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REVIEW ARTICLE

The Hitchhiker's Guide to Nucleocytoplasmic Trafficking in Neurodegeneration

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Abstract

The widespread nature of nucleocytoplasmic trafficking defects and protein accumulation suggests distinct yet overlapping mechanisms in a variety of neurodegenerative diseases. Detailed understanding of the cellular pathways involved in nucleocytoplasmic transport and its dysregulation are essential for elucidating neurodegenerative pathogenesis and pinpointing potential areas for therapeutic intervention. The transport of cargos from the nucleus to the cytoplasm is generally regulated by the structure and function of the nuclear pore as well as the karyopherin α/β, importin, exportin, and mRNA export mechanisms. The disruption of these crucial transport mechanisms has been extensively described in the context of neurodegenerative diseases. One common theme in neurodegeneration is the cytoplasmic aggregation of proteins, including nuclear RNA binding proteins, repeat expansion associated gene products, and tau. These cytoplasmic aggregations are partly a consequence of failed nucleocytoplasmic transport machinery, but can also further disrupt transport, creating cyclical feed-forward mechanisms that exacerbate neurodegeneration. Here we describe the canonical mechanisms that regulate nucleocytoplasmic trafficking as well as how these mechanisms falter in neurodegenerative diseases.

Keywords ALS/FTD · Huntington's disease · Alzheimer's disease · Proteinopathy · TDP-43 · FUS

Introduction

Neurodegenerative diseases describe a highly heterogeneous set of disorders where progressive and irreversible neuronal death leads to detrimental symptoms dependent upon the subset of neurons that are impacted. While patients will exhibit various degrees of heterogeneity in regard to the specific brain regions involved in their disease as well as the severity of symptom onset and progression, for most patient populations, the progression of these diseases will slowly cost patients their independence and ultimately their lives. While promising clinical trials appear to be on the horizon, very few existing therapeutics have disease modifying effects. A substantial hurdle to developing effective therapeutics is the lack of causative and targetable cellular pathways that directly lead to neurodegenerative phenotypes. Therefore, comprehensive understanding of the pathogenesis of neurodegenerative diseases is critical for finding successful therapies. Here we review the possible mechanisms leading to neurodegeneration in the context of altered nucleocytoplasmic transport.

The movement of proteins, RNA and other cargos between the nucleus and the cytoplasm broadly encompass the cellular mechanisms controlled by nucleocytoplasmic transport machinery. There is substantial evidence to suggest an association of nucleocytoplasmic trafficking deficits with neurodegeneration [1–5]. Furthermore, an overarching theme that has emerged in recent years is that the failure of cargo transport across the nuclear envelope is not unique to a single neurodegenerative disease, nor one genetic insult [1–5]. However, more work remains to be completed to determine, precisely, how nucleocytoplasmic trafficking deficits may cause neurodegeneration. To properly survey the disrupted landscape of nucleocytoplasmic trafficking in neurodegeneration, it is essential to understand how a

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healthy cell manages nucleocytoplasmic transport. Thus, we first review the principles and components of canonical nucleocytoplasmic transport, and then follow with how these highly regulated transport mechanisms are proposed to be disrupted in several neurodegenerative diseases.

Canonical Nucleocytoplasmic Transport

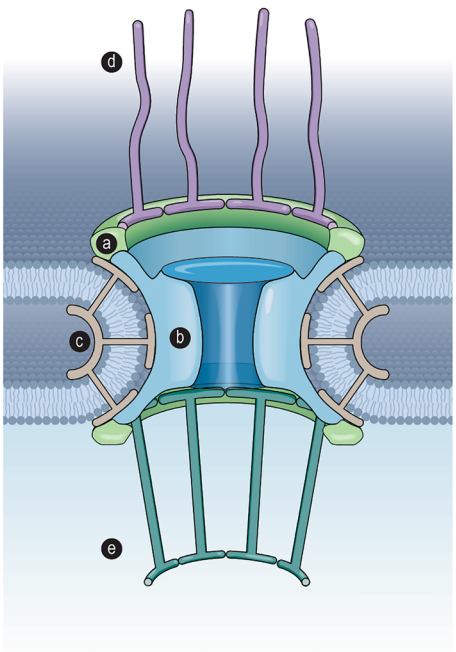
Structure and Architecture of the Nuclear Pore

The nuclear membrane acts as a critical divide between the nuclear and cytoplasmic compartments of all eukaryotic cells [6, 7]. Its structural architecture provides a semi-permeable separation between the nucleus and the cytoplasm, which allows cytoplasmic access to genetic material, creating opportunities for complex gene regulation responses to stimuli outside of the nucleus. Unknown structures identified on the nuclear envelope using electron microscopy in the 1950s were first hypothesized to be regularly interspaced fusions of the inner and outer nuclear membrane and were later termed "nuclear pores" [8, 9]. These highly conserved structures are present in all eukaryotes, and are likely the result of early evolutionary events [10]. At the center of each nuclear pore is the massive, ~ 125,000 kDa nuclear pore complex (NPC) [8, 10–12]. The NPC displays eight-fold symmetry around a central channel, and additionally exhibits symmetry on an axis perpendicular to the central channel; where there exists a symmetrical nuclear and cytoplasmic ring on either side of the inner ring complex [12–14]. Additionally, asymmetric components of the NPC, the nuclear basket and cytoplasmic filaments, extend processes out from the NPC [14].

Each NPC consists of ~ 500–1000 proteins, which—due to the symmetry of the NPC—are comprised of multiple copies of only about 30 unique proteins known as nucleoporins (Nups) [14–16]. These ~ 30 unique Nups can be categorized into 5 groups, based on their structural role in the NPC: the coat nucleoporins, inner ring nucleoporins, transmembrane nucleoporins, cytoplasmic filament nucleoporins, and the nuclear basket nucleoporins (Fig. 1). Although the structure of nucleoporins and NPCs are highly conserved, they display poor genetic conservation, suggesting that broad structures not conserved by genetic sequence still perform similar functions across species [17, 18]. Figure 1 provides the human nomenclature, structural component, and the potential role in disease for each Nup [14, 19–24].

Coat Nucleoporins

This subset of Nups dictates the structure of the nuclear and cytoplasmic rings (Fig. 1a). In humans this "Y" shaped structure is composed of: Sec13, Seh1, Nup96, Nup75, Nup107, Nup160, Nup133, Nup37, Nup43, and ELYS [25,



Nucleoporin	Component of the NPC	FG-Repeats	Disease Associations				References
			ALS	HD	AD	Others	
Sec13	Coat Nups					R	[12,19,22,31]
Seh1	Coat Nups		↑				[12,19,31,114]
Nup96	Coat Nups						[12,19,31]
Nup75	Coat Nups						[12,19,31]
Nup107	Coat Nups		↓ M T			Δ R	[12,19,22,31,89,92,113,114]
Nup160	Coat Nups		↓ M			Δ P	[12,19,22,31]
Nup133	Coat Nups					Δ R	[12,19,22,32]
Nup37	Coat Nups					Δ	[12,19,22,32]
Nup43	Coat Nups						[12,19]
ELYS	Coat Nups						[12,32,20]
NDC1	Transmembrane Nups		↑			M Δ	[12,19,22,31,87]
POM210	Transmembrane Nups		M			Δ R	[12,19,22]
POM121	Transmembrane Nups		M			Δ	[12,20,22,92]
Nup205	Inner Ring Nups		M T			Δ	[12,19,22,31,89,92]
Nup188	Inner Ring Nups					Δ R	[12,20,22,31]
Nup93	Inner Ring Nups		↑ M			R P	[12,21,22,31,92,114]
Nup155	Inner Ring Nups		↓ M			Δ	[12,21,22,31,92,114]
Nup53	Inner Ring Nups		M				[12,19,92]
Nup54	Inner Ring Nups	✓				Δ R	[12,22,19,31]
Nup58	Inner Ring Nups	✓	M			Δ	[12,19,22,31,92]
Nup62	Inner Ring Nups/Cytoplasmic Filaments	✓	↑ M T	M T	T	Δ PP	[12,21,22,31,90,92,114,196,197]
Nup98	Inner Ring Nups/Cytoplasmic Filaments	✓	↓ M		T	Δ R	[12,21,22,23,31,92,113]
Rae1	Cytoplasmic Filaments					R	[12,21,22,31]
Nup42	Cytoplasmic Filaments	✓					[12,23,31]
Nup88	Cytoplasmic Filaments		M T	M		R	[12,20,22,31,90,92,196]
Nup214	Cytoplasmic Filaments	✓	M			Δ R	[12,20,22,31,92]
DDX19	Cytoplasmic Filaments						[12]
Gle1	Cytoplasmic Filaments		↑ M			Δ	[12,22,24,31,113]
RanBP2	Cytoplasmic Filaments		M			Δ R	[12,19,22,92]
Nup153	Nuclear Basket	✓	↑ M T			Δ R P	[12,19,22,92,113,196]
Nup50	Nuclear Basket	✓	↓↑				[12,20,31,113,114]
Tpr	Nuclear Basket		↑			Δ R	[12,20,22,31,114]

Genetic Modifiers: ↑ = enhancers, ↓ = suppressors; Mislocalization: T = Tissue, M = Disease Model; Expressioin: PP = Protein, RR = RNA; Mutation = Δ

Fig. 1 The structure and architecture of the nuclear pore complex. The structure of the human nuclear pore complex is comprised of 5 major groups. Coat nucleoporins (*a*), inner ring nucleoporins (*b*), transmembrane nucleoporins (*c*), cytoplasmic filaments (*d*), and the nuclear basket (*e*). Right panel: structural grouping of the human nucleoporins and the associations with ALS, AD, HD, and other non-neurological diseases

26]. 16 coat nucleoporin complexes make up each of the nuclear and cytoplasmic rings by intricate interlocking of the upper arm with the center stalk of the "Y" region [12, 27]. The topographical structure of these complexes has been somewhat conserved, but species divergence does exist [25, 27].

