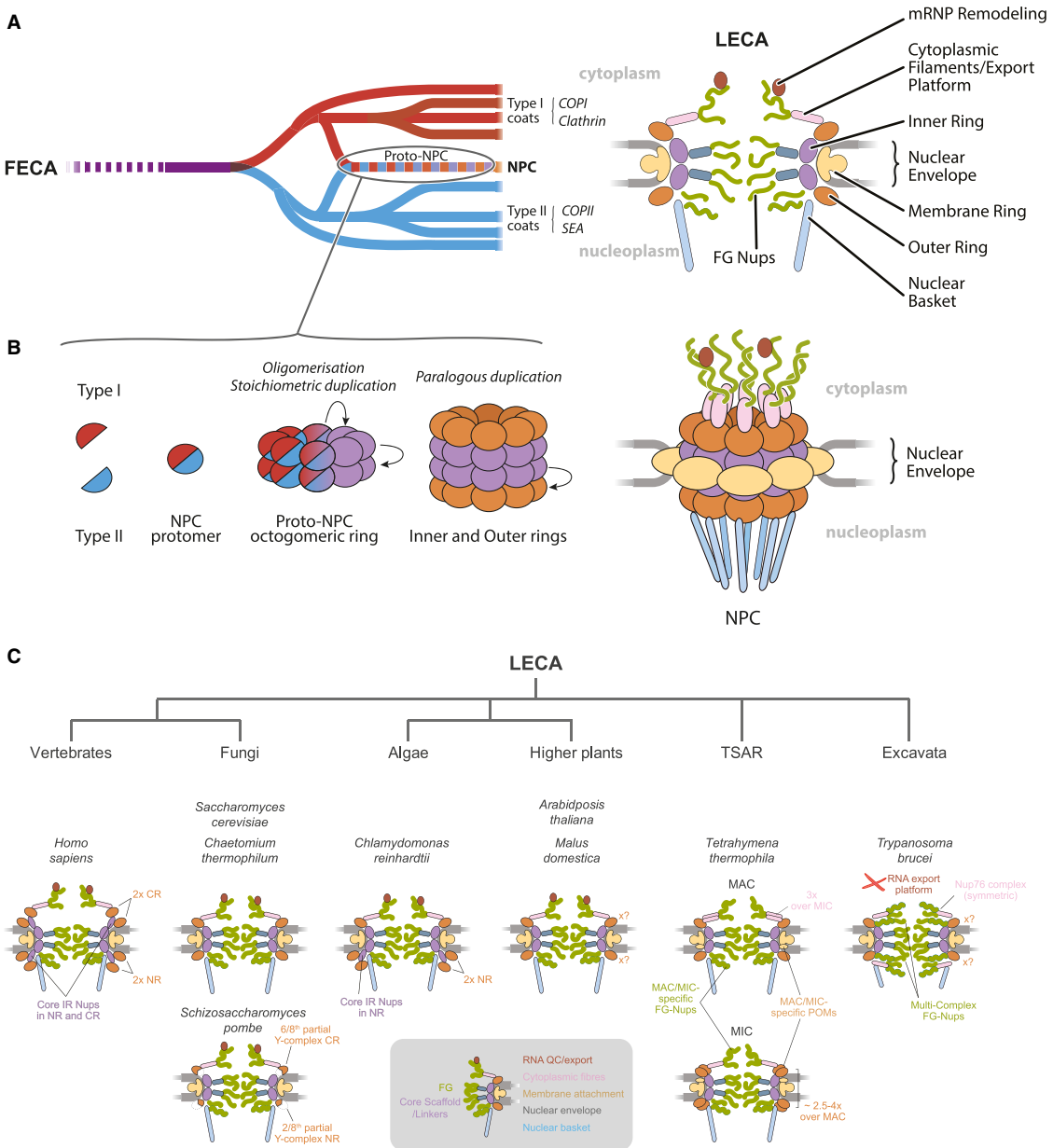


Figure 1. Evolutionary origins of the NPC.

(**A**) Protocoatomer hypothesis states that a single protocoatomer (left, dark purple line) originated sometime early in eukaryotic evolution and gave rise to the two major coat protein families — type I (red lines) and type II (blue lines). Type I and type II coat proteins jointly formed the Proto-NPC — progenitor of the LECA NPC (right) — the concentric assembly of octagonally symmetric inner (IR, purple and dark blue), outer (OR, orange) and membrane (MR, beige) rings anchored in the NE pore that houses the nuclear basket (light blue), the cytoplasmic export platform (pink and burgundy) and FG-repeat nucleoporins (green). (**B**) An assembly of type I and II coat proteins formed an NPC protomer that populated the double inner ring via oligomerisation and stoichiometric duplication, and further — via paralogous duplication — the outer rings of LECA NPC. (**C**) Further diversification post-LECA gave rise to many NPC architectures in major taxa of eukaryotic tree (top) that principally differ in the stoichiometry and symmetry of outer cytoplasmic and nucleoplasmic rings (CR and NR), the manner of outer ring attachment to the inner rings and presence and symmetry of specific FG-nup and MR elements.

Molecular data are available for NPCs from many lineages, including metazoa (*Homo sapiens*) [5–8], fungi (*S. cerevisiae*, *Schizosaccharomyces pombe*, *Chaetomium thermophilum*) [9–16], algae (*Chlamydomonas reinhardtii*) [17], higher plants (*Arabidopsis thaliana, Malus domestica*) [18,19], alveolates (*Tetrahymena thermophila*) [20] and excavates (*Trypanosoma brucei*) [21]. While not particularly deep in taxon coverage, these data span a considerable proportion of eukaryotic diversity. Here we consider the structural variations that are evident between NPCs, consider export mechanisms for transiting the pore and how these systems co-evolved.

Stoichiometry in outer rings

The outer rings anchor the cytoplasmic export platform and nuclear basket [6,10,11,22–24]. The Nup85 (yeast nomenclature) or Y-complex is the outer ring building block and contains up to nine components. All are β-propeller, α-solenoid or β-propeller/α-solenoid proteins, archetypal for membrane coating complexes and thus likely to share a common evolutionary origin [1,25–27]. At least six Y-complex components are broadly conserved and five are scaffold nucleoporins, orthologs of HsNup75, HsNup96, HsNup107, HsNup133 and HsNup160 [28]. Notable exceptions are *T. brucei* and *T. thermophila*; each possess novel or highly divergent β/α-proteins TbNup109 [21] and TtNup185 (albeit with some similarity to HsNup133, 155 and 160), respectively [20]. The remaining components are β-propeller nucleoporins, the widely conserved Sec13, Seh1 (absent from excavates), HsNup43 (absent from fungi and TSAR) and HsNup37 (restricted to animals and some fungi); all of which suggests considerable evolutionary flexibility (Figure 2).

Notably, a pronounced difference in NPC organisation across taxa lies in the number of these rings and stoichiometry of Y-complex components. While *S. cerevisiae* has a single CR and NR [10], *H. sapiens* enjoys two copies of each [6] and curiously *C. reinhardtii* has two NR but only one CR [17] (Figures 1C and 2). Additional components are present for interconnection of these duplicate rings, including the vertebrate-specific CF component RanBP2/Nup358 [7,29] and inner ring components — Nup155, 188 and 205 [6–8,17,29]. Deviating further are *S. pombe* and *T. thermophila* (Figures 1C and 2). *S. pombe* rings are 'split': SpNup107 and SpNup132 localise exclusively to the NR, while six remaining nucleoporins, including SpNup131 (diverse paralog of SpNup132), are CR-exclusive. Deepening this uneven distribution is the overall comparatively low abundance of SpNup107 and SpNup131 [12,13]. *T. thermophila* NPCs, by contrast, demonstrate an uneven distribution but with comparatively higher abundance of 2.5- to 4-fold for Y-complex subunits in the micronucleus (MIC) [20]. Whether this is related to the transcriptional activity differences is yet to be explored. *M. domestica* (apple) differs from *Arabidopsis* as it possesses multiple Y-complex subunit paralogs (albeit likely results of genome duplication) [18,19] (Figure 2), while *T. brucei* acquired an additional complex of three FG-Nups, symmetrically positioned on both sides of the NE [21]; the exact symmetry and stoichiometry of the outer ring in plants and excavates remains to be established.

ELYS, a β/α-nucleoporin with a nucleosome-binding domain, originally identified as a transcription factor [30], interacts with both the Y-complex and pore membrane proteins (POMs). ELYS mediates post-mitotic NPC assembly/anchoring, chromatin compaction and NPC/lamina associations, but most of these functions are metazoan and/or open mitosis-specific [31,32]. Paradoxically ELYS is present in most taxa, except excavates or TSAR, suggesting a probable ancient origin [12,17–21]. As neither plants nor fungi share a lamina with metazoa, ELYS functions are unclear [33,34]. Even more unusual is that the *S. pombe* ELYS ortholog is located on the cytoplasmic side of the NPC, potentially precluding obvious nucleoplasmic roles [13].

Expansion, loss and diversification in the inner core

Similar to ORs, main IR components are large α-solenoid (Nup93/Nic96, Nup188, Nup205 orthologs) or β-propeller/α-solenoid proteins (Nup155 orthologs) and again with clear origins in membrane coating complexes [1,25–27]. The IR is well conserved but sports surprising functional diversity (Figure 2). IR in some taxa rely on nucleoporins containing a membrane-binding domain (MMB) for anchorage to the pore membrane. Of these ScNup53 and ScNup59 in *S. cerevisiae* and MdNup35a and MdNup35b in *M. domestica* exemplify a paralog duplication absent elsewhere and presumably occurred independently. However, *T. brucei* sports TbNup65, a single ortholog to ScNup53/59, possessing a conventional *trans*-membrane domain instead of an amphipathic lipid-packing sensor (ALPS) MMB present in ScNup53/59 [21]. *T. thermophila*, by contrast, has no identifiable orthologs to ScNup53/59 and possibly relies on TtNup155, an ortholog to ScNup157/170, and additional POMs for membrane anchoring [20].

Connections between the IR, OR, NB and CFs are also variant. In *S. cerevisiae* connector FG-nups ScNup145N/ScNup116/ScNup100 asymmetrically connect IR with outer rings, with ScNup145N extending

PORTLAND PRESS

Biochemical Society Transactions (2021) **49** 1601–1619
https://doi.org/10.1042/BST20200570

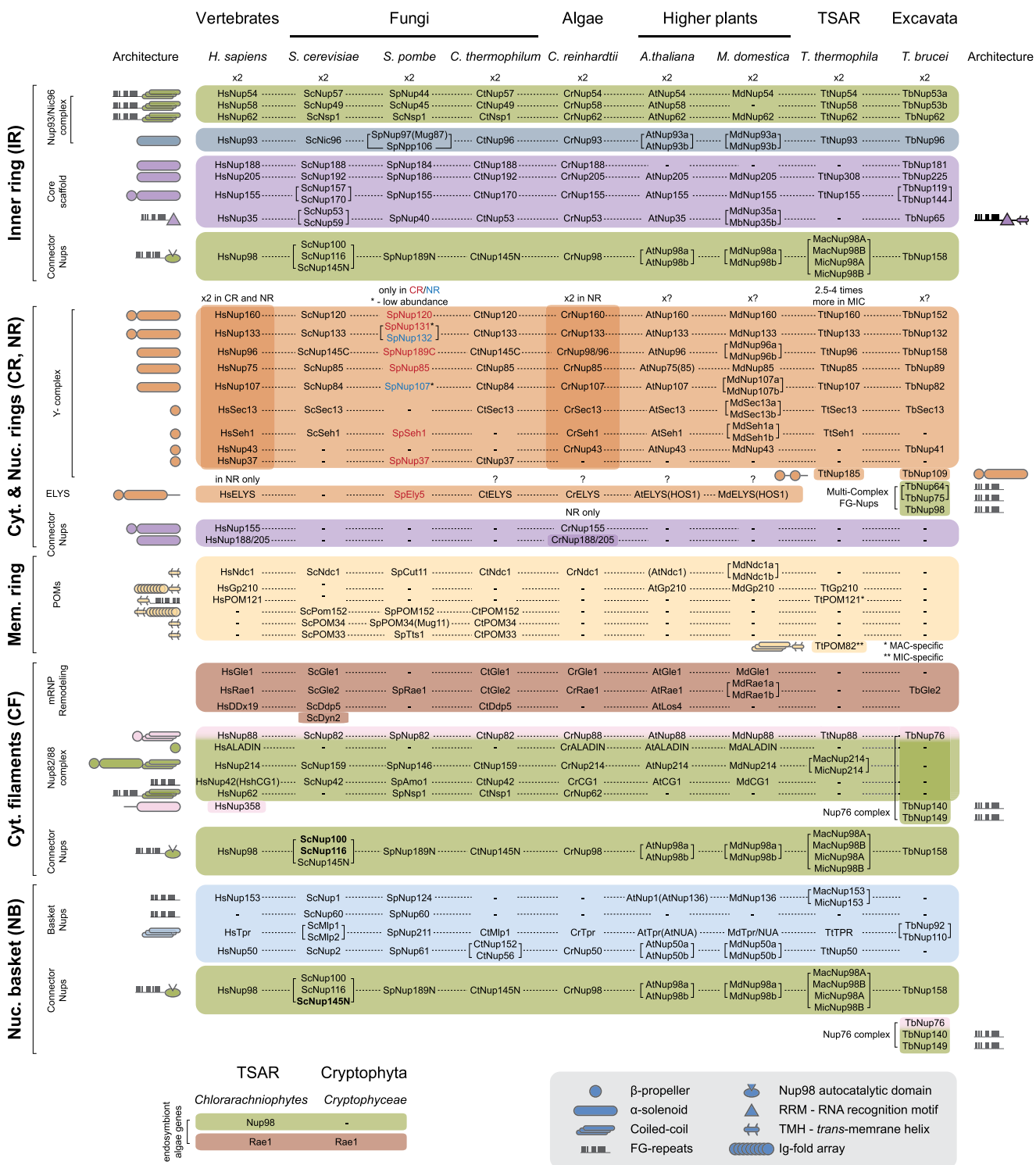


Figure 2. Comparisons of nucleoporins across species in selected taxa. Part 1 of 2

