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Inherited and Sporadic Amyotrophic Lateral Sclerosis and Fronto-Temporal Lobar Degenerations arising from Pathological Condensates of Phase Separating Proteins.

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ABSTRACT

Recent work on the biophysics of proteins with low complexity, intrinsically disordered domains that have the capacity to form biological condensates has profoundly altered the concepts about the pathogenesis of inherited and sporadic neurodegenerative disorders associated with pathological accumulation of these proteins. In the present review, we use the FUS, TDP-43 and A11 proteins as examples to illustrate how missense mutations and aberrant post-translational modifications of these proteins cause amyotrophic lateral sclerosis (ALS) and fronto-temporal lobar degeneration (FTLD).

ABBREVIATIONS

ADMA FUS: asymmetrically di-methylated arginine FUS;
EWS: Ewing sarcoma protein
fALS: familial amyotrophic lateral sclerosis;
FTLD: frontotemporal lobar degeneration
FUS: fused in sarcoma protein
hnRNP: heterogeneous nuclear ribonucleoprotein
PTM: post-translational modification.
PY-NLS: proline tyrosine nuclear localisation signal
QGSY: glutamine glycine serine and tyrosine repeats motif
RGG: arginine glycine glycine repeat motif
RRM: RNA recognition Motif
SMN: survival motor neuron
TAF15: TATA box binding protein 15
TDP-43: transactive response DNA binding protein 43
TNPO1: transportin 1/karyopherin β2
ANXA11: annexin 11
PSD95: postsynaptic density 95

Key words
Amyotrophic lateral sclerosis; frontotemporal dementia; biological condensates; phase separation; gelation; hydrogels; local RNA translation; stress granules; neuronal transport granules; biological condensates; FUS; TDP-43; ANXA11.

Amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease or motor neurone disease) and frontotemporal lobar degeneration (FTLD, also known as frontotemporal dementia - FTD) are well recognised neurodegenerative diseases. In ALS, the predominant clinical features reflect the progressive degeneration of corticospinal motor neurons and spinal motor neurons, with progressive weakness together with variable degrees of spasticity, hyperreflexia, flaccidity, hyporeflexia and muscle atrophy affecting both limb and cranial motor systems. In FTLD, the disease is characterised by progressive degeneration of neurons in the hippocampus, temporal lobe and frontal lobe. These neuropathological changes are accompanied by changes in behaviour, personality, frontal executive function and language. Based on clustering of these clinical features, FTLD is clinically classified into behavioural FTD (with cognitive decline and behavioural dysfunction) and primary progressive aphasia (comprising semantic dementia and progressive non-fluent aphasia (1). However, while ALS and FTLD are conventionally considered as nosologically distinct, there is increasing evidence that they represent a spectrum (2-4). Thus, careful examination of patients presenting with predominantly a motor phenotype can elicit clinical evidence of frontotemporal dysfunction, and vice versa. Moreover, mutations in several genes are associated with both disorders, and in some families, affected individuals may present with quite different degrees of motor and cortical deficits.

Both disorders are encountered in typical community clinical practices. ALS has an incidence of approximately 2 per 100,000 per year. It is usually rapidly progressive, leading to death within 3-5 years (but with some notable exceptions)(5, 6). FTLD is the second most common form of early-onset dementia, affecting up to 15 per 100,000 (1, 7, 8). The reader is referred to several excellent reviews on the epidemiology, clinical features and neuropathology of these disorders (ALS (9-12); FTLD: (13-19)).

Genetics of ALS and FTLD: focussing on genes involved in RNA binding and transport.

ALS and FTLD are etiologically heterogeneous disorders, displaying both inherited and apparently sporadic forms. Approximately 10% of ALS cases are familial (9, 11, 20, 21). Up to 40% of FTLD cases have a positive family history, and in about one third of these cases, the disorder is inherited as an autosomal dominant trait (16, 18, 19, 22, 23). Figure 1 contains a list of these genes.

Prevalent amongst these phase separating proteins are RNA binding proteins, such as fused in sarcoma (FUS)(24, 25), transactive response DNA binding protein 43 (TDP-43)(26, 27), heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and A2/B1(28), T-cell restricted intracellular antigen 1 (TIA1) (29). However, they also include other proteins such as annexin A11 (ANXA11) involved in intracellular transport of biological condensates such as stress granules (SG) and other ribonucleoprotein (RNP) granules enriched in these RNA binding proteins

A common, although not universal neuropathological feature of ALS and FTLD associated with mutations in these phase-separating proteins, is aberrant deposition of visible aggregates of the corresponding proteins in neurons and glia (30-34). These aggregates differ from conventional amyloid aggregates such as Aβ, tau and α-synuclein because they typically stain poorly with amyloidophyllic dyes such as thioflavin T, and are partially soluble in urea (18, 30-32). Furthermore, the mechanisms by which they cause neurotoxicity appear to be distinct from those of conventional amyloids (35-38).

As is apparent from the list of genes associated with these diseases, ALS and FTLD can be caused by disruption of multiple unrelated biochemical processes. Some of these biochemical processes and their underlying genes/proteins have been the subject of recent reviews (e.g. SOD1(39-42), C9orf72(43-45)). The current review will therefore focus on emerging work on a unique subset of ALS and FTLD-related proteins that can *reversibly* transition between: i) a dispersed (mixed) phase; ii) a condensed phase-separated state as liquid protein droplets suspended within a liquid (visually like oil and water); or iii) more condensed states such as hydrogels (like jelly dessert), or into fibrillary or glass-like solids (Figures 1,2).

A Short Review of the Biophysics of Biological Condensates

The physics of phase transition of synthetic polymers into droplets and gels are well known in materials science. As a result, the terminology used in the emerging field of phase transition of biological polymers (typically proteins and nucleic acids) borrows heavily from the material science field. Thus, condensation of polymers from a dispersed state into two-phase state with liquid droplets suspended in a liquid has been termed “liquid: liquid phase separation”

or “coacervation” or “de-mixing” (46, 47). Polymers can also undergo phase transition from a dispersed or liquid droplet state into more solid states with different viscoelastic properties including “hydrogels” or “networked liquids” similar to jelly dessert, or into more viscous liquid “glass-like” states (Figure 2). In contrast to most soluble intracellular proteins (e.g. classical enzymes, which exist in a limited number of well-defined three-dimensional shapes (“folds”) that are necessary for the functional properties, the assembly of polymers into liquid droplets, hydrogels, or glasslike states does not require formation of highly ordered structured complexes. Instead, they represent a metastable (nonequilibrium) ensemble of polymers in different conformations all interacting with each other. As such, these two-dimensional and three-dimensional condensates of biological polymers typically form either as free-standing membraneless organelles in the nucleus (e.g. nucleolus) or cytoplasm (e.g. ribonucleoprotein granules). They can also form on the surface of intracellular membranes (e.g. the postsynaptic density 95 PSD95 complex on postsynaptic membranes(48), synapsin on presynaptic vesicles(49, 50) and annexin A11-mediated molecular tethering of RNP granules to lysosomes)(51).

A crucial feature that drives phase transition of both synthetic and biological polymers is their ability to form multiple interactions with other polymers of the same type (homotypic) or of a different type (heterotypic, composed of either protein X:protein Y or protein: RNA interactions) (46, 52-56). These “multivalent” interactions can arise between conventional structurally-ordered domains, or more commonly, between disordered domains within the polymers. These intra- and inter-polymer interactions reduce the free energy of the polymer-solute system by encouraging condensation of the polymers into a distinct, phase-separated volume within the solute, which then becomes depleted of the biological polymer (Figure 2).