Inner Ring Nucleoporins

Forming the central channel of the NPC, the inner ring structure contains Nup205, Nup188, Nup93, Nup155, Nup53, Nup54, Nup58, Nup62, Nup98 (Fig. 1b) [15, 28]. These Nups crystal structures have revealed that flexible linkers mediate the interaction of this complex, allowing for the flexibility of the inner ring [29]. The flexibility of these Nups and their ability to interact with cargos play crucial roles in proper trafficking through the nuclear pore [12].

Transmembrane Nucleoporins

The pore membrane proteins (POMs) act as anchors to secure the NPC in position by utilizing a transmembrane domain to interact with the nuclear envelope (Fig. 1c). The human nuclear pore contains NDC1, POM210, and POM121 [12, 15, 30]. These components are some of the most poorly characterized features of the NPC with regard to their structure and function [12, 30]. While the sequence conservation of POMs is particularly poor, it is likely that the function of transmembrane nucleoporins is conserved [12, 30]. POMs contain large often unstructured regions that have been hypothesized to interact either with the nuclear envelope or with the soluble components of the NPC [30]. While little is known about the function and regulation of human transmembrane nucleoporins, deletion of POMs in fungi produces no overt survival phenotype and they have been shown to be nonessential for cellular function [31, 32]. However, some studies indicate that these proteins play an important role in NPC assembly and nuclear membrane structure. Indeed, evidence suggests POM121 and NDC1 are important during NPC biogenesis and nuclear membrane homeostasis [33, 34].

Cytoplasmic Filaments and the Nuclear Basket

These two components of the nuclear pore are considered asymmetric because they have rotational symmetry like the components above, but not symmetry across the nuclear envelope. The cytoplasmic filaments are long projections that reach into the cytoplasm from the NPC (Fig. 1d) [35]. They consist of the following nucleoporins: Rae1, Nu42, Nup88, Nup214, DDX19, Gle1, and RanBP2 (also commonly referred to as Nup358) [12]. A principal role of these Nups is the spatial restriction of DDX19 and RanBP2, which are utilized for bulk mRNA and protein export, respectively, across the NPC to the cytoplasm [12, 36–38]. On the opposite side of the nuclear membrane, the human nuclear basket nucleoporins (Nup153, Nup50, and Tpr) also play roles in the organization of transport machinery across the nuclear pore by facilitating the recognition and binding of nuclear import and export factors in the nucleus (Fig. 1e) [12, 36].

Passive Diffusion Through the Nuclear Pore

The NPC regulates trafficking through many mechanisms. The first degree of regulation of transport through the nuclear pore is the diffusion barrier created by the intrinsically disordered phenylalanine—glycine (FG)-rich repeats [10, 12]. FG-repeats are common structural components of different nucleoporins spanning different structural regions and play multiple roles in regulating trafficking through the NPC. These FG-repeats form an intrinsically disordered domain in the central channel of the NPC that allows for the passive diffusion of small molecules (< 40 kDa) but creates an energetically inefficient method of transport for larger molecules [39]. It is interesting to note that the majority of the FG-repeat Nups are not essential, except for Nup98 which constitutes the largest contribution to the diffusion barrier [40, 41].

Facilitated Transport Through the Nuclear Pore

The Ran Gradient

The direction of—and energy for—cargo transport through the NPC is regulated by the gradient of Ras-related nuclear protein (Ran) [42]. This small GTPase adopts different conformations in its GTP or GDP bound state, modulating its affinity for transport factors [42]. The concentration of RanGTP, with higher levels in the nucleus, and RanGDP, with higher levels in the cytoplasm, is a tightly regulated, cyclical process resulting in an actively maintained Ran gradient in which the concentration of RanGTP to RanGDP mediates nucleocytoplasmic transport [42]. Cytoplasmic levels of RanGDP are maintained by the strictly cytoplasmic localization of Ran GTPase-activating protein (RanGAP) and Ran binding proteins (RanBPs) [43–45]. RanGAP together with RanBPs contribute to higher cytoplasmic levels of RanGDP by facilitating the hydrolysis of RanGTP that enters the cytoplasm (Fig. 2e, l, and q) [43–45]. Cytoplasmic RanGDP is shuttled into the nucleus by a dedicated transporter, nuclear transport factor 2 (NTF2) (Fig. 2f, m) [46]. Once in the nucleus, Ran's chromatin-associated guanine nucleotide exchange factor (RCC1) promotes the exchange of GDP for GTP on Ran, increasing the concentration of nuclear RanGTP and completing the cyclical mechanism for

