The nuclear pore complex: bridging nuclear transport and gene regulation

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Abstract | Although the nuclear pore complex (NPC) is best known for its primary function as the key regulator of molecular traffic between the cytoplasm and the nucleus, a growing body of experimental evidence suggests that this structure participates in a considerably broader range of cellular activities on both sides of the nuclear envelope. Indeed, the NPC is emerging as an important regulator of gene expression through its influence on the internal architectural organization of the nucleus and its apparently extensive involvement in coordinating the seamless delivery of genetic information to the cytoplasmic protein synthesis machinery.

Nuclear periphery
The region of the nucleus comprised of the nuclear envelope and its associated structures, including the NPC and the nuclear components found in the neighbourhood.

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Subcellular compartmentalization by membrane systems, such as the nuclear envelope or the endoplasmic reticulum (ER), pre-dates the emergence of the major modern eukaryotic lineages (reviewed in REF. 1). Although this adaptation offers advantages, such as allowing cellular functions to be separated in specialized organelles and affording more complex means of functional regulation, it also poses considerable logistical challenges made necessary by the need to exchange material between separate organelles. The nuclear envelope separates the cellular genome from the rest of the cell and is composed of two distinct membranes, the nucleoplasm-facing inner nuclear membrane and the cytoplasm-facing outer nuclear membrane, that are separated by a perinuclear lumenal space. Traffic between the nucleus and the cytoplasm is accomplished through specialized, circular apertures that occur at sites where the inner and outer nuclear membranes join together. These apertures are filled with cylindrical macromolecular assemblies termed nuclear pore complexes (NPCs).

NPCs, with a molecular mass of ~50 mDa, are among the largest proteinaceous assemblies in the cell and are constructed of multiple copies of ~30 different proteins called nucleoporins (Nups). The structure of the NPC consists of two main functional regions: the NPC central structure, which is embedded in the plane of the nuclear envelope, and the NPC peripheral structures, which extend the reach of the NPC towards both the nuclear interior and the cytoplasm (FIG. 1). The NPC centre consists of an eight-fold symmetrical cylindrical assembly, which encases the main nuclear transport channel and functions as a molecular sieve to regulate the bidirectional transport of macromolecules and small metabolites (FIG. 2). The peripheral NPC extensions consist of asymmetrical filamentous structures that connect the NPC core structure to its molecular milieu either inside the nucleus or in the cytoplasm (FIG. 3). Within the nucleus, the nuclear basket structure (hereafter referred to as the basket) connects the NPC to aspects of nuclear metabolism, such as mRNA biogenesis and genome maintenance. On the opposite side of the membrane, specialized filaments project out towards the cytoplasm and channel export cargo towards the protein synthesis machinery while funneling incoming cargo from the cytoskeleton towards the nuclear interior (FIG.1).

Work in recent years has caused the field to re-evaluate the view of the NPC as an insular entity, the only role of which is nucleocytoplasmic transport. In fact, the NPC seems to be the central unit of a network of proteins and ribonucleoproteins (RNPs) positioned along the gene expression 'path' (FIG. 3). This network spreads from the basket into a region commonly referred to as the nuclear periphery and interconnects neighbouring NPCs to form a multifunctional platform beneath the nucleoplasmic side of the nuclear envelope. Here, unique sets of macromolecular complexes assemble to ensure the efficient control of gene expression at the transcriptional and post-transcriptional levels (reviewed in REFS 2,3). On the cytoplasmic side, filaments extend from the NPC and connect to the protein synthesis machinery and the cytoskeleton to facilitate the close coupling between messenger RNP (mRNP) export and translation initiation. This network of protein–protein and protein–RNA interactions is required for the correct flow of information into and out of the nucleus, and ensures that nuclear cargoes can carry out their function on both sides of the nuclear envelope with as little impediment as possible along their route (FIG. 3).

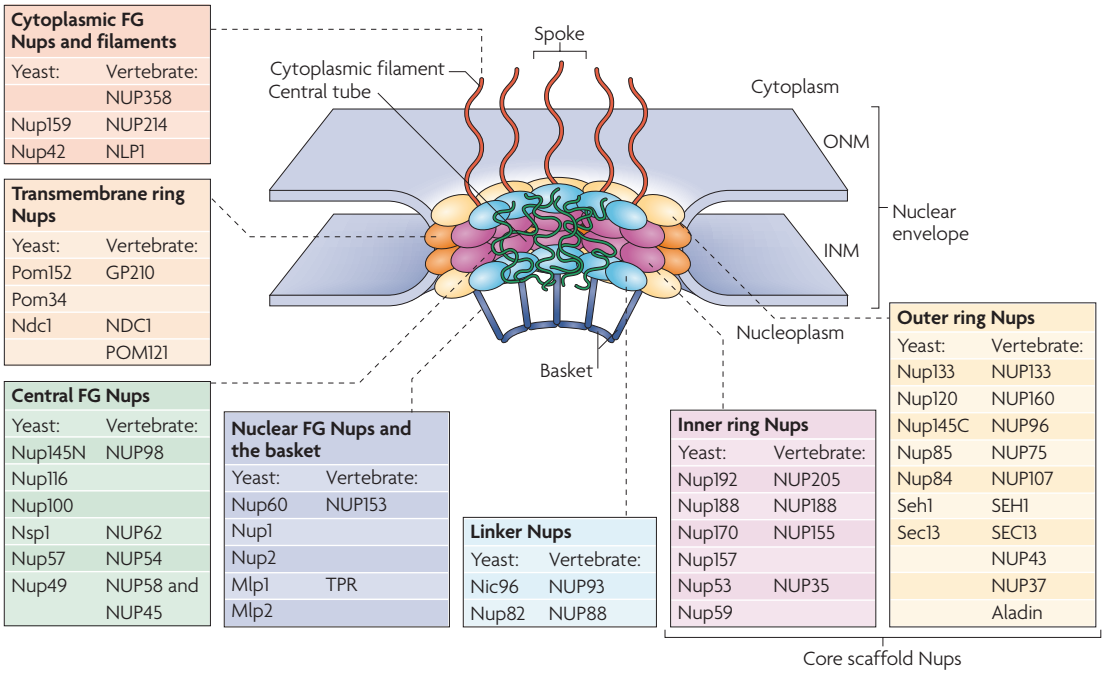

Figure 1 | **Nuclear pore complex structure.** Each nuclear pore complex (NPC) is a cylindrical structure comprised of eight spokes surrounding a central tube that connects the nucleoplasm and cytoplasm. The outer and inner nuclear membranes (ONM and INM, respectively) of the nuclear envelope join to form grommets in which the NPC sits. The NPC is anchored to the nuclear envelope by a transmembrane ring structure that connects to the core scaffold and comprises inner ring and outer ring elements. Linker nucleoporins (Nups) help anchor the Phe-Gly (FG) Nups such that they line and fill the central tube. NPC-associated peripheral structures consist of cytoplasmic filaments, the basket and a distal ring. The Nups that are known to constitute each NPC substructure are listed, with yeast and vertebrate homologues indicated. Both inner and outer ring Nups are known to form biochemically stable NPC subcomplexes, which are thought to have a role in NPC biogenesis and nuclear envelope assembly. GP210, glycoprotein 210; Mlp, myosin-like protein; Ndc1, nuclear division cycle protein 1; Nic96, Nup-interacting component of 76 kDa; NLP1, Nup-like protein 1; Pom, pore membrane protein; Seh1, SEC13 homologue 1; TPR, translocated promoter region.