Early studies in the field of biological condensates focused upon proteins which contained at least one “low complexity domain” (LCD) composed of repetitive stretches of amino acids that are typically enriched in a subset of amino acids with: i) polar side chains (glycine, glutamine, asparagine and serine); ii) nonpolar side chains (proline); iii) positive side chains (arginine, lysine); iv) negative side chains (aspartate, glutamate); or v) aromatic side chains (phenylalanine, tryptophan and tyrosine). Hydrophobic residues are typically underrepresented in these low complexity domains. This unusual amino acid content and the repetitive amino acid sequence of these LCDs permit weak intra- and inter-polymer interactions based on: charged interactions (e.g. glutamate - arginine); cation-π interactions (between positively charged side

chains of arginines or lysines with the free electrons in the aromatic rings of tyrosines, tryptophans or phenylalanines); dipole interactions (between glycine, glutamine, asparagine and serine residues), as well as π-π interactions (formed by stacking of aromatic rings or between the guanidino moiety of arginines and the rings of aromatic amino acids) (57). Proteins or protein domains with these features typically do not fold into well-defined three-dimensional structures, and are therefore often described as "intrinsically disordered". However, while these features do not support a traditional fixed three-dimensional fold, they do underpin a crucial property of these polymers, namely their ability to form *networks* of intra- and inter-molecular interactions within and between phase-separating polymers (53, 55, 58). The propensity polymers to condense into phase-separated states is driven by: i) the number (or "valence") of interactions (e.g. the number of cation-π interactions); and ii) the affinity of these interactions (which are individually often week, but become biologically significant because of their high valence numbers). Crucially, the condensation of these disordered proteins is not dependent on any individual residue, or on precise three-dimensional spatial relationships and assumes a metastable rather than equilibrium state, which allows for reversibility of the ensemble (59, 60).

Recent studies have revealed that similar multivalency can also be achieved through oligomerisation of structured domains of proteins forming biological condensates. For instance, dimerization of G3BP1 is required for the assembly of stress granules (61). Similarly, phase separation of TDP-43 is driven by its C-terminal LCD and facilitated by oligomerisation of the N-terminus and/or oligomerisation of a 30 residue αhelical domain in the C-terminus of TDP-43 (62, 63).

Condensation of phase separating proteins into more solid ("hard") condensates such as hydrogels and into stable fibrillar condensates likely follows similar rules but with some differences. For instance, intermolecular hydrogen bonding of β-sheet domains and the presence of glutamate and serine residues appears to be important in "hardening" or "gelation" of condensates, while glycine residues promote fluidity (64). The molecular and biophysical details of this hardening process are currently under intense scrutiny because they may give insight into the formation of pathological, irreversible fibrillar gels. However, electron microscopic, solid state nuclear magnetic resonance and x-ray diffraction studies of fibrillar condensates of FUS have shown that, in contrast to conventional amyloids, they display : i) short (<5 residues) β-sheet domains; ii) few hydrophobic residues but multiple hydrophilic residues (which reduce full condensation); and iii) the presence of motifs with "kinks" at glycine,

proline or aromatic residues (e.g. residues [37]-SYSGYS-[42] and [54]-SYSSYGQS-[61], which minimize formation of stable, steric zippers characteristic of conventional amyloids (65, 66). These motifs have been termed "low-complexity aromatic-rich kinked segments" or LARKS (65).

Functional implications of physiological phase separation and gelation

The biophysical properties of proteins forming biological condensates allows them to form reversible 2-dimensional or 3-dimensional molecular scaffolds that underpin formation and function of variety of intracellular membraneless organelles, such as nucleoli, P-bodies, Cajal bodies and RNP granules (see reviews (67-69).

These scaffolds are metastable, dynamic structures that can be rapidly assembled / disassembled by: i) altering the relative stoichiometries of co-partitioning scaffolds (e.g. changing the relative abundance of mRNA and FUS polymers in FUS RNP granules(70); ii) altering the post-translational state (e.g. arginine methylation, serine phosphorylation) which changes the multivalency and/or the binding affinity of scaffolds; or iii) by the introduction of small molecule modifiers and hydrotropes such as calcium (51) and ATP (71).

The scaffolds can also recruit "cargo" or "clients" such as other proteins or nucleic acids. These client molecules can diffuse in/out of the condensate, attach to the scaffolds either by binding to the LCD, or to structured domains elsewhere in the scaffold proteins (e.g. RNA recognition motifs). The binding can be modulated by scaffold/client stoichiometry and by post-translational modifications that manipulate client: substrate affinity. The ability of the scaffold polymers in membraneless organelles to pick up and locally concentrate functional client molecules underpins the role of membraneless organelles t in a wide range of biological functions such as transport, storage and local concentration of components of intracellular signalling or metabolic machinery. As an example, stress granules allow sequestration of translationally-stalled mRNA transcripts during cellular stress (72). Neuronal transport granules sequester and transport key cargo elements involved in specialised local protein translation in axon terminals and dendrites (73-75). The presynaptic synapsin scaffold in neurons allows the physical collation of molecules necessary for assembly and fast release of presynaptic vesicles (49, 50). The postsynaptic PSD95 scaffold assembles molecules close to the postsynaptic membrane, and for rapid signalling downstream postsynaptic receptors (48).

Consequences of pathological phase separation and gelation

Recent work has shown that ALS/FTLD-associated mutations and aberrant ALS/FTLD-associated post-translational modifications of phase-separating proteins can cause their accelerated condensation into pathologically stable, β-sheet rich, intermolecular hydrogen bonded, fibrillary gel assemblies (29, 62, 76-81). These pathologically "hardened" condensates lose their ability to relax back to liquid droplet or dispersed states (29, 62, 76-81). Such defective biophysicsfunction of these proteins would be predicted to cause: i) failure of formation of the condensate (e.g. RNP granule); ii) abnormal partitioning and binding of clients into the condensate; iii) abnormal transport of condensate; and/or iv) dysregulated release of clients. The rest of this review uses FUS, TDP43 and ANXA11 to illustrate these deleterious effects.

FUS

FUS is a 526 amino acid heterologous nuclear ribonucleoprotein (hnRNP), and a member of the **F**US, **E**wing sarcoma Breakpoint region 1 (EWS/EWSR1) and **T**ATA box binding protein 15 (TAF15) (**FET**) family of RNA binding proteins (82, 83). It is composed of an N-terminal intrinsically disordered LCD region (residues 1-214) which contains multiple glutamine, glycine, serine and tyrosine (QGSY) repeats. In its middle and C-terminal domains, FUS has a well-conserved RNA recognition motif (RRM), a zinc finger domain, two domains enriched in arginine, glycine, glycine (RGG) motifs, and an atypical proline tyrosine nuclear localisation sequence (PY-NLS) (82-86). FUS is predominantly located in the nucleus, where it is involved in both DNA repair as well as RNA transcription and processing (82, 83). FUS is also present in RNP granules in the cytoplasm in axons and dendrites, where it supports regulated local synthesis of proteins involved in synaptic biology and plasticity (Figure 3) (83, 87-89). FUS is normally post-translationally modified both by asymmetric dimethylation of arginine by protein arginine methyl transferases (90), by deamination by protein arginine deiminases(60, 91) and by serine phosphorylation by DNA protein kinase (66, 92).

FUS undergoes physiological reversible phase separation and gelation in a FUS protein and RNA concentration-dependent manner (18, 60, 66, 76-78, 80, 93). The condensation arises from multivalent cation-π interactions between arginine residues in the C-terminus (n=37) and tyrosine residues (n=36) that are predominantly located at the N-terminus of FUS(60). These condensates are stabilized by intermolecular hydrogen bonded β-sheets (66, 94). This propensity to form biological condensates is tuned by post-translational modification of FUS (60, 66, 91). Thus, post-translational modification arginines modulates the strength of the cation-π interactions (60). Post-translational phosphorylation of serine strongly inhibits phase separation,

presumably by disrupting the packing of the LC domains (66).

Aberrant intracytoplasmic inclusions of FUS aggregates are observed in neurons of patients with ALS and/or FTLD. Approximately 1-4% of familial ALS (fALS) cases arise from missense or frameshift mutations in the C-terminus of FUS (residues 495 and 526), or in the N-terminal LCD (22, 95). Approximately 10% of sporadic FTLD cases (neuronal intermediate filament inclusion body disease (NIFID) and basophilic inclusion body disease (BIBD)) are associated with abnormal hypomethylation of arginine residues (33, 96).

Several non-mutually exclusive mechanisms have been proposed for how FUS aggregates induce fALS-FUS FTLD-FUS. However, recent work by several groups showing that FUS undergoes phase separation and gelation provide a compelling new theory summarised in Figure 3 (18, 60, 66, 76-78, 80, 93). This emerging insights suggests that missense and truncating mutations associated with fALS-FUS disrupt binding by the TNPO1 chaperone, and/or increase the intrinsic propensity of FUS to condense into irreversible intracytoplasmic fibrillar condensates. A similar effect arises from pathological post-translational modification of FUS. Thus, mutations in the PAD4 protein deiminase cause fALS by abrogating the capacity to reduce cation-π interactions by conversion of FUS arginines into citrullines (91). Sporadic FTLD-FUS appears to arise from either failure to asymmetrically di-methylate FUS or excessive protein arginine demethylation (85, 86, 97, 98).

