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Multifunctionality of F-rich nucleoporins

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Abstract

Nucleoporins (Nups) represent a range of proteins most known for composing the macromolecular assembly of the nuclear pore complex (NPC). Among them, the family of intrinsically disordered proteins (IDPs) phenylalanine-glycine (FG) rich Nups, form the permeability barrier and coordinate the high-speed nucleocytoplasmic transport in a selective way. Those FG-Nups have been demonstrated to participate in various biological processes besides nucleocytoplasmic transport. The high number of accessible hydrophobic motifs of FG-Nups potentially gives rise to this multifunctionality, enabling them to form unique microenvironments. In this review, we discuss the multifunctionality of disordered and F-rich Nups and the diversity of their localizations, emphasizing the important roles of those Nups in various regulatory and metabolic processes.

Introduction

The nuclear pore complex (NPC) is one of the largest macromolecular machineries in eukaryotic cells, composed of multiple copies of ~30 proteins called nucleoporins (Nups) [1,2]. Nups can be roughly classified into scaffold Nups, anchoring the NPC in the nuclear envelope (NE), and intrinsically disordered protein (IDP) Nups known as FG-Nups, containing phenylalanine-glycine (FG) repeats. The FG repeat is a weakly interacting hydrophobic motif, typically separated by more soluble linkers which probably counterbalance any tendency to bury the motif from the aqueous solvent. The intrinsic high dynamics and surface accessibility of the FG-Nups provide a means to expose those motifs rapidly when needed.

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Since the NPC is the gateway of the nucleus, FG-Nups are first and foremost known for their role in nucleocytoplasmic transport. However, more and more recent evidence shows the involvement of FG-Nups in a wide range of transport-independent cellular processes. Some FG-Nups are highly mobile and even found distant from the NPC (Figure 1A). In this review we will discuss their broad multifunctionality and versatility to summarize how FG-Nups contribute to various distinct cellular functions (Figure 2). How such huge diversity can be achieved by proteins that seem to have a simple architecture — in layman's term, they might be compared to spaghetti with multiple sticky patches — is the mechanism that we are only starting to grasp. By giving a brief tour through the different cellular functions that FG-Nups have been found in, we hope to inspire future research to find one or multiple functional concepts encoded into FG-Nup's and other intrinsically disordered protein sequences with similar architectures/designs. Although we focus and speculate on the possible contribution of disordered and F-rich domains, we want to stress that many Nups are multi-domain proteins (Figure 1B) and only partially disordered. To which extent all of the discussed mechanisms are related to the F-rich domain, is an open question in many cases and part of future research. We thus do not suggest that all functionalities described below can be accounted only towards being a disordered protein containing F's only, but we believe that understanding how such disordered but sticky chains could work, would unravel a fundamental working mechanisms to grasp multifunctionality concepts in biology.

FG-Nups form a permeability barrier for selective nucleocytoplasmic transport

The canonical role of FG-Nups is to constitute a selective permeability barrier in the central channel of the NPC. This barrier serves as a programmed gate for molecule exchange between nucleus and cytoplasm, and maintains certain microenvironments at both sides of the NE. The canonical function of FG-Nups has been reviewed multiple times, and we refer the reader to several extensive reviews [3,4]. Here we will only briefly review some key facts and existing questions.

The NPC obstructs the passage of large macromolecules where the passive diffusion gradually decreases with increasing molecular size [5]. The nucleocytoplasmic transport of larger cargos is typically facilitated by nuclear transport receptors (NTRs, e.g. karyopherins) through the interactions with the FG-Nup matrix, and generally but not necessarily coupled with the energy input from RanGTPase. It is still debated how the dense barrier of the NPC can maintain its high selectivity and simultaneously mediate the translocation of large cargo-complexes at high rates (~1000 molecules per second [6]). We note that human FG-Nups have more than twice as many Fs than FG repeats, and the role of the G likely adds to conformational freedom contributing further flexibility [7,8]. In that sense, the term FG-Nup can be misleading as in many models of nuclear transport only the number of FGs and not F's are considered. Some open questions around FG-Nups in the NPC are: What is the function/role of different motif flavors (F/FG/GLFG/FXFG/etc.) and what is the spatial localization and geometry of different FG-Nups? Are they brushes, gels or liquids [9–11]? Are their dynamic interactions obligatory? What is the role of glycosylation in metazoans which is absent in e.g. yeast? Can a single NPC import and export simultaneously equally

well? Are NTRs just temporary clients during active transport or integral parts of the barrier matrix [12,13]? How does the interaction between FG-Nups and the cargo complexes dilate/constrict the nuclear pore?