In this Review, we discuss apparent points of integration between the NPC and the greater cellular environment, and explore the emerging role of this complex in the coordination of diverse nuclear and cytoplasmic processes beyond transport.

Structure of the NPC

The morphology of the NPC was first studied in detail in vertebrates, although it seems that most features are conserved throughout eukaryotes[4]. The vertebrate NPC consists of a ~125-nm diameter core structure, which contains eight spokes in a radially symmetrical arrangement. The spokes join to form three main rings surrounding a central tube (FIG. 1). The inner ring (also known as the inner spoke ring or central ring) at the NPC's equator is sandwiched between two outer rings, one on the cytoplasmic side and one on the nucleoplasmic side, with each ring closely following the curved inner surface of the pore membrane (FIG. 1). This triple-ring framework of the NPC creates a central channel with a minimum diameter of ~35 nm, which has been inferred mainly from the size of artificial transport cargo (reviewed in REFS 5,6).

A detailed architectural map of the yeast NPC was recently determined using a computational approach that combined proteomic, biophysical and imaging data[7,8]. This map agrees with a large body of complementary data in both vertebrates and yeast (reviewed in REF. 9) and allows each Nup to be assigned to particular substructures in the NPC. Nups can thus be subdivided into four classes (FIG. 1): transmembrane, core scaffold (inner ring and outer ring), linker and Phe-Gly (FG). In yeast and vertebrates, three transmembrane Nups span the pore membrane and constitute an outer transmembrane ring (also known as the lumenal ring) that anchors the NPC to the nuclear envelope. Around a dozen core scaffold Nups form the outer and inner rings, which together comprise the core scaffold of the NPC. This scaffold encases the central transport tube of the NPC and is formed from several biochemically stable and conserved NPC subcomplexes that seem to have a key role as building blocks during NPC biogenesis (for example, the conserved *Saccharomyces cerevisiae* Nup84 and Nup170 complexes and their respective metazoan homologues, the NUP170–NUP160, and the NUP35–NUP155 complexes)[10–12]. Anchored to the core scaffold are a dozen largely unfolded FG Nups, which line the surface of the central tube from the nuclear to the cytoplasmic face. Finally, linker Nups create a bridge between the core scaffold and FG Nups.

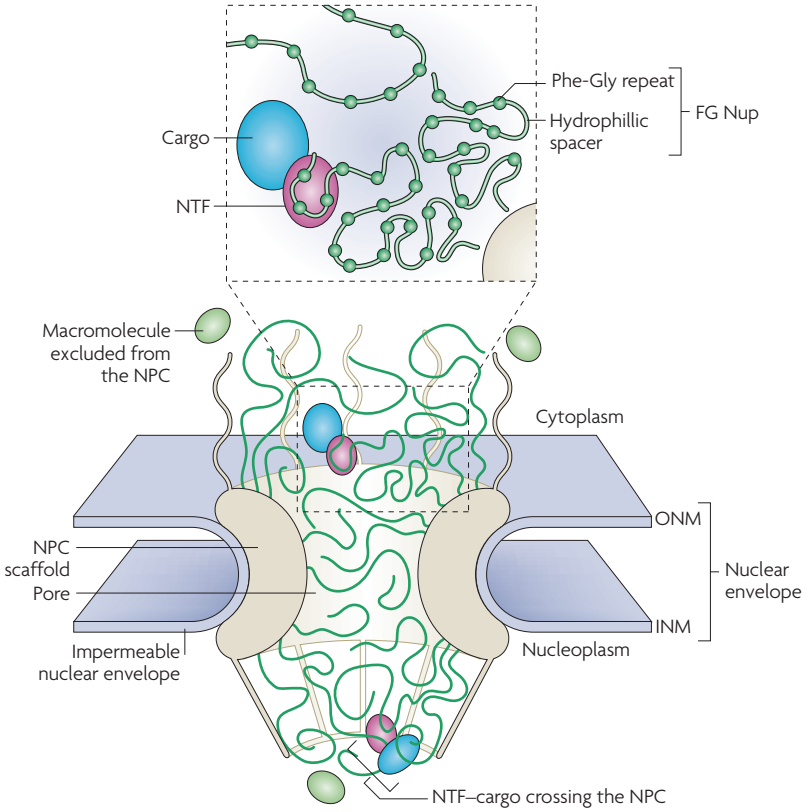

Figure 2 | **The nuclear pore complex functions as a 'virtual gate'.** The outer and inner nuclear membranes (ONM and INM, respectively) of the nuclear envelope join to form a ring-shaped pore where the nuclear pore complex (NPC) resides. At the NPC, the nucleus and cytoplasm are connected by a channel, which is filled with flexible, filamentous Phe-Gly nucleoporins (FG Nups). Spurious macromolecules are physically excluded from entering the densely packed FG Nup meshwork. Nuclear transport factor (NTF)-bound cargo can enter the channel from either its cytoplasmic or nucleoplasmic side and hop between binding sites on the FG Nups until they return to the original compartment or reach the opposite side of the NPC.

Surprisingly, the Nups collectively contain only a few fold types[9,13,14]. Mapping these folds onto the NPC architecture[8] reveals that the NPC's core scaffold consists of cage-like structures containing Nups composed entirely of either a β-propeller fold, an α-solenoid (or helix-turn-helix repeat) fold, or a distinctive arrangement of both — a combination that is characteristic of a particular family of membrane coating proteins represented by components of the clathrin, coat protein I (COPI) and COPII vesicle-coating complexes (reviewed in REF. 15). This similarity supports the 'protocoatomer hypothesis', which posits a simple membrane-curving module, composed primarily of β-propeller and α-solenoid folds, was the ancient common ancestor for NPCs and coated vesicles[8,14]. This protocoatomer allowed cells to form and manipulate internal membrane systems, and so led to the evolution of the ER, Golgi and nucleus[1]. Over the past few years, striking atomic-resolution pictures of Nups have also been generated, which show the strong similarity of the core scaffold Nups to coating complex proteins and are helping to piece together the architecture of the NPC at the atomic scale (reviewed in REF. 9).

β-Propeller
A compact structural protein domain of similarly sized β-sheets, which are stacked into a cylinder to resemble the blades of a propeller.

α-Solenoid
A structural protein domain composed of numerous pairs of antiparallel α-helices that are stacked to form a solenoid.

Mechanism of nucleocytoplasmic transport

The NPC is freely permeable to small molecules, metabolites and ions, but acts as a highly efficient molecular sieve for macromolecules; this being its main function. Transport of almost all macromolecules into and out of the nucleus is achieved through a common active mechanism that requires the assistance of soluble nuclear transport factors (NTFs) and transport signals, which together form the 'soluble phase' of nuclear transport (reviewed in REFS 16,17). Most NTFs, but not all, belong to the karyopherin (Kap) family of proteins (BOX 1).