The overarching net effect of both FUS mutations and of altered FUS posttranslational modification in fALS-FUS and FTLD-FUS, is an increased propensity for FUS to form irreversible fibrillary condensates that accumulate in neuronal cytoplasm. These irreversible aggregates abrogate transport and release of FUS RNP granule cargo involved in local RNA translation and metabolism in distal neurites, thereby attenuating new protein synthesis in these critical neuronal compartments (60, 78, 99).

TDP-43

TDP-43 accumulates as ubiquitinated intraneuronal inclusions, in a significant proportion of ALS and FTLD(100, 101). TDP-43 is a multi-domain RNA binding protein that plays important roles in RNA metabolism including transcription, splicing, mRNA and microRNA processing, expression and transport (102-104). TDP-43 specifically binds (UG)-rich RNA sequences

through the two highly conserved RNA recognition motifs RRM1 and RRM2. TDP-43 contains an N-terminal domain (NTD), tandem RNA recognition motifs (RRM1 and RRM2), and an intrinsically disordered C-terminal domain (CTD). The N-terminal domain is involved in multimer-formation, which is critical for its function in RNA splicing (105). The NTD can undergo liquid-liquid phase separation when induced by single strand DNA (ssDNA)(106).

Liquid-liquid phase separation of TDP-43 *in vitro* (62, 107) and in cells (108) is driven by its intrinsically disordered glycine-rich CTD (residues 267-414). An α-helical structure (residues 321-340) located between 2 disordered domains in the TDP-43 CTD, plays critical role in phase separation. Several ALS-associated mutations occur in this region, and affect phase separation(A321G, Q331K and M337V impair liquid-liquid phase separation and enhance formation of aggregates(62); N345K and A382T impair monomer exchange between TDP-43 droplet and monodispersed protein in solution (108). Mutations in hydrophobic residues within CTD (W334G) affect phase separation of CTD (109, 110). The 312-317 segment forms reversible weak cross-β interactions during gelation, and ALS-causing mutations (A315E and A315T) and phosphorylation of the segment can strengthen these normally reversible interaction into stable irreversible interactions, causing pathogenic aggregation (111).

The mechanism of neurotoxicity for TDP-43 are still the subject of intense study. Like FUS mutations, pathological mutant aggregates are associated with impaired axonal transport of TDP-43 RNP granules(112, 113). However, RNA binding of TDP-43 is modulates its toxicity. Mutations that eliminate TDP-43 binding to RNA abrogates TDP-43-mediated neurodegeneration (114-116). TDP-43 toxicity is also modulated by several other genes which cause ALS/FTLD, including: p62 (sequestrome 1)(117-119); valosin containing protein (VCP) (120-123); and ataxin 2 (124-126).

ANXA11

Annexin 11 (ANXA11) encodes a widely expressed, 505 amino acid, calcium-dependent phospholipid-binding protein. Like other members of the annexin protein family, ANXA11 contains four highly conserved annexin domains at the C-terminus, which form calcium-dependent complexes with negatively charged membrane phosphatidylinositols such as PI(3,5)P2. Atypically amongst annexins, ANXA11 also has a 196 amino acid, structurally disordered, low complexity domain at its N terminus. Biophysical experiments both in

biochemical preparations and in cells, confirm that ANXA11 can undergo reversible phase transition into liquid droplets and hydrogels in a process that requires the N-terminal LCD(51). Missense mutations in ANXA11 are associated with ALS with or without FTLD (127, 128).

Recent work has shown the dual biophysical properties of ANXA11 protein allow it to act as a molecular tether that binds neuronal stress granules (and possibly other RNP granules) to lysosomes(51). ANXA11 attaches to RNP granules via its structurally disordered N-terminal domain, and to lysosomes via the C-terminal annexin repeats. The biophysics of the attachment to RNP granules and the mechanisms that control the reversible assembly of the RNP granule-ANXA11-lysosome complex are under investigation. However the RNP granule: ANXA11 binding likely requires co-partitioning of the ANX11 LCD into the RNP granule when it can form co-scaffolds with intrinsically disordered domains of other RNP granule proteins such as G3BP1(51).

Prior to these discoveries, it was unclear how RNP granules, which lack motor protein attachments, were transported to the sites of local protein synthesis in remote synaptic compartments of neurons. The observation of ANXA11-mediated molecular tethering of RNP granules to a subset of LAMP1 -positive cytoplasmic vesicles provides an elegant answer to that longstanding enigma, and is fully congruent with the observation of new protein synthesis on the surface of endosomes in axons (89).

Crucially, ALS-associated missense mutations in either the N-terminal LCD or in the annexin repeat domain of ANXA11 disrupt formation of the molecular tether and are associated with impaired delivery of mRNA to axon terminals for local protein synthesis (51). This result is congruent with the observation that spinal cord neurons of ALS patients with ANXA11 mutations have abundant cytoplasmic aggregates of ANXA11 (127).

DISCUSSION

The review describes arapidly emerging area of cell biology related to the previously poorly-recognised, but critical role biological condensates in membraneless organelles such as stress granules, neuronal transport granules and other RNP granules. The basic biophysics of phase separation and gelation and the effect of pathogenic mutations/post-translational modifications on these crucial cellular processes is now becoming clearer. There are obvious functional similarities between the pathobiological mechanisms underlying neurodegeneration

associated with mutations and abnormal post-translational modifications of FUS, TDP-43 and ANXA11. These commonalities suggest opportunities for therapeutic interventions that may have broad implications across multiple genetic and sporadic forms of ALS/FTLD associated with defective function of phase separating proteins.

The next phase of this work will need to focus on understanding how normal assembly and relaxation/disassembly of biological condensates is physiologically regulated in response to cellular metabolic state, particularly in neurons. The discovery of a new class of proteins (e.g. ANXA11, synapsin and PSD95) that adjoin membrane- and membrane-free biology presents the additional opportunity to investigate functional interactions between membrane-bound organelles and phase-separated structures in the cytosol This future work may also provide some tractable molecular targets for novel approaches to prevent, halt or reverse abnormal phase separation of intrinsically disordered proteins, such as FUS, TDP-43 and ANXA11.

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CONTRIBUTIONS

All authors contributed to the writing of this review manuscript.

FIGURE LEGENDS

Figure 1

Genetic overlap between ALS and FTLD. Genetic profiling of familial and sporadic cases of ALS and FTLD have revealed a striking level of overlap between genes linked to each disease. The shared genetic basis for these seemingly distinct clinical syndromes suggests a common core pathophysiology. Most genes linked to either disease cluster into one of three groups: proteostasis and sorting, cytoskeleton and transport, or RNA-binding. Additionally, several genes across these functional groups encode proteins that form biological condensates involved in RNA transport and translation in remote neuronal compartments, strongly linking this biophysical phenomenon to disease pathogenesis.

Figure 2

Biological condensates form free droplets and membrane-associated superstructures. In the dispersed state, protein scaffolds (green circles) and cargo/client RNA molecules (red lines) are intermixed with solute molecules (black circles). Under appropriate conditions, protein scaffolds can phase separate to form a liquid droplet enriched in the scaffold protein and client RNA. Some phase separating proteins, such as A11, can also assemble as 2D- and 3D condensates on membrane surfaces

(Edited to correspond to panel labels)

A – Monodisperse FUS

B – FUS condensates

C – Annexin A11 enables the attachment of biological condensates to membranes. Liposomes (blue), ANXA11 (red), G3BP1 RNPs (green).

D– In the dispersed state, protein scaffolds (green dots) and cargo/client RNA molecules (magenta dots) are intermixed with solute molecules (grey dots).

E - Under appropriate conditions, protein scaffolds can phase separate to form liquid droplets enriched in the scaffold protein and client RNA. Owing to their lack of delimiting membranes, these structures can fuse with each other to form larger condensates

F – Some phase separating proteins, such as annexin A11(orange dots), can assemble as 2D and 3D condensates on membranes, enabling the scaffolding of non-lipid-binding condensates

Figure 3.

