As the first genome-wide screen in the mammalian brain, the study by Wertz et al. (2020) also raises intriguing questions that could to be further pursued. First, it is interesting that by comparing the screening results of HD mice versus the WT control mice, they surprisingly found that KD of 426 genes in R6/2 mice and 494 genes in zQ175 mice appear "protective" in HD mice, i.e., the shRNA elements were recovered more frequently in HD mice compared to WT controls. This list contains interesting genes, including complement factor C3, dopamine receptor D2, etc. However, since the screen is designed to recover shRNA or sgRNA depletion due to neuronal loss, and there is no known mechanism to amplify such elements. Indeed, about 270 of the 494 genes found as "protective" in zQ175 appear to be essential for neuronal survival in WT mice, but not in zQ175 mice. Thus, an alternative explanation is that the HD mouse backgrounds might dampen the sensitivities to the KD of a distinct subset of genes that are essential to MSNs in WT mice and further investigating the underlying mechanisms could be a fruitful pursuit. Another issue is our lack of understanding of how the striatal MSNs degenerate in the HD patient brains, and studies in existing HD mouse models provide few clues to this mystery due to the minimal or lack of neuronal cell loss in these models. Thus, much work remains to be done to define the precise mechanisms of SLIC-mediated striatal neuronal loss and to validate them in the HD brains.

In neuroscience, new tools often spur the pace of discovery. It is our rational optimism to anticipate that these powerful new genome-wide screening tools in the mammalian brain will be impactful in the field of molecular neuroscience and brain disease research.

DECLARATION OF INTERESTS

X.W.Y. is on the Scientific Advisory Board of Mitokinin and was a paid consultant for Roche, Nuredis, and Voyager Therapeutics.

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The Sense of Targeting Nonsense-Mediated Decay in C9-ALS/FTD

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Defective nucleocytoplasmic transport contributes to C9-ALS/FTD, but an inventory of proteins that become redistributed has remained elusive. In this issue of *Neuron*, Ortega et al. (2020) catalog these redistributed proteins and pinpoint nonsense-mediated decay as a therapeutic target for C9-ALS/FTD.

Several studies have linked defective nucleocytoplasmic trafficking to fatal neurodegeneration (Moore et al., 2020). For example, the (G_4C_2) hexanucleotide-repeat expansion in *C9orf72* (C9-HRE) and its associated gene products found in amyotrophic lateral sclerosis/frontotemporal dementia (C9-ALS/FTD) disrupt nucleocytoplasmic transport via

non-mutually exclusive mechanisms. First, the extended C9-RNAs can bind and sequester RNA-binding proteins (RBPs) and nuclear-transport factors in nuclear RNA foci (Moore et al., 2020). Second, arginine-rich dipeptide-repeat proteins (DPRs) translated from the C9-HRE, poly-(PR) and poly-(GR), directly interfere with cargo loading onto nuclear-import receptors (Hayes et al., 2020). Additionally, poly-(PR) and poly-(GR) interact with several intrinsically aggregation-prone proteins with prion-like domains (PrLDs), triggering aberrant phase separation in the nucleus and the cytoplasm (Moore et al., 2020). Nuclear depletion, cytoplasmic mislocalization, and subsequent aggregation of many disease-linked RBPs with PrLDs, like TDP-43, can lead to sequestration of nuclear-transport factors and nucleoporins in cytoplasmic inclusions (Moore et al., 2020). This sequestration exacerbates defects in RNA export, RNA metabolism, and nucleocytoplasmic transport and can even disrupt nuclear pore integrity (Moore et al., 2020). Previous studies have focused on elucidating the roles of particular loss- or gain-of-function events related to nucleocytoplasmic deficits in neurodegenerative disease (Moore et al., 2020). However, the vast majority of redistributed proteins and the pathways that are affected by their redistribution have remained largely enigmatic.