Data collated for *H. Sapiens* [8], *S. cerevisiae* [10], *S. pombe* [12], *C. thermophilum* [14], *C. reinhardtii* [17], *A. thaliana* [18], *M. domestica* [19], *T. thermophila* [20], *T. brucei* [21] species and *Chlorarachniophytes* and *Cryptophyceae* species clades [125]. Nucleoporins are listed according to their complex and ring disposition in NPC compartments: the inner ring (IR), outer cytoplasmic and nucleoplasmic rings (CR and NR), membrane ring (Mem. Ring, MR), cytoplasmic filaments (Cyt. Filaments, CF) and nuclear basket (Nuc. Basket, NB). Nucleoporins are additionally coloured by type: FG-nups and linker FG-nups in IR, CR and NR, CF and NB in swamp green; Nup93/Nic96 complex core in IR in blue; core IR scaffold Nups in purple (also present in ORs as connector Nups); OR Nups in orange; pore membrane proteins (POMs) in MR in beige; Cytoplasmic Export platform

Figure 2. Comparisons of nucleoporins across species in selected taxa. Part 2 of 2

scaffold in CF in pink; mRNP Remodelling in CF in burgundy, and basket scaffold Nups in NB in light blue. Each row represents an orthologous nucleoporin group. Nucleoporins absent in an organism are indicated by a dash (-). In cases of paralogous duplication within an organism — multiple nucleoporins are listed in square brackets, i.e. [ScNup167 ScNup170]. Alternative nucleoporin names are listed in round brackets, i.e. HsNup42 (HshCG1). Ring stoichiometry noted for IR and ORs. Exclusive Y-complex component distribution in *S. pombe* is additionally indicated by colour: red for CR-specific and blue for NR-specific. Macronuclei/micronuclei- (MAC-/MIC-) specificity for nucleoporins in *Tetrahymena* is denoted by Mac- or Mic-prefixes in gene names or * and ** for POMs. *Trypanosoma*-specific Multi-Complex FG-Nup and Nup76 complexes are shown by additional brackets. In 'architecture' column (left) given are the schematic protein fold architectures common for the orthologous Nup groups. Additional unique architectures are indicated for TbNup65 that sports a *trans*-membrane helix uniquely in its orthologous group (demonstrated on the right), and for lineage-specific Nups TtPOM82, TtNup185, TbNup64, TbNup75, TbNup98, TbNup140 and TbNup149.

from the central NPC towards NR and NB, and ScNup116 and ScNup100 binding IR to CR and the export complex [10] (Figure 2). Furthermore, ScNup157 and 170 stabilise the spokes within the inner ring, while ScNup188 and ScNup192 act as buttresses within the spokes and neither interact with the OR. However, to facilitate connections with duplicate ORs in *H. sapiens* and *C. reinhardtii* eight additional copies of HsNup155/CrNup155 and HsNup188/CrNup188 (respective orthologs of ScNup157/170 and ScNup188) form pillars, one per spoke, on each side of the NE when there are duplicate ORs [6–8,17]. Interestingly, in the *Xenopus laevis* NPC XlNup205 (ortholog of ScNup192) replaces Nup188 [29], which suggests considerable flexibility, even within vertebrates.

Unsurprisingly, excavate and TSAR NPCs are organised distinctly to metazoan and fungal complexes (Figure 2). *T. brucei* has a paralog pair, TbNup144 (orthologous to ScNup157/170) and TbNup119 (similar to ScNup170) [21]. Notably, pullouts revealed TbNup144 to have weak interactions with TbNup89, the ortholog of HsNup75/ScNup85 of the Y-complex. In contrast TbNup119 pulled down the entire *T. brucei* OR as well as the IR core α-solenoid TbNup225, the ortholog of HsNup205/ScNup192. Notably, neither TbNup225, nor other components of the T. *brucei* IR interact with the OR, suggesting that TbNup119 bridges IR and OR and is more akin to ScNup145N/ScNup116/ScNup100. Simultaneously no data exists on whether TbNup158, an ortholog to HsNup98/(ScNup145N/ScNup116/ScNup100), is present in the IR or contributes to IR-OR connection. Instead, TbNup158 appears as a constituent component of the Y-complex in *T. brucei* anchoring the TbNup76 complex and multi-complex FG-Nups in the ORs [21]. Finally, T. *thermophila* sports single orthologs to each HsNup155/(ScNup157/ScNup170)/(TbNup144/TbNup199) and HsNup205/ScNup192/TbNup225, but up to four orthologs to HsNup98/(ScNup145N/ScNup116/ScNup100)/TbNup158. Two localise exclusively to the macronuclei and carry GLFG repeats, while two are micronuclei-specific and carry NIFN repeats, likely functioning to differentially regulate MAC/MIC-specific transport [20].

Pore membrane proteins

The MR is an integral structure of the NE [35], composed of POMs [9] and is possibly the least conserved NPC subcomplex (Figure 2). Only three POMs are candidates for a LECA origin: Ndc1 and Gp210, present broadly but partially lost from TSAR and excavates, with Gp210 also lost from algae and fungi [36]; and the more widely found POM121 [20,36]. All other POMs appear narrowly conserved, leading to structural deviation: Metazoan and fungal NPC ultrastructures are very different within the NE lumen, despite being comprised of structurally homologous domains of Gp210 or POM152, respectively [10,37–39], and no such similarities can be expected in algae or excavates lacking Gp210 [17,21]. Similarly, a particular function is difficult to assign to the core structural POMs. POM152, and the closed-mitosis fungai-specific POM34 [36], form a complex interaction network with NPC components but are non-essential in yeast [40]. However, Gp210 is not expressed in several tissues of mice and human primary fibroblasts [41,42] and reports are conflicting on the phenotype of Gp210 depletions in vertebrates and nematodes [43,44]. POM121 is dispensable in somatic human cell lines but critical in *Xenopus* embryos [44,45]. In *Tetrahymena*, POM121 is MAC-specific and distributes towards the NR side, but it is unknown if POM121 acts with MIC- and/or cytoplasmic side-specific TtPOM82 to alter OR stoichiometry [20]. Ndc1 depletion causes severe NPC assembly defects in metazoa and yeast, where it additionally functions in embedding spindle pole bodies into the NE [46–49]. Thus, the MR is a highly divergent structure and may lack a single defined architecture or function.

Biochemical Society Transactions (2021) **49** 1601–1619
https://doi.org/10.1042/BST20200570

The nuclear basket and mRNA export

The NB in Metazoa consists of three highly conserved nucleoporins (Figures 2 and 3): Tpr, Nup50 and Nup153 [50], with yeast Mlp1/2, Nup2 and Nup1/Nup60 as respective homologues [51–53]. Tpr/Mlp proteins are involved in RNA biogenesis, spindle pole assembly, regulation of transcription, chromatin remodelling and mRNA export [54]. Although highly conserved within Metazoa, NB proteins suffered a significant diversification in other taxa, suggesting adaptations to organismal-specific roles. Tpr/Mlp homologues are more conserved than Nup153 and Nup50 and several organisms bear lineage-specific NB proteins [55–57].

Tpr, a coiled-coil homodimer, is the NB scaffold [58]. Nup153, a protein with RNA-binding properties [59], anchors to Tpr and the FG-repeats of Nup153 can reach into the NPC core [56,60]. It is unclear if Nup153 is essential for Tpr attachment to the NPC as data are conflicting [56,60–62]. It is likely that Nup153 is essential for Tpr recruitment during post-mitotic NPC assembly but not for anchoring Tpr already NPC localised, suggesting that an additional Tpr-binding site is present [63]. *A. thaliana* and *M. domestica* present divergent Tpr homologues (Tpr/NUA) [64–66] and also possess AtNup1/136, homologue of yeast Nup1/human Nup153 [18,67]. AtNup1 and AtTpr localise in the nuclear periphery during interphase with AtTpr localised in the vicinity of the spindle in pro-metaphase [66]. Significantly, C. *thermophilum* possesses two novel NB proteins, CtNup152 and CtNup56, which bear partial similarity to metazoan Nup50 and yeast Nup2 [14]. In trypanosomes, and conserved across kinetoplastids, TbNup92 and TbNup110 (coiled-coil proteins) constitute the NB and likely evolved independently from Mlp/Tpr [21].

The NB is a platform for the initial stages of RNA export, docking mRNPs and facilitating transport [10,68]. Tpr is crucial for recruiting TREX-2 complex components and hence efficient export of mRNA [61,63]. In yeast, Mlp1/Mlp2 provide docking sites for mRNPs and nuclear export adaptors, while Nup60 bears quality control capacity as Nup60 deletion causes Mlp1 mislocalisation and defects to pre-mRNAs retention [69]. In *Arabidopsis*, AtTpr and AtNup1 participate in polyA transcript export [18,64–67,70]. In *T. brucei*, NB proteins impact transcription of some RNA-binding proteins but precise roles remain undetermined [71].

The Mex67–Mtr2 family

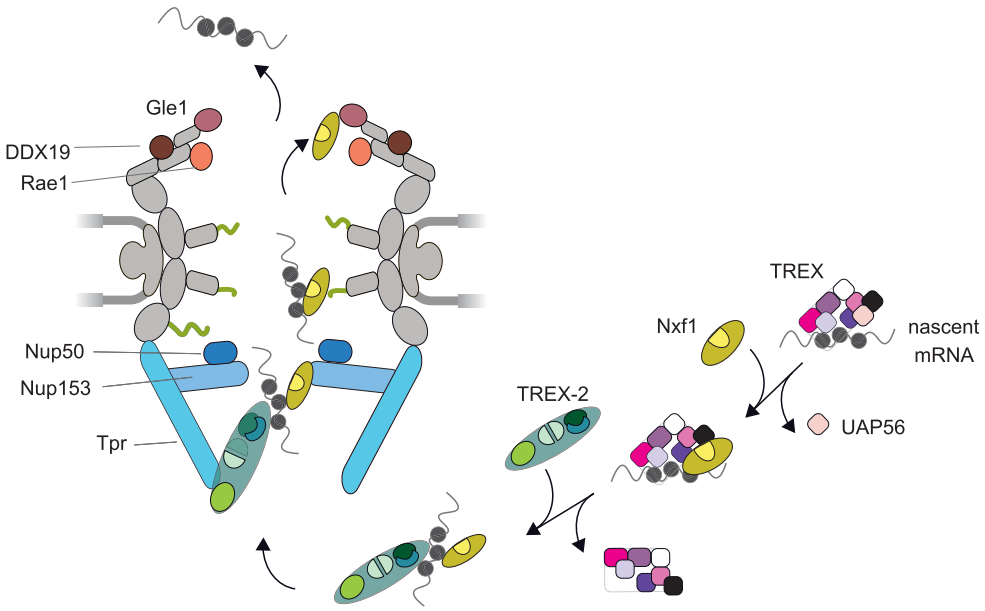
mRNA export pathways employ primarily the Nxf1–Nxt1 complex in mammals and their orthologs Mex67–Mtr2 in yeast. Sequence conservation between NFX1–NXT1 and Mex67–Mtr2 is low, albeit retaining functional complementarity [72,73]. Mex67–Mtr2 is the main mature mRNP carrier, an interaction essential for export [74,75]. Translocation of mRNPs across the NPC is mediated by interactions between Mex67–Mtr2 and FG-Nups [76–78]. Localisation of Mex67 at the NB is highly dependent on FG-repeats and deletions cause severe loss of Mex67 from the nuclear periphery [74]. Moreover, in humans, NXF1 presents additional tissue-specific isoforms [79] and ubiquitination regulates recruitment of Mex67 to nascent transcripts [80], adding further complexity to the regulation of mRNA export.

Mex67 has an RNA-recognition motif (RRM), a leucine-rich repeat (LRR), a nuclear transport factor 2-like (NTF2L) and ubiquitin-associated (UBA) domains [74,75,81]. The RRM binds interactors such as the TREX complex [82] and splicing factors [83,84]. Mtr2 also possesses an NTF2L domain, which contacts the Mex67 NTF2L region, forming a heterodimer. Although Mtr2 colocalises with the NPC, it does not directly contact the FG-repeats [76,77,85].

Orthologs of Mex67 and Mtr2 are both present in trypanosomes and essential for mRNA export [21,86–90]. Trypanosome Mex67 retains the LRR and the NTF2L domains, but has a divergent N-terminus with a CCCH-type Zn^{2+} finger replacing the typical RRM domain [21,91], while TbMtr2 also retains an NTF2L domain [21,91]. Additional TbMex67 paralogs are also present but their functions are unknown [90]. Importantly, TbMex67 interacts directly with Ran, suggesting that mRNA export is Ran-dependant and hence mechanistically distinct from animals and fungi [90,91]. In *T. gondii*, TgZnf2, a nuclear protein containing a C_2H_2 zinc finger, functions in mRNA export and cell cycle regulation and is highly conserved across Apicomplexa. TgZnf2 interacts with TgNtx1, a probable ortholog of Mtr2 [92]. In plants, Mex67 has not been found.