The roles of proteins forming biological condensates in the transport and local translation of RNAs in remote synaptic compartments in neurons. These proteins form RNP granule scaffolds for binding of RNA and RNA translation machinery, and for the subsequent long-range intracellular transport of these granules to distal neuronal compartments such as dendritic spines and axon terminals (green arrows). Disease associated mutations and pathological posttranslational modification of these proteins result in the formation of irreversible aggregates that sequester RNP granule cargo, and/or failure of intra-neuronal transport of the RNP granules (red arrows).

REFERENCES

1 Sivasathiaseelan, H., Marshall, C.R., Agustus, J.L., Benhamou, E., Bond, R.L., van Leeuwen, J.E.P., Hardy, C.J.D., Rohrer, J.D. and Warren, J.D. (2019) Frontotemporal Dementia: A Clinical Review. *Semin Neurol*, **39**, 251-263.
2 Couratier, P., Corcia, P., Lautrette, G., Nicol, M. and Marin, B. (2017) ALS and frontotemporal dementia belong to a common disease spectrum. *Rev Neurol (Paris)*, **173**, 273-279.
3 Ng, A.S., Rademakers, R. and Miller, B.L. (2015) Frontotemporal dementia: a bridge between dementia and neuromuscular disease. *Ann N Y Acad Sci*, **1338**, 71-93.
4 Lomen-Hoerth, C., Anderson, T. and Miller, B. (2002) The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. *Neurology*, **59**, 1077-1079.
5 van Es, M.A., Hardiman, O., Chio, A., Al-Chalabi, A., Pasterkamp, R.J., Veldink, J.H. and van den Berg, L.H. (2017) Amyotrophic lateral sclerosis. *Lancet*, **390**, 2084-2098.
6 Al-Chalabi, A. and Hardiman, O. (2013) The epidemiology of ALS: a conspiracy of genes, environment and time. *Nat Rev Neurol*, **9**, 617-628.
7 Raffaele, F., Claudia, M. and John, H. (2019) Genetics and molecular mechanisms of frontotemporal lobar degeneration: an update and future avenues. *Neurobiol Aging*, **78**, 98-110.
8 Rabinovici, G.D. and Miller, B.L. (2010) Frontotemporal lobar degeneration: epidemiology, pathophysiology, diagnosis and management. *CNS Drugs*, **24**, 375-398.
9 Ghasemi, M. and Brown, R.H., Jr. (2018) Genetics of Amyotrophic Lateral Sclerosis. *Cold Spring Harb Perspect Med*, **8**.
10 Saberi, S., Stauffer, J.E., Schulte, D.J. and Ravits, J. (2015) Neuropathology of Amyotrophic Lateral Sclerosis and Its Variants. *Neurol Clin*, **33**, 855-876.
11 Swinnen, B. and Robberecht, W. (2014) The phenotypic variability of amyotrophic lateral sclerosis. *Nat Rev Neurol*, **10**, 661-670.
12 Tiryaki, E. and Horak, H.A. (2014) ALS and other motor neuron diseases. *Continuum (Minneap Minn)*, **20**, 1185-1207.
13 Neumann, M. and Mackenzie, I.R.A. (2019) Review: Neuropathology of non-tau frontotemporal lobar degeneration. *Neuropathol Appl Neurobiol*, **45**, 19-40.
14 Olney, N.T., Spina, S. and Miller, B.L. (2017) Frontotemporal Dementia. *Neurol Clin*, **35**, 339-374.
15 Finger, E.C. (2016) Frontotemporal Dementias. *Continuum (Minneap Minn)*, **22**, 464-489.
16 Bang, J., Spina, S. and Miller, B.L. (2015) Frontotemporal dementia. *Lancet*, **386**, 1672-1682.
17 Seltman, R.E. and Matthews, B.R. (2012) Frontotemporal lobar degeneration: epidemiology, pathology, diagnosis and management. *CNS Drugs*, **26**, 841-870.
18 Mann, D.M.A. and Snowden, J.S. (2017) Frontotemporal lobar degeneration: Pathogenesis, pathology and pathways to phenotype. *Brain Pathol*, **27**, 723-736.
19 Lashley, T., Rohrer, J.D., Mead, S. and Revesz, T. (2015) Review: an update on clinical, genetic and pathological aspects of frontotemporal lobar degenerations. *Neuropathol Appl Neurobiol*, **41**, 858-881.
20 Chia, R., Chio, A. and Traynor, B.J. (2018) Novel genes associated with amyotrophic lateral sclerosis: diagnostic and clinical implications. *Lancet Neurol*, **17**, 94-102.
21 Corcia, P., Couratier, P., Blasco, H., Andres, C.R., Beltran, S., Meininger, V. and Vourc'h, P. (2017) Genetics of amyotrophic lateral sclerosis. *Rev Neurol (Paris)*, **173**, 254-262.
22 Rainero, I., Rubino, E., Michelerio, A., D'Agata, F., Gentile, S. and Pinessi, L. (2017) Recent advances in the molecular genetics of frontotemporal lobar degeneration. *Funct Neurol*, **32**, 7-16.

23 Pottier, C., Ravenscroft, T.A., Sanchez-Contreras, M. and Rademakers, R. (2016) Genetics of FTLD: overview and what else we can expect from genetic studies. *J Neurochem*, **138 Suppl 1**, 32-53.
24 Kwiatkowski, T.J., Jr., Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vanderburg, C.R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E.J., Munsat, T. *et al.* (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science*, **323**, 1205-1208.
25 Vance, C., Rogelj, B., Hortobagyi, T., De Vos, K.J., Nishimura, A.L., Sreedharan, J., Hu, X., Smith, B., Ruddy, D., Wright, P. *et al.* (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science*, **323**, 1208-1211.
26 Sreedharan, J., Blair, I.P., Tripathi, V.B., Hu, X., Vance, C., Rogelj, B., Ackerley, S., Durnall, J.C., Williams, K.L., Buratti, E. *et al.* (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science*, **319**, 1668-1672.
27 Kabashi, E., Valdmanis, P.N., Dion, P., Spiegelman, D., McConkey, B.J., Vande Velde, C., Bouchard, J.P., Lacomblez, L., Pochigaeva, K., Salachas, F. *et al.* (2008) TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet*, **40**, 572-574.
28 Kim, H.J., Kim, N.C., Wang, Y.D., Scarborough, E.A., Moore, J., Diaz, Z., MacLea, K.S., Freibaum, B., Li, S., Molliex, A. *et al.* (2013) Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature*, **495**, 467-473.
29 Mackenzie, I.R., Nicholson, A.M., Sarkar, M., Messing, J., Purice, M.D., Pottier, C., Annu, K., Baker, M., Perkerson, R.B., Kurti, A. *et al.* (2017) TIA1 Mutations in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia Promote Phase Separation and Alter Stress Granule Dynamics. *Neuron*, **95**, 808-816 e809.
30 Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M. *et al.* (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*, **314**, 130-133.
31 Neumann, M., Rademakers, R., Roeber, S., Baker, M., Kretzschmar, H.A. and Mackenzie, I.R. (2009) A new subtype of frontotemporal lobar degeneration with FUS pathology. *Brain*, **132**, 2922-2931.
32 Rademakers, R., Neumann, M. and Mackenzie, I.R. (2012) Advances in understanding the molecular basis of frontotemporal dementia. *Nat Rev Neurol*, **8**, 423-434.
33 Mackenzie, I.R. and Neumann, M. (2016) Molecular neuropathology of frontotemporal dementia: insights into disease mechanisms from postmortem studies. *J Neurochem*, **138 Suppl 1**, 54-70.
34 Hirsch-Reinshagen, V., Pottier, C., Nicholson, A.M., Baker, M., Hsiung, G.R., Krieger, C., Sengdy, P., Boylan, K.B., Dickson, D.W., Mesulam, M. *et al.* (2017) Clinical and neuropathological features of ALS/FTD with TIA1 mutations. *Acta Neuropathol Commun*, **5**, 96.
35 Kim, H.J. and Taylor, J.P. (2017) Lost in Transportation: Nucleocytoplasmic Transport Defects in ALS and Other Neurodegenerative Diseases. *Neuron*, **96**, 285-297.
36 Lagier-Tourenne, C., Polymenidou, M. and Cleveland, D.W. (2010) TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. *Hum Mol Genet*, **19**, R46-64.
37 Coady, T.H. and Manley, J.L. (2015) ALS mutations in TLS/FUS disrupt target gene expression. *Genes Dev*, **29**, 1696-1706.
38 Bowden, H.A. and Dormann, D. (2016) Altered mRNP granule dynamics in FTLD pathogenesis. *J Neurochem*, **138 Suppl 1**, 112-133.
39 Sangwan, S. and Eisenberg, D.S. (2016) Perspective on SOD1 mediated toxicity in Amyotrophic Lateral Sclerosis. *Postepy Biochem*, **62**, 362-369.