The NTFs specifically bind transport signals found on their cognate substrates and translocate them through the NPC channel. The best-studied transport signals are found on nuclear protein cargoes. Such signals consist of short amino acid sequences called nuclear localization sequences (NLSs; for import) or nuclear export sequences (NESs; for export). A consensus three-step mechanism for macromolecular cargoes has provided the framework for all subsequent investigations into the molecular details of nuclear transport[18]. First, nuclear transport substrates are recognized and bound by NTFs. Second, this NTF–cargo complex docks to the NPC by binding to FG Nups and translocates through the NPC. Third, on reaching its target compartment (either the nucleoplasm or cytoplasm), the complex dissociates. Because molecules lacking effective NLSs or NESs cannot bind NTFs and are thus not afforded passage through the NPC, this model also explains the mechanism governing the barrier function of the NPC. In the case of Kap-mediated transport, directionality is enforced by the distribution of the GTP- and GDP-bound states of the small GTPase RAN, which is essential for the nuclear transport of RNA and proteins[19,20]. RAN–GTP is predominantly found in the nucleus and drives the release of import cargo inside the nuclear compartment by binding to importing Kaps. In the case of nuclear export, the presence of RAN–GTP increases the affinity of exporting Kaps for NES-containing cargo. On reaching the other side of the NPC, GTP hydrolysis induces the release of cargo into the cytoplasm. The resulting NTF and RAN–GDP are subsequently recycled back into the transport pathway. Thousands of cargo types pass through the NPC using this mechanism, making a detailed discussion of this topic beyond the scope of this Review.

FG Nups (reviewed in REF. 21) make up as much as one-third of the molecular mass of the NPC and have a pivotal role in determining the mechanism of nuclear transport as they contain the docking sites for most NTF–cargo complexes *en route* through the NPC[22,23]. FG Nups are characterized by regions of multiple Phe-Gly repeats separated by hydrophilic spacer sequences of 5–30 amino acids[24]. FG Nups can be classified into two groups: symmetric, which are found on both sides of the NPC (for example NUP62), and asymmetric, which are found further from the central plane of the nuclear envelope and have a clear nuclear or cytoplasmic bias (for example NUP153)[8,25,26] (FIG. 1). Most available evidence supports the view that

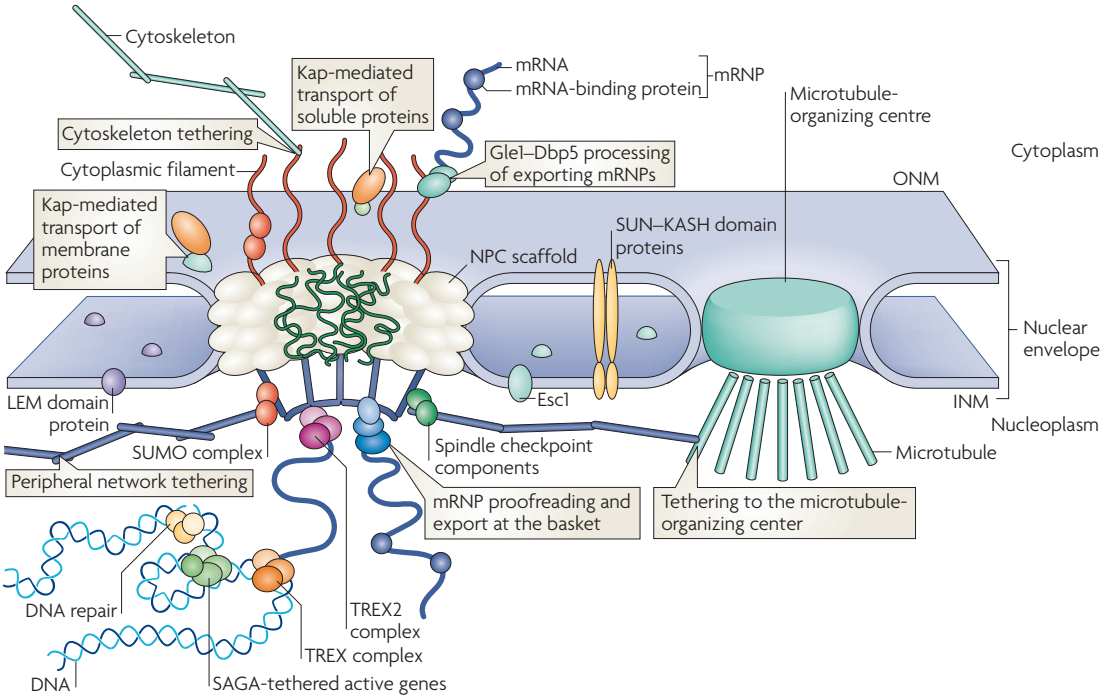

Figure 3 | **The function of the nuclear pore complex peripheral structures.** The nuclear pore complex (NPC) associates with numerous molecules and structures in the cytoplasm and nucleoplasm through its cytoplasmic filaments and nuclear basket, respectively. This enables the NPC to be involved in diverse functions in addition to the import and export of soluble and membrane proteins, which requires nuclear transport factors such as karyopherins (Kaps). The basket is part of an interconnected and highly dynamic molecular platform on the nucleoplasmic face of the nuclear envelope. In *Saccharomyces cerevisiae* and *Drosophila melanogaster*, this molecular platform couples transcriptional regulation (through interactions between the SAGA chromatin remodelling complex and active genes, interaction of the NPC with components of the TREX2 complex, and interactions between small ubiquitin-related modifier (SUMO) regulatory complexes and the proofreading machinery of exporting ribonucleoproteins (RNPs)[105]), chromatin stability (through interactions with the transcription-coupled DNA repair machinery)[3] and chromosome handling during mitosis (through interactions with spindle checkpoint proteins and the spindle)[154]. On the other side of the nuclear envelope, cytoplasmic filaments link these processes to the protein synthesis machinery and cytoskeleton. These filaments interact with the Gle1–DEAD box protein 5 (Dbp5) RNA helicase complex to ensure close spatial and temporal coordination between the final phases of messenger RNP export and the initiation of mRNA translation at ribosomes[56]. Cytoplasmic filaments also interact with cytoskeletal structures to direct traffic in and out of the nucleus to the appropriate cellular 'highways' in the cytoplasm[64]. The network of protein–protein interactions extending from the NPC also includes integral inner nuclear membrane (INM) proteins such as the yeast establishment of silenced chromatin protein 1 (Esc1)[92] and the highly conserved LEM domain[167] and SUN domain[164] proteins. SUN domain proteins are thought to link cytoplasmic microtubules with chromatin through direct interactions with KASH domain proteins in the nuclear envelope lumen[172], thus underscoring the existence of an extended communication network spanning the nuclear envelope at the NPC and across both the INM and outer nuclear membrane (ONM).

LEM domain
(LAP2, emerin and MAN1 domain). A domain that is present in a family of evolutionarily conserved integral membrane proteins of the INM, which participate in chromatin organization, gene expression regulation and nuclear envelope biogenesis.