Transcription-export complex 2

The TREX-2 complex has a crucial role in genome stability and transcription, providing an essential platform to recruit the mRNA processing machinery [93]; subunit deletions lead to nuclear mRNA accumulation [94]. TREX-2 is loaded onto mRNPs to aid translocation to the NPC and facilitates export by increasing the entry



		Vertebrates	Fungi	Plants	TSAR		Excavata
		H. sapiens	*S. cerevisiae*	*A. thaliana*	*T. thermophila*	*T. gondii*	*T. brucei*
Nucleus	TREX-2	GANP	Sac3	Sac3A & B**	ni	ni	ni
		PCID2	Thp1	Thp1	ni	ni	ni
		ENY2	Sus1	ENY2**	ni	ni	ni
		Centrin2	Cdc31	Cen1	ni	◌	ni
		DSS1	Sem1	DSS1	ni	ni	ni
	Mex67/ Nxf1	Nxf1/Tap	Mex67	ni	ni	ZNF2	Mex67
		Nxt1/p15	Mtr2	ni	ni	◌	Mtr2
	Basket Nups	Tpr	Mlp1 & 2	Tpr/NUA	Tpr	ni	Nup92 & 110**
		Nup153	Nup1 & Nup60	Nup136/1	Nup153	ni	ni
		Nup50	Nup2	Nup50a & 50b	Nup50	ni	ni
	TREX-THO	THOC1	HPR1	HPR1/Tho1/EMU	ni	ni	ni
		THOC2	Tho2	THO2	ni	ni	ni
		THOC3	Tex1	TEX1/THO3	◌	◌	◌
		THOC5	Thp2	THO5A	ni	ni	ni
		THOC6	-	THO6	ni	ni	ni
		THOC7	Mft1	THO7A	ni	ni	ni
		-	-	THO5B	ni	ni	ni
		-	-	THO7B	ni	ni	ni
	TREX-Helicase	UAP56/ DDX39B	Sub2	UAP56A	ni	UAP56	Sub2
				UAP56B	ni	ni	ni
	TREX-Adaptor	ALY/REF/ THOC4	Yra1	ALY1	ni	ni	ni
				ALY2-4	ni	ni	ni
Cytoplasm	Cytoplasmic Nups	Gle1	Gle1	Gle1	ni	ni	ni
		Rae1	Gle2	Rae1	ni	Rae1	Gle2
		DDX19	Dbp5	LOS4	ni	ni	ni

Figure 3. mRNA export machinery evolution. Part 1 of 2

(**A**) Schematic summarising interactions and participants in the global RNA-independent mRNA export machinery. Nascent mRNA complexes with THO/TREX and after displacement of UAP56, export factor Nxf1 is recruited. mRNP is delivered to TREX-2 which facilitates the entry to the NPC. Nxf1 interacts with FG-repeats along the NPC and components at the

Biochemical Society Transactions (2021) **49** 1601–1619
https://doi.org/10.1042/BST20200570

Figure 3. mRNA export machinery evolution. Part 2 of 2

cytoplasmic face catalyse the release of mRNA. (**B**) TREX and TREX-2 complexes, export factors, nuclear basket and cytoplasmic NPC components across taxa are summarised. Compared components are shown on the left, depicted with small coloured circles. All the experimental characterised components are written. With a dash, components are certainly absent, dotted circles, components identified *in silico*, but not experimentally characterised yet. Components marked with a double black star have been identified, but participation in mRNA export has not been proven. n.i. component not identified yet.

efficiency of mRNPs into the NPC transport channel [93,95]. In metazoa, TREX-2 interacts with the NPC in a highly stable manner [61].

In *S. cerevisiae* TREX-2 consists of six subunits (Sac3, Thp1, Sus1, Cdc31 and Sem1) with Sac3 acting as the scaffold [93]. In animals, the complex consists of a GANP scaffold, PCID2, two copies of ENY2, Centrin2 and DSS1 [96] as respective homologues. TREX-2, through FG-repeats in Sac3, interacts with the mRNP and Mex67/Mtr2, and through Sac3–Sus1–Cdc31 with Nup1 [96–99]. Homologues of TREX-2 components are present in *A. thaliana*, including Thp1, two Sac3 paralogs and orthologs of Cdc31 and Sem1 with physical interactions between them [67]. However, the Sus1 ortholog, AtENY2, is not a TREX-2 constituent [99,100] and rather is a component of the SAGA transcription complex [101]. As in other systems, depletion of Sac3 and Thp1 in *A. thaliana* causes mRNA [67] and miRNA accumulation [102]. To our knowledge, no components of TREX-2 have been functionally characterised in other taxa, with the possibility that they are present but too divergent to be identified by sequence comparison. In Apicomplexa, a potential Cdc31 has only been identified *in silico* [103].

TREX

The TREX complex is co-transcriptionally recruited to nascent mRNAs and regulates splicing and export [104]. TREX consists of the multi-subunit THO complex, a conserved DEAD-box RNA helicase Sub2 and an export adaptor Yra1 (Figure 3), to which other components assemble. Sub2 has conserved functions promoting splicing, mRNA export and recruitment of Yra1. In the assembled TREX-mRNP complex, Sub2 together with Yra1 may load Mex67–Mtr2. Remarkably, although THO complex composition is clearly divergent between animals and fungi (Figure 3), recent data suggests that the overall tertiary structure, multimerization and flexibility of TREX are strikingly conserved [105]. Yra1 is essential in *S. cerevisiae* [106], but not metazoa [107,108] and overexpression in *A. thaliana* lacks an obvious phenotype [109]. This highlights that, although overall structure and mechanisms seem conserved, adaptations can contribute towards evolutionary context-dependent essentiality.

THO is conserved in plants but *A. thaliana* possesses additional THO and Yra1 paralogs (Figure 3), indicating complex diversity [110,111]. Trypanosomes have a Sub2 ortholog, an essential protein associated with mRNA transcription/processing sites and export [112], while in *T. gondii* a highly divergent ortholog has been characterised [113]. The remainder of the TREX complex in these organisms awaits discovery [114].

Cytoplasmic filaments and mRNA export

At the cytoplasmic face of the NPC in yeast lies the Nup82 complex (Nup82, Nup159, Nsp1), a part of the export platform and attached to the cytoplasmic OR facing the central channel. Nup82 also helps Gle1, Dbp5 and Nup42 in organising the last stages of export. Dbp5 is an RNA-binding DEAD-box helices involved in transcription, mRNA export and termination of transcription [115,116]. During mRNA export, Dbp5 triggers remodelling of mRNAs emerging into the cytoplasm in the final export steps. Yeast Dbp5 is regulated by Gle1, an interaction stabilised by inositol-hexakisphosphate (IP_6), which catalyses the release of RNA-binding proteins to ensure directional transport from the nucleus [117,118]. However, in humans IP_6 binding may be dispensable [119], suggesting diverse mechanisms. Adding additional levels of regulation of mRNA export pathways, multiple Gle1 isoforms have been found [120].

In *Arabidopsis*, Gle1 is highly conserved [121], essential [122] and stimulates the Dbp5 homologue AtLOS4 [18,122]. Interestingly, in *Lotus japonicus*, Gle1 has evolved to promote a symbiotic relationship with mycorrhiza *Mesorhizobium loti* for symbiotic nitrogen fixation [123]. Trypanosomes lack orthologs of Gle1 and Dbp5 indicating a distinct mechanism [21] and consistent with simplified *trans*-splicing.

Gle2 (mammal/plant Rae1) is involved in mRNA export as inactivation/mislocalisation leads to nuclear accumulation of mRNA [124]. Interestingly, Gle2 is retained in trypanosomes and nucleomorph nuclei [21,125] (Figures 2 and 3), suggesting an ancient origin in the eukaryotic lineage and possibly a central role.

Functional and genomic constraints moulding NPC evolution

The NPC is a remarkable example of co-evolution as mutations could result in complex effects produced by impacting many interacting partners [126,127]. Interestingly, Nups show different evolutionary rates which may reflect distinct evolutionary pressures [128]. Distinct functional constraints (Table 1) may influence this process and include protein–protein and protein–nucleic acid interactions [127,129]. Moreover, ubiquitously expressed proteins tend to evolve slower than tissue-specific proteins [129,130] and HsNup50, Tpr and Gp210 show differential mRNA and protein expression levels in different tissues, suggesting altered NPC composition between cell types [5,131].

An interesting further example is the autocatalytic domain of HsNup98/Sc145C. HsNup98 and HsNup96 are expressed as a single fusion protein which undergoes autoproteolytic processing [132], an event essential for localisation [133]. Interestingly, this mechanism is conserved in *S. cerevisiae* Nup145 [134], but absent from other organisms such as trypanosomes [21].

Dynamic properties of the NPC

The inner and outer rings are the most conserved NPC subcomplexes and the α-solenoids, β-propellers and coiled-coil bundles within the ring nucleoporins account for the majority of mass density in cryo-EM structures. Conserved architectures trace NPC core evolution back to type I and type II coats (Figure 4A), but NPC modularity permits a variety of architectures (Figure 4B). It is important to understand that the NPC core is dynamic and there is a noticeable variation of the central channel diameter between 60 and 40 nm, respectively, depending on species and imaging method [7,10,17,135,136], representing the capacity of the NPC for constriction/dilation in response to environment [137], presence of transport factors/cargo [138–140], cell cycle stage

Table 1. Functional and genomic constraints with potential impacts on NPC evolution

Constraint class	Constraint	Possible impact	Examples within the NPC
Functional	Structural environment of catalytic amino acids	Residues within the catalytic core are under greater pressure of being conserved. Residues are substituted in ways that the overall stabilities of structure are maintained [127,129]	Nups with enzymatic activity: Gle1, helicases Dpb5/DHX9. Autoproteolytic domain found in human Nup98/Nup96 (yeast 145N/145C) [135]
	Protein–protein interactions	Mould a complex network of protein : protein and subcomplex : subcomplex interactions. Restraints for the acceptance of amino acid substitutions based on interfaces contacts [127]	Subcomplexes within NPC. Different NPC stoichiometry across different tissues [5] Regulation of DHX9 helicase activity/distribution by Nup98 [136]
	Protein–nucleic acid interactions	Protein–nucleic acid recognition/interfaces tend to be highly conserved [127]	Rae1, Elys, Nup153 and ScNup157 capacity to bind nucleic acid [32,59,137]
Genomic	Gene expression: differential expression in tissues	Ubiquitously expressed proteins tend to evolve slower than tissue-specific proteins [129,130]	HsNup50, Tpr, Nup214, Aladin, Gp210, Pom121 and Nup37 levels (transcript and protein) are different in different tissues, suggesting rearrangements of NPC stoichiometry across cell types [5,131]
	Epigenetics: chromatin remodelling	Chromatin remodelling and epigenetic marks impact gene expression and therefore, protein evolution [126,130]	Nuclear basket roles in chromatin remodelling and epigenetic regulation (reviewed in [138])

Biochemical Society Transactions (2021) **49** 1601–1619
https://doi.org/10.1042/BST20200570

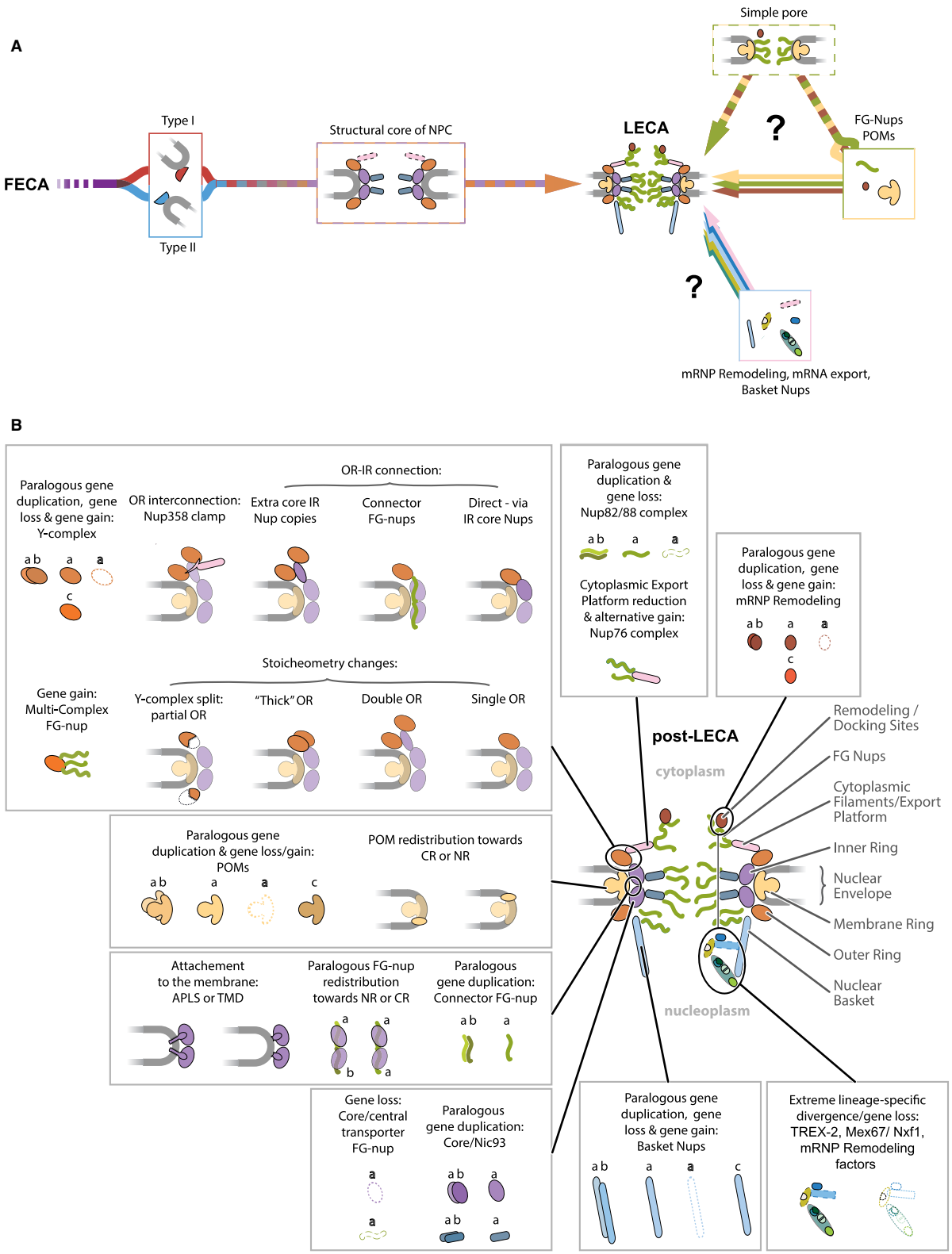


Figure 4. Outstanding questions in NPC evolution and known NPC diversifications. Part 1 of 2