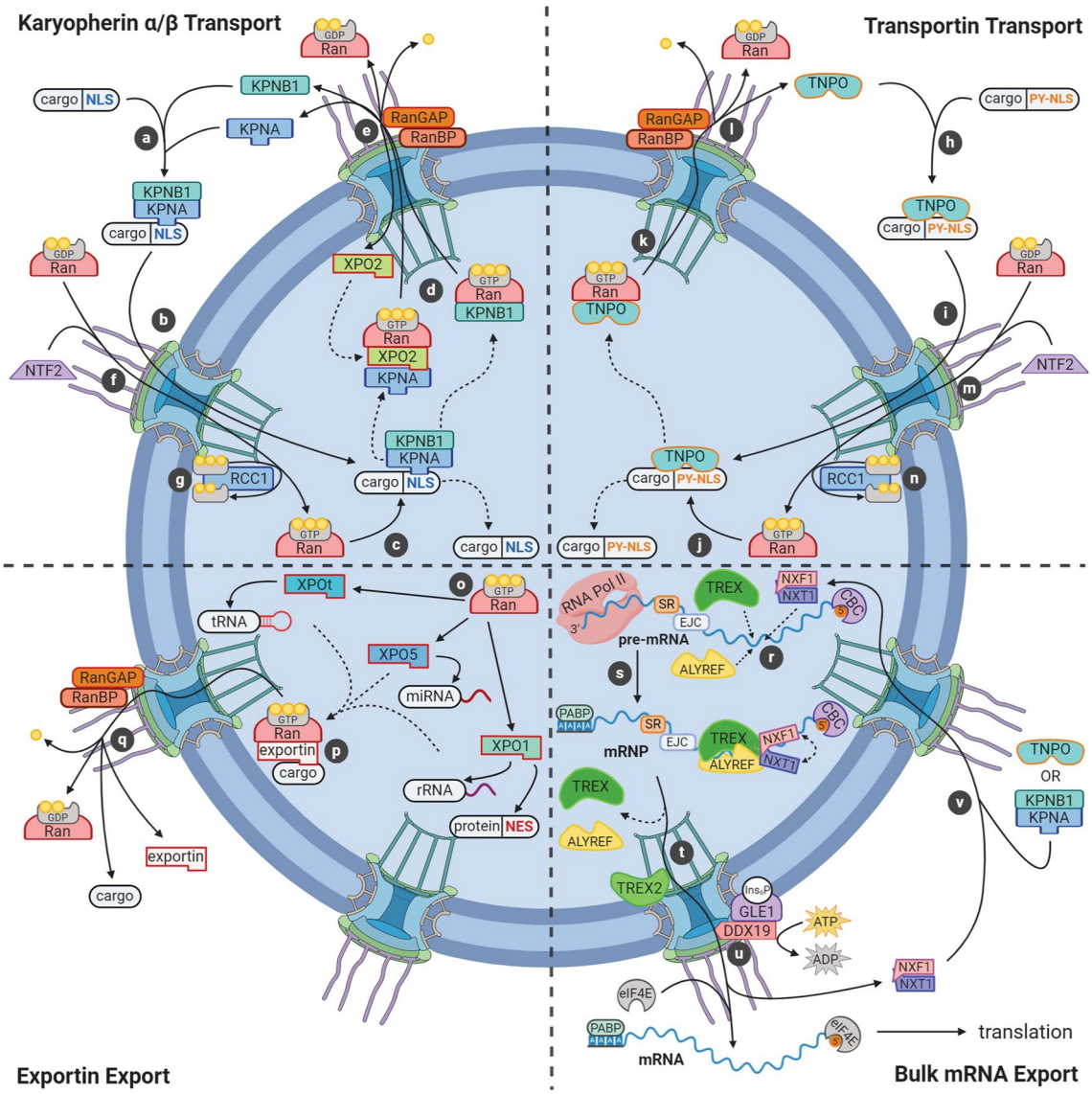


Fig. 2 Canonical nucleocytoplasmic trafficking mechanisms. Top left: Karyopherin α/β mediated import. KPNA binding NLS containing cargo with KPNB binding (*a*). The tripartite complex is translocated through the NPC (*b*) and dissociated by RanGTP binding to KPNB1 (*c*). KPNA recycling to the cytoplasm by XPO2 bound to RanGTP, while KPNB1 recycling is facilitated by its direct binding to RanGTP (*d*). On the cytoplasmic side of the NPC, the hydrolysis of RanGTP via RanGAP and RanBP dissociates KPNB1 and KPNA export complexes, producing RanGDP and releasing the karyopherins in the cytoplasm (*e*). RanGDP is shuttled to the nucleus by NTF2 (*f*), where RCC1 exchanges Ran's GDP for GTP (*g*). Top right: *Transportin.* Cargo with a PY-NLS requires TNPO binding (*h*) and is transported through the NPC (*i*). Cargo is released after RanGTP binds TNPO (*j*). TNPO is recycled to the nucleus bound to RanGTP (*k*). TNPO and RanGTP dissociation is facilitated by RanGAP and RanBP (*l*), and the Ran gradient is maintained with NFT2 and RCC1 (*m*, *n*). Bottom left: *Exportin.* In the presence of RanGTP, exportins bind tRNA, miRNA, rRNA or NES containing protein as cargo (*o*). The resulting RanGTP and cargo bound exportin (*p*) is translocated through the NPC and dissociates after hydrolysis of RanGTP (*q*). Bottom right: Bulk mRNA transport. RNA export to the cytoplasm begins during transcription as the nascent strand emerges from RNA Pol II where the TREX complex and ALYREF bind the RNA between the EJC and the 5′ end of the pre-mRNA (*r*). This promotes the recruitment and conformational change of NXF1-NXT1, essential to the hand-off of RNA (*r*). After the dissociation of RNA Pol II and the binding of PABP to the 3′ polyA tail, the mRNP proceeds to the NPC (*s*). Releasing TREX and ALYREF, the mRNP is drawn through the NPC where NXF1 may interact with TREX2 as it enters the channel (*t*). On the cytoplasmic side of the NPC, DDX19, powered by ATP hydrolysis and stimulated by GLE1 bound to Ins_6P, promotes the dissociation of the mRNP into its mRNA and export factor components (*u*). NXF1-NXT1 is recycled to the nucleus by either the TNPO or the KPNA/KPNB1 import pathways (*v*)

the maintenance of the Ran gradient, essential for nuclear import and export (Fig. 2g, n) [47, 48].

Karyopherin α/β Transport

Large protein complexes destined for the nucleus that struggle to passively diffuse across the FG-repeat domain of the NPC typically contain a nuclear localization signal (NLS) or a nuclear export signal (NES) [49–51]. These specific nuclear transport peptide sequences facilitate the binding of nuclear transport factors which promote transport through the NPC. In what is considered the "classical" transport pathway, karyopherin α (KPNA) binds directly to cargo containing a classical NLS [52]. Seven KPNA isoforms are thought to exist in humans and while there is some interesting specificity of the different isoforms, KPNA2 is considered to be the primary import protein for cargo with a classic NLS [53]. KPNA interacts directly with karyopherin β (KPNB1) through KPNA's potent NLS domain, creating a tripartite cargo-KPNA-KPNB1 nuclear import complex (Fig. 2a) [16, 53]. Additionally, KPNB1 can also bind directly to some NLS sequences without the KPNA adaptor protein [16]. Once a cargo is bound to a nuclear import complex, the entire complex is guided through the NPC by KPNB1 due to its interactions with the intrinsically disordered FG-repeat domain of the central channel Nups (Fig. 2b) [54, 55]. After successful passage across the nuclear pore and into the nucleus, the import complex dissociates when RanGTP binds KPNB1 (Fig. 2c). KPNB1 recycling and return to the cytoplasm requires binding of RanGTP, while KPNA recycling requires RanGTP dependent nuclear export factor, exportin-2 (XPO2) (Fig. 2d) [56]. Immediately following translocation through the intrinsically disordered FG-repeats, the coordination of RanGAP with RanBPs promotes the innate GTPase activity of Ran, leading to hydrolysis of RanGTP, and disassembly of the karyopherin export complex, thereby releasing the export cargo (Fig. 2e) [57, 58].

Transportin Transport

A different nuclear transport mechanism was discovered by the identification of a new type of NLS, the PY-NLS, which is not recognized by the classical karyopherin α/β machinery, but instead by another group of nuclear transport receptors called transportins (TNPO) [59–62]. Consisting of Transportin1, 2A, and 2B, this transport family functions in a similar manner to the classical transport machinery described above, in which transportin binds to its cargo (Fig. 2h), mediates the interaction with FG-repeat Nups to allow for translocation through the NPC (Fig. 2i), dissociates from its cargo in a RanGTP dependent manner (Fig. 2j), is exported back to the cytoplasm in complex with RanGTP (Fig. 2k) and freed to bind new cargo following hydrolysis of RanGTP by RanGAP (Fig. 2l) [63, 64]. The difference in cargo recognition appears to be largely mediated by transportins' recognition of cargo with the PY-NLS, unique from the classical NLS [65]. However, experimental evidence suggests that there are transportin dependent cargos that do not contain a PY-NLS, suggesting multiple recognition motifs for transportin mediated nuclear import [63, 66]. Additionally, post-translational modifications near the PY-NLS can alter transportin mediated transport [63].

Exportin Transport

Export of proteins and certain types of RNA from the nucleus, is mediated by seven exportin proteins in humans [67]. Exportins load their cargo in a RanGTP dependent manner, whereby the RanGTP binding increases exportins affinity for its cargo (Fig. 2o) [42]. Translocation of the exportin-cargo-RanGTP complex (Fig. 2p) through the NPC is mediated by exportin's interactions with the FG-repeat Nups of the central channel. Once in the cytoplasm dissolution of the export complex and unloading of the cargo occurs through the hydrolysis of RanGTP via RanGAP, thus decreasing exportin's affinity for its cargo (Fig. 2q) [57, 58]. Exportin 1 (XPO1) is the most prevalent and heavily utilized export receptor, which recognizes protein cargo with an NES [42]. Interestingly, XPO1 can also mediate rRNA, snRNA, and some mRNA export by binding to adaptor RNA binding proteins [42]. Different exportin isoforms have different targets. For example, XPOt is responsible for nuclear export of tRNA, and XPO5 is responsible for miRNA and rRNA export (Fig. 2o) [68, 69].

mRNA Transport

mRNA transport through the nuclear pore occurs through a different mechanism from the Ran mediated nucleocytoplasmic transport mechanism described above [69, 70]. Nuclear export of mRNA is a dynamic process where messenger ribonucleoprotein (mRNP) undergo various remodeling events from biogenesis to maturation and export [69]. The transcription and export (TREX) complex is a key player that initially associates with nascent RNA from transcribing RNA Pol II (Fig. 2r) [71–73]. During mRNA processing, recruitment of a TREX subunit, the RNA export factor ALYREF, is essential for nuclear export after RNA maturation (Fig. 2r) [74]. TREX and ALYREF contain binding sites that mediate the binding of the nuclear RNA export factor 1 (NXF1)—nuclear transport factor 2 like export factor 1 (NXT1) heterodimer [75, 76]. The NXF1-NXT1 interaction with ALYREF triggers the transfer of RNA from ALYREF to NXF1 (Fig. 2r) [77]. This hand-off of RNA is essential because NXF1 has the ability to drive the translocation

through the FG-repeats of the nuclear pore [77]. These binding events signal the maturation of pre-mRNA into an NXF1-NXT1-mRNP complex (Fig. 2s), which then translocates to the nuclear basket, a process that may be conducted by TREX2 whose scaffolding component has been shown to directly interact with NXF1 (Fig. 2t) [78–80]. Once at the nuclear basket, recognition, docking, and subsequent translocation of the mRNP proceeds through interactions between FG-repeat Nups and NXF1 (Fig. 2u) [81]. As an mRNP passes through the NPC and enters the cytoplasm, its cargo mRNA and receptor complexes are dissociated via the ATP-dependent RNA helicase activity of DDX19 (human ortholog of Dbp5), in association with Nup214 stimulated by GLE1, which is bound to its co-factor inositol hexaphosphate (IP6) (Fig. 2u) [82]. The remodeling concludes when the cap-binding protein, eukaryotic translation initiation factor 4E (eIF4E), replaces the cap binding complex (CBC) added during the pre-mRNA stage, preparing the transcript for translation [83]. NXF1-NXT1 can be recycled to the nucleus by either the TNPO or the KPNA/KPNB1 pathway (Fig. 2v). Nuclear export of mRNA appears to be a relatively inefficient process. Studies have indicated that only about 30% of mRNPs that interact with the NPC are successfully passed through the diffusion barrier [84, 85]. While still unclear, it is postulated that the export receptor's capacity for hydrophobic FG-repeat interactions is tempered by the size and composition of the mRNP, a balance that determines the solubility of the cargo-receptor complex in the hydrogel meshwork and, thus, its propensity to translocate through the nuclear pore [39, 86].