SUN domain
(Sad1 and UNC84 domain). A conserved C-terminal amino acid sequence found in integral membrane proteins of the INM. These proteins act with members of the KASH domain-containing protein family to form a molecular 'velcro', which is thought to mediate several processes requiring nuclear repositioning, such as fertilization, establishment of polarity, division and differentiation.

Brownian motion
The seemingly random movement of particles suspended in a liquid or gas, which is driven by the kinetic energy of the particles in the system.

the Phe-Gly repeat regions have a natively unfolded structure, forming flexible polypeptide strings that can dynamically convert between random conformations owing to Brownian motion[27–29]. In yeast, it has been shown that around 160 individual FG Nups line the walls of the transport channel in each NPC[8,25]. Thus, the NPC can be thought of as a passage filled with FG Nups forming a continual interacting surface for NTF–cargo complexes throughout the pore (FIG. 2). Remarkably, it was recently shown that an 'artificial NPC' — a simple fabricated nanotube filled with the Phe-Gly repeat region from an FG Nup — was capable of reproducing many of the key characteristics of nucleocytoplasmic transport, including efficiently passing NTFs and NTF–cargo complexes that specifically bind FG Nups, and inhibiting the passage of proteins that do not[30].

Many different models have been proposed to explain the ability of the NPC to function as a selective macromolecular transporter. Initial models (based on early structural evidence) advocated the existence of two iris-like assemblies that open asynchronously to regulate access to either end of the NPC transport channel[31] or an affinity gradient of binding sites that drive directional shuttling of NTFs along the transporter channel[32,33]. These hypotheses were subsequently abandoned for various reasons. The absence of motor proteins among NPC components excludes the presence of mechanochemical doors regulating nuclear access[25,26], whereas the mainly symmetrical distribution of FG Nups with respect to the nuclear envelope plane[25], the dispensable nature of most asymmetrically disposed NTF-binding sites on the NPC[34] and the observation

Box 1 | **The karyopherin family of nuclear transport factors**

The karyopherin (Kap) family of proteins in yeast is thought to comprise fourteen members (reviewed in REF. 156). Some Kaps, known as importins, specialize in transporting cargoes into the nucleus, and others, known as exportins, ferry cargoes out of the nucleus (reviewed in REF. 157). For example, in *Saccharomyces cerevisiae*, the nuclear transport factor (NTF) Kap123 is known to ferry ribosomal proteins into the nucleus, and one of the many jobs of chromosome region maintenance 1 (CRM1) is to help ferry pre-60S ribosomal subunits out of the nucleus. In addition to ribosomal RNAs, other types of RNAs are transported by Kaps. For example, *S. cerevisiae* loss of suppression 1 (Los1) is recruited by tRNAs and promotes their nuclear export, whereas CRM1 is known to modulate the export of unspliced or partially spliced viral RNAs and might be involved with the regulated export of important mRNA species during specific developmental stages (reviewed in REF. 158). Nevertheless, not all NTFs belong to the Kap family[157]. The most notable exceptions are nuclear RNA export factor 1 (NXF1) and NTF2-like export factor 1 (NXT1) and their respective *S. cerevisiae* homologues, mRNA export factor of 67 kDa (Mex67) and mRNA transport protein 2 (Mtr2). These proteins are responsible for the nuclear export of mature messenger ribonucleoproteins and have no obvious structural resemblance to the Kap family (reviewed in REF. 16).

that the direction of nuclear transport can be reversed by modifying the cellular distribution of nucleotide-bound RAN states[19], exclude an affinity gradient as the main driving force of transport.

The conceptual framework of 'virtual gating'[25,35] was introduced in order to contrast it with the mechanical gate and affinity gradient concepts. Within this framework "local stochastic molecular interactions within the NPC ensure selective gating without the need for individual structural elements to move with respect to each other"[36]. In other words, the NPC is not a physical gate that opens and shuts but is constructed to behave like one (FIG. 2). Indeed, there is now a general consensus that both the actual transport step through the NPC and the exclusion of nonspecific macromolecules from this step are largely mediated by the milieu formed by the FG Nups[36]. In and around the central tube of the NPC there is a dense network of intertwined Phe-Gly repeat filaments that form an obstacle to the passive diffusion of most macromolecules, leading to their exclusion from the NPC. Transport factors overcome this exclusion through their capacity to bind Phe-Gly repeats, moving from binding site to binding site across the NPC. However, the details of exactly how the FG Nups carry out this selective gating function in molecular terms is still a matter of vigorous debate. The FG Nups may exhibit a polymer brush-like behaviour, pushing away nonspecific macromolecules[25], or they may collapse on binding, providing temporary passage through the meshwork[28,37]. In contrast, it has been suggested that FG Nups may be cross linked by amyloid-like interactions[38–42] to form "a hydrogel-based, sieve-like permeability barrier that allows rapid entry of nuclear transport receptors"[42]; the Phe-Gly repeats may form a lining to the central tube, which NTFs can access but other macromolecules cannot[36,43]. A combination of these behaviours may be at work[27,29,44,45]. At the same time, competition for binding sites and limited space in this region also seem to be important considerations, leading to NTFs acting not only as carriers of cargo but also as 'bouncers' that compete with nonspecific macromolecules for NPC access and thus contribute greatly to the selective filtering process[30,46,47].

Beyond transport: cytoplasmic functions

Several recent lines of evidence indicate that the NPC acts as a key regulator of events that occur on either side of the nuclear envelope. For example, early work conducted primarily with amphibian oocytes suggested that the NPC participates in the organization of interphase chromatin as well as in nucleocytoplasmic communication[48,49], whereas work in mammalian cells suggested that cytoplasmic filaments might be involved in the release of export cargo into the cytoplasm[50,51]. These events are required for the seamless transfer of genetic information from the nucleus to the cytoplasm and at the same time for relaying extranuclear and extracellular signals to the nuclear genome. Such regulatory functions are carried out primarily by peripheral structures situated on the cytoplasmic and nuclear faces of the NPC (FIG. 1; FIG. 3).

On the cytoplasmic side, eight filaments project from the NPC surface to interface with the protein synthesis machinery and the cytoskeleton. These cytoplasmic filaments are thought to be formed from extended domains, including the Phe-Gly domains, of Nups found at the cytoplasmic face of the NPC (for example, NUP214 and NUP358 (also known as RANBP2) in vertebrates and Nup42 and Nup159 in *S. cerevisiae)*. Recent evidence has shed some light on the mysterious structure of such filaments[52]. The yeast dynein light chain (Dyn2) has been seen to physically interact with Nup159 and might contribute to the formation of a 'pearls on a string', rigid and rod-like filament, capable of projecting the Phe-Gly repeat region of Nup159 outwards from the NPC core. Nups that form cytoplasmic filaments contain numerous binding sites with diverse functions related to the termination of several export reactions and the handing over of transport cargo for further processing in the cytoplasm[53], as well as possibly funnelling import cargo into the NPC[54,55] (FIG. 1; FIG. 3).