(**A**) While the structural core of LECA NPC — its inner and outer rings — can be convincingly traced to type I and type II coat proteins (left), it is yet unclear how the NPC acquired its other components — responsible for mRNA processing and general permselective function (right). As individual FG-nups/transport factor combinations were shown sufficient to execute partial per-selective function, and POMs — to form pores in lipid bilayers,

Figure 4. Outstanding questions in NPC evolution and known NPC diversifications. Part 2 of 2

the possibility arises that these components formed a separate simple pore before incorporation into NPC. (**B**) Summary of principal variations and evolutionary events in NPC architectures post-LECA. Principal architectural changes are named for each compartment. Principal evolutionary events are named and indicated. Paralogous duplication events indicated by a — a,b pairs with slight colour change. Gene loss — by dashed lines and letters. Gene gain — by a — c pairs and colour change. Changes in stoichiometry — shown schematically. Principal complex gains are named.

[141] and mechanical force [142]. Such flexibility is thought to depend on several structural characteristics of the NPC. Firstly, within the central channel allosteric coupling exists between structured and intrinsically disordered FG-Nup domains in interactions with transport factors [139]. Furthermore, the lateral spoke interconnections within the inner ring are small [10] and contributing to the stability of the inner ring are 'flexible connectors', intrinsically disordered sequences interconnect NPC subcomplexes. For example, in *S. cerevisiae* these connectors are present in Nic96 that bridges Nup192 and the FG-Nups of the Nsp1 complex. Furthermore, Nup145N/Nup100/Nup116 interconnects inner and outer rings [16]. Notably, these interactions can be allosterically coupled by transport factors: Nup53 interaction with Kap121 destabilises Nic96 and Nup157 binding [140], potentially contributing to loosening of the inner ring and pore dilation to allow transport of cargo otherwise excluded from the NPC. Conservation of these interaction sites and allosteric interactions between animals and fungi [16,140] suggest such mechanisms are intrinsic to NPC function.

The NPC during mitosis

Eukaryotes perform cell division by closed, open or semi-open mitosis [143]; in all cases, nucleoporins affect mitotic progression. Metazoans and higher plants (here represented by *H. Sapiens*, *A. thaliana* and *M. domestica*) undergo open mitosis that includes full breakdown of NE (NEBD) and NPC disassembly [143,144]. The remaining organisms, i.e. *C. reinhardtti*, *T. thermophila* and *T. brucei* perform close mitosis [145–147] which leaves the NE intact during cell division.

Although these models of nuclear division seem radically different, the mechanisms for disassembly of the NPC are strikingly similar, occurring in a highly synchronised manner. In animal cells, NPC disassembly occurs during NEBD and after phosphorylation of Nup98, while in *S. pombe* NPCs are gradually lost from the anaphase bridge connecting daughter nuclei. In open and closed division peripheral Nups are disassembled first, followed by the central scaffold and finally POMs [148–151]. If differences in NPC architecture influence the mitotic mode is unknown.

Moonlighting NPC components

Moonlighting, or multi-functionality, is common amongst NPC and NPC-associated components and inevitably impacts selection constraints. NPC components are involved in a variety of pathways, located in additional compartments as individual nucleoporins or entire NPC subcomplexes. Sec13 (Y-complex) is a COPII component [152] and, together with Seh1 (also Y-complex) [153], are part of the SEA vacuolar complex. Significantly Nup62 and Nup188 are at mammalian centrosomes [154,155], and the entire mammalian Y-complex at kinetochores and, together with Seh1, recruit the chromosomal passenger complex [156–160]. Multiple nucleoporins promote chromatin decondensation, transcriptional activation [161–166] or epigenetic silencing [163] via direct localisation in the nucleoplasm or tethering chromosomal regions to assembled NPCs [167–173]. Very little is known in terms of conservation of moonlighting functions, although it is clear that trypanosomes do have similar processes, albeit with likely distinct evolutionary origins, suggesting possible convergence.

One emerging example of NPC component moonlighting is the 'ciliary pore complex' [174]. NPC components can localise at the base of the cilia and in human cells, these are the cytoplasmic filament Nup214, inner ring Nup35, Nup62 and Nup93, the outer ring Nup37 [174] and Nup75/Nup85 [175]. Additionally, Nup93 and multi-ring Nup188 [176] and, potentially, Nup205 [177] are thought to localise to the cilia base in *Xenopus*. These structures appear to support embryonic cilia formation and intraflagellar transport (IFT). Additional similarities between NPC and IFT, such as the ciliary localisation signal (CLS) likeness to NLS [178] and the requirement of CLS-recognising nuclear transport factor importin-β2 and a RanGTP/GDP gradient for the ciliary transport of several proteins [179,180], have added to the concept of a ciliary pore complex [174]. However, structural details and ubiquity remain to emerge and imaging of Nup188 in *Xenopus* shows structures incompatible with typical NPC organisation [174]. Among FG-nups, only Nup98 was found at the cilia [175] and ciliary

Biochemical Society Transactions (2021) **49** 1601–1619
https://doi.org/10.1042/BST20200570

transport appears insensitive to classic inhibitors of the NPC barrier [181]. The similarity in form and function between the NPC and ciliary pore complex is, therefore, unclear; however, the constraints applied by potential co-evolution between the nuclear and ciliary transport are intriguing. Interestingly, there is further mutual moonlighting between the NPC and cilia as centrin2, critical for centriole/centrosome, and thus cilia organisation [182], is also present in animal and fungal NPCs, contributing to mRNA and protein export [183].

NPC evolution: a minimal pore?

The predictive and experimental structural analysis finds that the ∼30 proteins of the NPC core are composed predominantly of just eight structural fold types [25,26]. Of these, the three most frequent, α-solenoid, FG-repeat and β-propeller, account for >80% of residues. Notably, while sequence similarity is generally low, comparative genomics has identified over 20 nucleoporins from all three groups as conserved across all eukaryotic taxa [36] and thus likely represented in the LECA. Assuming that the NPC evolved incrementally with increased complexity, what forms did the NPC take during the transition from the FECA to the LECA (Figure 4A)? Phylogenetic and genetic analysis may be currently insufficient to resolve this question, but insights from other studies are valuable here.

Firstly, nucleomorphs, drastically reduced nuclei from red and green algae endosymbiotically absorbed into chlorarachniophytes and cryptophyceans [125], reveal a near-complete secondary reduction in algae NPC genes. The only NPC proteins that could participate in pore formation [184] are orthologs of Nup98 (in chlorarachniophytes) and Rae1 (in chlorarachniophytes and cryptophyceans) (Figure 2). However, there is no ultrastructural evidence for a pore-associated structure at the nucleomorph membrane and the retention of these subunits may indicate recruitment to other processes. Secondly, attempts to create an artificial pore, using truncated versions of yeast Nsp1 and Nup100 [185] or a designer 'NupX' [186], produced pores with selective permeability, suggesting minimal requirements to achieve gating. A self-assembling pore of just two POMs, Ndc1 and an FG-containing Pom121 in a lipid bilayer has also been achieved [187]. While these artificial or derived states almost definitely do not recapitulate the NPC during the FECA to LECA transition, they do indicate considerable potential for flexibility and great simplicity in mediating selective nucleocytoplasmic transport.

Perspectives

- There are examples of clear divergence in NPC structure and likely functions across eukaryotes; more examples are needed to understand the basis for these changes.
- Mapping structure to function remains a major goal for NPC research, but understanding diversity and how these connect to biological aspects are also critical.
- Gaining deeper insight in the organisation of nuclear pores in nucleomorphs and into pore formation by POMs may aid reconstructions of the origins of permeability-selective NPCs.
- There is an urgent need to characterise mRNA export platforms (TREX, Mex67) and the manner in which these interface with the NPC, especially in non-canonical organisms.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contributions

A.A.M. and N.E.P.-M.; investigation and preparation of manuscript and figures. A.A.M., N.E.P.-M. and M.C.F.; writing, review and editing; M.C.F.; conceptualisation and supervision.

Acknowledgements

Work in our laboratory is supported by the Wellcome Trust (204697/Z/16/Z to M.C.F.).

Abbreviations

CF, cytoplasmic filament; IFT, intraflagellar transport; LRR, leucine-rich repeat; MIC, micronucleus; MMB, membrane-binding domain; MR, membrane ring; NB, nuclear basket; NPC, nuclear pore complex; NTF2L, nuclear transport factor 2-like; POMs, pore membrane proteins; RRM, RNA-recognition motif.