In this issue of *Neuron*, Ortega, Daley, et al. provide valuable insight into protein redistribution in C9-HRE carrier cells via an unbiased, proteome-wide approach (Ortega et al., 2020). They first expressed a control or disease-associated number of (G_4C_2)-repeat sequences (8 or 58, respectively) in HEK293 cells. Constructs contained a canonical start site (ATG) in frame with poly-(GP)-GFP. Importantly, cells carrying the $(G_4C_2)_{58}$ repeat also produced poly-(GR) via repeat-associated non-ATG (RAN) translation (Ortega et al., 2020). Cells with GFP fluorescence were sorted by fluorescence-activated cell sorting and then nuclear and cytoplasmic fractions were generated and analyzed by semiquantitative mass spectrometry (MS). This approach yielded several important findings. First, C9x58-HRE carrier cells undergo a significant global protein redistribution, with a pronounced shift of proteins accumulating in the cytoplasm. Second, an inventory of 126 unique proteins with a significantly altered nucleocytoplasmic ratio (N/C) was identified, with an enrichment for proteins involved in RNA metabolism and protein translation. Third, the majority of redistributed proteins contained nuclear localization/export signals and were larger than 40 kDa, implicating their transport through the nuclear pore complex (NPC). Finally, when the authors compared their list of proteins to those reported to interact with C9-RNA and C9-DPRs, they found a strong overlap indicating that sequestration of proteins by toxic C9-HRE products may contribute to their redistribution. Collectively, these findings illuminate the nature of redistributed proteins and pave the way for discovering novel pathways linked to C9-associated neurodegeneration (Ortega et al., 2020).

Based on the MS analysis, one could ask whether protein redistribution is due to impaired trafficking in C9-HRE carrier cells or whether it is a protective cellular response. To examine these possibilities in an intact nervous system, Ortega, Daley, et al. utilized a transgenic C9-fly model, expressing either 30 or 36 (G_4C_2) repeats, which has been reported to elicit eye degeneration (Ortega et al., 2020; Xu et al., 2013). Of the 126 redistributed proteins identified by MS, the authors selected 6 proteins that had the most pronounced change in localization and then crossed RNAi lines of each of these proteins with the C9-fly model. Intriguingly, all six proteins—eukaryotic translation termination factor 1 (eRF1), RBP SRSF1, arginine methyltransferase PRMT1, mRNA-transport factor ENY2, nuclear-import receptor TNPO3, and chaperone CCT8—were potent genetic modifiers of C9-associated toxicity (Ortega et al., 2020). eRF1 stood out as the strongest modifier. Reduced eRF1 expression exacerbated C9 toxicity, whereas eRF1 overexpression suppressed C9 toxicity in flies (Ortega et al., 2020). Knockdown or overexpression of eRF1 correlated with high or low levels of poly-(GR), respectively. Thus, eRF1 modulated C9 toxicity by regulating the expression levels of toxic C9-DPRs (Ortega et al., 2020).

Consistent with its role in translation and nonsense-mediated decay (NMD; a translation-coupled process that eliminates mRNAs containing premature stop codons), eRF1 is normally found in the cytoplasm. However, in C9-HRE carrier cells, eRF1 is enriched in the nuclear fraction (Ortega et al., 2020). Importantly, eRF1 had a much higher N/C ratio in both ALS patient induced pluripotent stem cell (iPSC)-derived motor neurons (MNs) and ALS postmortem tissue (Ortega et al., 2020). In fact, eRF1 was found to specifically redistribute to the cytoplasmic side of nuclear-envelope invaginations, which also correspond to sites of active protein translation (Ortega et al., 2020). These findings linked eRF1 redistribution to a role in modulating C9-HRE toxicity.

eRF1 regulates a balance between protein translation termination and NMD of mRNAs. Stop codons are recognized by eRF1, which triggers release of nascent polypeptides from the ribosome. However, if eRF1 detects a premature stop codon (e.g., in an unspliced intron), it recruits upframeshift protein 1 (UPF1), the master regulator of NMD. UPF1 endonuclease activity is unleashed after phosphorylation by a serine/threonine-protein kinase, SMG1 (Wolin and Maquat, 2019). eRF1 knockdown in healthy MNs reduced the rate of protein synthesis, confirming that eRF1 regulates translation (Ortega et al., 2020). However, in C9-HRE carrier cells and ALS patient iPSC-derived MNs, the rate of translation was already reduced, and eRF1 knockdown only had a modest effect on *de novo* protein synthesis (Ortega et al., 2020). Thus, how is eRF1 knockdown contributing to toxicity in C9-fly models? The authors hypothesized that in C9-ALS/FTD, eRF1 modulates the levels of NMD instead. Indeed, C9-ALS patient MNs and C9-HRE carrier cells had elevated levels of NMD, as indicated by elevated levels of phosphorylated UPF1 (pUPF1) and SMG1 (Ortega et al., 2020). Importantly, eRF1 knockdown resulted in reduced pUPF1 levels. Thus, in C9-ALS/FTD, likely as a protective mechanism, neurons upregulate NMD in an eRF1-dependent manner.