In one example involving primarily NUP214 (also known as CAN and homologous to *S. cerevisiae* Nup159) and Nup-like protein 2 (NUPL2; also known as GC1 and homologous to *S. cerevisiae* Nup42), mRNPs in transit towards the cytoplasm encounter the RNA helicase DEAD box protein 19B (DDX19B; also known as DBP5) and the NPC accessory protein and RNA export mediator homologue GLE1, which is bound to its cofactor inositol hexakisphosphate while attached to the cytoplasmic fibrils of the NPC (reviewed in REF. 53). Yeast Dbp5 and Gle1 are thought to catalyse the release of mRNP-bound proteins, such as the mRNA shuttling cofactor nuclear polyadenylated RNA-binding protein 2 (Nab2) and the mRNA export factor Mex67, from mature mRNPs as they leave the NPC transport channel and enter the cytoplasm. It is thought that this release essentially terminates export, driving the directionality of transport and readying the mRNA for engagement with the ribosomal translation apparatus in the cytoplasm[56].

NUP358 has multiple domains, including a cyclophilin homology domain, zinc finger domains, a binding site for the small ubiquitin-related modifier (SUMO) ligase ubiquitin-conjugating protein 9 (UBC9; also known as UBE2I), and sites mediating interaction with both the GTP- and GDP-bound forms of the RAN GTPase and RAN GTPase-activating protein (GAP)[57–59], and it has been implicated in multiple functions that require close coordination between nuclear and cytoplasmic events. For example, NUP358 is probably responsible for helping exportins release their nuclear export cargo into the cytoplasm[50] while promoting the recruitment of import cargo to the NPC[55]. This latter function is usurped by HIV-1, whereby the regulator of virion expression (Rev) protein of HIV-1 uses NUP358 for NPC docking[55]. In addition, NUP358 is thought to be involved in the sumoylation of nuclear transport protein cargoes as they pass through the NPC, thus helping to modulate their protein–protein interaction properties[60]. Moreover, the distal tips of the NUP358 filaments also bind to microtubules, pointing to a role for NUP358 in regulating microtubule assembly, stability and dynamics during interphase. Several types of nuclear transport cargoes (including incoming viruses[61]) have been seen travelling along microtubules to[62] and from the NPC[63], and anchoring of the NPC to the microtubule cytoskeleton may also help to keep *bona fide* cargoes 'on track' as they cross the nuclear envelope[64,65].

Beyond transport: nuclear functions

In 1989, Hans Ris presented the first electron microscopy (EM) images of the nuclear basket[66]. This filamentous nuclear structure, which resembles a fish trap or a woven basket, projects from the nuclear face of the NPC core scaffold towards the nucleoplasm[67,68]. Since then, the basket has been the focus of intense research, leading to the discovery of both its structure and function, and thus shedding more light on how the NPC-associated structures help to coordinate gene expression.

Nuclear basket: structure and potential roles. The detailed architecture of the basket has remained elusive owing to the fact that ultrastructural images obtained by EM vary considerably depending on the preparation technique and growth conditions[69–71]. The prevailing view depicts the basket as consisting of eight proteinaceous filaments protruding ~60–80 nm from the nuclear face of the NPC into the nucleoplasm and converging in a distal ring structure[67–69,72] (FIG. 1; FIG. 3). Strikingly, large mRNPs, such as the Balbiani ring particles of *Chironomus* spp. salivary gland cells, induce dramatic rearrangements of the basket's distal ring on nuclear export, suggesting that the basket is a dynamic and flexible structure that can regulate access to the NPC core transport channel[73–75]. Fibrils 8–10 nm in diameter — similar in size to the basket filaments — emanate from the basket and interconnect to form a meshwork, extending both in parallel and perpendicularly to the nuclear envelope plane[68,69]. The most striking images of this basket-associated meshwork were obtained in *Triturus* spp. and *Xenopus laevis*, revealing a regular filamentous lattice interlinking the distal rings of the baskets, termed the nuclear envelope lattice[69]. Similar structures were also seen in yeast[72], suggesting that a basket-associated platform might be a conserved feature of eukaryotes. Recent studies indicate that this platform might also play a big part in excluding unwanted macromolecular assemblies, including heterochromatin, from the vicinity of the NPC entrance in order to ensure specific, efficient and blockage-free nuclear transport[49,76,77].

Owing to the limited understanding of the basket's composition and organization, its function has also remained a matter of debate. Early work suggested that the basket might be composed of FG Nups, such as NUP153 in humans and Nup60 and Nup1 in *S. cerevisiae*. However, recent immuno-EM experiments have demonstrated that the highly conserved nuclear protein translocated promoter region (TPR)[78,79], which is homologous to *Drosophila melanogaster* Megator[80,81] and myosin-like protein 1 (Mlp1) and Mlp2 in *S. cerevisiae*[82], is instead the main molecular component of the basket, at least in vertebrates[83,84]. Consistently, all TPR-related proteins studied to date are long, filamentous, coiled-coil dimers that are capable of forming the basket and the nuclear envelope lattice[81,82,85,86]. Multiple lines of evidence indicate that the basket or proteins associated with the basket are involved in multiple nuclear envelope-associated functions, such as transcriptional regulation, RNA biogenesis, regulation of SUMO homeostasis, chromatin maintenance and the control of cell division[75,76,81,87–101]. These findings suggest that an important and highly conserved role of the basket is to link the nuclear transport channel to an extended, highly dynamic protein–protein interaction network that forms a platform between NPCs. This platform, together with the basket itself, is involved in the recruitment of RNA processing and transport factors to the nuclear periphery, regulation of macromolecular access to the nuclear face of the NPC, epigenetic regulation of gene expression, and maintenance and intranuclear organization of chromatin during interphase and mitosis (FIG. 3).

Post-transcriptional control of gene expression. The production of translationally competent mRNAs requires transcription, post-transcriptional processing, NPC docking and translocation across the NPC (reviewed in REF. 102). The past years of accumulated evidence places the basket at the centre of these pivotal and closely coupled processes[75,76,103]. In particular, basket components seem to be involved in the mechanism of mRNA surveillance, which prevents defective mRNAs, such as unspliced or partially spliced polyadenylated RNA molecules, from reaching the cytoplasm (reviewed in REF. 104). Furthermore, many of the factors involved in mRNP maturation and export have also been implicated in the recruitment of active genes to the NPC, thus suggesting a connection between gene expression and mRNA metabolism[105,106] (see next section and FIG. 4).

Eukaryotic cells possess several mechanisms for recognizing and targeting aberrant RNA species for degradation[107]. Both steps require specific surveillance factors and can lead either to retention of the mRNA

Heterochromatin
A highly condensed form of chromatin that is either genetically inactive or transcriptionally repressed. It is predominantly located near the nuclear envelope and includes centromeres, telomeres and silenced genes.

SUMO homeostasis
The overall level of proteins modified by the covalent attachment of SUMO. It is balanced through the regulated activities of sumoylating ligases and desumoylating proteases.