What we know is that the multivalency of the interaction between FG-Nups and NTRs is key to function. A single F binding to a NTR has very low binding affinity (hundreds of μM to low mM regime), hardly distinguishable from what might commonly be considered 'non-specific sticking' [11,14,15]. However, as FG-Nups have multiple Fs and are very dynamic (Figure 1C), unbinding of one F can easily be followed by binding of another F. This effective higher local concentration can substantially increase the binding strength. On top, NTRs like Importin- β , also have multiple binding sites, further increasing the multivalency in the system [14–17]. Due to the multivalency, FG-Nups can also phase separate (Figure 1D). *In vitro*-formed FG-Nup hydrogels [18,19] were previously shown to exhibit NPC-like permeability properties. Recently on a microfluidic chip the liquid state of FG-Nup phase-separated droplets was captured and reconstituted key features of NPC transport properties as well [11]. Though the technology to directly visualize the functional state of FG-Nups inside the NPC is still lacking, these *in vitro* discoveries can provide clues into the transport machinery of the NPC.

FG-Nups in cilia function

Cilia are membrane-bound microtubule-containing projections with important roles in sensory functions, signaling, motility, and cellular development [20]. To maintain the ciliary compartment for functioning and regulate the shuttling of cytoplasmic molecules, a ciliary pore complex (CPC) might be assembled at the ciliary base [21], where several FG-Nups including Nup62 and Nup98, were reported to localize [22,23]. siRNA-mediated knockdown of Nup98 altered protein transport into the cilia, resulting in shorter cilia due to increased tubulin efflux from the ciliary matrix [21]. However, a wide range of Nups could not be reliably detected at the bases of cilia using mAb414 (recognizing several FG-containing Nups) and other Nup-specific antibodies [24,25]. Moreover, 3D super-resolution images revealed two barrel-like Nup188 structures encasing the basal body and daughter centriole, but not residing in the transition zone of ciliary bases where protein entry into the cilium lumen is believed to be regulated [25]. Further dissections of the structures and dynamics of FG-Nups at the ciliary base will be helpful to elucidate whether they are the building and functional blocks of the ciliary diffusion barrier or have other functions.

FG-Nups in membraneless organelles and functional bodies

P granules and P bodies

P granules are messenger ribonucleoprotein (mRNP) granules formed by liquid-liquid phase separation (LLPS) in the germline of *C. elegans* [26]. Interestingly, the *C. elegans* homologue of the vertebrate Nup98 (CeNup98) was found to be a pivotal factor for maintaining the integrity of P granules [27]. CeNup98 associates with maternal P granule-enriched *nos-2* mRNA, engaging in its natural translational repression. Processing bodies (P bodies), another class of liquid droplet-forming mRNP granules, act as both mRNA decay and storage sites in eukaryotic cells [28]. siRNA-mediated depletion of Nup358

led to an impaired P body assembly [29]. Taken together, these findings emphasize the important roles of FG-Nups in membraneless organelle formation, mRNA regulation and gene regulation (as discussed below).