References

1 Fernandez-Martinez, J. and Rout, M.P. (2021) One ring to rule them all? Structural and functional diversity in the nuclear pore complex. *Trends Biochem. Sci.* **46**, 595–607. https://doi.org/10.1016/j.tibs.2021.01.003
2 Field, M.C. and Rout, M.P. (2019) Pore timing: the evolutionary origins of the nucleus and nuclear pore complex. *F1000Res* **8**, F1000 Faculty Rev-369 https://doi.org/10.12688/f1000research.16402.1
3 Dacks, J.B. and Robinson, M.S. (2017) Outerwear through the ages: evolutionary cell biology of vesicle coats. *Curr. Opin. Cell Biol.* **47**, 108–116 https://doi.org/10.1016/j.ceb.2017.04.001
4 Faini, M., Beck, R., Wieland, F.T. and Briggs, J.A. (2013) Vesicle coats: structure, function, and general principles of assembly. *Trends Cell Biol.* **23**, 279–288 https://doi.org/10.1016/j.tcb.2013.01.005
5 Ori, A., Banterle, N., Iskar, M., Andres-Pons, A., Escher, C., Khanh Bui, H. et al. (2013) Cell type-specific nuclear pores: a case in point for context-dependent stoichiometry of molecular machines. *Mol. Syst. Biol.* **9**, 648 https://doi.org/10.1038/msb.2013.4
6 Bui, K.H., von Appen, A., DiGuilio, A.L., Ori, A., Sparks, L., Mackmull, M.T. et al. (2013) Integrated structural analysis of the human nuclear pore complex scaffold. *Cell* **155**, 1233–1243 https://doi.org/10.1016/j.cell.2013.10.055
7 von Appen, A., Kosinski, J., Sparks, L., Ori, A., DiGuilio, A.L., Vollmer, B. et al. (2015) In situ structural analysis of the human nuclear pore complex. *Nature* **526**, 526140–526143 https://doi.org/10.1038/nature15381
8 Kosinski, J., Mosalaganti, S., von Appen, A., Teimer, R., DiGuilio, A.L., Wan, W. et al. (2016) Molecular architecture of the inner ring scaffold of the human nuclear pore complex. *Science* **352**, 363–365 https://doi.org/10.1126/science.aaf0643
9 Alber, F., Dokudovskaya, S., Veenhoff, L.M., Zhang, W., Kipper, J., Devos, D. et al. (2007) The molecular architecture of the nuclear pore complex. *Nature* **450**, 695–701 https://doi.org/10.1038/nature06405
10 Kim, S.J., Fernandez-Martinez, J., Nudelman, I., Shi, Y., Zhang, W., Raveh, B. et al. (2018) Integrative structure and functional anatomy of a nuclear pore complex. *Nature* **555**, 475–482 https://doi.org/10.1038/nature26003
11 Kim, S.J., Fernandez-Martinez, J., Sampathkumar, P., Martel, A., Matsui, T., Tsuruta, H. et al. (2014) Integrative structure-function mapping of the nucleoporin Nup133 suggests a conserved mechanism for membrane anchoring of the nuclear pore complex. *Mol. Cell. Proteom.* **13**, 2911–2926 https://doi.org/10.1074/mcp.M114.040915
12 Asakawa, H., Yang, H.J., Yamamoto, T.G., Ohtsuki, C., Chikashige, Y., Sakata-Sogawa, K. et al. (2014) Characterization of nuclear pore complex components in fission yeast *Schizosaccharomyces pombe*. *Nucleus* **5**, 149–162 https://doi.org/10.4161/nucl.28487
13 Asakawa, H., Kojidani, T., Yang, H.J., Ohtsuki, C., Osakada, H., Matsuda, A. et al. (2019) Asymmetrical localization of Nup107-160 subcomplex components within the nuclear pore complex in fission yeast. *PLoS Genet.* **15**, e1008061 https://doi.org/10.1371/journal.pgen.1008061
14 Amlacher, S., Sarges, P., Flemming, D., van Noort, V., Kunze, R., Devos, D.P. et al. (2011) Insight into structure and assembly of the nuclear pore complex by utilizing the genome of a eukaryotic thermophile. *Cell* **146**, 277–289 https://doi.org/10.1016/j.cell.2011.06.039
15 Thierbach, K., von Appen, A., Thoms, M., Beck, M., Flemming, D. and Hurt, E. (2013) Protein interfaces of the conserved Nup84 complex from *Chaetomium thermophilum* shown by crosslinking mass spectrometry and electron microscopy. *Structure* **21**, 1672–1682 https://doi.org/10.1016/j.str.2013.07.004
16 Fischer, J., Teimer, R., Amlacher, S., Kunze, R. and Hurt, E. (2015) Linker Nups connect the nuclear pore complex inner ring with the outer ring and transport channel. *Nat. Struct. Mol. Biol.* **22**, 774–781 https://doi.org/10.1038/nsmb.3084
17 Mosalaganti, S., Kosinski, J., Albert, S., Schaffer, M., Strenkert, D., Salome, P.A. et al. (2018) In situ architecture of the algal nuclear pore complex. *Nat. Commun.* **9**, 2361 https://doi.org/10.1038/s41467-018-04739-y
18 Tamura, K., Fukao, Y., Iwamoto, M., Haraguchi, T. and Hara-Nishimura, I. (2010) Identification and characterization of nuclear pore complex components in *Arabidopsis thaliana*. *Plant Cel* **22**, 4084–4097 https://doi.org/10.1105/tpc.110.079947
19 Zhang, C., An, N., Jia, P., Zhang, W., Liang, J., Zhang, X. et al. (2020) Genomic identification and expression analysis of nuclear pore proteins in *Malus domestica*. *Sci. Rep.* **10**, 17426 https://doi.org/10.1038/s41598-020-74171-0
20 Iwamoto, M., Osakada, H., Mori, C., Fukuda, Y., Nagao, K., Obuse, C. et al. (2017) Compositionally distinct nuclear pore complexes of functionally distinct dimorphic nuclei in the ciliate Tetrahymena. *J. Cell Sci.* **130**, 1822–1834 https://doi.org/10.1242/jcs.199398
21 Obado, S.O., Brillantes, M., Uryu, K., Zhang, W., Ketaren, N.E., Chait, B.T. et al. (2016) Interactome mapping reveals the evolutionary history of the nuclear pore complex. *PLoS Biol.* **14**, e1002365 https://doi.org/10.1371/journal.pbio.1002365
22 Drin, G., Casella, J.F., Gautier, R., Boehmer, T., Schwartz, T.U. and Antonny, B. (2007) A general amphipathic alpha-helical motif for sensing membrane curvature. *Nat. Struct. Mol. Biol.* **14**, 138–146 https://doi.org/10.1038/nsmb1194
23 Shi, Y., Fernandez-Martinez, J., Tjioe, E., Pellarin, R., Kim, S.J., Williams, R. et al. (2014) Structural characterization by cross-linking reveals the detailed architecture of a coatomer-related heptameric module from the nuclear pore complex. *Mol. Cell. Proteom.* **13**, 2927–2943 https://doi.org/10.1074/mcp.M114.041673
24 Nordeen, S.A., Turman, D.L. and Schwartz, T.U. (2020) Yeast Nup84-Nup133 complex structure details flexibility and reveals conservation of the membrane anchoring ALPS motif. *Nat. Commun.* **11**, 6060 https://doi.org/10.1038/s41467-020-19885-5
25 Devos, D., Dokudovskaya, S., Alber, F., Williams, R., Chait, B.T., Sali, A. et al. (2004) Components of coated vesicles and nuclear pore complexes share a common molecular architecture. *PLoS Biol.* **2**, e380 https://doi.org/10.1371/journal.pbio.0020380

26 Devos, D., Dokudovskaya, S., Williams, R., Alber, F., Eswar, N., Chait, B.T. et al. (2006) Simple fold composition and modular architecture of the nuclear pore complex. *Proc. Natl Acad. Sci. U.S.A.* **103**, 2172–2177 https://doi.org/10.1073/pnas.0506345103
27 DeGrasse, J.A., DuBois, K.N., Devos, D., Siegel, T.N., Sali, A., Field, M.C. et al. (2009) Evidence for a shared nuclear pore complex architecture that is conserved from the last common eukaryotic ancestor. *Mol. Cell. Proteom.* **8**, 2119–2130 https://doi.org/10.1074/mcp.M900038-MCP200
28 Lutzmann, M., Kunze, R., Buerer, A., Aebi, U. and Hurt, E. (2002) Modular self-assembly of a Y-shaped multiprotein complex from seven nucleoporins. *EMBO J.* **21**, 387–397 https://doi.org/10.1093/emboj/21.3.387
29 Huang, G., Zhang, Y., Zhu, X., Zeng, C., Wang, Q., Zhou, Q. et al. (2020) Structure of the cytoplasmic ring of the *Xenopus laevis* nuclear pore complex by cryo-electron microscopy single particle analysis. *Cell Res.* **30**, 520–531 https://doi.org/10.1038/s41422-020-0319-4
30 Kimura, N., Takizawa, M., Okita, K., Natori, O., Igarashi, K., Ueno, M. et al. (2002) Identification of a novel transcription factor, ELYS, expressed predominantly in mouse foetal haematopoietic tissues. *Genes Cells* **7**, 435–446 https://doi.org/10.1046/j.1365-2443.2002.00529.x
31 Rasala, B.A., Orjalo, A.V., Shen, Z., Briggs, S. and Forbes, D.J. (2006) ELYS is a dual nucleoporin/kinetochore protein required for nuclear pore assembly and proper cell division. *Proc. Natl Acad. Sci. U.S.A.* **103**, 17801–17806 https://doi.org/10.1073/pnas.0608484103
32 Rasala, B.A., Ramos, C., Harel, A. and Forbes, D.J. (2008) Capture of AT-rich chromatin by ELYS recruits POM121 and NDC1 to initiate nuclear pore assembly. *Mol. Biol. Cell* **19**, 3982–3996 https://doi.org/10.1091/mbc.e08-01-0012
33 Padilla-Mejia, N.E., Makarov, A.A., Barlow, L.D., Butterfield, E.R. and Field, M.C. (2021) Evolution and diversification of the nuclear envelope. *Nucleus* **12**, 21–41 https://doi.org/10.1080/19491034.2021.1874135
34 Koreny, L. and Field, M.C. (2016) Ancient eukaryotic origin and evolutionary plasticity of nuclear lamina. *Genome Biol. Evol.* **8**, 2663–2671 https://doi.org/10.1093/gbe/evw087
35 Akey, C.W. and Radermacher, M. (1993) Architecture of the Xenopus nuclear pore complex revealed by three-dimensional cryo-electron microscopy. *J. Cell Biol.* **122**, 1–19 https://doi.org/10.1083/jcb.122.1.1
36 Neumann, N., Lundin, D. and Poole, A.M. (2010) Comparative genomic evidence for a complete nuclear pore complex in the last eukaryotic common ancestor. *PLoS One* **5**, e13241 https://doi.org/10.1371/journal.pone.0013241
37 Upla, P., Kim, S.J., Sampathkumar, P., Dutta, K., Cahill, S.M., Chemmama, I.E. et al. (2017) Molecular architecture of the major membrane ring component of the nuclear pore complex. *Structure* **25**, 434–445 https://doi.org/10.1016/j.str.2017.01.006
38 Hao, Q., Zhang, B., Yuan, K., Shi, H. and Blobel, G. (2018) Electron microscopy of Chaetomium pom152 shows the assembly of ten-bead string. *Cell Discov.* **4**, 56 https://doi.org/10.1038/s41421-018-0057-7
39 Zhang, Y., Li, S., Zeng, C., Huang, G., Zhu, X., Wang, Q. et al. (2020) Molecular architecture of the luminal ring of the *Xenopus laevis* nuclear pore complex. *Cell Res.* **30**, 532–540 https://doi.org/10.1038/s41422-020-0320-y
40 Wozniak, R.W., Blobel, G. and Rout, M.P. (1994) POM152 is an integral protein of the pore membrane domain of the yeast nuclear envelope. *J. Cell Biol.* **125**, 31–42 https://doi.org/10.1083/jcb.125.1.31
41 Eriksson, C., Rustum, C. and Hallberg, E. (2004) Dynamic properties of nuclear pore complex proteins in gp210 deficient cells. *FEBS Lett.* **572**, 261–265 https://doi.org/10.1016/j.febslet.2004.07.044
42 Olsson, M., Scheele, S. and Ekblom, P. (2004) Limited expression of nuclear pore membrane glycoprotein 210 in cell lines and tissues suggests cell-type specific nuclear pores in metazoans. *Exp. Cell Res.* **292**, 359–370 https://doi.org/10.1016/j.yexcr.2003.09.014
43 Cohen, M., Feinstein, N., Wilson, K.L. and Gruenbaum, Y. (2003) Nuclear pore protein gp210 is essential for viability in HeLa cells and *Caenorhabditis elegans*. *Mol. Biol. Cell* **14**, 4230–4237 https://doi.org/10.1091/mbc.e03-04-0260
44 Stavru, F., Nautrup-Pedersen, G., Cordes, V.C. and Gorlich, D. (2006) Nuclear pore complex assembly and maintenance in POM121- and gp210-deficient cells. *J. Cell Biol.* **173**, 477–483 https://doi.org/10.1083/jcb.200601002
45 Antonin, W., Franz, C., Haselmann, U., Antony, C. and Mattaj, I.W. (2005) The integral membrane nucleoporin pom121 functionally links nuclear pore complex assembly and nuclear envelope formation. *Mol. Cell* **17**, 83–92 https://doi.org/10.1016/j.molcel.2004.12.010
46 Winey, M., Hoyt, M.A., Chan, C., Goetsch, L., Botstein, D. and Byers, B. (1993) NDC1: a nuclear periphery component required for yeast spindle pole body duplication. *J. Cell Biol.* **122**, 743–751 https://doi.org/10.1083/jcb.122.4.743
47 West, R.R., Vaisberg, E.V., Ding, R., Nurse, P. and McIntosh, J.R. (1998) Cut11(+): a gene required for cell cycle-dependent spindle pole body anchoring in the nuclear envelope and bipolar spindle formation in *Schizosaccharomyces pombe*. *Mol. Biol. Cell* **9**, 2839–2855 https://doi.org/10.1091/mbc.9.10.2839
48 Eisenhardt, N., Redolfi, J. and Antonin, W. (2014) Interaction of Nup53 with Ndc1 and Nup155 is required for nuclear pore complex assembly. *J. Cell Sci.* **127**, 908–921 https://doi.org/10.1242/jcs.141739
49 Stavru, F., Hulsmann, B.B., Spang, A., Hartmann, E., Cordes, V.C. and Gorlich, D. (2006) NDC1: a crucial membrane-integral nucleoporin of metazoan nuclear pore complexes. *J. Cell Biol.* **173**, 509–519 https://doi.org/10.1083/jcb.200601001
50 Frosst, P., Guan, T., Subauste, C., Hahn, K. and Gerace, L. (2002) Tpr is localized within the nuclear basket of the pore complex and has a role in nuclear protein export. *J. Cell Biol.* **156**, 617–630 https://doi.org/10.1083/jcb.200106046
51 Ashkenazy-Titelman, A., Shav-Tal, Y. and Kehlenbach, R.H. (2020) Into the basket and beyond: the journey of mRNA through the nuclear pore complex. *Biochem. J.* **477**, 23–44 https://doi.org/10.1042/BCJ20190132
52 Strambio-de-Castillia, C., Blobel, G. and Rout, M.P. (1999) Proteins connecting the nuclear pore complex with the nuclear interior. *J. Cell Biol.* **144**, 839–855 https://doi.org/10.1083/jcb.144.5.839
53 Kosova, B., Pante, N., Rollenhagen, C., Podtelejnikov, A., Mann, M., Aebi, U. et al. (2000) Mlp2p, a component of nuclear pore attached intranuclear filaments, associates with nic96p. *J. Biol. Chem.* **275**, 343–350 https://doi.org/10.1074/jbc.275.1.343
54 Niepel, M., Molloy, K.R., Williams, R., Farr, J.C., Meinema, A.C., Vecchietti, N. et al. (2013) The nuclear basket proteins Mlp1p and Mlp2p are part of a dynamic interactome including Esc1p and the proteasome. *Mol. Biol. Cell* **24**, 3920–3938 https://doi.org/10.1091/mbc.e13-07-0412
55 Makise, M., Mackay, D.R., Elgort, S., Shankaran, S.S., Adam, S.A. and Ullman, K.S. (2012) The Nup153-Nup50 protein interface and its role in nuclear import. *J. Biol. Chem.* **287**, 38515–38522 https://doi.org/10.1074/jbc.M112.378893
56 Duheron, V., Chatel, G., Sauder, U., Oliveri, V. and Fahrenkrog, B. (2014) Structural characterization of altered nucleoporin Nup153 expression in human cells by thin-section electron microscopy. *Nucleus* **5**, 601–612 https://doi.org/10.4161/19491034.2014.990853

