A PR plug for the nuclear pore in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive and uniformly fatal neurodegenerative disease characterized by loss of motor neurons in the brain and spinal cord (1). ALS overlaps clinically and genetically with other adult-onset neurodegenerative disorders, most often frontotemporal dementia (FTD), suggesting that the disease may represent one manifestation of a broader clinical spectrum. Mounting evidence suggests that impairment of nucleocytoplasmic transport contributes to the most common genetic form of ALS and FTD, although the underlying mechanism has remained obscure. In PNAS, a study by Shi et al. (2) provides evidence that toxic disease-related peptides directly obstruct the central channel of the nuclear pore complex. Fittingly, this study appears on the 50th anniversary of Gall’s seminal electron microscopy study (3) that revealed the architecture of the nuclear pore.

ALS usually presents sporadically, with no clear family history of motor neuron disease. Nevertheless, substantial evidence points to a strong genetic component to the disease. About 10% of cases of ALS are transmitted within families, almost always as dominant traits, and frequently with high penetrance (4). Recently, the most frequent genetic cause of both ALS and FTD was determined to be microsatellite expansion in the gene C9ORF72 (5, 6). The offending mutation is an expansion of an intronic hexanucleotide repeat, GGGGCC (G4C2). Unaffected individuals typically have between two and 23 G4C2 repeats, whereas people with C9ORF72-related ALS and FTD (hereafter referred to as C9-ALS/FTD) have hundreds or even thousands of repeats. This mutation is very common, accounting for up to 40% of familial cases and ~5–10% of sporadic cases of ALS-FTD (4).

The primary driver of disease downstream of G4C2 repeat expansion appears to be toxic gain of function arising from repeat-containing transcripts of C9ORF72 that accumulate in the brain and spinal cord of patients with C9-ALS/FTD (6–9). One possibility is that these RNAs sequester RNA-binding proteins and disturb their function, although evidence of this phenomenon is limited. On the other hand, substantial evidence has accrued indicating that poly-dipeptides encoded by these transcripts are toxic. Poly-dipeptides are produced by repeat-associated non-AUG (RAN) translation, which occurs in the absence of an initiating AUG codon and produces peptides from all reading frames from both sense and antisense transcripts (10).

RAN translation of repeat-containing transcripts from C9ORF72 produces five different poly-dipeptides: glycine-alanine (GA) and glycine-arginine (GR) from sense G4C2-containing transcripts, proline-arginine (PR) and proline-alanine (PA) from antisense C4G2-containing transcripts, and glycine-proline (GP) from both sense and antisense transcripts. All five of these poly-dipeptides are produced in patients with C9-ALS/FTD and accumulate in cytoplasmic and intranuclear inclusions in the brain and spinal cord (7–9). Of the five poly-dipeptides, the arginine-containing poly-dipeptides (GR and PR) appear to be particularly toxic. Expression of either GR or PR in cultured cells (including neurons) causes defects in RNA processing and subsequent cell death, whereas expression of GA, GP, or PA poly-dipeptides is comparatively well tolerated (11–13). Consistent results have been found in transgenic Drosophila engineered to express each of the five poly-dipeptides individually (13, 14). Although the mechanism of cellular dysfunction and death remains obscure, genetic model systems have pointed a finger directly at the process of nucleocytoplasmic transport. For example, a comprehensive unbiased screen for genetic modifiers of C4G2 repeat-expanded toxicity in Drosophila identified 18 genes centered on the nuclear pore complex and nucleocytoplasmic trafficking (14). A separate unbiased screen that specifically sought genetic modifiers of PR dipeptide toxicity in yeast also identified numerous genes encoding components of the nuclear pore complex and effectors of nucleocytoplasmic trafficking (15).

By conventional confocal microscopy, exogenously expressed PR poly-dipeptides have been found to accumulate in several subcellular sites, including nucleoli, stress granules, and other ribonucleoprotein bodies (11–13, 16). Digging deeper, Shi et al. (2) examined the

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The central channel of the nuclear pore is composed of Nup proteins harboring low-complexity sequence domains (LCDs) that are enriched in phenylalanine and glycine residues, and are therefore known as “FG Nups.” These flexible LCDs project into the central channel, where they interact and assemble into a gel-like structure that defines the permeability barrier of the pore (19). McKnight and coworkers examined FG domains of two Nups (Nup54 and Nup98) by transmission electron microscopy and X-ray diffraction, revealing assembly into amyloid-like fibrils composed of a cross-beta structure, similar to assemblies observed in previous studies (20) of related FG Nups. Quite unlike prototypical amyloids, which are quite stable, these FG domain fibrils were readily dissociated by even low concentrations of an anionic detergent, indicating significant lability. This property is likely critical to the function of the central channel of the nuclear pore, because FG domains also bind nuclear transport receptors to enable selective transport through this dynamic barrier.

For one of these FG domains (Nup54), the investigators used a chemical footprinting approach to map both exposed and buried residues in the fibrillar (i.e., polymeric) vs. monomeric conformation. The footprinted region was further investigated by rational mutagenesis to identify key phenylalanine residues that are required for fibril assembly. With this insight, and key mutants in hand, the investigators carried out a crucial series of experiments indicating that PR binds selectively to labile, amyloid-like fibrils of the FG domains, but not to the monomeric forms. Consistent with this observation, fluorescently tagged PR was found to decorate the surface of fibrils composed of Nup54 and Nup98. Importantly, PR binding appeared to stabilize fibrils of Nup54 and Nup98, as evidenced by partial resistance to depolymerization in the presence of the aliphatic alcohol 1,6-hexanediol. Taken together, these observations strongly imply that PR binding influences the structure and dynamics of the central channel through direct interaction with FG domains, thus impairing nucleocytoplasmic transport.

The study by Shi et al. (2) not only provides a satisfying explanation for the nucleocytoplasmic transport defect detected in model systems of C9-ALS/FTD but may also illuminate the molecular basis of ALS-FTD pathogenesis more broadly. Two recent studies used proteomic approaches to identify the cellular protein targets of the toxic, arginine-containing poly-dipeptides GR and PR (16, 21). Both studies revealed that GR and PR have a strong propensity to interact with proteins harboring LCDs, including Nups, intermediate filament proteins, and RNA-binding proteins. Notably, among these targets were the ALS-associated RNA-binding proteins TDP-43, FUS, hnRNPA1, and hnRNPA2B1. The LCDs in these RNA-binding proteins mediate assembly into a spectrum of higher order structures ranging from liquid droplets to labile fibrils composed of a cross-beta structure (20); moreover, binding of PR (or GR) to these LCDs promotes the formation and stability of these higher order structures (16, 21). ALS and FTD can also be caused by missense mutations directly impacting the LCDs of TDP-43, FUS, hnRNPA1, and hnRNPA2B1 (1). Disease mutations also promote the formation and stability of these higher order structures (22–26). Thus, a common feature underlying the molecular defect in ALS-FTD appears to be enhanced assembly and increased stability of structures assembled from LCDs.

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