40 Silverman, J.M., Fernando, S.M., Grad, L.I., Hill, A.F., Turner, B.J., Yerbury, J.J. and Cashman, N.R. (2016) Disease Mechanisms in ALS: Misfolded SOD1 Transferred Through Exosome-Dependent and Exosome-Independent Pathways. *Cell Mol Neurobiol*, **36**, 377-381.
41 Peters, O.M., Ghasemi, M. and Brown, R.H., Jr. (2015) Emerging mechanisms of molecular pathology in ALS. *J Clin Invest*, **125**, 1767-1779.
42 Boillee, S., Vande Velde, C. and Cleveland, D.W. (2006) ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron*, **52**, 39-59.
43 Cook, C. and Petrucelli, L. (2019) Genetic Convergence Brings Clarity to the Enigmatic Red Line in ALS. *Neuron*, **101**, 1057-1069.
44 Balendra, R. and Isaacs, A.M. (2018) C9orf72-mediated ALS and FTD: multiple pathways to disease. *Nat Rev Neurol*, **14**, 544-558.
45 Gendron, T.F. and Petrucelli, L. (2018) Disease Mechanisms of C9ORF72 Repeat Expansions. *Cold Spring Harb Perspect Med*, **8**.
46 Shin, Y. and Brangwynne, C.P. (2017) Liquid phase condensation in cell physiology and disease. *Science*, **357**.
47 Jain, A. and Vale, R.D. (2017) RNA phase transitions in repeat expansion disorders. *Nature*, **546**, 243-247.
48 Zeng, M., Shang, Y., Araki, Y., Guo, T., Huganir, R.L. and Zhang, M. (2016) Phase Transition in Postsynaptic Densities Underlies Formation of Synaptic Complexes and Synaptic Plasticity. *Cell*, **166**, 1163-1175 e1112.
49 Milovanovic, D., Wu, Y., Bian, X. and De Camilli, P. (2018) A liquid phase of synapsin and lipid vesicles. *Science*, **361**, 604-607.
50 Snead, D. and Eliezer, D. (2019) Intrinsically disordered proteins in synaptic vesicle trafficking and release. *J Biol Chem*, **294**, 3325-3342.
51 Liao, Y.C., Fernandopulle, M., Guozhen Wang, G., Choi, H., Hao, L., Drerup, C.M., Qamar, S., Nixon-Abell, J., Shen, Y., Meadows, W.P. *et al.* (2019) RNA granules hitchhike on lysosomes for long-distance transport, using annexin A11 as a molecular tether. *Cell*, **https://ssrn.com/abstract=3312723**.
52 Bergeron-Sandoval, L.P., Safaee, N. and Michnick, S.W. (2016) Mechanisms and Consequences of Macromolecular Phase Separation. *Cell*, **165**, 1067-1079.
53 Brangwynne, C.P., Tompa, P. and Pappu, R.V. (2015) Polymer physics of intracellular phase transitions. *Nat Phys*, **11**, 899-904.
54 Pak, C.W., Kosno, M., Holehouse, A.S., Padrick, S.B., Mittal, A., Ali, R., Yunus, A.A., Liu, D.R., Pappu, R.V. and Rosen, M.K. (2016) Sequence Determinants of Intracellular Phase Separation by Complex Coacervation of a Disordered Protein. *Mol Cell*, **63**, 72-85.
55 Wei, M.T., Elbaum-Garfinkle, S., Holehouse, A.S., Chen, C.C., Feric, M., Arnold, C.B., Priestley, R.D., Pappu, R.V. and Brangwynne, C.P. (2017) Phase behaviour of disordered proteins underlying low density and high permeability of liquid organelles. *Nat Chem*, **9**, 1118-1125.
56 Banani, S.F., Lee, H.O., Hyman, A.A. and Rosen, M.K. (2017) Biomolecular condensates: organizers of cellular biochemistry. *Nat Rev Mol Cell Biol*, **18**, 285-298.
57 Vernon, R.M., Chong, P.A., Tsang, B., Kim, T.H., Bah, A., Farber, P., Lin, H. and Forman-Kay, J.D. (2018) Pi-Pi contacts are an overlooked protein feature relevant to phase separation. *Elife*, **7**.
58 Harmon, T.S., Holehouse, A.S., Rosen, M.K. and Pappu, R.V. (2017) Intrinsically disordered linkers determine the interplay between phase separation and gelation in multivalent proteins. *Elife*, **6**.
59 Lin, Y., Currie, S.L. and Rosen, M.K. (2017) Intrinsically disordered sequences enable modulation of protein phase separation through distributed tyrosine motifs. *J Biol Chem*, **292**, 19110-19120.

60 Qamar, S., Wang, G., Randle, S.J., Ruggeri, F.S., Varela, J.A., Lin, J.Q., Phillips, E.C., Miyashita, A., Williams, D., Strohl, F. *et al.* (2018) FUS Phase Separation Is Modulated by a Molecular Chaperone and Methylation of Arginine Cation-pi Interactions. *Cell*, **173**, 720-734 e715.
61 Tourriere, H., Chebli, K., Zekri, L., Courselaud, B., Blanchard, J.M., Bertrand, E. and Tazi, J. (2003) The RasGAP-associated endoribonuclease G3BP assembles stress granules. *J Cell Biol*, **160**, 823-831.
62 Conicella, A.E., Zerze, G.H., Mittal, J. and Fawzi, N.L. (2016) ALS Mutations Disrupt Phase Separation Mediated by alpha-Helical Structure in the TDP-43 Low-Complexity C-Terminal Domain. *Structure*, **24**, 1537-1549.
63 Wang, A., Conicella, A.E., Schmidt, H.B., Martin, E.W., Rhoads, S.N., Reeb, A.N., Nourse, A., Ramirez Montero, D., Ryan, V.H., Rohatgi, R. *et al.* (2018) A single N-terminal phosphomimic disrupts TDP-43 polymerization, phase separation, and RNA splicing. *EMBO J*, **37**.
64 Wang, J., Choi, J.M., Holehouse, A.S., Lee, H.O., Zhang, X., Jahnel, M., Maharana, S., Lemaitre, R., Pozniakovsky, A., Drechsel, D. *et al.* (2018) A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins. *Cell*, **174**, 688-699 e616.
65 Hughes, M.P., Sawaya, M.R., Boyer, D.R., Goldschmidt, L., Rodriguez, J.A., Cascio, D., Chong, L., Gonen, T. and Eisenberg, D.S. (2018) Atomic structures of low-complexity protein segments reveal kinked β sheets that assemble networks. *Science*, **359**, 698-701.
66 Murray, D.T., Kato, M., Lin, Y., Thurber, K.R., Hung, I., McKnight, S.L. and Tycko, R. (2017) Structure of FUS Protein Fibrils and Its Relevance to Self-Assembly and Phase Separation of Low-Complexity Domains. *Cell*, **171**, 615-627 e616.
67 Gomes, E. and Shorter, J. (2019) The molecular language of membraneless organelles. *J Biol Chem*, **294**, 7115-7127.
68 Alberti, S. and Carra, S. (2018) Quality Control of Membraneless Organelles. *J Mol Biol*, **430**, 4711-4729.
69 Boeynaems, S., Alberti, S., Fawzi, N.L., Mittag, T., Polymenidou, M., Rousseau, F., Schymkowitz, J., Shorter, J., Wolozin, B., Van Den Bosch, L. *et al.* (2018) Protein Phase Separation: A New Phase in Cell Biology. *Trends Cell Biol*, **28**, 420-435.
70 Maharana, S., Wang, J., Papadopoulos, D.K., Richter, D., Pozniakovsky, A., Poser, I., Bickle, M., Rizk, S., Guillen-Boixet, J., Franzmann, T.M. *et al.* (2018) RNA buffers the phase separation behavior of prion-like RNA binding proteins. *Science*, **360**, 918-921.
71 Patel, A., Malinovska, L., Saha, S., Wang, J., Alberti, S., Krishnan, Y. and Hyman, A.A. (2017) ATP as a biological hydrotrope. *Science*, **356**, 753-756.
72 Protter, D.S. and Parker, R. (2016) Principles and Properties of Stress Granules. *Trends Cell Biol*, **26**, 668-679.
73 Shigeoka, T., Jung, H., Jung, J., Turner-Bridger, B., Ohk, J., Lin, J.Q., Amieux, P.S. and Holt, C.E. (2016) Dynamic Axonal Translation in Developing and Mature Visual Circuits. *Cell*, **166**, 181-192.
74 Holt, C.E. and Bullock, S.L. (2009) Subcellular mRNA localization in animal cells and why it matters. *Science*, **326**, 1212-1216.
75 Holt, C.E. and Schuman, E.M. (2013) The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron*, **80**, 648-657.
76 Han, T.W., Kato, M., Xie, S., Wu, L.C., Mirzaei, H., Pei, J., Chen, M., Xie, Y., Allen, J., Xiao, G. *et al.* (2012) Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. *Cell*, **149**, 768-779.
77 Kato, M., Han, T.W., Xie, S., Shi, K., Du, X., Wu, L.C., Mirzaei, H., Goldsmith, E.J., Longgood, J., Pei, J. *et al.* (2012) Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. *Cell*, **149**, 753-767.