Next, Ortega, Daley, et al. investigated eRF1/UPF1-dependent NMD targets in C9-ALS/FTD. Given the high GC content and retention of the first intron in *C9orf72*, C9-HRE could be a potential target for NMD (Ortega et al., 2020). Thus, they looked at C9-ALS MNs and uncovered a strong positive correlation

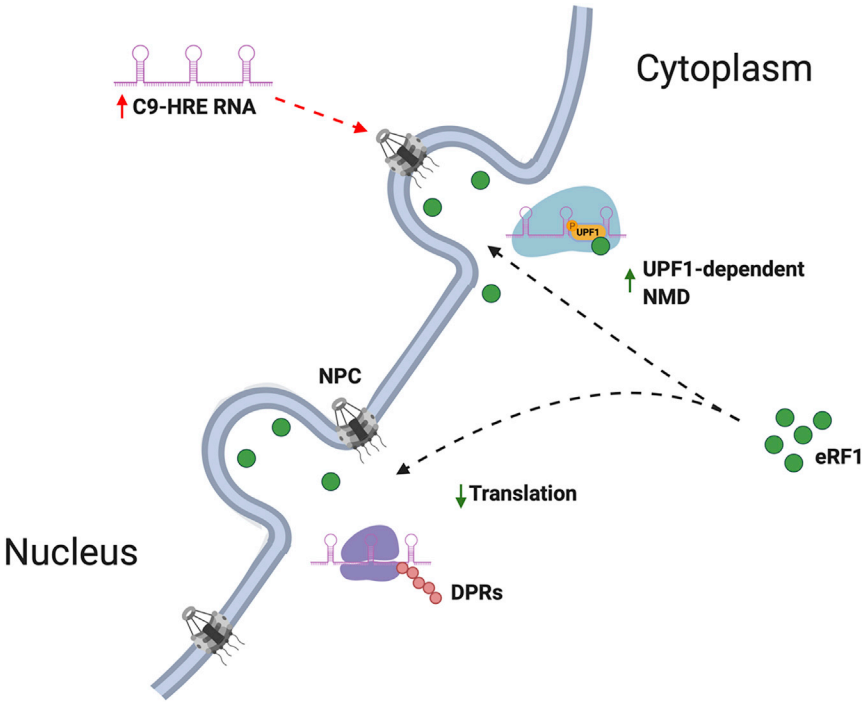

Figure 1. Protective eRF1/UPF1-Dependent NMD in C9-ALS/FTD
In *C9orf72* mutant neurons expressing C9-HRE RNA, eRF1 is redistributed from the cytoplasm to invaginations in the outer nuclear envelope. eRF1 redistribution safeguards neurons from toxicity by targeting C9-HRE RNAs to UPF1-dependent NMD as soon as they emerge from the nucleus. This degradation halts production of toxic DPRs by RAN translation. Strategies to upregulate eRF1 or UPF1 could have therapeutic utility in C9-ALS/FTD.

between the enrichment of eRF1 in nuclear-envelope invaginations and the presence of nuclear C9-HRE RNA foci. This finding suggested that eRF1 redistribution to nuclear-envelope invaginations might safeguard neurons from C9-RNAs by inhibiting their export through NPCs or that, upon nuclear export, C9-RNAs might be rapidly degraded by eRF1/UPF1-dependent NMD. In line with the second possibility, Ortega, Daley, et al. established that eRF1 knockdown in C9-ALS MNs caused an enrichment of cytoplasmic C9-RNA foci and increased C9 intron retention levels, suggesting that once eRF1-dependent NMD is impaired, the C9-RNAs escape NMD surveillance (Ortega et al., 2020). UPF1 knockdown also exacerbated C9 intron retention, further establishing that the C9-HRE is a target of UPF1-dependent NMD (Ortega et al., 2020).

Finally, in the C9-fly model, UPF1 knockdown enhanced neurotoxicity, whereas UPF1 overexpression suppressed it (Ortega et al., 2020). Critically, UPF1 knockdown in C9 flies also increased poly-(GR) levels, phenocopying the effects of eRF1 knockdown (Ortega et al., 2020). These data imply that knockdown of eRF1 or UPF1 results in deficits in NMD, which enables the escape of C9-HRE RNAs into the cytoplasm. This escape allows RAN translation and production of toxic C9-DPRs (especially poly-(GR)) (Ortega et al., 2020). Conversely, eRF1 or UPF1 overexpression activates NMD, which degrades C9-HRE RNA upon nuclear export and reduces toxicity. Thus, agents that raise eRF1 or UPF1 expression in vulnerable neurons could have therapeutic utility for C9-ALS/FTD (Figure 1).