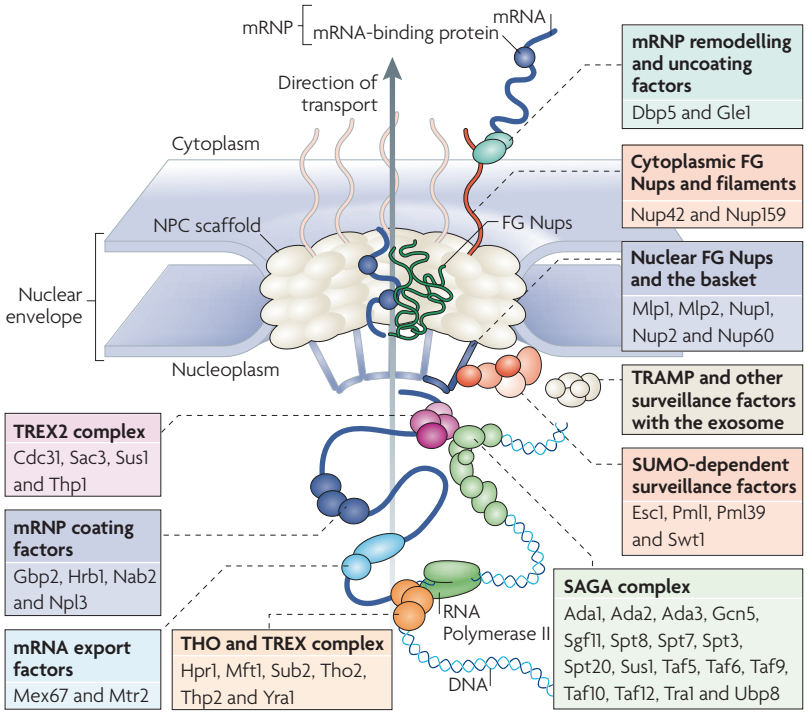

Figure 4 | **The gene expression path traverses the NPC.** The movement of mRNAs from the transcription site inside the nucleus towards the cytoplasmic protein synthesis machinery involves several extensively coupled steps, in which overlapping factors coordinate a closely orchestrated 'dance' by accompanying the transcripts[173]. The precise order of events is poorly understood, but a consensus model for how this process might take place is depicted[174]. The SAGA chromatin remodelling complex is recruited to the promoter of a subset of inducible genes and promotes their transcription. Crosstalk between SAGA and the nuclear pore complex (NPC)-associated TREX2 complex might aid relocation of transcriptionally active genes to the nuclear periphery, resulting in the phenomenon of gene gating[49] (whereby transcription and export machineries cooperate to 'gate' genes to the nuclear pore). Active transcription of gated genes produces nascent transcripts that recruit shuttling mRNA-coating factors[175], THO, TREX and, subsequently, the mRNA export factors Mex67 and mRNA transport protein 2 (Mtr2), resulting in the formation of an export-competent ribonucleoprotein (mRNP)[176]. Thus, the association of the maturing mRNPs with components of the basket is strengthened in preparation for nuclear translocation, favouring mRNP surveillance mechanisms carried out by a basket-associated machinery[104]. After translocation, the release of mRNA export factors from mRNPs is induced by the combined action of DEAD box protein 5 (Dbp5p) and Gle1, which are docked to NPC cytoplasmic filaments through interactions with nucleoporin of 42 kDa (Nup42) and Nup159, respectively, and are thought to act as mRNP remodelling factors[53]. It is presumed that this process drives the directionality of mRNP export[56] while priming mRNAs for translation initiation. Ada, transcriptional adaptor; Cdc31, cell division cycle protein 31; Esc1, establishes silent chromatin protein 1; GBP2, GTP-binding protein 2; Hpr1, hyper-recombination protein 1; Mlp, myosin-like protein; Mft1, mitochondrial fusion targeting protein 1; Nab2, nuclear polyadenylated RNA-binding protein 2; Npl3, nucleolar protein 3; Pml, pre-mRNA leakage; Sgf11, SAGA-associated factor 11; Spt, Suppressor of Ty; Swt1, synthetically lethal with TREX protein 1; Taf, TBP-associated factor; Tra1, transcription-associated protein 1; Ubp8, ubiquitin-specific processing protease 8.

for further processing or degradation of the mRNA by highly specialized RNases. mRNA surveillance is among the best studied of these quality control pathways and it requires surveillance factors[108] such as the TRAMP complex, nuclear pre-mRNA downregulation protein 1 (Nrd1)–Nab3 complex or ribosomal RNA-processing protein 6 (Rrp6). Once identified, defective pre-mRNAs are targeted to the exosome complex to be degraded[108]. A growing number of factors are implicated in the retention and subsequent degradation of unspliced or malformed mRNPs in the vicinity of the NPC[87,91], including the nuclear envelope-associated establishes silent chromatin protein 1 (Esc1), the peripheral nuclear proteins pre-mRNA leakage protein 1 (Pml1), Pml39, the conserved PIN domain endonuclease synthetically lethal with Trex protein 1 (Swt1) and Nab2 (REFS 87,92,93,98,109). Although all of these proteins have been either genetically or functionally linked to the basket, the details about their individual roles remain unclear. Interestingly, the same basket-associated group of factors also seems to be involved in maintaining a normal level and distribution of the desumoylating enzyme ubiquitin-like protein 1 (Ulp1) at the nuclear periphery[88,92,94]. As cells lacking Ulp1 show altered protein sumoylation patterns in the cell and increased pre-mRNA leakage to the cytoplasm, these findings suggest that Ulp1-mediated desumoylation of unspliced pre-mRNA-coating factors might mark mRNPs for retention (and perhaps degradation) at the nuclear periphery[92,94].

Epigenetic control of gene expression. Chromatin is not uniformly spread throughout the nucleus but instead is organized into separate functional microenvironments or domains that are associated with the control of gene expression and the determination of cell identity. Chromatin organization is usually preserved across entire tissues and can even be conserved between species[110]. Among the best-characterized chromatin domains are those found at the nuclear periphery (reviewed in REF. 2). Association of chromatin with the nuclear envelope is generally thought to promote transcriptional repression, as heterochromatin or silenced loci can be found in association with the nuclear envelope across a diverse range of eukaryotic cell types, from budding yeast to avian erythrocytes[111,112]. However, recent evidence supports early hypotheses[49] and shows that, in some cases, the nuclear periphery can also promote gene activation[113]. Specifically, individual NPCs seem to represent regions of transition between transcriptionally repressive zones that are involved in chromatin stabilization and permissive zones organized around the basket that are conducive to mRNA biogenesis and transcription-coupled repair[99–101,114–118].

Initial evidence for this phenomenon emerged from studies of chromatin boundaries[114,116,119] and the rapid reactivation of inducible yeast genes. Such genes, which include inositol 1-phosphate synthase (*INO1*) and galactokinase (*GAL1*), localize throughout the nucleoplasm when repressed, but seem to be recruited to the vicinity of individual NPCs on activation[89,116,120,121]. Interestingly, when such genes are repressed again after a period of active transcription, they seem to retain a peripheral association — a sort of short-term memory — that facilitates faster re-activation. This is achieved through the specific interaction between gene recruitment sequences in promoters and the basket, by a mechanism that requires transcription-dependent memory gene loops and Gal1, and is dependent on the

switching deficient–sucrose non-fermenting (SWI–SNF) chromatin-modifying complex and the non-canonical histone variant H2A.Z[99,100,118,121,122].