78 Murakami, T., Qamar, S., Lin, J.Q., Schierle, G.S., Rees, E., Miyashita, A., Costa, A.R., Dodd, R.B., Chan, F.T., Michel, C.H. *et al.* (2015) ALS/FTD Mutation-Induced Phase Transition of FUS Liquid Droplets and Reversible Hydrogels into Irreversible Hydrogels Impairs RNP Granule Function. *Neuron*, **88**, 678-690.
79 Molliex, A., Temirov, J., Lee, J., Coughlin, M., Kanagaraj, A.P., Kim, H.J., Mittag, T. and Taylor, J.P. (2015) Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization. *Cell*, **163**, 123–133.
80 Patel, A., Lee, H.O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M.Y., Stoynov, S., Mahamid, J., Saha, S., Franzmann, T.M. *et al.* (2015) A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. *Cell*, **162**, 1066-1077.
81 Schmidt, H.B. and Rohatgi, R. (2016) In Vivo Formation of Vacuolated Multi-phase Compartments Lacking Membranes. *Cell Rep*, **16**, 1228-1236.
82 Schwartz, J.C., Cech, T.R. and Parker, R.R. (2015) Biochemical Properties and Biological Functions of FET Proteins. *Annu Rev Biochem*, **84**, 355-379.
83 Ratti, A. and Buratti, E. (2016) Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins. *J Neurochem*, **138 Suppl 1**, 95-111.
84 Dormann, D., Madl, T., Valori, C.F., Bentmann, E., Tahirovic, S., Abou-Ajram, C., Kremmer, E., Ansorge, O., Mackenzie, I.R., Neumann, M. *et al.* (2012) Arginine methylation next to the PY-NLS modulates Transportin binding and nuclear import of FUS. *EMBO J*, **31**, 4258-4275.
85 Dormann, D., Rodde, R., Edbauer, D., Bentmann, E., Fischer, I., Hruscha, A., Than, M.E., Mackenzie, I.R., Capell, A., Schmid, B. *et al.* (2010) ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import. *EMBO J*, **29**, 2841-2857.
86 Suarez-Calvet, M., Neumann, M., Arzberger, T., Abou-Ajram, C., Funk, E., Hartmann, H., Edbauer, D., Kremmer, E., Gobl, C., Resch, M. *et al.* (2016) Monomethylated and unmethylated FUS exhibit increased binding to Transportin and distinguish FTLD-FUS from ALS-FUS. *Acta Neuropathol*, **131**, 587-604.
87 Sephton, C.F. and Yu, G. (2015) The function of RNA-binding proteins at the synapse: implications for neurodegeneration. *Cell Mol Life Sci*, **72**, 3621-3635.
88 Dormann, D. and Haass, C. (2013) Fused in sarcoma (FUS): an oncogene goes awry in neurodegeneration. *Mol Cell Neurosci*, **56**, 475-486.
89 Cioni, J.M., Lin, J.Q., Holtermann, A.V., Koppers, M., Jakobs, M.A.H., Azizi, A., Turner-Bridger, B., Shigeoka, T., Franze, K., Harris, W.A. *et al.* (2019) Late Endosomes Act as mRNA Translation Platforms and Sustain Mitochondria in Axons. *Cell*, **176**, 56-72 e15.
90 Rappsilber, J., Friesen, W.J., Paushkin, S., Dreyfuss, G. and Mann, M. (2003) Detection of arginine dimethylated peptides by parallel precursor ion scanning mass spectrometry in positive ion mode. *Anal Chem*, **75**, 3107-3114.
91 Tanikawa, C., Ueda, K., Suzuki, A., Iida, A., Nakamura, R., Atsuta, N., Tohnai, G., Sobue, G., Saichi, N., Momozawa, Y. *et al.* (2018) Citrullination of RGG Motifs in FET Proteins by PAD4 Regulates Protein Aggregation and ALS Susceptibility. *Cell Rep*, **22**, 1473-1483.
92 Deng, Q., Holler, C.J., Taylor, G., Hudson, K.F., Watkins, W., Gearing, M., Ito, D., Murray, M.E., Dickson, D.W., Seyfried, N.T. *et al.* (2014) FUS is phosphorylated by DNA-PK and accumulates in the cytoplasm after DNA damage. *J Neurosci*, **34**, 7802-7813.
93 Schwartz, J.C., Wang, X., Podell, E.R. and Cech, T.R. (2013) RNA seeds higher-order assembly of FUS protein. *Cell Rep*, **5**, 918-925.
94 Monahan, Z., Ryan, V.H., Janke, A.M., Burke, K.A., Rhoads, S.N., Zerze, G.H., O'Meally, R., Dignon, G.L., Conicella, A.E., Zheng, W. *et al.* (2017) Phosphorylation of the FUS low-complexity domain disrupts phase separation, aggregation, and toxicity. *EMBO J*, in press.
95 Kapeli, K., Martinez, F.J. and Yeo, G.W. (2017) Genetic mutations in RNA-binding proteins and their roles in ALS. *Hum Genet*, **136**, 1193-1214.