A Delicate Relationship Between Neurodegenerative Diseases and Nucleocytoplasmic Transport

The cytoplasmic aggregation of nuclear RNA binding proteins (RBPs), repeat expansion associated gene products, tau, and other proteins is often a common hallmark of neurodegenerative diseases. Emerging evidence indicates that the dysfunction of the dynamic and structural components of nucleocytoplasmic transport may be intricately tied to pathological protein aggregation [1, 87–90]. However, the exact mechanisms underlying this relationship and its implications on neurodegeneration are not fully understood. Disrupted RNA metabolism, as a downstream effect of aberrant RBP localization and aggregation, has become a widely accepted and commonly observed disease phenotype of ALS/FTD, although there has been limited evidence to support the idea that it directly leads to neuronal degeneration [1, 87, 91–93]. Similarly, although evidence suggests that aggregates of tau and repeat expanded gene products in Alzheimer's Disease (AD) and Huntington's Disease (HD), respectively, may directly impede nucleocytoplasmic transport and damage NPC machinery, it is not clear whether their presence is a driving force or a downstream by-product of disease progression. Here we will review proposed nucleocytoplasmic trafficking abnormalities across different neurodegenerative diseases, their relationship to aberrant protein aggregation, and how this complex dynamic may contribute to neuronal cell death.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that leads to the progressive loss of motor function [94]. Due to overlapping symptoms and genetics ALS is now commonly accepted as a spectrum disorder with frontotemporal dementia (FTD), where patients experience cognitive impairment due to the degeneration of the frontal and temporal lobes leading to social and behavioral changes [95–97]. The exact mechanisms leading to ALS/FTD remain unclear but nucleocytoplasmic trafficking abnormalities appear to play a central role in disease pathogenesis.

Chromosome 9 Open Reading Frame 72 (C9orf72)

The etiology of ALS/FTD is diverse and growing with over 40 genetic abnormalities currently associated with ALS [98, 99]. One of the most well characterized genetic abnormalities associated with the ALS/FTD spectrum to date is the chromosome 9 open reading frame 72 (*C9orf72*) repeat expansion [95–97]. First identified in 2011 the GGGGCC (G_4C_2) hexanucleotide repeat expansion in the first intron of the *C9orf72* gene is responsible for ~ 50% of all familial ALS and ~ 20% of sporadic ALS cases [95–97]. Healthy individuals have less than 23 repeats of the intronic G_4C_2 sequence of *C9orf72*, while patients with *C9orf72*-mediated ALS/FTD can exhibit anywhere from 30 to several hundred repeats [95–97]. Three commonly accepted but not mutually exclusive hypotheses exist to explain the neurodegenerative phenotype introduced by *C9orf72* repeat expansion: 1) C9orf72 haploinsufficiency, 2) accumulation of G_4C_2 sense and C_4G_2 antisense RNA-foci, and 3) the production of dipeptide repeats (DPRs) via repeat-associated non-AUG (RAN) translation (Fig. 3b) [100–112]. The direct mechanism for how the *C9orf72* repeat expansion causes ALS/FTD pathogenesis remains elusive but is currently being extensively investigated.

Early genetic screens in *S. cerevisiae* and *D. melanogaster* identified crucial components of the nucleocytoplasmic transport machinery as modifiers of *C9orf72* disease models [113–115]. Specifically, in regards to the NPC, the coat nucleoporins, Nup107 and Nup160, the nuclear basket component, Nup50, as well as inner ring components, Nup155 and Nup98, were found to be suppressors of *C9orf72*

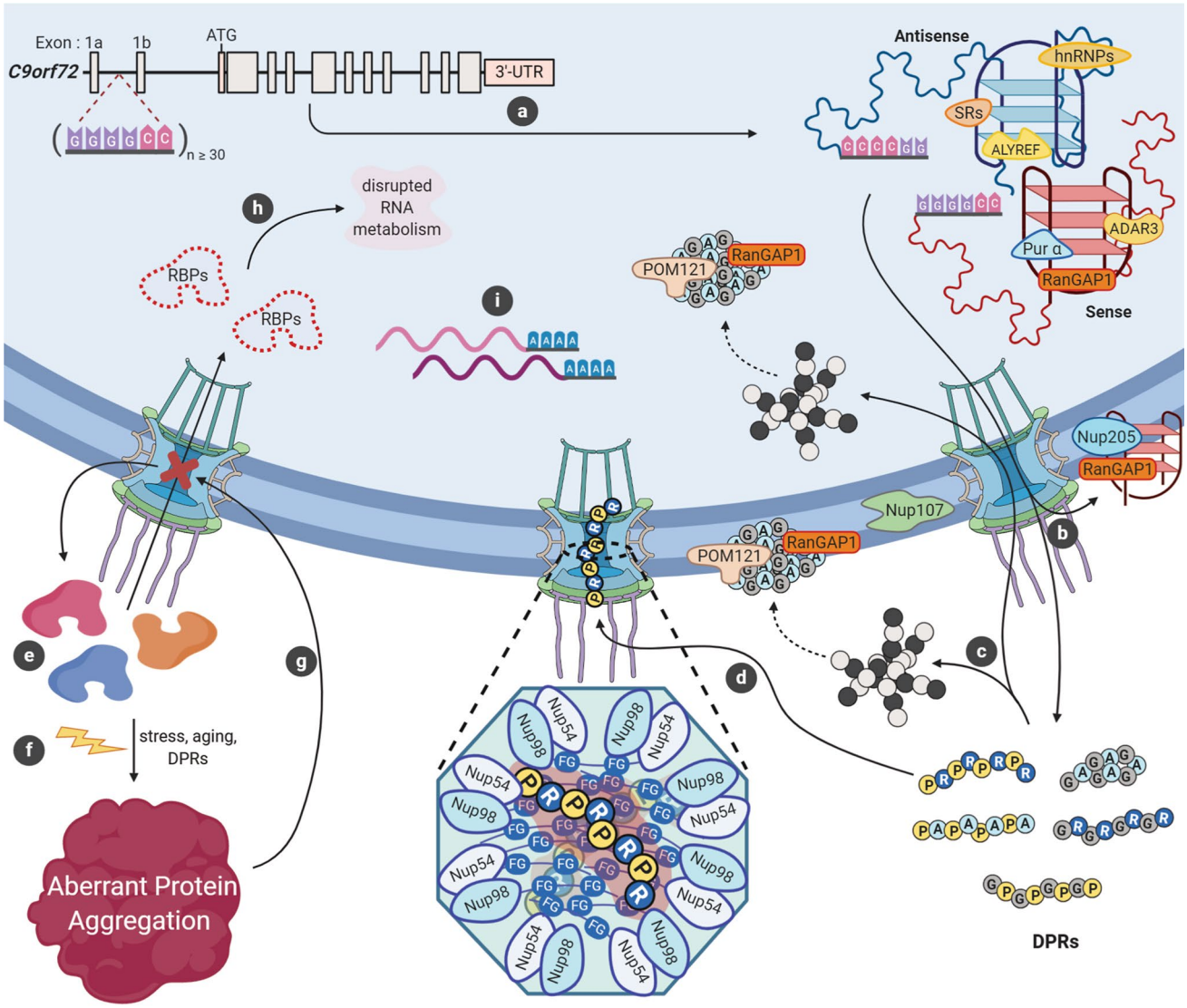


Fig. 3 Nucleocytoplasmic trafficking deficits caused by the C9orf72 repeat expansion. The sense and antisense transcription of the (G_4C_2) expansion of the *C9orf72* gene between exon 1a and 1b leads the formation of sense and antisense RNA foci found to sequester Pur α, RanGAP1, ADAR3, SRs, hnRNPs, and other proteins not shown (*a*). Expanded *C9orf72* RNA results in cytoplasmic/perinuclear RNA foci, which colocalize with RanGAP1 and Nup205, and produce DPRs via RAN translation (*b*). DPRs aggregate in the cytoplasm and nucleus, where polyGA aggregates may cause the mislocalization of POM121 and RanGAP1 (*c*). PolyPR clogs the NPC due to strong interactions with the FG-repeats of the central channel nucleoporins, Nup54 and Nup98, whereby it may promote the conversion of the FG hydrogel sieve to a more solid, fibrillar state (*d*). Impaired import and subsequent accumulation of soluble RBPs (*e*) coupled with factors like stress, aging, and DPR interaction (*f*) leads to the formation of insoluble protein aggregates that further disrupt nucleocytoplasmic transport (*g*), resulting in a feed forward mechanism of toxicity. In addition, the depletion of RBPs from the nucleus has a detrimental effect on RNA metabolism (*h*). PolyA RNA retention in the nucleus (*i*) is another common feature of *C9orf72* ALS/FTD models and may be a result of disrupted RNA metabolism and NPC defects

phenotype (Fig. 1) [113, 114]. In contrast, coat nucleoporin, Seh1, inner ring components, Nup62 and Nup93, nuclear basket components, Nup50, Nup153 and Tpr, the cytoplasmic filament component, GLE1, and the transmembrane nucleoporin, NDC1, were found to be enhancers of the observed *C9orf72* phenotype (Fig. 1) [113–115]. Additionally, components central to nucleocytoplasmic transport, such as protein transport factors, Ran, RanGAP, RCC1, transportins, exportins, and karyopherin α and β, and RNA export factors, NXF1, GLE1, and ALYREF, were shown to be modifiers of C9orf72-phenotypes [113, 115].