Several models have been proposed to explain the initial recruitment and subsequent retention of active genes at the nuclear periphery (reviewed in REF. 2). The emerging consensus is that they require a cascade of spatially linked and functionally interdependent phases, each involving overlapping sets of proteins (FIG. 4). Thus, initial NPC anchoring might precede transcriptional initiation and depend on the interaction of the NPC with the 3′ untranslated region and promoter of specific genes[116,123]. Subsequently, the SAGA histone acetyltransferase complex might be recruited to the promoter, strengthening the attachment of the activated gene to the NPC by the multifunctional TREX2 complex[106,115,124–127]. The active gene–NPC tethering seems to also be aided by components of the THO complex and the TREX complex, which are involved in post-transcriptional pre-mRNP processing and export[106,115,128], and recent studies have shed further light on the molecular mechanism of these phenomena[129–131]. In particular, the crystallographic structure of a TREX2 subcomplex composed of the central region of Sac3 together with Sup1 and cell division cycle protein 31 (Cdc31), was recently obtained[129]. The results of this study indicate that Sac3 provides the central scaffold for the assembly of the entire TREX2 complex and that it might help relocalize SAGA-regulated genes to the NPC while working in concert with THO and TREX to load Mex67–Mtr2 (mRNA transport protein 2) onto maturing mRNPs in preparation for exit through the NPC. Although data is still limited, the recruitment of active genes to the basket might be a conserved mechanism of gene regulation throughout eukaryotic cells[101,103,125,132,133]. Thus, a basket-associated protein network would anchor a subset of active genes to the NPC, allowing the organization of an optimal microenvironment for the modulation of gene expression[91,99–101,103,123] in a model that is reminiscent of the classic gene gating hypothesis[49]. At the same time, this microenvironment could also function as an entry point for chromatin remodelling factors that effectively block the propagation of heterochromatin formation from adjacent nuclear envelope-associated repressive zones[106,121] and thereby establish an 'active barrier'[134] to gene silencing. This would enable the basket to maintain differential organization of chromatin at the nuclear periphery while avoiding overcrowding and keeping the translocation channel open for efficient transport[75,76,82,103]. As well as active genes approaching the vicinity of the NPC, it seems that a subset of Nups is capable of forming complexes on active genes in the nuclear interior, far from the nuclear periphery and NPCs, which might have a role in their transcriptional activation during development and cell cycle progression[135,136].

Chromatin maintenance and repair. A role has also been indicated for the NPC in the stabilization or repair of DNA ends, such as those found at telomeres or double-stranded DNA breaks (DSBs; reviewed in REF. 3). In yeast, there is a network of genetic interactions between DNA replication and repair factors on the one hand and components of the Nup84 complex on the other[137]. Furthermore, Mlp mutations increase the propensity for DNA damage and result in aberrations of telomere length[88]. Not surprisingly, Mlps genetically interact with the SUMO ligase methyl methanesulphonate sensitivity protein 21 (Mms21), which is part of the structural maintenance of chromosomes (SMC) complex involved in both DNA repair and chromatin organization[138] (FIG. 3). Moreover, NPC anchoring of the SUMO protease Ulp1 (REF. 88) helps to prevent the accumulation of DNA damage and has been linked to defects in the sumoylation pattern of cellular substrates such as the yeast Ku protein of 80 kDa (yku80) DNA repair factor[93]. Importantly, the re-establishment of correct Ulp1 levels at the nuclear envelope suppresses both aberrant SUMO modification patterns and DNA damage accumulation. A recent study has also revealed the involvement of the NPC in the SUMO-dependent DNA repair pathway[139], with several Nups found to genetically and physically interact with the synthetic lethal of unknown function protein 5 (Slx5)–Slx8 SUMO-dependent ubiquitin ligase complex[140]. Real-time imaging and chromatin immunoprecipitation have confirmed an association between the NPC and stalled DNA replication forks, which accumulate in the presence of artificially induced DSBs[139]. Taken together, these results indicate that persistent DSBs are recruited to the basket for an NPC-anchored, SUMO-dependent 'last resort' DNA damage repair pathway[141]. Research examining eroded telomere ends in senescing yeast cells has provided additional evidence that the NPC helps to direct damaged DNA to peripheral repair machineries[142]. This work showed that uncapped artificial telomeres recruit DNA replication, damage checkpoint and repair factors, such as radiation sensitive mutant protein 52 (Rad52), into peripheral foci resembling those formed during the DSB response. Strikingly, these foci were specifically associated with the NPC, unlike wild-type telomeric clusters.

To understand why the NPC might play a part in chromosome stability and repair (see also BOX 2), it is important to consider that actively transcribed loci are highly susceptible to DNA damage and require surveillance mechanisms in order to prevent widespread genome instability. Nucleotide-excision repair (NER) is a highly conserved means of DNA damage repair that recognizes severely distorted DNA structures, including intra-strand cross links arising from ultraviolet exposure[143]. Aside from global genome surveillance, NER is also responsible for transcription-coupled repair, which takes place on the transcribed strand of active genes[144]. In eukaryotes, the precise mechanism of transcription-coupled repair remains elusive, even though members of the SWI–SNF family of chromatin-associated helicases have been implicated in this process[145]. In addition, these mechanisms may involve the ubiquitylation and proteasome-dependent degradation of damage-stalled RNA polymerase II complexes[146]. Thus, the NPC seems to establish microenvironments at the nuclear periphery that bring together the DNA repair machinery and DNA most susceptible to damage (that is, transcriptionally active sites; see BOX 2).

TRAMP complex
(Trf4 or Trf5, Air1 or Air2 and Mtr4 polyadenylation complex). A protein complex that functions in RNA processing, degradation and surveillance. It polyadenylates various aberrant nuclear RNAs and thus labels them for processing or degradation by the exosome complex.

Exosome complex
A complex of several exonucleases arranged in a ring structure that, assisted by RNA helicases, degrade RNAs in the nucleus and cytoplasm.

SAGA histone acetyltransferase complex
(Spt, Ada, Gcn5 and acetyltransferase histone acetyltransferase complex). A large and highly conserved multiprotein complex required for the normal transcription of many genes.

TREX2 complex
(Transcription–export complex 2). TREX2 comprises Thp1, Sac3, Cdc31 and the Sus1 subunit of the SAGA complex involved in chromatin remodelling and transcriptional activation. TREX2 interacts with the NPC and is thought to have an important role in coupling SAGA-dependent gene expression to mRNA export.

THO complex
A multiprotein complex conserved among yeast and metazoans that is involved in mRNP biogenesis and export. In *S. cerevisiae* it consists of Hpr1, Mft1, Tho2 and Thp2. The human counterpart consists of the THO complex proteins THOC1–THOC7.

TREX complex
(Transcription–export complex). A complex that consists of components of the THO complex together with Yra1 (homologous to human THOC4) and Sub2 (homologous to human BAT1). The TREX complex interacts with the NPC through the non-Kap NTFs Mex67 and Mtr2, helping to anchor active genes to the nuclear periphery.