96 Hortobagyi, T. and Cairns, N.J. (2017) Amyotrophic lateral sclerosis and non-tau frontotemporal lobar degeneration. *Handb Clin Neurol*, **145**, 369-381.
97 Neumann, M., Valori, C.F., Ansorge, O., Kretzschmar, H.A., Munoz, D.G., Kusaka, H., Yokota, O., Ishihara, K., Ang, L.C., Bilbao, J.M. *et al.* (2012) Transportin 1 accumulates specifically with FET proteins but no other transportin cargos in FTLD-FUS and is absent in FUS inclusions in ALS with FUS mutations. *Acta Neuropathol*, **124**, 705-716.
98 Troakes, C., Hortobagyi, T., Vance, C., Al-Sarraj, S., Rogelj, B. and Shaw, C.E. (2013) Transportin 1 colocalization with Fused in Sarcoma (FUS) inclusions is not characteristic for amyotrophic lateral sclerosis-FUS confirming disrupted nuclear import of mutant FUS and distinguishing it from frontotemporal lobar degeneration with FUS inclusions. *Neuropathol Appl Neurobiol*, **39**, 553-561.
99 Lopez-Erauskin, J., Tadokoro, T., Baughn, M.W., Myers, B., McAlonis-Downes, M., Chillon-Marinas, C., Asiaban, J.N., Artates, J., Bui, A.T., Vetto, A.P. *et al.* (2018) ALS/FTD-Linked Mutation in FUS Suppresses Intra-axonal Protein Synthesis and Drives Disease Without Nuclear Loss-of-Function of FUS. *Neuron*, **100**, 816-830 e817.
100 Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., Mori, H., Mann, D., Tsuchiya, K., Yoshida, M., Hashizume, Y. *et al.* (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem. Biophys. Res. Commun.*, **351**, 602-611.
101 Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M. *et al.* (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*, **314**, 130-133.
102 Lee, E.B., Lee, V.M.Y. and Trojanowski, J.Q. (2012) Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. *Nature Reviews Neuroscience*, **13**, 38-50.
103 Ratti, A. and Buratti, E. (2016) Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins. *J. Neurochem.*, **138**, 95-111.
104 Kapeli, K., Martinez, F.J. and Yeo, G.W. (2017) Genetic mutations in RNA-binding proteins and their roles in ALS. *Hum. Genet.*, **136**, 1193-1214.
105 Afroz, T., Hock, E.M., Ernst, P., Foglieni, C., Jambeau, M., Gilhespy, L.A.B., Laferriere, F., Maniecka, Z., Pluckthun, A., Mittl, P. *et al.* (2017) Functional and dynamic polymerization of the ALS-linked protein TDP-43 antagonizes its pathologic aggregation. *Nature Communications*, **8**.
106 Wang, L., Kang, J., Lim, L.Z., Wei, Y.Y. and Song, J.X. (2018) TDP-43 NTD can be induced while CTD is significantly enhanced by ssDNA to undergo liquid-liquid phase separation. *Biochem. Biophys. Res. Commun.*, **499**, 189-195.
107 Molliex, A., Temirov, J., Lee, J., Coughlin, M., Kanagaraj, A.P., Kim, H.J., Mittag, T. and Taylor, J.P. (2015) Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization. *Cell*, **163**, 123-133.
108 Schmidt, H.B. and Rohatgi, R. (2016) In Vivo Formation of Vacuolated Multi-phase Compartments Lacking Membranes. *Cell Reports*, **16**, 1228-1236.
109 Li, H.R., Chiang, W.C., Chou, P.C., Wang, W.J. and Huang, J.R. (2018) TAR DNA-binding protein 43 (TDP-43) liquid-liquid phase separation is mediated by just a few aromatic residues. *J. Biol. Chem.*, **293**, 6090-6098.
110 Li, H.R., Chen, T.C., Hsiao, C.L., Shi, L., Chou, C.Y. and Huang, J.R. (2018) The physical forces mediating self-association and phase-separation in the C-terminal domain of TDP-43. *Biochimica Et Biophysica Acta-Proteins and Proteomics*, **1866**, 214-223.
111 Guenther, E.L., Cao, Q., Trinh, H., Lu, J.H., Sawaya, M.R., Cascio, D., Boyer, D.R., Rodriguez, J.A., Hughes, M.P. and Eisenberg, D.S. (2018) Atomic structures of TDP-43

LCD segments and insights into reversible or pathogenic aggregation. *Nat. Struct. Mol. Biol.*, **25**, 463-+.

112 Alami, Nael H., Smith, Rebecca B., Carrasco, Monica A., Williams, Luis A., Winborn, Christina S., Han, Steve S.W., Kiskinis, E., Winborn, B., Freibaum, Brian D., Kanagaraj, A. *et al.* (2014) Axonal Transport of TDP-43 mRNA Granules Is Impaired by ALS-Causing Mutations. *Neuron*, **81**, 536-543.

113 Liu-Yesucevitz, L., Lin, A.Y., Ebata, A., Boon, J.Y., Reid, W., Xu, Y.F., Kobrin, K., Murphy, G.J., Petrucelli, L. and Wolozin, B. (2014) ALS-linked mutations enlarge TDP-43-enriched neuronal RNA granules in the dendritic arbor. *J Neurosci*, **34**, 4167-4174.

114 Flores, B.N., Li, X.L., Malik, A.M., Martinez, J., Beg, A.A. and Barmada, S.J. (2019) An Intramolecular Salt Bridge Linking TDP43 RNA Binding, Protein Stability, and TDP43-Dependent Neurodegeneration. *Cell Reports*, **27**, 1133-1150.

115 Ihara, R., Matsukawa, K., Nagata, Y., Kunugi, H., Tsuji, S., Chihara, T., Kuranaga, E., Miura, M., Wakabayashi, T., Hashimoto, T. *et al.* (2013) RNA binding mediates neurotoxicity in the transgenic Drosophila model of TDP-43 proteinopathy. *Hum. Mol. Genet.*, **22**, 4474-4484.

116 Voigt, A., Herholz, D., Fiesel, F.C., Kaur, K., Muller, D., Karsten, P., Weber, S.S., Kahle, P.J., Marquardt, T. and Schulz, J.B. (2010) TDP-43-Mediated Neuron Loss In Vivo Requires RNA-Binding Activity. *Plos One*, **5**.

117 Rubino, E., Rainero, I., Chio, A., Rogaeva, E., Galimberti, D., Fenoglio, P., Grinberg, Y., Isaia, G., Calvo, A., Gentile, S. *et al.* (2012) SQSTM1 mutations in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Neurology*, **79**, 1556-1562.

118 Brady, O.A., Meng, P., Zheng, Y.Q., Mao, Y.X. and Hu, F.H. (2011) Regulation of TDP-43 aggregation by phosphorylation andp62/SQSTM1. *J. Neurochem.*, **116**, 248-259.

119 Tanji, K., Zhang, H.X., Mori, F., Kakita, A., Takahashi, H. and Wakabayashi, K. (2012) p62/sequestosome 1 binds to TDP-43 in brains with frontotemporal lobar degeneration with TDP-43 inclusions. *J. Neurosci. Res.*, **90**, 2034-2042.

120 Kushimura, Y., Tokuda, T., Azuma, Y., Yamamoto, I., Mizuta, I., Mizuno, T., Nakagawa, M., Ueyama, M., Nagai, Y., Yoshida, H. *et al.* (2018) Overexpression of ter94, Drosophila VCP, improves motor neuron degeneration induced by knockdown of TBPH, Drosophila TDP-43. *Am J Neurodegener Dis*, **7**, 11-31.

121 Ritson, G.P., Custer, S.K., Freibaum, B.D., Guinto, J.B., Geffel, D., Moore, J., Tang, W., Winton, M.J., Neumann, M., Trojanowski, J.Q. *et al.* (2010) TDP-43 mediates degeneration in a novel Drosophila model of disease caused by mutations in VCP/p97. *J Neurosci*, **30**, 7729-7739.

122 Gitcho, M.A., Strider, J., Carter, D., Taylor-Reinwald, L., Forman, M.S., Goate, A.M. and Cairns, N.J. (2009) VCP mutations causing frontotemporal lobar degeneration disrupt localization of TDP-43 and induce cell death. *J Biol Chem*, **284**, 12384-12398.

123 Rodriguez-Ortiz, C.J., Hoshino, H., Cheng, D., Liu-Yescevitz, L., Blurton-Jones, M., Wolozin, B., LaFerla, F.M. and Kitazawa, M. (2013) Neuronal-specific overexpression of a mutant valosin-containing protein associated with IBMPFD promotes aberrant ubiquitin and TDP-43 accumulation and cognitive dysfunction in transgenic mice. *Am J Pathol*, **183**, 504-515.

124 Elden, A.C., Kim, H.J., Hart, M.P., Chen-Plotkin, A.S., Johnson, B.S., Fang, X.D., Armakola, M., Geser, F., Greene, R., Lu, M.M. *et al.* (2010) Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature*, **466**, 1069-U1077.

125 Becker, L.A., Huang, B., Bieri, G., Ma, R., Knowles, D.A., Jafar-Nejad, P., Messing, J., Kim, H.J., Soriano, A., Auburger, G. *et al.* (2017) Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. *Nature*, **544**, 367-371.

126 Bakthavachalu, B., Huelsmeier, J., Sudhakaran, I.P., Hillebrand, J., Singh, A., Petrauskas, A., Thiagarajan, D., Sankaranarayanan, M., Mizoue, L., Anderson, E.N. *et al.* (2018) RNP-Granule Assembly via Ataxin-2 Disordered Domains Is Required for Long-Term Memory and Neurodegeneration. *Neuron*, **98**, 754-766 e754.

127 Smith, B.N., Topp, S.D., Fallini, C., Shibata, H., Chen, H.J., Troakes, C., King, A., Ticozzi, N., Kenna, K.P., Soragia-Gkazi, A. *et al.* (2017) Mutations in the vesicular trafficking protein annexin A11 are associated with amyotrophic lateral sclerosis. *Sci Transl Med*, **9**.