Stress granules

Stress granules (SG) are cytoplasmic condensates formed by association of proteins and mRNAs released from ribosomes upon stress. Using a combination of experiment and modeling, recent studies provide an exciting mechanistic insight into the relationship between SGs and LLPS [30–32]. Several FG-Nups and NTRs have been found to localize to SGs [33–35], which seem to disrupt nucleocytoplasmic transport. Interestingly, the localization of FG-Nups in SGs depends on the stress inducer. Sodium arsenite and tubercidin are compounds that can trigger a cellular stress response. These chemical agents have distinct profiles of action — arsenite exposure leads to oxidative stress, while tubercidin gets incorporated into DNA and RNA to affect protein synthesis — however, both inducers cause eIF2 α phosphorylation with following inhibition of translation and SG assembly. Nup153 was found in SGs under tubercidin stress, but not under arsenite stress [35]. Moreover, Nup358, Nup214 and Pom121 do not equally localize to SGs upon different conditions of arsenite-induced stress. Further analysis about the molecular network in different SGs would help to reveal how the inhibition of nucleocytoplasmic transport help cells cope with various stress conditions. On the other hand, the mislocalization and aggregation of FG-Nups in SGs induced by protein misfolding can sometimes be detrimental, which will be discussed in section FG-Nups in DNA repair.

Annulate lamellae

Annulate lamellae (ALs) are cytoplasmic organelles of stacked ER membrane sheets that are penetrated by structures containing most Nups, called AL-NPCs [36]. ALs are found to be abundant in gametes and embryonic cells and were proposed to serve as NPC storages for early development [37]. However, ALs are also represented in cancer, somatic and plant cells and their general function might thus go beyond NPC storage but still remains elusive.

During AL biogenesis, AL-NPC formation occurs on ER membranes. A recent study highlights the important role of Nup358 in AL-NPC assembly via distinct mechanisms proceeding in the absence of a nuclear compartment [38], where various granules with different compositions are transported along microtubules and regulated by Nup358 in concert with Ran and NTRs. Remarkably, the AL-NPC assembly proceeds from Nup358-containing granules rather than soluble Nups. These granules recapitulate many hallmarks of biomolecular condensates — membraneless organelles driven by phase separation. Other known membraneless organelles, such as P bodies and SGs were observed to locate close to ALs containing Nup358 and the dynamic interplay between mRNP granules and Nup358-positive ALs was visualized with live cell imaging [29]. Nup358 is also involved in association of the miRNA-induced silencing complex (miRISC) with its target mRNA and ALs might thus be one of the sites where gene regulation via the miRNA pathway takes place.

FG-Nups as key players in genome organization, maintenance and gene expression

FG-Nups regulate gene expression

The earliest hypothesis of ‘gene gating’ goes back to 1985 and postulated a model in which tethering of genes to the NPC coordinates transcription, mRNA maturation, and export [39]. Since then, many studies provided evidence that NPCs and their components indeed contribute to gene regulation. In yeast, several inducible genes localize to NPCs upon activation as well as the heat-shock locus *hsp-16.2* in *C. elegans* (reviewed in [40,41]). The depletion of e.g. Nup1 leads to the loss of *GALI* tethering in yeast [42]. Recently, Scholz et al. also provided evidence [43] of a (pathological) gene gating mechanism in mammalian systems: the oncogenic super-enhancer (SE) for *MYC* expression associates with NPCs and enables rapid export of *myc* mRNA in colon cancer cells.

In general, the mechanisms underlying gene regulation at the NPC and by NPC-independent Nup activity are quite diverse and not only coupled to transport. Interaction of the NPC/Nups with the genome can e.g. be mediated through interaction with chromatin-remodeling proteins or histone modifiers (reviewed in [44]), enhancers — and specifically, cell fate-determining SEs [45] —, transcription factors ([46], among others), polycomb domains [47] and possibly by mediating chemical modification as e.g. desumoylation of other factors (reviewed in [41]). Remarkably, in most of these processes not only nuclear envelope associated Nups are involved and tethering to the NPC for gene regulation is not observed in all cases. On the contrary, in some cases dynamic Nups, which are able to shuttle off the pore [48], interact with the genome in the nucleoplasm, adding another layer of spatiotemporal complexity. As an example, Nup98 as well as Nup50 and Nup62 has been shown to interact with genomic sites — highly enriched in developmental or cell cycle genes — in the nucleoplasm and at the NPC in *Drosophila* [49]. Expression levels of Nup98 had a clear impact on gene expression in the case of nucleoplasmic interaction, whereas expression levels of Nup98 did not have a strong impact on gene expression for genes tethered to the NPC, suggesting that Nups might have different functionalities in gene regulation depending on the spatial localization and local context as interaction partners like e.g. helicases [50] or histone-modifiers [51].