In agreement with the idea that nucleocytoplasmic transport is altered in *C9orf72*-mediated ALS/FTD, RanGAP1, was shown to directly bind to the *C9orf72* repeat

expansion (Fig. 3a), and was later shown to form intranuclear inclusions [89, 116]. These findings suggest that RanGAP1′s ability to regulate the Ran gradient is altered in *C9orf72*-mediated ALS/FTD [89, 116]. Not surprisingly, disruptions of the Ran gradient have been observed in the presence of the *C9orf72* repeat expansion, a phenotype that is rescued with overexpression of RanGAP1 [89]. Further evidence of disrupted nucleocytoplasmic transport components has been shown in mice overexpressing the GA dipeptide repeat, which form cytoplasmic and intranuclear aggregates that colocalize with RanGAP1 and POM121 (Fig. 3c) [117].

In addition to RNA-foci interaction with RanGAP1, evidence also suggests that *C9orf72* RNA-foci sequester RNA-binding proteins, including ALYREF, Adenosine Deaminase Acting on RNA (ADAR3), heterogeneous nuclear ribonucleoproteins (hnRNPs), Pur-alpha, and serine arginine (SR) proteins (Fig. 3a) [116, 118, 119]. Additionally, the disruption of proper RBP function may be exacerbated by DPRs. GR and PR overexpression models in HEK293T cells identified over a hundred interactors of the arginine containing DPRs, including disease causing RBPs with low-complexity domains (LCD) [120, 121]. DPR interaction with LCD containing proteins reduces the capacity of membrane-less organelles for liquid–liquid phase separation (LLPS), a characteristic essential for the dynamic nature of RBPs, stress granules, nucleoli, and the diffusion barrier of the nuclear pore complex [120, 121]. The LLPS of proteins, a normally reversible process whereby proteins shift from a soluble or "de-mixed" state to liquid droplet state, can be a seeding point for the deposition of protein aggregates—commonly seen in ALS/FTD pathology—when aging, stress, and DPR interaction are in play (Fig. 3f) [122, 123]. Through similar mechanisms, PR repeat proteins have been shown to promote the fibrillization of the FG-repeats on Nup98 and Nup54, thereby clogging the NPC and reducing nuclear transport of RNA and proteins (Fig. 3d, i) [120, 124]. However, a recent study from Vanneste and colleagues provides evidence that these arginine containing DPRs do not directly interfere with nucleocytoplasmic transport and highlights a need for additional studies clarifying the role DPRs may play in the pathogenesis of C9orf72-mediated ALS/FTD [125].

Taken altogether, it is clear that nucleocytoplasmic transport can be disrupted in *C9orf72* mediated ALS/FTD, through mechanisms leading to the sequestration of transport machinery and aberrant cytoplasmic RBP accumulation and aggregation (Fig. 3e–g). Aside from direct impairment of nuclear import and export, the nuclear depletion of RBPs causes a widespread disruption of RNA metabolism (Fig. 3h) that will be described in the context of TDP-43 and FUS below.

Transactive Response DNA Binding Protein (TARDBP)

Considering the diversity in the etiology and patient presentation within the ALS/FTD disease spectrum, it is surprising that there may be shared mechanisms leading to neurodegeneration in many patient subgroups [126–128]. Indeed, cytoplasmic accumulation and nuclear depletion of the RNA binding protein, encoded by the TARDBP gene, TAR-DNA binding protein-43 (TDP-43) is found in ~ 95% of all ALS cases and ~ 45% of FTD cases (FTD-TDP), irrespective of etiology [126, 127, 129, 130]. Notably, TDP-43 proteinopathies are not limited to ALS and FTD with TDP-43 pathology. In fact, TDP-43 positive inclusions are also a characteristic of certain cases of HD, AD, Parkinson's disease, chronic traumatic encephalopathy, and certain inclusion body myopathies [126, 130–133]. Despite extensive research, it is unclear whether cytoplasmic TDP-43 accumulation leads to a nuclear loss of function or a cytoplasmic toxic gain of function, or a combination of both [93, 129, 134, 135]. Whether wild-type TDP-43 inclusions are drivers of disease progression, or simply a by-product of a degenerating neuron, remains to be seen. Considering that aberrant TDP-43 inclusions are a shared occurrence in multiple diseases we will discuss TDP-43′s functional role in cellular homeostasis and its potential contribution to neurodegenerative disease pathogenesis in more detail below.

TDP-43 is an essential RNA binding protein involved in many steps of RNA metabolism, including: transcription, splicing, maturation, stability, transport, translation, and micro and long non-coding RNA processing [134]. It has further been proposed to be involved in the formation and maintenance of stress granules, and the regulation of stress granule nucleating protein expression [136]. TDP-43 binds to RNA with high specificity via its two RNA recognition motifs (RRMs). Containing both an NLS and an NES, TDP-43 plays roles in both the nucleus and the cytoplasm and, therefore, its trafficking across the nuclear membrane is essential for its proper function. Nuclear import of TDP-43 is facilitated by the classical KPNA/KPNB1 pathway (Fig. 2a–e) [137]. Interestingly, expression of the nuclear export factor XPO2, which is required for KPNA recycling (Fig. 2d), is reduced in the brains of FTD-TDP patients, potentially contributing to the cytoplasmic accumulation of TDP-43 in these patients [137]. The nuclear export of TDP-43 has been predicted to be recognized as an NES-containing cargo by XPO1. Indeed TDP-43 contains two NES sequences that have been predicted to interact with XPO1 [138]. However, recent studies have suggested these two NES sequences are neither necessary nor sufficient for nuclear export, indicating they may be nonfunctional and that TDP-43 may not be actively transported out of the nucleus [138]. In support of this hypothesis, pharmacological and genetic disruption of facilitated nuclear export

pathways (Fig. 2o–q), suggest that TDP-43 is not exported by any of the major export pathways (XPO1, XPO5 or with mRNA through bulk RNA export) [138, 139]. Instead, export of TDP-43 is thought to be mediated through passive diffusion [138].

Considering the prevalence of TDP-43 mislocalization it is essential to understand how the aberrant cytoplasmic accumulations (Fig. 4l) form and how these accumulations are involved in nucleocytoplasmic trafficking defects. The aggregation of TDP-43 is most commonly attributed to its LCD, housed within the C-terminus of the protein. The LCD is home to the majority of ALS associated *TARDBP* mutations [140]. In addition to being unusually disordered and aggregation prone, the C-terminus of TDP-43, in fragmented form, is a highly toxic and prevalent component of the cytoplasmic inclusions seen in ALS pathology [141, 142]. It seems possible that the ability of TDP-43 to undergo LLPS, may create opportunities for TDP-43 to form insoluble aggregates (Fig. 4n) [123, 143, 144]. Indeed, under stress, with age, and in mutated forms, cytoplasmic TDP-43 droplets become more solid and gel like, facilitating aggregation (Fig. 4p) [123, 144–146]. The specific mechanism detailing how endogenous TDP-43 aggregations form in neurodegenerative diseases remains unknown. Artificial introduction of TDP-43 inclusions recruit pathogenic species that mimic the TDP-43 inclusions observed in patients [147]. The low complexity domain on TDP-43 drives the formation of these neurotoxic TDP-43 inclusions and promotes further recruitment of nuclear TDP-43 to such inclusions [147]. Interestingly, aberrant TDP-43 liquid–liquid phase separation can be rescued by enhancing TDP-43 interaction with RNA [147], suggesting that the aggregation of TDP-43 in the cytoplasm may be the result of impaired mRNA export from the nucleus [147]. Mann *et. al* suggest the possibility that increased nuclear retention of RNA renders cytoplasmic TDP-43 more aggregation prone due to reduced cytoplasmic RNA [147].

TDP-43 aggregation may contribute to neurodegeneration by disrupting the nucleocytoplasmic transport machinery [92]. Overexpression of TDP-43 C-terminal fragments either co-aggregate with, or cause the mislocalization of coat Nups (Nup107 and Nup160), inner ring Nups (Nup35, Nup58, Nup62, Nup93, Nup98, Nup205), transmembrane Nups (POM121 and POM210), cytoplasmic filament Nups (Gle1, Nup88, Nup214, RanBP2) nuclear basket Nups (Nup153 and Nup155), and nuclear transport components (XPO5, NXF1) (Fig. 4r). This indicates that cytoplasmic aggregation of TDP-43 is highly disruptive to the NPC (Fig. 4t) [92]. While, the overexpressed system employed in these experiments may exaggerate the degree of NPC interaction, these studies strongly suggest that TDP-43 cytoplasmic inclusions have the capacity to disrupt the NPC and its components (Fig. 4t) [92]. Notably, despite the substantial dysregulation of the NPC and nucleocytoplasmic transport, the Ran gradient appears normal in these experiments [92].