Box 2 | **NPC-associated microenvironments promote chromatin stability**

Recent work links telomeres and unrepaired or slowly repaired double-stranded DNA breaks to the SUN domain inner-nuclear envelope integral membrane protein, monopolar spindle protein 3 (Mps3)[141,159,160], which is required for spindle pole body (SPB) duplication, sister chromatid cohesion and meiotic bouquet formation[161]. This suggests that there is a specific perinuclear mechanism for handling unprotected DNA ends, in which Mps3-dependent recruitment of wayward, broken chromosomal ends to the nuclear periphery might have an important regulatory role — determining whether such ends are either recognized as telomeres to be capped by the telomerase machinery and stably anchored at inter-nuclear pore complex (NPC) zones of the nuclear periphery[162], or identified as damaged and shunted towards the basket to be repaired[163]. Interestingly, human SUN1 was found to have a role in NPC distribution in the plane of the nuclear envelope[164], thus reinforcing the idea of a close inter-talk between the NPC and other nuclear envelope components. The notion that this inter-talk might contribute to the formation of a nuclear envelope- and NPC-associated functional microenvironment that promotes chromatin stability was recently reinforced by observations involving ribosomal DNA and nucleolar structure[165], indicating that anchoring of the rDNA silencing machinery to the nuclear envelope by interactions with the spliced mRNA and cell cycle regulated protein 1 (Src1) LEM domain protein[166] is required for peripheral localization and stabilization of highly repetitive yeast rDNA sequences. Interestingly, human SUN1 was found to have a role in NPC distribution in the plane of the nuclear envelope[164], and Src1 was also found to participate in both the repression of sub-telomeric gene expression and the close coordination between transcriptional regulation and messenger ribonucleoprotein export[167], thus underscoring the existence of a functional interface between DNA repair, chromatin organization and the NPC. Further evidence of this connection comes from studies of the THO, TREX and TREX2 complexes. Lack of THO leads to a strong transcription-associated hyper-recombination phenotype and defects in nucleotide-excision repair[168,169], and several lines of evidence implicate the TREX and TREX2 complexes together with mRNA export factors and the NPC in preserving the integrity of actively transcribed DNA regions[146,170,171].

Coordination of cell cycle progression. NPCs have a finite lifespan, undergoing temporally coordinated assembly (and in many cell types, disassembly) in a manner that depends on cell cycle progression and the life cycle of the nuclear envelope (reviewed in REF. 147). In addition, the interplay between the nuclear envelope and the mitotic machinery is becoming increasingly clear[148]. In particular, the basket might have a role in orchestrating the rapid chromatin segregation events that occur during mitosis in both *S. cerevisiae* and metazoans. In *S. cerevisiae*, Mlps specifically interact with the core components of the spindle pole body (SPB) and have a poorly understood role in its assembly[90]. In the same species, the NPC acts as a docking site for the spindle assembly checkpoint (SAC) proteins mitotic arrest-deficient protein 1 (Mad1) and Mad2 (REFS 149,150) (FIG. 3). The association between Mad1 and the NPC depends on Nup60 and Mlps[151], although the functional relevance of this interaction is still unclear[152]. In addition, evidence for the basket's involvement in regulating cell cycle progression has been seen in human, *D. melanogaster* and *Aspergillus nidulans* cells[95–97,153,154]. In human cells, TPR was found to directly bind MAD1 and MAD2 (REF. 95), and small interfering RNA depletion of TPR disrupted the interphase localization of MAD1 and MAD2 at the NPC and led to impaired anaphase. In *D. melanogaster*, Megator is in a complex with MAD1, MAD2 and MPS1, localizes to the vicinity of the spindle and regulates SAC response, chromosome movement in anaphase and mitotic progression[97]. During mitotic exit, MAD1 and MAD2 are re-imported into the nucleus, and their re-association with the nuclear envelope occurs only after Megator has been recruited to the nascent NPC[153]. The functional relevance of the connection between the basket and the SAC was further underscored by observations indicating that TPR is responsible for faithful chromosome segregation during mitosis through its binding to dynein light chain[154]. These data indicate that TPR functions as a spatial and temporal regulator of the SAC, ensuring the efficient recruitment of MAD1 and MAD2 to the molecular motor dynein to promote proper anaphase progression. A similar role for TPR was also seen in *A. nidulans*[96], confirming the conservation of this basket function among opisthokonts (that is, fungi and metazoans). Why such a clearly important and highly conserved connection between the NPC and the control of cell cycle progression exists is a matter of debate, although important clues might derive from findings linking defective SACs with widespread genomic instability and cancer[155].

Gene gating hypothesis
The hypothesis in which "the nuclear pore complexes are envisioned to serve as gene-gating organelles capable on interacting specifically with expanded (transcribable) portions of the genome"[49].

Spindle pole body (SPB)
The only microtubule organizing centre found in *S. cerevisiae*. SPBs are embedded in the nuclear envelope throughout the yeast life cycle and their functions include chromosome segregation during mitosis and meiosis, and intracellular trafficking.

Spindle assembly checkpoint (SAC)
The SAC monitors the correct attachment of kinetochores to spindle microtubules before anaphase. Unattached kinetochores activate this checkpoint and cause cell-cycle arrest through the inhibition of the anaphase-promoting complex.

Conclusions

Although it was initially seen as little more than a container for the genome, our view has evolved to recognize the nuclear envelope and its associated structures as key players in nuclear organization and gene regulation. Recent findings suggest that there is an extensive network of interactions, stretching out from the basket and interlinking neighbouring NPCs to establish a far-reaching molecular platform that ties together gene expression, nuclear division and genome stability (FIG. 3). At the same time, the NPC also coordinates the interaction of nuclear contents with the surrounding cytoskeleton by way of its cytoplasmic filaments. Why might the NPC be involved in such disparate nuclear functions? It is possible that active chromatin loops are recruited to the NPCs so that their transcripts can be efficiently exported by their physical proximity to the NPC transport channel. This seems less likely, however, as many active genes function efficiently away from the NPC and the rate of intra-nuclear transcript diffusion does not seem to be a rate-limiting factor in gene expression. However, given that the nucleus is so devoid of physical 'signposts' that are not directly connected to chromatin, it could be that the baskets of NPCs are convenient markers where increased local concentrations of specific factors can induce the

formation of highly dynamic microenvironments. Chromatin might converge onto such microenvironments by loop formation for efficient transcriptional regulation, post-transcriptional processing and the stabilization and repair of damage arising during transcription or replication. Indeed, the NPC, together with the nuclear envelope and the nuclear periphery, can be viewed as just one essential component of a large cellular machine that regulates the passage of information from DNA out of the nucleus to control and maintain the rest of the cell.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
GAL1 | *INO1*
UniProtKB: http://www.uniprot.org
Cdc31 | DDX19B | Dyn2 | Esc1 | GLE1 | ku80 | Mad1 | Mad2 | Mex67 | Mms21 | Mtr2 | Nab2 | Nab3 | Nrd1 | Nup1 | Nup42 | Nup60 | NUP62 | Nup84 | NUP153 | Nup159 | Nup170 | NUP214 | NUP214 | NUP358 | NUPL2 | Pml1 | Pml39 | Rad52 | RAN | Rrp6 | Sac3 | Slx5 | Slx8 | Sup1 | Swt1 | TPR | UBC9 | Ulp1

FURTHER INFORMATION
Michael P. Rout and Caterina Strambio-De-Castillia's homepage: www.rockefeller.edu/labheads/rout

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