Nup-mediated regulation of cell development (reviewed in [52,53] among others) is a recurrent theme as also the mammalian Nup153 binds cell-type-specific SEs in the nuclear periphery [45], controls differentiation of neural progenitor cells in a bimodal fashion [46] and upholds pluripotency of mouse embryonic stem cells [54]. The regulation of cell differentiation by Nups is timing sensitive and correlates with the progression of differentiation. The expression levels of Nup153 are lower in neuronal progenitor cells than in embryonic stem cells, and again lower in neurons [46,54], whereas in human embryonic stem cells Nup98-binding patterns of cell-type-specific genomic sites as well as NPC association depends on the developmental stage [55].

Posttranslational modification of Nups themselves also regulates gene expression. In budding yeast cell development and division depend on deacetylation of NPCs in daughter

cells [56]: deacetylation of the central channel Nups increases the nuclear concentration of the transcription factor Whi5, and deacetylation of Nup60 and Nup49 promotes tethering of the *CLN2* locus to the NPC, prolonging G1 phase. Additionally, phosphorylation of Nups regulates gene association/dissociation with the NPC (reviewed in [41]). Although Nups and especially their FG-domains are heavily glycosylated in multicellular organisms, the role of Nup glycosylation and if it is only antagonistic to phosphorylation in gene regulation so far remains elusive [57].

Except for regulation of inducible and developmental genes, tethering of genes to NPCs in yeast serves transcriptional memory and depends on Nup100, whereas in mammals it depends on the Nup100 homologue Nup98. Additionally, Nup98 promotes antiviral gene expression in *Drosophila* [58].

Although several studies report on an essential contribution of Nups to gene regulation and some also provide a molecular model, in many cases the question as to where the interaction of the genome or transcriptional/regulatory factors with the Nups takes place is unclear. Thus, if in general there is one or multiple underlying common principles for gene regulation by Nups — potentially based on the F-rich disordered domain of many Nups and their ability to phase separate — remains an open question (discussed also in [59]). As discussed above, multiple interaction partners have been identified for different Nups and although FG-Nups indeed play an important role in gene regulation, this is also true for structured and membrane-bound Nups. In which cases the disordered FG-region is crucial to gene regulation and in which cases the NPC or structured domains of FG-Nups are key elements remains to be investigated.

FG-Nups in DNA repair

Another striking example of NPCs as platforms for genome organization is the relocation of DNA double-strand breaks (DSB) to yeast NPCs for repair (reviewed in [60]). Several Nups have been identified as factors that might be involved in DNA repair as their depletion leads to hypersensitivity to DNA damage. Among those Nups, not only structured scaffold Nups have been identified, but also disordered FG-Nups like Nup60 [61], and Nup49 (reviewed in [60]). Although in mammalian cells there is no such dramatic genome rearrangement upon DNA damage [62], the involvement of Nups in DNA damage response is conserved. Notably, a recent study linked all central channel FG-Nups, in particular Nup54, to DNA repair [63]. The nuclear basket proteins Nup153, Nup50, in concert with the structured basket protein Tpr have also been associated with transport-independent functions in DNA repair, specifically sumoylation of the repair factor 53BP1 through localization of SENP1 to the NPC by Nup153 [64], as well as targeting 53BP1 to DNA repair foci by both Nup153 and Nup50 [65]. Nup-mediated sumoylation of DNA repair proteins is a mechanism that has also been observed in yeast, where Nup60 in concert with the structured Nup84 is crucial for the sumoylation of Yku70 [61]. Although most of the studies at this point do not provide a comprehensive picture of how Nups contribute on a mechanistic and molecular level to DNA damage repair, further studies could provide information on whether the role of FG-Nups in DNA repair is linked to their ability to phase separate and form microenvironments that facilitate function of the repair machinery. We also note that the yeast nuclear basket

proteins MLP1 and MLP2 have been implicated in the prevention of pathological R-loop formation — three-stranded DNA:RNA hybrids — by NPC tethering in *Saccharomyces cerevisiae* [66].