57 Lin, D.H. and Hoelz, A. (2019) The structure of the nuclear pore complex (an update). *Annu. Rev. Biochem.* **88**, 725–783 https://doi.org/10.1146/annurev-biochem-062917-011901
58 Krull, S., Thyberg, J., Bjorkroth, B., Rackwitz, H.R. and Cordes, V.C. (2004) Nucleoporins as components of the nuclear pore complex core structure and Tpr as the architectural element of the nuclear basket. *Mol. Biol. Cell* **15**, 4261–4277 https://doi.org/10.1091/mbc.e04-03-0165
59 Ball, J.R., Dimaano, C., Bilak, A., Kurchan, E., Zundel, M.T. and Ullman, K.S. (2007) Sequence preference in RNA recognition by the nucleoporin Nup153. *J. Biol. Chem.* **282**, 8734–8740 https://doi.org/10.1074/jbc.M608477200
60 Soop, T., Ivarsson, B., Bjorkroth, B., Fomproix, N., Masich, S., Cordes, V.C. et al. (2005) Nup153 affects entry of messenger and ribosomal ribonucleoproteins into the nuclear basket during export. *Mol. Biol. Cell* **16**, 5610–5620 https://doi.org/10.1091/mbc.e05-08-0715
61 Umlauf, D., Bonnet, J., Waharte, F., Fournier, M., Stierle, M., Fischer, B. et al. (2013) The human TREX-2 complex is stably associated with the nuclear pore basket. *J. Cell Sci.* **126**(Pt 12), 2656–2667 https://doi.org/10.1242/jcs.118000
62 Walther, T.C., Fornerod, M., Pickersgill, H., Goldberg, M., Allen, T.D. and Mattaj, I.W. (2001) The nucleoporin Nup153 is required for nuclear pore basket formation, nuclear pore complex anchoring and import of a subset of nuclear proteins. *EMBO J.* **20**, 5703–5714 https://doi.org/10.1093/emboj/20.20.5703
63 Aksenova, V., Smith, A., Lee, H., Bhat, P., Esnault, C., Chen, S. et al. (2020) Nucleoporin TPR is an integral component of the TREX-2 mRNA export pathway. *Nat. Commun.* **11**, 4577 https://doi.org/10.1038/s41467-020-18266-2
64 Zhang, B., You, C., Zhang, Y., Zeng, L., Hu, J., Zhao, M. et al. (2020) Linking key steps of microRNA biogenesis by TREX-2 and the nuclear pore complex in Arabidopsis. *Nat. Plants* **6**, 957–969 https://doi.org/10.1038/s41477-020-0726-z
65 Jacob, Y., Mongkolsiriwatana, C., Veley, K.M., Kim, S.Y. and Michaels, S.D. (2007) The nuclear pore protein AtTPR is required for RNA homeostasis, flowering time, and auxin signaling. *Plant Physiol.* **144**, 1383–1390 https://doi.org/10.1104/pp.107.100735
66 Xu, X.M., Rose, A., Muthuswamy, S., Jeong, S.Y., Venkatakrishnan, S., Zhao, Q. et al. (2007) NUCLEAR PORE ANCHOR, the Arabidopsis homolog of Tpr/Mlp1/Mlp2/megator, is involved in mRNA export and SUMO homeostasis and affects diverse aspects of plant development. *Plant Cell* **19**, 1537–1548 https://doi.org/10.1105/tpc.106.049239
67 Lu, Q., Tang, X., Tian, G., Wang, F., Liu, K., Nguyen, V. et al. (2010) Arabidopsis homolog of the yeast TREX-2 mRNA export complex: components and anchoring nucleoporin. *Plant J.* **61**, 259–270 https://doi.org/10.1111/j.1365-313X.2009.04048.x
68 Lee, E.S., Wolf, E.J., Ihn, S.S.J., Smith, H.W., Emili, A. and Palazzo, A.F. (2020) TPR is required for the efficient nuclear export of mRNAs and lncRNAs from short and intron-poor genes. *Nucleic Acids Res.* **48**, 11645–11663 https://doi.org/10.1093/nar/gkaa919
69 Galy, V., Gadal, O., Fromont-Racine, M., Romano, A., Jacquier, A. and Nehrbass, U. (2004) Nuclear retention of unspliced mRNAs in yeast is mediated by perinuclear Mlp1. *Cell* **116**, 63–73 https://doi.org/10.1016/S0092-8674(03)01026-2
70 Bi, X., Cheng, Y.J., Hu, B., Ma, X., Wu, R., Wang, J.W. et al. (2017) Nonrandom domain organization of the Arabidopsis genome at the nuclear periphery. *Genome Res.* **27**, 1162–1173 https://doi.org/10.1101/gr.215186.116
71 Holden, J.M., Koreny, L., Obado, S., Ratushny, A.V., Chen, W.M., Chiang, J.H. et al. (2014) Nuclear pore complex evolution: a trypanosome Mlp analogue functions in chromosomal segregation but lacks transcriptional barrier activity. *Mol. Biol. Cell* **25**, 1421–1436 https://doi.org/10.1091/mbc.e13-12-0750
72 Katahira, J., Strasser, K., Podtelejnikov, A., Mann, M., Jung, J.U. and Hurt, E. (1999) The Mex67p-mediated nuclear mRNA export pathway is conserved from yeast to human. *EMBO J.* **18**, 2593–2609 https://doi.org/10.1093/emboj/18.9.2593
73 Chen, S., Wang, R., Zheng, D., Zhang, H., Chang, X., Wang, K. et al. (2019) The mRNA export receptor NXF1 coordinates transcriptional dynamics, alternative polyadenylation, and mrna export. *Mol. Cell* **74**, 118–131.e7 https://doi.org/10.1016/j.molcel.2019.01.026
74 Aibara, S., Katahira, J., Valkov, E. and Stewart, M. (2015) The principal mRNA nuclear export factor NXF1:NXT1 forms a symmetric binding platform that facilitates export of retroviral CTE-RNA. *Nucleic Acids Res.* **43**, 1883–1893 https://doi.org/10.1093/nar/gkv032
75 Aibara, S., Valkov, E., Lamers, M.H., Dimitrova, L., Hurt, E. and Stewart, M. (2015) Structural characterization of the principal mRNA-export factor Mex67-Mtr2 from *Chaetomium thermophilum*. *Acta Crystallogr. F Struct. Biol. Commun.* **71**(Pt 7), 876–888 https://doi.org/10.1107/S2053230X15008766
76 Ben-Yishay, R., Mor, A., Shraga, A., Ashkenazy-Titelman, A., Kinor, N., Schwed-Gross, A. et al. (2019) Imaging within single NPCs reveals NXF1's role in mRNA export on the cytoplasmic side of the pore. *J. Cell Biol.* **218**, 2962–2981 https://doi.org/10.1083/jcb.201901127
77 Derrer, C.P., Mancini, R., Vallotton, P., Huet, S., Weis, K. and Dultz, E. (2019) The RNA export factor Mex67 functions as a mobile nucleoporin. *J. Cell Biol.* **218**, 3967–3976 https://doi.org/10.1083/jcb.201909028
78 Stewart, M. (2010) Nuclear export of mRNA. *Trends Biochem. Sci.* **35**, 609–617 https://doi.org/10.1016/j.tibs.2010.07.001
79 Li, Y., Bor, Y.C., Fitzgerald, M.P., Lee, K.S., Rekosh, D. and Hammarskjold, M.L. (2016) An NXF1 mRNA with a retained intron is expressed in hippocampal and neocortical neurons and is translated into a protein that functions as an Nxf1 cofactor. *Mol. Biol. Cell* **27**, 3903–3912 https://doi.org/10.1091/mbc.E16-07-0515
80 Eyboulet, F., Jeronimo, C., Cote, J. and Robert, F. (2020) The deubiquitylase Ubp15 couples transcription to mRNA export. *eLife* **9**, e61264 https://doi.org/10.7554/eLife.61264
81 Aibara, S., Valkov, E., Lamers, M. and Stewart, M. (2015) Domain organization within the nuclear export factor Mex67:Mtr2 generates an extended mRNA binding surface. *Nucleic Acids Res.* **43**, 1927–1936 https://doi.org/10.1093/nar/gkv030
82 Viphakone, N., Hautbergue, G.M., Walsh, M., Chang, C.T., Holland, A., Folco, E.G. et al. (2012) TREX exposes the RNA-binding domain of Nxf1 to enable mRNA export. *Nat. Commun.* **3**, 1006 https://doi.org/10.1038/ncomms2005
83 Huang, Y., Gattoni, R., Stevenin, J. and Steitz, J.A. (2003) SR splicing factors serve as adapter proteins for TAP-dependent mRNA export. *Mol. Cell* **11**, 837–843 https://doi.org/10.1016/S1097-2765(03)00089-3
84 Muller-McNicoll, M., Botti, V., de Jesus Domingues, A.M., Brandl, H., Schwich, O.D., Steiner, M.C. et al. (2016) SR proteins are NXF1 adaptors that link alternative RNA processing to mRNA export. *Genes Dev.* **30**, 553–566 https://doi.org/10.1101/gad.276477.115
85 Fribourg, S., Braun, I.C., Izaurralde, E. and Conti, E. (2001) Structural basis for the recognition of a nucleoporin FG repeat by the NTF2-like domain of the TAP/p15 mRNA nuclear export factor. *Mol. Cell* **8**, 645–656 https://doi.org/10.1016/S1097-2765(01)00348-3
86 Kramer, S., Kimblin, N.C. and Carrington, M. (2010) Genome-wide in silico screen for CCCH-type zinc finger proteins of *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*. *BMC Genomics* **11**, 283 https://doi.org/10.1186/1471-2164-11-283