In addition to the gain-of-toxic function due to TDP-43 aggregates, it is also possible that the nuclear depletion and loss of function of TDP-43 is a toxic contributor resulting from TDP-43 mislocalization to the cytoplasm [127]. These two mechanisms describing cellular stress from TDP-43 proteinopathy are likely not mutually exclusive. As stated above, TDP-43 is a master regulator of splicing and it has been shown to act as a splicing repressor for cryptic exons [148–150]. Proper TDP-43 function has been shown to prevent the inclusion of specific exons, termed cryptic exons, by negatively regulating splicing at these sites [150]. Loss of function of TDP-43 due to its depletion leads to the inclusion of these cryptic exons which could allow for improper handling throughout an RNAs translation and maturation often leading to the decay of the host transcript (Fig. 4u) [148–150]. Indeed, downregulated genes from RNA sequencing datasets of samples with depleted TDP-43 are enriched for cryptic exons [148]. The inclusion of cryptic exons has been observed in C9orf72-mediated ALS/FTD [150]. Interestingly, cryptic exons are highly variable in different cell types, a finding that may provide clues to explain selective vulnerability in neurodegeneration [149]. Considering that TDP-43 interacts with thousands of transcripts, substantial work remains to be completed to understand how the inclusion of cryptic exons is the result of TDP-43 mislocalization and what role they play in the pathogenesis of neurodegenerative diseases. One such possibility is TDP-43′s altered regulation of stathmin-2 in ALS [151, 152]. Depletion of TDP-43, mimicking loss of nuclear TDP-43, leads to the pre-polyadenylation of stathmin-2 and decreased protein expression [151, 152]. Interestingly, stathmin-2 is thought to play a role in neurite outgrowth and microtubule dynamics [151, 152]. The loss of stathmin-2 following depletion of TDP-43, causes failure of neuronal regeneration after axotomy, a phenotype that is rescued by stathmin-2 transduction and pharmacological inhibition of c-Jun N-terminal kinase [151, 152].

Fused in Sarcoma (FUS)

Another widely studied, dysregulated RBP implicated in neurodegenerative disease is the FUS protein [135, 153–157]. Historically, FUS pathology was considered to be a rare observation, only attributed to the abnormal aggregation of mutant FUS in ~ 5% of ALS cases (ALS-FUS), and the abnormal aggregation of wild-type FUS in ~ 10% of FTD patients (FTD-FUS) [153, 154, 158, 159]. While initial studies were focused primarily on pathological FUS inclusions, more recent work provides evidence that the spinal motor neurons of sporadic ALS patients display a diffuse cytoplasmic mislocalization of FUS, with no coinciding

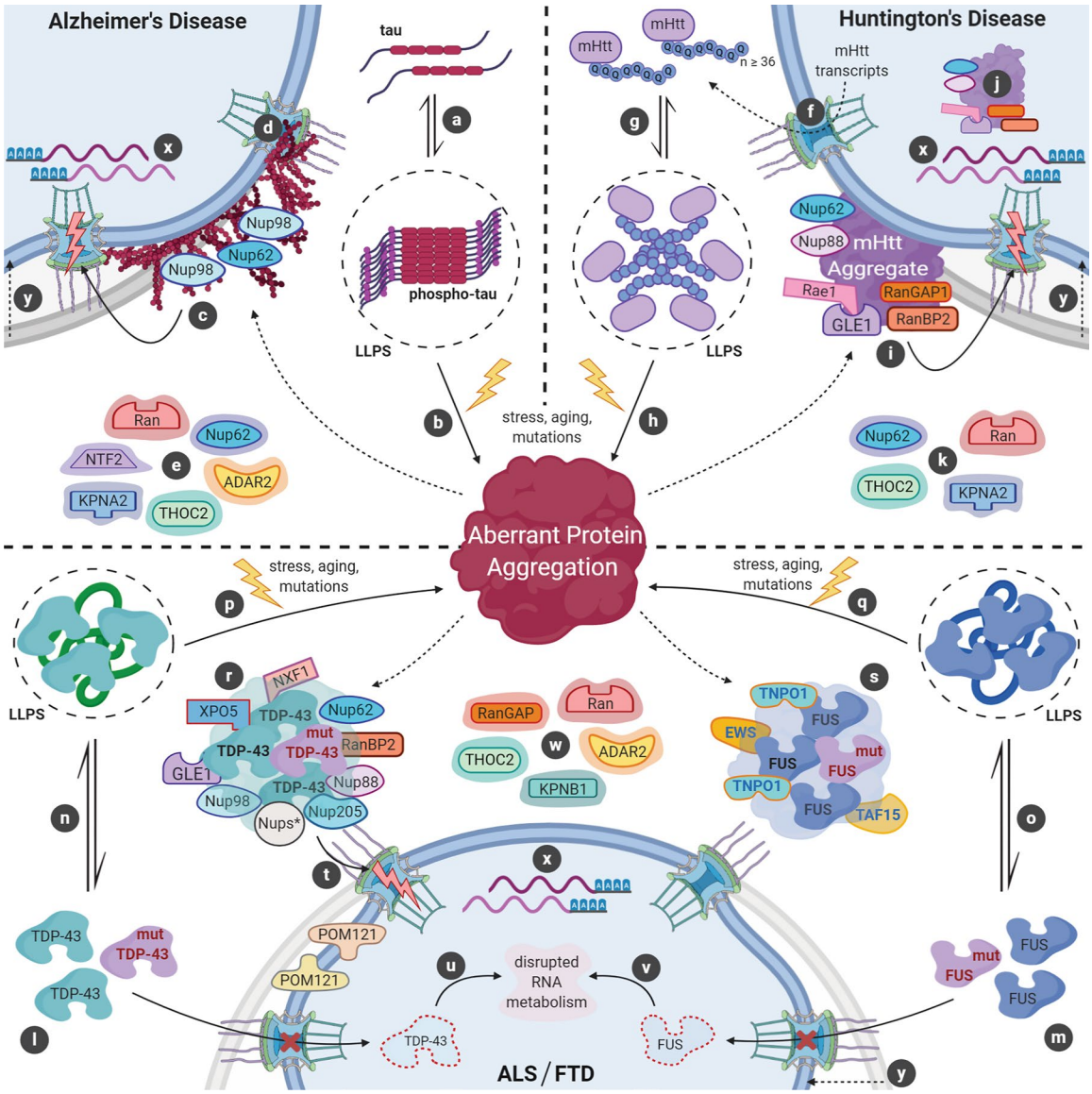


Fig. 4 Nucleocytoplasmic trafficking deficits in neurodegenerative diseases. Top Left: Alzheimer's disease. The phosphorylation state of tau protein determines its capacity for LLPS, where hyperphosphorylation promotes a more inseparable phase (*a*). Dysregulation of tau phosphorylation from stress, aging, and/or mutations cause it to aggregate into NFTs (*b*). Phospho-tau positive NFTs interact with the nuclear envelope, where they disrupt the NPC, and co-aggregate with Nup98 and Nup62 (*c*). NFT's physical occlusion of the NPC (*d*). Impaired Ran gradient, mislocalized or aggregated Nups, RBPs and transport factors act as additional evidence of nucleocytoplasmic trafficking disruptions (*e*). Top Right: Huntington's disease. mHtt harbors over 36 glutamine repeats (*f*). The polyQ tract within mHtt promotes its LLPS (*g*) which can lead to its pathological aggregation through aging and cellular stress (*h*). Toxic perinuclear aggregates of mHtt disrupt the NPC, evidenced by their sequestration of Nups, Ran-related proteins, and other transport factors (*i*). Nuclear mHtt aggregates are also common in Huntington's patients although their contribution to disease progression may be less severe (*j*). Similar to tau in AD, mHtt can lead to an impaired Ran gradient and the mislocalization and/or aggregation of proteins (*k*). Bottom: *ALS/FTD*. A common feature of ALS/FTD is the impaired import cytoplasmic accumulation of TDP-43 (*l*) and FUS (*m*). Both of these proteins can undergo LLPS distinct from stress granules, mediated by their LCDs (*n*, *o*). With aging, stress, and/or mutations in these LCDs, TDP-43 or FUS can shift to an aggregated state (*p*, *q*). TDP-43 aggregates can include at least 14 Nups as well as several nuclear transport factors (*r*). TDP-43 aggregates lead to further disruption of the NPC and induce mislocalization of POM121 and POM210 to perinuclear puncta (*t*). The binding interactions of FUS aggregates are less elucidated, although FTD-FUS co-aggregates with other FET proteins and TNPO1 (*s*, blue font). AD, HD, and ALS/FTD display a disrupted Ran gradient, and mislocalized RBPs and transport components (*w*). Nuclear loss of TDP-43 or FUS disrupts RNA metabolism (*u*, *v*). Nuclear accumulation of polyA-RNA (*x*) and distortions in the nuclear envelope (*y*)

aggregation [157]. In agreement with these results, mice with mutation-induced cytoplasmic mislocalization of FUS without detectable cytoplasmic aggregation, exhibit age dependent progressive motor and cognitive deficits [160]. These recent studies suggest that the cytoplasmic inclusions of FUS observed in postmortem tissue may not be the only toxic contribution provided by FUS dysregulation [160]. Considering FUS may have a wider contribution to ALS/FTD pathogenesis than once predicted, it is essential to understand the mechanisms by which FUS dysregulation occur, how these differ between ALS-FUS and FTD-FUS patients, and how they are distinct from the mechanisms driving TDP-43 pathology.