FG-Nups are crucial players in mitosis

Beyond the role in gene regulation and DNA repair, FG-Nups also regulate mitosis via various mechanisms at different stages, promoting the accurate progression of cell division. RanBP2/Nup358 binds to the Bicaudal D2 (BICD2) motor adaptor in late G2 phase [67] and thus participates in the positioning of the nucleus and the centromeres before and during mitotic entry for correct assembly of the bipolar mitotic spindle and chromosome segregation. Nup358 and Nup153 are also known to recruit the coatomer complex COPI to both sides of the NPC for efficient NE breakdown at prometaphase [68]. Additionally, Nup358 in complex with the transport factor RanGAP1 plays a crucial role in the stabilization of microtubule-kinetochore association [69].

FG-Nups also contribute to the spindle assembly checkpoint (SAC) that prevents chromosome missegregation and aneuploidy in the daughter cells by delaying the metaphase-anaphase transition until all chromosomes are revised for the proper attachment to the spindle microtubules. Only then the anaphase-promoting complex (APC/C) should become active leading to the release of sister chromatids for their following separation. In this process, the Nup98-Rae1 complex modulates the activity of the APC/C [70]. Alternatively, Nup98 fusion oncoproteins (as discussed in the next section) were revealed to keep APC/C in an active state [71]. Furthermore, both overexpression and depletion of Nup153 negatively affected cell division. A model was proposed to explain the obtained results: high levels of Nup153 lead to hypophosphorylation of spindle checkpoint mediator Mad1 that disturbs initial SAC function, whereas low levels cause hyperactivation of SAC which results in cytokinesis abnormalities [72]. In yeast, where closed mitosis proceeds without NE breakdown, FG-containing Nup53p is associated with Mad1 and Mad2 checkpoint proteins at the NPC throughout the cell cycle [73]. It has been shown that after SAC activation Mad1 cycles between NPC-binding sites (Nup53p-containing subcomplex, MLP foci) and kinetochores, whereas Mad2 translocates to kinetochores [73–75]. Interestingly, Mad1 cycling affects nuclear import through the NPC during SAC arrest via Kap121p transport inhibitory pathway [76]. Nup53p binds to Kap121p with relatively high affinity by means of an allosteric effect modulated by the C-terminal intrinsically disordered region and RRM domain [77]. However, such Nup53p conformation appears to be not available at the NPC structure, where Nup53p interacts with Mad1 and neighbor Nup157p and Nup170p. Release of Mad1 leads to NPC structural rearrangements following high-affinity interaction between Nup53p and Kap121p that blocks Kap121p-cargo passage through the pore. Such transport inhibition might be useful to exclude factors from the nucleus that antagonize SAC arrest, as it was demonstrated for Glc7p phosphatase [76].

At the final stage of mitotic progression, FG-Nups are also involved in mitosis regulation. The deletion of FG-domains in Nup153 results in severe defects in cytokinesis, highlighting the importance of Nup153 FG-domain for proper cytokinesis realization [78]. Additionally, Nup62 is colocalized with centrosomes [79] and Nup58 — with centrosomes and midbodies

[80]. Depletion of these proteins led to aberrant spindle formation, defective cytokinesis, and delayed abscission.