87 Dean, S., Sunter, J.D. and Wheeler, R.J. (2017) Tryptag.org: a trypanosome genome-wide protein localisation resource. *Trends Parasitol.* **33**, 80–82 https://doi.org/10.1016/j.pt.2016.10.009
88 Aslett, M., Aurrecoechea, C., Berriman, M., Brestelli, J., Brunk, B.P., Carrington, M. et al. (2010) TriTrypDB: a functional genomic resource for the Trypanosomatidae. *Nucleic Acids Res.* **38**, D457–D462 https://doi.org/10.1093/nar/gkp851
89 Dostalova, A., Kaser, S., Cristodero, M. and Schimanski, B. (2013) The nuclear mRNA export receptor Mex67-Mtr2 of *Trypanosoma brucei* contains a unique and essential zinc finger motif. *Mol. Microbiol.* **88**, 728–739 https://doi.org/10.1111/mmi.12217
90 Schwede, A., Manful, T., Jha, B.A., Helbig, C., Bercovich, N., Stewart, M. et al. (2009) The role of deadenylation in the degradation of unstable mRNAs in trypanosomes. *Nucleic Acids Res.* **37**, 5511–5528 https://doi.org/10.1093/nar/gkp571
91 Rink, C. and Williams, N. (2019) Unique interactions of the nuclear export receptors TbMex67 and TbMtr2 with components of the 5S ribonuclear particle in *Trypanosoma brucei*. *mSphere* **4**, e00471-19 https://doi.org/10.1128/mSphere.00471-19
92 Gissot, M., Hovasse, A., Chaloin, L., Schaeffer-Reiss, C., Van Dorsselaer, A. and Tomavo, S. (2017) An evolutionary conserved zinc finger protein is involved in *Toxoplasma gondii* mRNA nuclear export. *Cell Microbiol.* **19**, e12644 https://doi.org/10.1111/cmi.12644
93 Stewart, M. (2019) Structure and function of the TREX-2 complex. *Subcell Biochem.* **93**, 461–470 https://doi.org/10.1007/978-3-030-28151-9_15
94 Jani, D., Lutz, S., Hurt, E., Laskey, R.A., Stewart, M. and Wickramasinghe, V.O. (2012) Functional and structural characterization of the mammalian TREX-2 complex that links transcription with nuclear messenger RNA export. *Nucleic Acids Res.* **40**, 4562–4573 https://doi.org/10.1093/nar/gks059
95 Dimitrova, L., Valkov, E., Aibara, S., Flemming, D., McLaughlin, S.H., Hurt, E. et al. (2015) Structural characterization of the *Chaetomium thermophilum* TREX-2 complex and its interaction with the mRNA nuclear export factor Mex67:Mtr2. *Structure* **23**, 1246–1257 https://doi.org/10.1016/j.str.2015.05.002
96 Jani, D., Valkov, E. and Stewart, M. (2014) Structural basis for binding the TREX2 complex to nuclear pores, GAL1 localisation and mRNA export. *Nucleic Acids Res.* **42**, 6686–6697 https://doi.org/10.1093/nar/gku252
97 Ellisdon, A.M., Dimitrova, L., Hurt, E. and Stewart, M. (2012) Structural basis for the assembly and nucleic acid binding of the TREX-2 transcription-export complex. *Nat. Struct. Mol. Biol.* **19**, 328–336 https://doi.org/10.1038/nsmb.2235
98 Gordon, J.M.B., Aibara, S. and Stewart, M. (2017) Structure of the Sac3 RNA-binding M-region in the *Saccharomyces cerevisiae* TREX-2 complex. *Nucleic Acids Res.* **45**, 5577–5585 https://doi.org/10.1093/nar/gkx158
99 Jani, D., Lutz, S., Marshall, N.J., Fischer, T., Kohler, A., Ellisdon, A.M. et al. (2009) Sus1, Cdc31, and the Sac3 CID region form a conserved interaction platform that promotes nuclear pore association and mRNA export. *Mol. Cell* **33**, 727–737 https://doi.org/10.1016/j.molcel.2009.01.033
100 Sorensen, B.B., Ehrnsberger, H.F., Esposito, S., Pfab, A., Bruckmann, A., Hauptmann, J. et al. (2017) The Arabidopsis THO/TREX component TEX1 functionally interacts with MOS11 and modulates mRNA export and alternative splicing events. *Plant Mol. Biol.* **93**, 283–298 https://doi.org/10.1007/s11103-016-0561-9
101 Pfab, A., Bruckmann, A., Nazet, J., Merkl, R. and Grasser, K.D. (2018) The adaptor protein ENY2 is a component of the deubiquitination module of the Arabidopsis SAGA transcriptional co-activator complex but not of the TREX-2 complex. *J. Mol. Biol.* **430**, 1479–1494 https://doi.org/10.1016/j.jmb.2018.03.018
102 Yang, Y., La, H., Tang, K., Miki, D., Yang, L., Wang, B. et al. (2017) SAC3B, a central component of the mRNA export complex TREX-2, is required for prevention of epigenetic gene silencing in Arabidopsis. *Nucleic Acids Res.* **45**, 181–197 https://doi.org/10.1093/nar/gkw850
103 Avila, A.R., Cabezas-Cruz, A. and Gissot, M. (2018) mRNA export in the apicomplexan parasite *Toxoplasma gondii*: emerging divergent components of a crucial pathway. *Parasit Vectors* **11**, 62 https://doi.org/10.1186/s13071-018-2648-4
104 Meinel, D.M., Burkert-Kautzsch, C., Kieser, A., O'Duibhir, E., Siebert, M., Mayer, A. et al. (2013) Recruitment of TREX to the transcription machinery by its direct binding to the phospho-CTD of RNA polymerase II. *PLoS Genet.* **9**, e1003914 https://doi.org/10.1371/journal.pgen.1003914
105 Puhringer, T., Hohmann, U., Fin, L., Pacheco-Fiallos, B., Schellhaas, U., Brennecke, J. et al. (2020) Structure of the human core transcription-export complex reveals a hub for multivalent interactions. *eLife* **9**, e61503 https://doi.org/10.7554/eLife.61503
106 Portman, D.S., O'Connor, J.P. and Dreyfuss, G. (1997) YRA1, an essential *Saccharomyces cerevisiae* gene, encodes a novel nuclear protein with RNA annealing activity. *RNA* **3**, 527–537 PMID: 9149233
107 Longman, D., Johnstone, I.L. and Caceres, J.F. (2003) The Ref/Aly proteins are dispensable for mRNA export and development in *Caenorhabditis elegans*. *RNA* **9**, 881–891 https://doi.org/10.1261/rna.5420503
108 Gatfield, D. and Izaurralde, E. (2002) REF1/Aly and the additional exon junction complex proteins are dispensable for nuclear mRNA export. *J. Cell Biol.* **159**, 579–588 https://doi.org/10.1083/jcb.200207128
109 Kammel, C., Thomaier, M., Sorensen, B.B., Schubert, T., Langst, G., Grasser, M. et al. (2013) Arabidopsis DEAD-box RNA helicase UAP56 interacts with both RNA and DNA as well as with mRNA export factors. *PLoS One* **8**, e60644 https://doi.org/10.1371/journal.pone.0060644
110 Yelina, N.E., Smith, L.M., Jones, A.M., Patel, K., Kelly, K.A. and Baulcombe, D.C. (2010) Putative Arabidopsis THO/TREX mRNA export complex is involved in transgene and endogenous siRNA biosynthesis. *Proc. Natl Acad. Sci. U.S.A.* **107**, 13948–13953 https://doi.org/10.1073/pnas.0911341107
111 Pfaff, C., Ehrnsberger, H.F., Flores-Tornero, M., Sorensen, B.B., Schubert, T., Langst, G. et al. (2018) ALY RNA-binding proteins are required for nucleocytosolic mRNA transport and modulate plant growth and development. *Plant Physiol.* **177**, 226–240 https://doi.org/10.1104/pp.18.00173
112 Serpeloni, M., Moraes, C.B., Muniz, J.R., Motta, M.C., Ramos, A.S., Kessler, R.L. et al. (2011) An essential nuclear protein in trypanosomes is a component of mRNA transcription/export pathway. *PLoS One* **6**, e20730 https://doi.org/10.1371/journal.pone.0020730
113 Serpeloni, M., Jimenez-Ruiz, E., Vidal, N.M., Kroeber, C., Andenmatten, N., Lemgruber, L. et al. (2016) UAP56 is a conserved crucial component of a divergent mRNA export pathway in *Toxoplasma gondii*. *Mol. Microbiol.* **102**, 672–689 https://doi.org/10.1111/mmi.13485
114 Serpeloni, M., Vidal, N.M., Goldenberg, S., Avila, A.R. and Hoffmann, F.G. (2011) Comparative genomics of proteins involved in RNA nucleocytoplasmic export. *BMC Evol. Biol.* **11**, 7 https://doi.org/10.1186/1471-2148-11-7
115 Rajakyla, E.K., Viita, T., Kyheroinen, S., Huet, G., Treisman, R. and Vartiainen, M.K. (2015) RNA export factor Ddx19 is required for nuclear import of the SRF coactivator MKL1. *Nat. Commun.* **6**, 5978 https://doi.org/10.1038/ncomms6978
116 Kaminski, T., Siebrasse, J.P. and Kubitscheck, U. (2013) A single molecule view on Dbp5 and mRNA at the nuclear pore. *Nucleus* **4**, 8–13 https://doi.org/10.4161/nucl.23386
117 Folkmann, A.W., Noble, K.N., Cole, C.N. and Wente, S.R. (2011) Dbp5, Gle1-IP6 and Nup159: a working model for mRNP export. *Nucleus* **2**, 540–548 https://doi.org/10.4161/nucl.2.6.17881

118 Arul Nambi Rajan, A. and Montpetit, B. (2021) Emerging molecular functions and novel roles for the DEAD-box protein Dbp5/DDX19 in gene expression. *Cell. Mol. Life Sci.* **78**, 2019–2030 https://doi.org/10.1007/s00018-020-03680-y
119 Lin, D.H., Correia, A.R., Cai, S.W., Huber, F.M., Jette, C.A. and Hoelz, A. (2018) Structural and functional analysis of mRNA export regulation by the nuclear pore complex. *Nat. Commun.* **9**, 2319 https://doi.org/10.1038/s41467-018-04459-3
120 Kendirgi, F., Barry, D.M., Griffis, E.R., Powers, M.A. and Wente, S.R. (2003) An essential role for hGle1 nucleocytoplasmic shuttling in mRNA export. *J. Cell Biol.* **160**, 1029–1040 https://doi.org/10.1083/jcb.200211081
121 Braud, C., Zheng, W. and Xiao, W. (2013) Identification and analysis of LNO1-like and AtGLE1-like nucleoporins in plants. *Plant Signal. Behav.* **8**, e27376 https://doi.org/10.4161/psb.27376
122 Lee, H.S., Lee, D.H., Cho, H.K., Kim, S.H., Auh, J.H. and Pai, H.S. (2015) InsP6-sensitive variants of the Gle1 mRNA export factor rescue growth and fertility defects of the ipk1 low-phytic-acid mutation in Arabidopsis. *Plant Cell* **27**, 417–431 https://doi.org/10.1105/tpc.114.132134
123 Imai, A., Ohtani, M., Nara, A., Tsukakoshi, A., Narita, A., Hirakawa, H. et al. (2020) The *Lotus japonicus* nucleoporin GLE1 is involved in symbiotic association with rhizobia. *Physiol. Plant.* **168**, 590–600 https://doi.org/10.1111/ppl.12996
124 Lee, J.Y., Lee, H.S., Wi, S.J., Park, K.Y., Schmit, A.C. and Pai, H.S. (2009) Dual functions of *Nicotiana benthamiana* Rae1 in interphase and mitosis. *Plant J.* **59**, 278–291 https://doi.org/10.1111/j.1365-313X.2009.03869.x
125 Irwin, N.A.T. and Keeling, P.J. (2019) Extensive reduction of the nuclear pore complex in nucleomorphs. *Genome Biol. Evol.* **11**, 678–687 https://doi.org/10.1093/gbe/evz029
126 Pazos, F. and Valencia, A. (2008) Protein co-evolution, co-adaptation and interactions. *EMBO J.* **27**, 2648–2655 https://doi.org/10.1038/emboj.2008.189
127 Gong, S., Worth, C.L., Bickerton, G.R., Lee, S., Tanramluk, D. and Blundell, T.L. (2009) Structural and functional restraints in the evolution of protein families and superfamilies. *Biochem. Soc. Trans.* **37**, 727–733 https://doi.org/10.1042/BST0370727
128 Bapteste, E., Charlebois, R.L., MacLeod, D. and Brochier, C. (2005) The two tempos of nuclear pore complex evolution: highly adapting proteins in an ancient frozen structure. *Genome Biol.* **6**, R85 https://doi.org/10.1186/gb-2005-6-10-r85
129 Worth, C.L., Gong, S. and Blundell, T.L. (2009) Structural and functional constraints in the evolution of protein families. *Nat. Rev. Mol. Cell Biol.* **10**, 709–720 https://doi.org/10.1038/nrm2762
130 Pal, C., Papp, B. and Lercher, M.J. (2006) An integrated view of protein evolution. *Nat. Rev. Genet.* **7**, 337–348 https://doi.org/10.1038/nrg1838
131 D'Angelo, M.A., Gomez-Cavazos, J.S., Mei, A., Lackner, D.H. and Hetzer, M.W. (2012) A change in nuclear pore complex composition regulates cell differentiation. *Dev. Cell* **22**, 446–458 https://doi.org/10.1016/j.devcel.2011.11.021
132 Rosenblum, J.S. and Blobel, G. (1999) Autoproteolysis in nucleoporin biogenesis. *Proc. Natl Acad. Sci. U.S.A.* **96**, 11370–11375 https://doi.org/10.1073/pnas.96.20.11370
133 Griffis, E.R., Xu, S. and Powers, M.A. (2003) Nup98 localizes to both nuclear and cytoplasmic sides of the nuclear pore and binds to two distinct nucleoporin subcomplexes. *Mol. Biol. Cell* **14**, 600–610 https://doi.org/10.1091/mbc.e02-09-0582
134 Teixeira, M.T., Fabre, E. and Dujon, B. (1999) Self-catalyzed cleavage of the yeast nucleoporin Nup145p precursor. *J. Biol. Chem.* **274**, 32439–32444 https://doi.org/10.1074/jbc.274.45.32439
135 Allegretti, M., Zimmerli, C.E., Rantos, V., Wilfling, F., Ronchi, P., Fung, H.K.H. et al. (2020) In-cell architecture of the nuclear pore and snapshots of its turnover. *Nature* **586**, 796–800 https://doi.org/10.1038/s41586-020-2670-5
136 Mahamid, J., Pfeffer, S., Schaffer, M., Villa, E., Danev, R., Cuellar, L.K. et al. (2016) Visualizing the molecular sociology at the HeLa cell nuclear periphery. *Science* **351**, 969–972 https://doi.org/10.1126/science.aad8857
137 Liashkovich, I., Meyring, A., Kramer, A. and Shahin, V. (2011) Exceptional structural and mechanical flexibility of the nuclear pore complex. *J. Cell Physiol.* **226**, 675–682 https://doi.org/10.1002/jcp.22382
138 Jaggi, R.D., Franco-Obregon, A., Muhlhausser, P., Thomas, F., Kutay, U. and Ensslin, K. (2003) Modulation of nuclear pore topology by transport modifiers. *Biophys. J.* **84**, 665–670 https://doi.org/10.1016/S0006-3495(03)74886-3
139 Koh, J. and Blobel, G. (2015) Allosteric regulation in gating the central channel of the nuclear pore complex. *Cell* **161**, 1361–1373 https://doi.org/10.1016/j.cell.2015.05.013
140 Blus, B.J., Koh, J., Krolak, A., Seo, H.S., Coutavas, E. and Blobel, G. (2019) Allosteric modulation of nucleoporin assemblies by intrinsically disordered regions. *Sci. Adv.* **5**, eaax1836 https://doi.org/10.1126/sciadv.aax1836
141 Feldherr, C.M. and Akin, D. (1990) The permeability of the nuclear envelope in dividing and nondividing cell cultures. *J. Cell Biol.* **111**, 1–8 https://doi.org/10.1083/jcb.111.1.1
142 Elosegui-Artola, A., Andreu, I., Beedle, A.E.M., Lezamiz, A., Uroz, M., Kosmalska, A.J. et al. (2017) Force triggers YAP nuclear entry by regulating transport across nuclear pores. *Cell* **171**, 1397–1410.e14 https://doi.org/10.1016/j.cell.2017.10.008
143 Guttinger, S., Laurell, E. and Kutay, U. (2009) Orchestrating nuclear envelope disassembly and reassembly during mitosis. *Nat. Rev. Mol. Cell Biol.* **10**, 178–191 https://doi.org/10.1038/nrm2641
144 Pradillo, M., Evans, D. and Graumann, K. (2019) The nuclear envelope in higher plant mitosis and meiosis. *Nucleus* **10**, 55–66 https://doi.org/10.1080/19491034.2019.1587277
145 Cross, F.R. and Umen, J.G. (2015) The Chlamydomonas cell cycle. *Plant J.* **82**, 370–392 https://doi.org/10.1111/tpj.12795
146 Ali, E.I., Loidl, J. and Howard-Till, R.A. (2018) A streamlined cohesin apparatus is sufficient for mitosis and meiosis in the protist Tetrahymena. *Chromosoma* **127**, 421–435 https://doi.org/10.1007/s00412-018-0673-x
147 Ogbadoyi, E., Ersfeld, K., Robinson, D., Sherwin, T. and Gull, K. (2000) Architecture of the *Trypanosoma brucei* nucleus during interphase and mitosis. *Chromosoma* **108**, 501–513 https://doi.org/10.1007/s004120050402
148 Champion, L., Linder, M.I. and Kutay, U. (2017) Cellular reorganization during mitotic entry. *Trends Cell Biol.* **27**, 26–41 https://doi.org/10.1016/j.tcb.2016.07.004
149 Dultz, E., Zanin, E., Wurzenberger, C., Braun, M., Rabut, G., Sironi, L. et al. (2008) Systematic kinetic analysis of mitotic dis- and reassembly of the nuclear pore in living cells. *J. Cell Biol.* **180**, 857–865 https://doi.org/10.1083/jcb.200707026
150 Dey, G., Culley, S., Curran, S., Schmidt, U., Henriques, R., Kukulski, W. et al. (2020) Closed mitosis requires local disassembly of the nuclear envelope. *Nature* **585**, 119–123 https://doi.org/10.1038/s41586-020-2648-3