In summary, Ortega, Daley, et al. unveil a compelling model linking C9-RNA expression to the activation of eRF1/UPF1-dependent NMD (Figure 1) (Ortega et al., 2020). Moreover, their work indicates that several other redistributed proteins could be significant modulators of toxicity. For example, the arginine methyltransferase PRMT1 was cytoplasmically enriched in C9-HRE cells and a significant modulator of toxicity in C9 flies (Ortega et al., 2020). Recent studies suggest that arginine methylation of poly-(GR) reduces toxicity (Gittings et al., 2020), indicating that the cytoplasmic redistribution of PRMT1 may be protective. Interestingly, deficits in nucleocytoplasmic trafficking and protein mislocalization are often linked to toxic events, which are intertwined in a cyclical feedforward loop (Moore et al., 2020). However, we learn from Ortega, Daley, et al. that a redistributed protein is not necessarily "mislocalized." Their discovery linking eRF1 redistribution to its function in the context of C9-ALS/FTD demonstrates that protein redistribution can occasionally take place as a protective mechanism to promote survival (Ortega et al., 2020).

The work by Ortega, Daley, et al. complements accumulating evidence that NMD is a therapeutic target for ALS/FTD. Indeed, upregulating UPF1 suppresses FUS and TDP-43 toxicity (Barmada et al., 2015; Jackson et al., 2015; Sun et al., 2011). Similarly, pharmacological activation of NMD mitigates C9-DPR toxicity (Xu et al., 2019). Could eRF1 or UPF1 be delivered as a gene therapy by adeno-associated viruses to degenerating neurons in patients (Jackson et al., 2015)? Or could brain-penetrant small-molecule drugs that specifically stimulate eRF1 or UPF1 activity or expression be developed (Xu et al., 2019)? Finally, could antisense oligonucleotides be designed to upregulate NMD in degenerating neurons? How the exciting findings of Ortega, Daley, et al. might translate into effective therapeutics awaits future exploration.

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Experience- and Context-Dependent Modulation of the Invertebrate Compass System

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How are head direction signals computed and maintained in neural circuits? In this issue of *Neuron*, Shiozaki et al. (2020) expand our understanding of the fly "compass" network, revealing context- and experience-dependent changes in the multiplexed encoding of head direction and steering maneuvers.

One of the main jobs of the brain is to move an animal through space. Perhaps it is not surprising, then, that representations of an animal's orientation and movement through space are widespread in the brain. Although the existence of such representations in vertebrates has been known for over a decade, the more recent discovery of heading direction selectivity in fruit fly neurons has made it possible to study the circuit mechanisms that generate these signals.

In a landmark paper, Seelig and Jayaraman (2015) first showed that a group of neurons in the fly Central Complex (CX)—so-called "compass" cells or E-PGs (Figure 1)—contains a representation of head direction. A "bump" of calcium activity moves through a ring of these neurons as the fly turns. The strength and position of this E-PG bump depend on both visual cues and the fly's own movements. A few years later, two groups reported the discovery of P-EN cells (Green et al., 2017; Turner-Evans et al., 2017) that encode angular velocity and rotate the E-PG heading bump. P-ENs provide a mechanism by which the fly can integrate its own movements to maintain an estimate of orientation in space. This process is thought to be critical for tasks such as path integration, where an animal keeps track of its orientation and distance relative to a nest after a long and circuitous outbound route (Stone et al., 2017). More recent modeling and connectomics studies support the hypothesis that the E-PG compass functions as a ring attractor, allowing it to select a single heading estimate despite complex visual input (Kim et al., 2017).

E-PGs and P-ENs are found in the ellipsoid body (EB), just one of many neuropils that make up the ancient and evolutionarily conserved CX. The fan-shaped body (FB), another CX neuropil, is anatomically similar to the EB. It possesses CX-intrinsic columnar neurons, akin to E-PGs and P-ENs, and tangential cells that provide extrinsic input. A prominent hypothesis in the field has been that FB columns function similarly to the columns of the EB, with activity in FB columnar neurons representing either heading or angular velocity.

In this issue of *Neuron*, new work from Shiozaki et al. (2020) tests this hypothesis directly. To examine whether FB columns also encode orientation, Shiozaki and colleagues monitored the wingbeat amplitude of tethered flies while recording calcium activity in subsets of FB columnar neurons. By comparing the stroke amplitude of the left and right wings, intended turning behavior was used to rotate a visual panorama, giving the fly sensory feedback consistent with having actually made a turn. This "closed loop" stimulus allows flies to navigate in a reduced virtual environment.

Under these conditions, Shiozaki et al. (2020) found that the middle layers of the FB encode heading direction. Importantly, coordinated activity was observed across the EB and FB, suggesting that both populations of columnar neurons carry related signals. Under pan-neuronal GCaMP expression, ventral FB layers