FG-Nups in diseases

Oncogenic fusion proteins

In rare but recurrent cases, FG-Nups are expressed as fusion proteins as a consequence of chromosomal translocations (see also excellent reviews in ref. [81–83]). A chromosomal translocation involving Nups usually goes along with hematological cancer in the affected patients — specifically acute myeloid leukemia (AML) in most cases — and poor prognosis and treatment outcome. Among the different Nups involved in cancerous diseases, Nup98 stands out: as much as 30 different fusion partners (e.g. transcription factors, histone-binding proteins) have been identified, which strikingly share a common pattern with C-terminal fusions of the partner gene to the N-terminal FG repeats of Nup98. In most leukemia-associated Nup214 fusion proteins the FG repeats are also preserved in the fusions [84]. The mechanism(s) by which FG-Nup-fusion proteins contribute to leukemogenesis are not yet entirely understood and most likely also vary with the (dys)functionality of the different partner genes. Computational studies also revealed a prevalence of disordered fragments in other oncogenic fusion proteins, e.g. the disordered N-terminal parts of FET family fusion proteins (FUS, EWSR1 and TAF15) are often present in leukemia [85]. It is therefore tempting to speculate about a common leukemogenic pathway in which the disorder provides the flexibility or platform for different fusion elements to synergistically disturb a wide set of regulatory and metabolic processes in the cell.

FG-Nups in neurodegenerative disease

Since Nups participate in diverse types of metabolic and regulatory processes, it is not surprising that dysregulated expression and/or a less dramatic mutation than a chromosomal translocation of Nups can lead to other severe conditions (nicely reviewed in [82,86]) such as neurodegenerative diseases. A typical phenomenon in neurodegenerative diseases is the aggregation of disease-associated proteins in the cytoplasm and/or nucleoplasm, as well as mRNA accumulation in the nucleus. It has been observed that Nups and NTRs can be mislocalized in neurodegenerative diseases, sometimes colocalizing with pathological protein aggregates. As discussed in detail by Hutten and Dormann [87], although Nups are involved in neurodegenerative diseases, the current state of research does not provide a profound mechanistic model and cannot answer the question if the dysfunction of Nups is a ‘cause or consequence’ of the disease. It can be speculated that impaired function and mislocalization of Nups and NTRs could lead to impaired nucleocytoplasmic transport and mislocalization of cargo proteins and mRNA, but also potentially of transcription factors necessary to uphold the non-pathological state of the cell. Another possible scenario takes into account the gene regulatory properties of Nups: their up- or downregulation as well as mutation or mislocalization might cause deleterious alterations in the expression of neuroprotective genes. For example, expression levels of Nup153 — whose transport-independent interaction with the transcription factor Sox2 has recently been shown to be essential for the regulation of the cellular state of neuronal stem cells (NSCs) [46] — are sensitive to oxidative and nitrosative stress as can be caused by A β accumulations, which

affects proliferative capacity, differentiation and electrophysiological properties of NSCs in an Alzheimer's disease mouse model [88]. The broad multifunctionality of Nups will make it interesting to further investigate how different Nups might contribute to different neuro-degenerative phenotypes and through which mechanism.

Viral-associated organelles

FG-Nups are widely associated with distinct viral interactions, promoting the development of the viral life cycle. Nuclear import of viruses (e.g. lentiviruses) implies the hijacking of the NPC transport machinery. Nup358 and Nup153 are known to mediate the nuclear translocation of the HIV-1 pre-integration complex through nuclear pores [89–91]. Interestingly, crystal structures show that HIV-1 capsid binds to Nup153 in a FG-dependent manner [91]. It is therefore tempting to speculate if the capsid can mimic NTRs like properties to facilitate its nuclear import. Besides the function of escorting viral particles/components across the pore, FG-Nups were shown to be required for various steps of viral biogenesis, such as integration into the host genome, regulation of transcription, viral replication, assembly and morphogenesis of viral particles and action in host defense evasion [92].