The relationship between TDP-43 and FUS is interesting considering their structural and functional similarities: both have RNA recognition motifs, an LCD, and an NES and NLS; both are predominantly nuclear, yet play roles in mRNA stability and translation in the cytoplasm; and both are involved in transcription and splicing [161–163]. Indeed, the LCD of FUS is thought to mediate its capacity for LLPS, disruptions of which may seed FUS aggregation similar to the manner by which TDP-43 is thought to aggregate (Fig. 4o, q) [164]. Also, the nuclear export of FUS appears to be similar to TDP-43, given FUS has 2 predicted XPO1-mediated NES sequences that do not mediate its nuclear export [138]. Instead FUS export is regulated by passive diffusion through the nuclear pore and not by facilitated transport pathway [138, 165]. However, they have key differences that might explain why TDP-43 and FUS inclusions are mutually exclusive [153, 154, 158, 159]. For example, they utilize different transport pathways. The nuclear import of TDP-43 is facilitated by the KPNA/KPNB1 pathway (Fig. 2a–e), while FUS nuclear import is conducted by Transportin-1 (TNPO1) via the TNPO pathway (Fig. 2h–l) [63, 65, 162, 166]. FUS mutations in ALS-FUS patients, have been shown to disrupt the binding of the TNPO1 receptor, which would lead to FUS's cytoplasmic accumulation in a manner independent from drivers of impaired TDP-43 nuclear import (Fig. 4m) [162, 166–170]. Interestingly, neuronal FUS has been shown to be dysregulated in conditions of stress due to failures of TNPO1 [165]. Intriguingly, astrocytes do not display FUS mislocalization under the same conditions, suggesting FUS may have cell-type specific contributions to disease [165]. Additionally, different manifestations of post-translational modifications of FUS, may play a role in its different presentations in ALS and FTD [171–179]. Specifically, the methylation of FUS residues adjacent to the PY-NLS can alter FUS's interaction with TNPO1 [171–179]. Dimethylation, characteristic of ALS-FUS, reduces this interaction, and hypomethylation, characteristic of FTD-FUS, increases it [171–179]. In support of these findings, FTD-FUS inclusions are positive for TNPO1 and other members of the FET protein family, EWS and TAF15, while ALS-FUS inclusions are not (Fig. 4s) [180]. These differences are intricately tied to the unique presentations of each disease and parsing out why these differences occur is important to characterize disease progression.

Similar to TDP-43, one consequence of FUS aggregation and mislocalization may also be a loss of function due to a nuclear depletion. While TDP-43 and FUS have similar roles in RNA processing and regulation of alternative splicing, FUS is thought to regulate a largely distinct set of transcripts in comparison to TDP-43 [181]. Depletion of FUS leads to broad misregulation of RNAs, including the dysregulation of expression for over 600 mRNAs as well as the dysregulation of over 350 splicing patterns (Fig. 4v) [181]. Interestingly, while both TDP-43 and FUS are important regulators of RNA metabolism, only 86 RNAs are regulated by both proteins, suggesting that the disruption to RNA metabolism due to FUS dysfunction impacts a different group of RNA compared to TDP-43 dysfunction [181]. These studies suggest that neurodegeneration resulting from FUS dysfunction may be distinct from neurodegeneration caused by TDP-43 dysfunction [181].

hnRNP A1 and hnRNP A2B1

Both TDP-43 and FUS are members of a large subclass of RNA-binding proteins known as hnRNPs, which have been shown to have diverse transcriptional control through their roles in RNA maturation and neuronal RNA transport granules. Additionally, these proteins have been associated with stress granule formation, which is critical to the cellular stress response [182–184]. Like TDP-43 and FUS, rare dominant mutations in the LCD of hnRNP A1 and hnRNP A2B1 have been associated with ALS [182–184]. Interestingly, these mutations are most frequently associated with multisystem proteinopathy, a rare inherited disease in which patients experience the degeneration of muscle, bone and/or the central nervous system [182–184]. The mutations in hnRNP A1 and hnRNP A2B1 have been shown to cause their mislocalization from the nucleus to the cytoplasm and to pathologically alter stress granule dynamics by enhancing their tendency to fibrillize and thereby disrupting their capacity to undergo LLPS [182–184]. These cytoplasmic inclusions have also been shown to colocalize with cytoplasmic TDP-43 aggregates and have been associated with TDP-43 nuclear depletion [182–184].

ADAR2

Our lab has shown that the RNA editing enzyme Adenosine Deaminase Acting on double-stranded RNA 2 (ADAR2) forms cytoplasmic inclusions in C9orf72-mediated ALS/FTD and in Alzheimer's Disease (Fig. 4e, w) [185]. ADAR2 utilizes KPNA1 and KPNA3 to traffic into the nucleus [186,

187]. While it is unclear how ADAR2 accumulations form in ALS and AD it is possible that dysregulation of KPNA1 or 3 would lead to abnormal cytoplasmic inclusions of ADAR2. In addition to its cytoplasmic accumulation we provided evidence to suggest aberrant function of A to I editing in C9orf72-mediated ALS/FTD with enrichment of A to I editing aberrations in the EIF2 pathway, which may lead to global translational inhibition [185]. ADAR2 is a nuclear enzyme and its presence in the cytoplasm alters the function of the enzyme, suggesting that the altered cellular localization in disease represents a mechanism for its dysregulation [185]. There has been a revolution in the understanding of the function of A to I editing in both normal cellular function and in disease [188]. Dysregulation of ADAR2 and its normal cellular function would lead to altered regulation of RNA transcripts that would have yet unknown cellular consequences. While many RNA editing abnormalities have been associated with neurodegeneration [188], due to the massive number of uncharacterized A to I editing sites further experiments are needed to establish the specific role of altered RNA A to I editing events in the pathogenesis of neurodegenerative diseases.

Superoxide Dismutase 1 (SOD1)

Mutations in the SOD1 gene were the first genetic association with ALS [189]. Patients with SOD1 mutations do not have TDP-43 or FUS pathology [190–193]. However, large SOD1 accumulations exist in patients with these SOD1 mutations [190–193]. The dichotomy that appears to exist between neurons with SOD1 accumulations compared to TDP-43 and FUS accumulations is interesting and suggests different mechanisms leading to neurodegeneration [190–193]. Further evidence to support the unique nature of SOD1 suggests that SOD1 exhibits a distinct transcriptome compared to that of C9orf72 patients [194]. It is possible that some components of the nuclear pore and nucleocytoplasmic transport are disrupted as a result of the SOD1 G93A mutation [195]. However, conflicting reports suggest that more experiments are required to understand nucleocytoplasmic transport deficits in SOD1 mediated ALS [92].

Sporadic ALS

In contrast to familial ALS, there have been few studies which describe nucleocytoplasmic transport deficits in sporadic ALS. Limited number of studies report impaired nucleoporins (Nup62) and KPNB1 in cells with TDP-43 aggregates [196, 197]. Considering the challenges associated with studying and modeling sporadic forms of ALS it is not surprising that few studies have begun to describe nucleocytoplasmic trafficking deficits. However, as stated above, TDP-43 aggregations have the capacity to disrupt the nucleocytoplasmic trafficking machinery and it is possible there will be future disruptions identified in sporadic ALS.

Huntington's Disease

The degeneration of striatal medium spiny projection neurons as well as cortical pyramidal neurons due to the presence of an expanded CAG repeat in the Huntingtin (*HTT*) gene is known as Huntington's disease [198]. Individuals with more than 39 CAG repeats in the *HTT* gene develop progressive motor, cognitive and psychiatric symptoms leading to a steady decline in physical health and quality of life culminating in death 10—30 years after disease onset [199]. Translation of *HTT* with an abnormal number of CAG repeats, leads to huntingtin protein with an expanded poly-glutamine (polyQ) tract (mHtt) (Fig. 4f). Due to this polyQ tract and proline-rich region, mHtt undergoes LLPS (Fig. 4g), potentially leading to cytoplasmic, perinuclear, and nuclear aggregates through factors such as aging and other stressors (Fig. 4h–j) [200]. There is substantial evidence that suggests these aggregates disrupt the NPC and nucleocytoplasmic trafficking in Huntington's disease.