151 Exposito-Serrano, M., Sanchez-Molina, A., Gallardo, P., Salas-Pino, S. and Daga, R.R. (2020) Selective nuclear pore complex removal drives nuclear envelope division in fission yeast. *Curr. Biol.* **30**, 3212–3222.e2 https://doi.org/10.1016/j.cub.2020.05.066
152 Kaiser, C.A. and Schekman, R. (1990) Distinct sets of SEC genes govern transport vesicle formation and fusion early in the secretory pathway. *Cell* **61**, 723–733 https://doi.org/10.1016/0092-8674(90)90483-U
153 Dokudovskaya, S., Waharte, F., Schlessinger, A., Pieper, U., Devos, D.P., Cristea, I.M. et al. (2011) A conserved coatomer-related complex containing Sec13 and Seh1 dynamically associates with the vacuole in *Saccharomyces cerevisiae*. *Mol. Cell. Proteom.* **10**, M110 006478 https://doi.org/10.1074/mcp.M110.006478
154 Itoh, G., Sugino, S., Ikeda, M., Mizuguchi, M., Kanno, S., Amin, M.A. et al. (2013) Nucleoporin Nup188 is required for chromosome alignment in mitosis. *Cancer Sci.* **104**, 871–879 https://doi.org/10.1111/cas.12159
155 Hashizume, C., Moyori, A., Kobayashi, A., Yamakoshi, N., Endo, A. and Wong, R.W. (2013) Nucleoporin Nup62 maintains centrosome homeostasis. *Cell Cycle* **12**, 3804–3816 https://doi.org/10.4161/cc.26671
156 Zuccolo, M., Alves, A., Galy, V., Bolhy, S., Formstecher, E., Racine, V. et al. (2007) The human Nup107-160 nuclear pore subcomplex contributes to proper kinetochore functions. *EMBO J.* **26**, 1853–1864 https://doi.org/10.1038/sj.emboj.7601642
157 Belgareh, N., Rabut, G., Bai, S.W., van Overbeek, M., Beaudouin, J., Daigle, N. et al. (2001) An evolutionarily conserved NPC subcomplex, which redistributes in part to kinetochores in mammalian cells. *J. Cell Biol.* **154**, 1147–1160 https://doi.org/10.1083/jcb.200101081
158 Loiodice, I., Alves, A., Rabut, G., Van Overbeek, M., Ellenberg, J., Sibarita, J.B. et al. (2004) The entire Nup107-160 complex, including three new members, is targeted as one entity to kinetochores in mitosis. *Mol. Biol. Cell* **15**, 3333–3344 https://doi.org/10.1091/mbc.e03-12-0878
159 Platani, M., Santarella-Mellwig, R., Posch, M., Walczak, R., Swedlow, J.R. and Mattaj, I.W. (2009) The Nup107-160 nucleoporin complex promotes mitotic events via control of the localization state of the chromosome passenger complex. *Mol. Biol. Cell* **20**, 5260–5275 https://doi.org/10.1091/mbc.e09-05-0377
160 Platani, M., Samejima, I., Samejima, K., Kanemaki, M.T. and Earnshaw, W.C. (2018) Seh1 targets GATOR2 and Nup153 to mitotic chromosomes. *J. Cell Sci.* **131**, jcs213140 https://doi.org/10.1242/jcs.213140
161 Davis, L.I. and Blobel, G. (1987) Nuclear pore complex contains a family of glycoproteins that includes p62: glycosylation through a previously unidentified cellular pathway. *Proc. Natl Acad. Sci. U.S.A.* **84**, 7552–7556 https://doi.org/10.1073/pnas.84.21.7552
162 Kuhn, T.M., Pascual-Garcia, P., Gozalo, A., Little, S.C. and Capelson, M. (2019) Chromatin targeting of nuclear pore proteins induces chromatin decondensation. *J. Cell Biol.* **218**, 2945–2961 https://doi.org/10.1083/jcb.201807139
163 Capelson, M., Liang, Y., Schulte, R., Mair, W., Wagner, U. and Hetzer, M.W. (2010) Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes. *Cell* **140**, 372–383 https://doi.org/10.1016/j.cell.2009.12.054
164 Kalverda, B., Pickersgill, H., Shloma, V.V. and Fornerod, M. (2010) Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm. *Cell* **140**, 360–371 https://doi.org/10.1016/j.cell.2010.01.011
165 Vaquerizas, J.M., Suyama, R., Kind, J., Miura, K., Luscombe, N.M. and Akhtar, A. (2010) Nuclear pore proteins nup153 and megator define transcriptionally active regions in the Drosophila genome. *PLoS Genet.* **6**, e1000846 https://doi.org/10.1371/journal.pgen.1000846
166 Franks, T.M., McCloskey, A., Shokirev, M.N., Benner, C., Rathore, A. and Hetzer, M.W. (2017) Nup98 recruits the Wdr82-Set1A/COMPASS complex to promoters to regulate H3K4 trimethylation in hematopoietic progenitor cells. *Genes Dev.* **31**, 2222–2234 https://doi.org/10.1101/gad.306753.117
167 Light, W.H., Freaney, J., Sood, V., Thompson, A., D'Urso, A., Horvath, C.M. et al. (2013) A conserved role for human Nup98 in altering chromatin structure and promoting epigenetic transcriptional memory. *PLoS Biol.* **11**, e1001524 https://doi.org/10.1371/journal.pbio.1001524
168 Ibarra, A., Benner, C., Tyagi, S., Cool, J. and Hetzer, M.W. (2016) Nucleoporin-mediated regulation of cell identity genes. *Genes Dev.* **30**, 2253–2258 https://doi.org/10.1101/gad.287417.116
169 Iglesias, N., Paulo, J.A., Tatarakis, A., Wang, X., Edwards, A.L., Bhanu, N.V. et al. (2020) Native chromatin proteomics reveals a role for specific nucleoporins in heterochromatin organization and maintenance. *Mol. Cell* **77**, 51–66.e8 https://doi.org/10.1016/j.molcel.2019.10.018
170 Gozalo, A., Duke, A., Lan, Y., Pascual-Garcia, P., Talamas, J.A., Nguyen, S.C. et al. (2020) Core components of the nuclear pore bind distinct states of chromatin and contribute to polycomb repression. *Mol. Cell* **77**, 67–81.e7 https://doi.org/10.1016/j.molcel.2019.10.017
171 Van de Vosse, D.W., Wan, Y., Lapetina, D.L., Chen, W.M., Chiang, J.H., Aitchison, J.D. et al. (2013) A role for the nucleoporin Nup170p in chromatin structure and gene silencing. *Cell* **152**, 969–983 https://doi.org/10.1016/j.cell.2013.01.049
172 Kehat, I., Accornero, F., Aronow, B.J. and Molkentin, J.D. (2011) Modulation of chromatin position and gene expression by HDAC4 interaction with nucleoporins. *J. Cell Biol.* **193**, 21–29 https://doi.org/10.1083/jcb.201101046
173 Smith, S., Galinha, C., Desset, S., Tolmie, F., Evans, D., Tatout, C. et al. (2015) Marker gene tethering by nucleoporins affects gene expression in plants. *Nucleus* **6**, 471–478 https://doi.org/10.1080/19491034.2015.1126028
174 Kee, H.L., Dishinger, J.F., Blasius, T.L., Liu, C.J., Margolis, B. and Verhey, K.J. (2012) A size-exclusion permeability barrier and nucleoporins characterize a ciliary pore complex that regulates transport into cilia. *Nat. Cell Biol.* **14**, 431–437 https://doi.org/10.1038/ncb2450
175 Endicott, S.J. and Brueckner, M. (2018) NUP98 sets the size-exclusion diffusion limit through the ciliary base. *Curr. Biol.* **28**, 1643–1650.e3 https://doi.org/10.1016/j.cub.2018.04.014
176 Del Viso, F., Huang, F., Myers, J., Chalfant, M., Zhang, Y., Reza, N. et al. (2016) Congenital heart disease genetics uncovers context-dependent organization and function of nucleoporins at cilia. *Dev. Cell* **38**, 478–492 https://doi.org/10.1016/j.devcel.2016.08.002
177 Marquez, J., Bhattacharya, D., Lusk, C.P. and Khokha, M.K. (2021) Nucleoporin NUP205 plays a critical role in cilia and congenital disease. *Dev. Biol.* **469**, 46–53 https://doi.org/10.1016/j.ydbio.2020.10.001
178 Nachury, M.V., Seeley, E.S. and Jin, H. (2010) Trafficking to the ciliary membrane: how to get across the periciliary diffusion barrier? *Annu. Rev. Cell Dev. Biol.* **26**, 59–87 https://doi.org/10.1146/annurev.cellbio.042308.113337
179 Dishinger, J.F., Kee, H.L., Jenkins, P.M., Fan, S., Hurd, T.W., Hammond, J.W. et al. (2010) Ciliary entry of the kinesin-2 motor KIF17 is regulated by importin-beta2 and RanGTP. *Nat. Cell Biol.* **12**, 703–710 https://doi.org/10.1038/ncb2073
180 Hurd, T.W., Fan, S. and Margolis, B.L. (2011) Localization of retinitis pigmentosa 2 to cilia is regulated by Importin beta2. *J. Cell Sci.* **124**(Pt 5), 718–726 https://doi.org/10.1242/jcs.070839
181 Breslow, D.K., Koslover, E.F., Seydel, F., Spakowitz, A.J. and Nachury, M.V. (2013) An in vitro assay for entry into cilia reveals unique properties of the soluble diffusion barrier. *J. Cell Biol.* **203**, 129–147 https://doi.org/10.1083/jcb.201212024

182 Salisbury, J.L., Suino, K.M., Busby, R. and Springett, M. (2002) Centrin-2 is required for centriole duplication in mammalian cells. *Curr. Biol.* **12**, 1287–1292 https://doi.org/10.1016/S0960-9822(02)01019-9
183 Resendes, K.K., Rasala, B.A. and Forbes, D.J. (2008) Centrin 2 localizes to the vertebrate nuclear pore and plays a role in mRNA and protein export. *Mol. Cell. Biol.* **28**, 1755–1769 https://doi.org/10.1128/MCB.01697-07
184 Ludwig, M. and Gibbs, S.P. (1989) Evidence that the nucleomorphs of chlorarachnion reptans (chlorarachniophyceae) are vestigial nuclei: morphology, division and DNA-DAPI fluorescence. *J. Phycol.* **25**, 385–394 https://doi.org/10.1111/j.1529-8817.1989.tb00135.x
185 Jovanovic-Talisman, T., Tetenbaum-Novatt, J., McKenney, A.S., Zilman, A., Peters, R., Rout, M.P. et al. (2009) Artificial nanopores that mimic the transport selectivity of the nuclear pore complex. *Nature* **457**, 1023–1027 https://doi.org/10.1038/nature07600
186 Fragasso, A., de Vries, H.W., Andersson, J., van der Sluis, E.O., van der Giessen, E., Dahlin, A. et al. (2021) A designer FG-Nup that reconstitutes the selective transport barrier of the nuclear pore complex. *Nat. Commun.* **12**, 2010 https://doi.org/10.1038/s41467-021-22293-y
187 Panatala, R., Barbato, S., Kozai, T., Luo, J., Kapinos, L.E. and Lim, R.Y.H. (2019) Nuclear pore membrane proteins self-assemble into nanopores. *Biochemistry* **58**, 484–488 https://doi.org/10.1021/acs.biochem.8b01179