FG-Nups localized outside the NPC were found to accumulate at sites of viral components. In hepatitis C virus (HCV)-infected cells, FG-Nups together with scaffold Nups and NTRs were shown to accumulate in special membrane structures, termed the membranous web [93–95]. These cytoplasmic compartments represent a special local environment for viral replication and assembly, containing essential factors for the viral life cycle and excluding host cell pattern recognition receptors [96]. The depletion of various Nups disturbed the HCV life cycle at different stages. In particular, Nup98- and Nup153-depleted cells showed lower intracellular and extracellular levels of viral RNA [93,94]. Proteomics studies also identified Nup98 as a component of HCV virions [94]. NLS-containing substrates were demonstrated to localize to the cytoplasmic regions occupied by the membranous web, thus implying the ability of Nups to create a permeability barrier between cytosol and viral-induced compartments in order to maintain a specialized microenvironment for viral replication and assembly [93,96]. In another study, Nup358 and Nup62 were recruited to vaccinia virus factories in a spatiotemporal manner, where Nup358 was involved in the targeting of Nup62 [97]. Recruitment of the translation regulator eIF4E was dependent on Nup358 levels. Nup358 knockdown led to a decrease of viral yield as well as a reduction of the size and amount of viral factories without a significant effect on viral DNA replication. The authors speculated that Nup358 might have an impact on the vaccinia virus morphogenesis and regulation of host and/or viral mRNA translation [97].

Concluding remarks

FG-Nups are astonishingly versatile in their functions (see also [98]) and regulate cellular metabolism in the cytoplasm as well as in the nucleoplasm. They can be very specific in their interactions and although their involvement in certain cellular processes is conserved across species, the actual mechanisms may vary from species to species. They seem to play a crucial role in determining cellular fate and in this their expression and action is highly

timing sensitive. As a consequence, a dysfunction of FG-Nups, due to mislocalization, mutation or fusion through chromosomal translocation leads to severe pathologies. Although FG-Nups can also contain structured domains which interact with specific partners, it is tempting to assume that for many of the above discussed mechanism an underlying common principle or mechanism exists that is related to the disorder and multivalency of F's occurring in FG-Nups. Observations that e.g. different FG-Nup transcription factor fusions for Nup98 and Nup214 all lead to very similar pathological phenotypes point into this direction, but then again, also other intrinsically disordered proteins fusions that do not have an F-rich domain can lead to similar phenotypes. The disordered nature of F-rich Nups is very likely able to create unique local environments, which might facilitate some interactions or enhance local concentrations of interaction partners. In future studies, it will be interesting to investigate to which extent their unique properties and behavior contribute to or are essential for correct cellular regulation processes and metabolism.

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Perspectives

- FG-Nups are disordered proteins with unique properties and behavior. While mainly known as constituents of the selective permeability barrier, they actually participate in a wide range of different cellular processes besides nucleocytoplasmic transport and can be found in the nucleoplasm as well as in other cellular compartments.
- FG-Nups are essential to regulate cellular functions and determine cell fate. Based on their disordered nature different models for underlying common interaction principles in the nucleoplasm and at the NPC have been hypothesized, but the understanding of how and to which extent multivalent interactions contribute is still limited.
- A sequence to function relationship is in general harder to establish for intrinsically disordered proteins than folded proteins. It will be interesting to investigate how different cellular processes involving FG-Nups are intertwined and how the disordered nature of FG-Nups contributes and guarantees the stable regulation of cellular metabolism and development. In context of diseases, studying dysfunctional F-rich Nups will be crucial.

Figure 1. Properties of FG-Nups.

(A) FG-Nups are not exclusively found in the nucleus, but also in other cellular compartments (marked in blue). AL: annulate lamellae, ER: endoplasmatic reticulum, NTR: nuclear transport receptor. (B) The architecture of a typical FG-Nup contains multiple structured domains with dedicated functions and a disordered F-rich stretch, containing multiple F and FG motifs (here drawn as blue dots). The human NPC contains >14 000 Fs and >5000 FG repeats in disordered Nups. (C) Due to their intrinsically disordered nature, FG-Nups have high intramolecular dynamics and conformational plasticity. The conformational state of FG-Nups at specific cellular sites is largely unknown. (D) FG-Nups contain multiple weak interaction sites and can undergo phase separation due to multivalent interactions with themselves and other proteins. In some phase separated states/droplets FG-Nups were also found to colocalize with other DNA/RNA or proteins.

Figure 2. Pie chart of different cellular functions of FG-Nups.

The yellow outer ring represents the localization of functions in cytoplasm, the blue inner ring — in nucleoplasm. TF, transcription factor; CR, chromatin remodeler; R, repressor.