Immune-electron microscopy performed on cells overexpressing mHtt show distortion of the nuclear membrane (Fig. 4y) and cytoplasmic inclusions of NPC proteins in cells with perinuclear inclusions of mHtt (Fig. 4k) [201]. Additionally, early analysis of protein interactions suggested that polyQ accumulations interact with nuclear pore components, specifically FG-repeat Nups found in the cytoplasmic filaments (DDX19, RanBP2, and Nup214), the nuclear basket (Nup153), and the inner ring nucleoporin Nup62 [202].

More recent studies elaborated on these previous publications, firmly associating nucleocytoplasmic trafficking abnormalities with HD [90, 203]. First Gasset-Rosa et al. confirmed that mutant huntingtin accumulations lead to abnormal nuclear envelope morphology [203]. Using a mHtt mouse model, an increasingly severe nuclear envelope pathology in the homozygous compared to heterozygous animals suggested a dose-dependent phenotype [203]. These findings were confirmed in human induced pluripotent neural progenitor cells and in human postmortem tissue further associating mutant huntingtin and nuclear membrane abnormalities with disease state [203]. In addition to nuclear membrane pathology, HD models and patients displays overt nucleocytoplasmic trafficking deficits [203]. mRNA export is dysregulated, as evidenced by increased mRNA levels in the nuclei of cortical regions in a mouse models and Huntington's disease human postmortem tissue (Fig. 4x) [203]. Concurrently, work done by Grima et al. showed a disruption of the Ran gradient in motor neurons differentiated from hiPSCs from HD patients [90]. Evidence for disruption of the NPC comes from the discovery in human post mortem tissue showing that patients with Huntington's disease exhibit a

mislocalization or aggregation of the Ran GTPase activating protein, RanGAP1, in the frontal cortex, striatum, cerebellum, and hiPSC neurons (Fig. 4k) [90]. Additionally, they found that Nup62, a component of the central channel of the inner ring of the NPC, was mislocalized only in the striatum and displayed a diffuse cytoplasmic pathology in human tissue and hiPSC neuronal models (Fig. 4k) [90]. The Ran gradient deficit can be rescued both by inhibition of nuclear export via Exportin-1 inhibition with KPT-350 treatment; and interestingly, inhibition of O-GlcNAcylation, a common post translational modification for nucleoporins [90].

Alzheimer's Disease

AD is the leading cause of dementia worldwide [204]. The age related progressive cognitive decline of episodic memory is the most common clinical feature of Alzheimer's disease [204]. Early studies utilizing electron microscopy identified a close association between neurofibrillary tangles (NFTs) and the NPC in neurons of post mortem tissue from patients with Alzheimer's disease (Fig. 4d) [205]. Furthermore, recent studies using iPSC-differentiated neurons have shown that tau accumulation disrupts nuclear lamina and nucleocytoplasmic transport [206]. Mechanisms for tau aggregation have parallels to RBP and mHtt aggregation—mutation and the phosphorylation states of tau have been implicated in its aberrant LLPS (Fig. 4a, b) [207, 208]. Given the prevalence of tau-pathology in AD and the emerging link between nucleocytoplasmic trafficking deficits and neurodegenerative diseases, elucidating the impact of tau inclusions on the NPC is critical to understanding disease pathogenesis.

Conflicting reports exist on the nature of Nup62s localization in Alzheimer's disease postmortem tissue [88, 209]. Early studies found that Nup62 staining on abnormal nuclei in neurons with NFTs was localized primarily to the nuclear membrane and identified no aberrant localization [209]. More recent studies have suggested that similar to Huntington's disease, Nup62 is mislocalized in neurons from AD patients (Fig. 4e) [88]. Due to difficulties in Nup62 labeling the authors suggest it is difficult to determine if cytoplasmic aggregation exists due to lipofuscin autofluorescence, but instead suggest diffuse cytosolic mislocalization and depletion at the nuclear membrane [88]. Nup414 labeling, which includes Nup62, further suggests the dysregulation of FG-repeat Nups [88]. Indeed, Nup98, the largest contributor to the FG-repeat diffusion barrier, interacts directly with phospho-tau and is mislocalized in AD hippocampal neurons [88]. Not surprisingly, the direct interaction of tau with the NPC leads to disruption of the NPC diffusion barrier and the Ran gradient (Fig. 4c) [88]. However, Nup54, POM121, Nup88, Nup153, Nup133, and Nup214 were shown to not have apparent differences in expression or distribution in neurons positive for tau tangles, which suggests normal function of the transmembrane, cytoplasmic filaments, nuclear basket, and coat nucleoporins, as well as some of the inner ring nucleoporins, indicating there is not a complete breakdown of the NPC [88, 209]. Nonetheless, similar to nuclear envelope aberrations found in other neurodegenerative diseases, neurons from patients with Alzheimer's disease exhibit aberrantly shaped nuclei (Fig. 4y) [209].

Neurons with and without NFTs also contain cytoplasmic accumulations of the RanGDP transporter, NTF2 (Fig. 4e) [209]. Failure of the nuclear import of RanGDP would lead to the disruption of the Ran gradient in AD and disruption of Ran mediated nucleocytoplasmic transport [210]. This supports the disruption of the Ran gradient in AD CA1 hippocampal tissue, with phospho-tau inclusions [88]. Considering that the interactions of the karyopherin family with NTF2 are thought to expedite transport kinetics, it would not be surprising that NTF2 accumulations accompany additional nuclear import deficits [211]. Interestingly, induced cytoplasmic artificial beta sheets that form amyloid-like aggregates, inhibit nuclear export of mRNA, likely due to their forced mislocalization and co-aggregation of KPNA2 and KPNA4, RanBP1, and THOC2 suggesting that the presence of beta-sheets in NFTs found in AD may further disrupt nuclear trafficking adding additional sources of cellular stress (Fig. 4e) [212, 213].

Therapeutically Targeting Nucleocytoplasmic Transport Defects

The prevalence of nucleocytoplasmic trafficking throughout neurodegenerative diseases creates an enticing opportunity for therapeutic intervention. To this point the nuclear export inhibitors KPT-350, KPT-335, and KPT-276 have been shown to have neuroprotective effects [89, 90, 92, 139]. However, the mechanism of this neuroprotection remains unclear [139]. Considering the recent evidence suggesting that TDP-43 and FUS are not targets of exportin mediated transport, it is unknown whether KPT compounds are directly modulating TDP-43 or FUS nucleocytoplasmic transport [138, 139, 165]. Biogen has recently initiated a Phase1 safety trial with an XPO1 inhibitor in ALS patients to be completed by the end of the fiscal year 2020 [214]. Interestingly though, low non-toxic concentrations of KPT compounds are not able to inhibit nuclear export, emphasizing even more that the detailed mechanisms for how KPT compounds might confer neuroprotection in neurodegenerative disease have yet to be identified [139].

Considering the widespread presence of protein aggregation in these diseases, removing these aberrant protein aggregates may alleviate the associated neurotoxic phenotypes [146, 215]. Interestingly, nuclear import receptors can

disassemble aberrant insoluble protein aggregates that have been associated with neurodegenerative diseases [216]. Specifically, KNPA and KNPB have been shown to be able to disaggregate TDP-43 aggregates and TNPO1 was able to disaggregate FUS aggregates as well as other aggregates of proteins with a PY-NLS [216]. These findings suggest that failure of nuclear import can be rescued by supporting the possibly overwhelmed endogenous nuclear import receptors (Ex. KNPA, KNPB, and TNPO1) [216]. If disaggregation of aberrant fibrils using nuclear import receptors can also restore normal transport of RNA binding proteins to the nucleus, it may be possible to rescue toxic cytoplasmic aggregates, alleviate nuclear depletion of RBPs and thereby protect against neuronal loss in neurodegenerative diseases [215, 216].

Conclusion

The widespread prevalence of impaired elements of nucleocytoplasmic trafficking machinery throughout instances of neurodegeneration suggest the existence of a common mechanism of dysfunction. The potential of overlapping causative disease mechanisms creates exciting opportunities for multifaceted therapeutic interventions. While an enormous body of work remains to fully understand the mechanisms behind possible therapeutic interventions in nucleocytoplasmic transport, early evidence suggests multiple exciting opportunities. These trafficking aberrations have been well established and their role in neurodegenerative diseases has been increasingly strengthened. However, the initiating factor that leads directly to detrimental nucleocytoplasmic trafficking deficits remains unclear. Nevertheless, it seems likely that regardless of how trafficking deficits begin, the consequences act in a feed-forward mechanism that will further disrupt nucleocytoplasmic trafficking resulting in more severe consequences. Once nucleocytoplasmic trafficking defects have taken place, how this cellular disruption causes neurodegeneration is still largely unknown. It is likely that nuclear depletion and increased cytoplasmic concentration leading to aberrant aggregations will contribute to cellular stress through feed-forward disruptions of RNA metabolism and nucleocytoplasmic transport. The identification of toxic species that result from trafficking deficits may result in additional opportunities for therapeutic interventions.

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