128 Zhang, K., Liu, Q., Liu, K., Shen, D., Tai, H., Shu, S., Ding, Q., Fu, H., Liu, S., Wang, Z. *et al.* (2018) ANXA11 mutations prevail in Chinese ALS patients with and without cognitive dementia. *Neurol Genet*, **4**, e237.

Figure 1

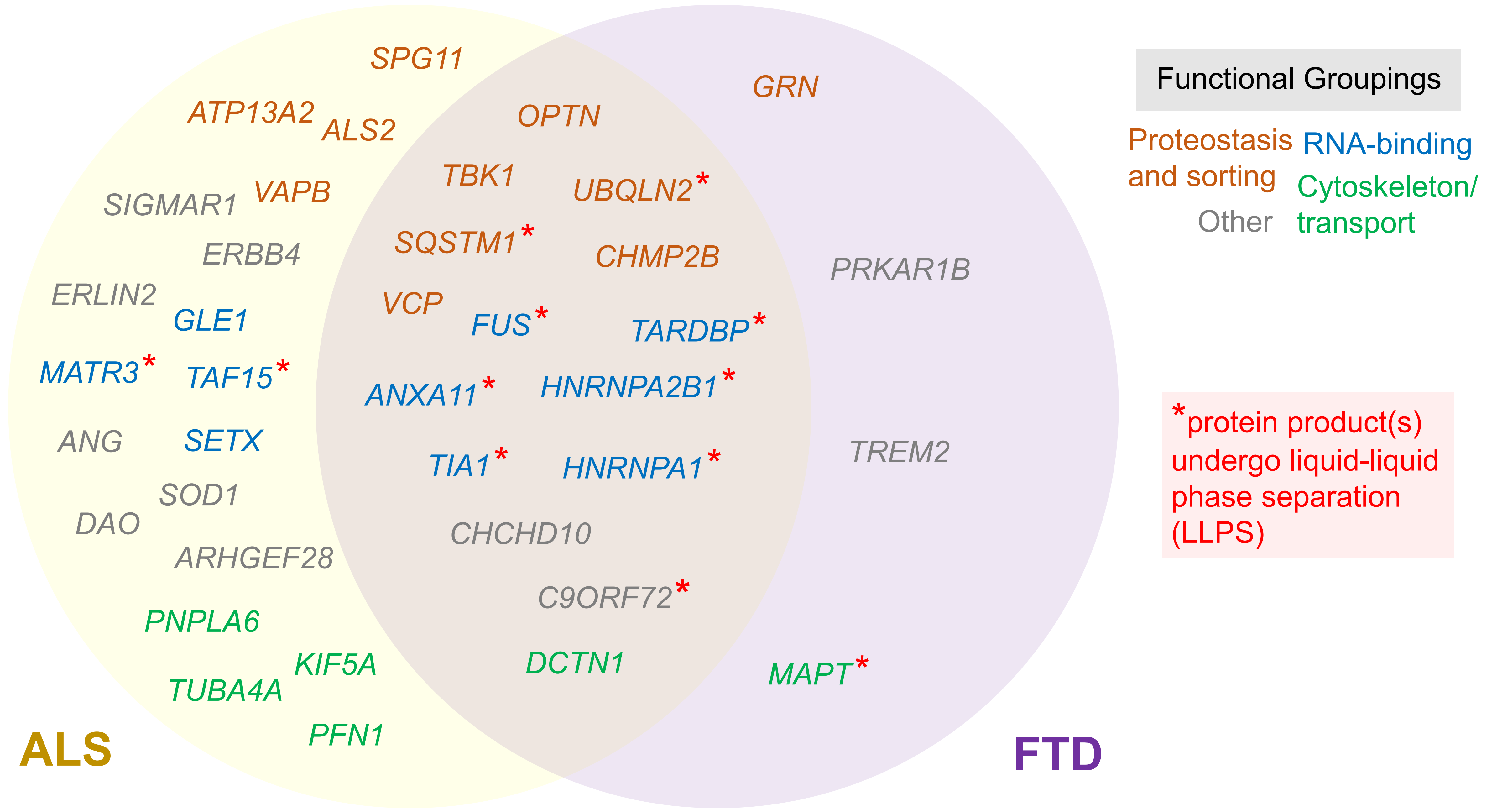

Figure 2

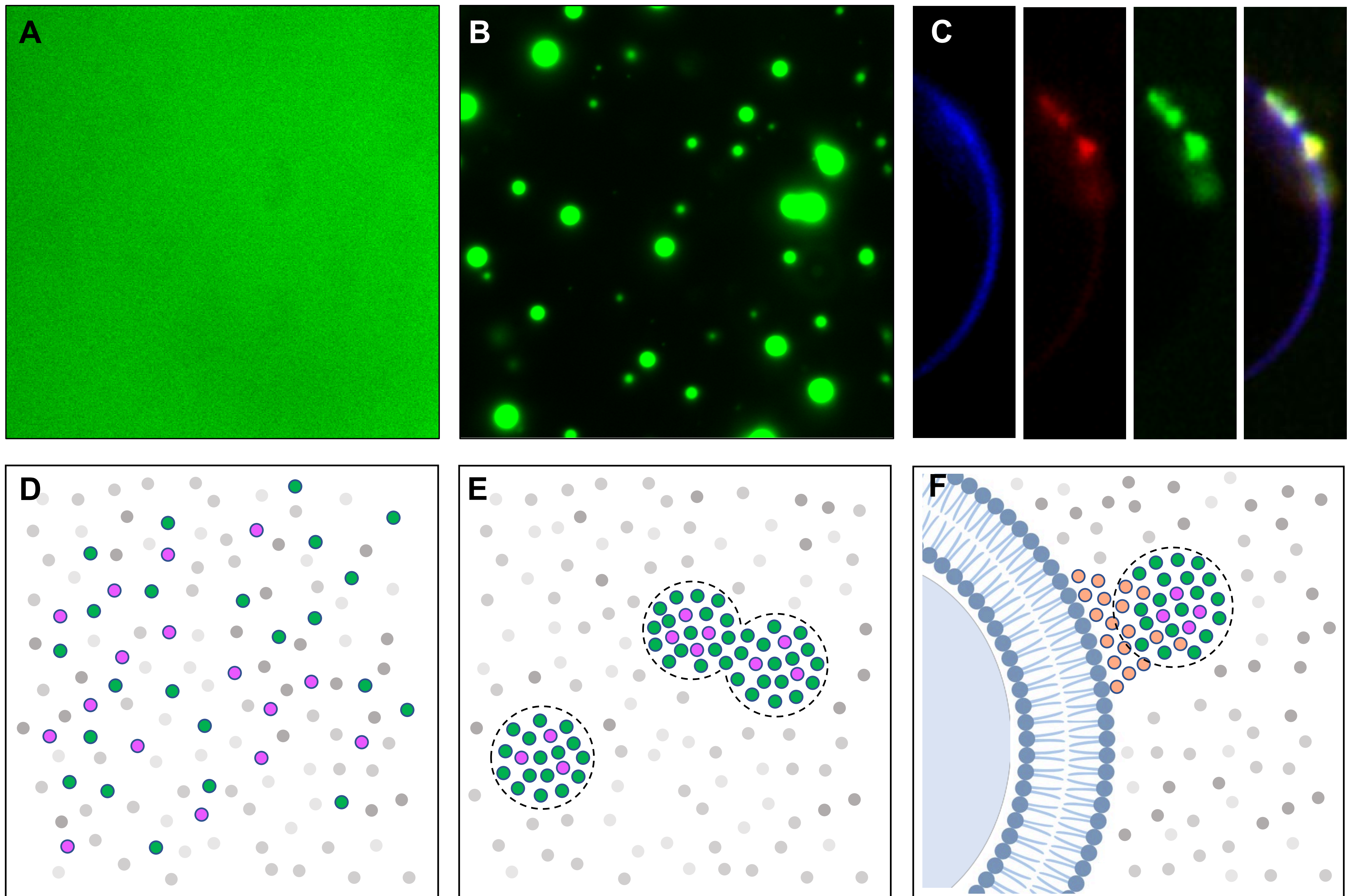

Figure 3