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Review

Protein disorder, prion propensities, and self-organizing macromolecular collectives ☆

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ABSTRACT

Eukaryotic cells are partitioned into functionally distinct self-organizing compartments. But while the biogenesis of membrane-surrounded compartments is beginning to be understood, the organizing principles behind large membrane-less structures, such as RNA-containing granules, remain a mystery. Here, we argue that protein disorder is an essential ingredient for the formation of such macromolecular collectives. Intrinsically disordered regions (IDRs) do not fold into a well-defined structure but rather sample a range of conformational states, depending on the local conditions. In addition to being structurally versatile, IDRs promote multivalent and transient interactions. This unique combination of features turns intrinsically disordered proteins into ideal agents to orchestrate the formation of large macromolecular assemblies. The presence of conformationally flexible regions, however, comes at a cost, for many intrinsically disordered proteins are aggregation-prone and cause protein misfolding diseases. This association with disease is particularly strong for IDRs with prion-like amino acid composition. Here, we examine how disease-causing and normal conformations are linked, and discuss the possibility that the dynamic order of the cytoplasm emerges, at least in part, from the collective properties of intrinsically disordered prion-like domains. This article is part of a Special Issue entitled: The emerging dynamic view of proteins: Protein plasticity in allostery, evolution and self-assembly.

1. Introduction

1.1. Bringing order to the cytoplasm

Living matter is staggeringly complex. Nothing epitomizes this better than the highly organized structure of the cytoplasm. The cytoplasm of eukaryotic cells is a complex landscape, permeated by a fibrous meshwork of cytoskeletal proteins and compartmentalized into numerous organelles and subcellular domains. What determines the shapes and sizes of these structures, why they form in particular locations, and how their architecture affects cellular function is largely unknown. Despite its complex appearance, however, the cytoplasm is organized by only one process: molecular self-assembly.

A biological structure is self-assembling if it is able to determine its own organization based on the interactions between its constituent components. Thus, the intrinsic properties of these components – their abilities to associate or their affinities for membranes – are the only factors that determine the final architecture of a self-assembling system. A system of disordered components can self-assemble into either static or dynamic structures. In static self-assembly, the structure is the product of a new thermodynamic equilibrium. In dynamic self-assembly, the structure is resulting from a steady state, which is dynamically maintained by dissipative processes. Dynamic structures are characteristic of biological systems and are also known as self-organized [1–4]. They are typically very robust and able to self-repair in response to even severe perturbations. Being decentralized and only reliant on the collective properties of their components, self-assembly and self-organization are simple but also very efficient ways of achieving cellular organization.

In principle, a self-organizing biological system has to fulfill only two requirements: it has to be dynamic to allow the continuous exchange of material and it has to be able to establish and maintain a stable configuration from initially disordered components. Large macroscopic structures, such as mitochondria, the endoplasmic reticulum, or the Golgi, rely on delimiting membranes to maintain their self-organized state. To manage a constant flux of material and retain their integrity over long timescales, they employ the same mechanisms of protein sorting and retrieval. However, despite the importance of membranes in shaping the overall architecture of eukaryotic cells, they are not essential for compartmentalization. Here, we focus on alternative mechanisms for cellular organization that operate in the absence of membranes.

Numerous membrane-less compartments have been identified, and with rising interest their number is likely to increase (Table 1). Examples of such compartments include centrosomes, inclusion bodies, and cytoplasmic RNP granules. Large membrane-free structures, however, are not limited to the cytoplasm. They have also been observed in the nucleus, with nucleoli, Cajal bodies, and PML bodies as prominent

Abbreviations: IDP, intrinsically disordered protein; IDR, intrinsically disordered region; PrD, prion domain; IPOD, insoluble protein deposit; JUNQ, juxtanuclear protein quality control; RNP, ribonucleoprotein

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Table 1
Nuclear and cytoplasmic protein and RNA bodies.

	Name	Other names	Localization	Function/description	Reference
Cytoplasmic bodies	Processing bodies (PBs)	GW (glycine and tryptophan-rich) bodies, Dcp-containing bodies	Cytoplasm of somatic cells	Assemble under stress; store translationally silenced and degrade decay-prone mRNA	[8,11,15,32,34]
	Stress granules (SGs)		Cytoplasm of somatic cells	Form under stress; keep mRNAs of housekeeping genes paused in translation initiation	[6,10,19]
	EGP bodies		Cytoplasm of somatic cells	Intermediate between PBs and polysomes; remodel degradative mRNPs from PBs into translational mRNPs *en route* to translation initiation	[14]
	Germ cell granules	Nuage, *Drosophila melanogaster*: Polar granules or Sponge bodies, *Caenorhabditis elegans*: P granules, mammals: inter-mitochondrial cement, or chromatoid body in spermatocytes	In the cytoplasm of germ cells, associated with nuclear envelope	Partitioned to prospective germ cells where they direct the timing of nascent maternal mRNA translation; probably needed for developmental progression	[18,27]
	Neuronal transport granules	Neuronal RNA granules, Dendritic P-body like structures (dlP-bodies), FMRP granules, Staufen granules	In the cytoplasm of neurons	Transport granules that store translationally repressed mRNA (also rRNA) to prevent translation and decay of mRNA until delivered to specific sites	[7,26]
	Metabolic bodies	Purinosomes	Cytoplasm of somatic cells	Protein storage granules in the quiescent state that serve as reservoirs for reentry into cell cycle when nutrients are available again	[5,28,39]
	Actin bodies		Cytoplasm of somatic cells	Store reorganized F-actin network components in the quiescence phase, which, like in metabolic bodies, can be used for F-actin formation after cell-cycle reentry	[30]
	JUNQ/IPOD = Inclusion bodies		In the yeast cytoplasm in proximity to the nucleus (JUNQ) and located in the peripheral perivacuolar location (IPOD)	Storage of soluble ubiquitinated misfolded proteins in juxtanuclear compartments (JUNQ) and terminally aggregated proteins in peripheral inclusions (IPOD)	[37]
	Proteasome storage granules			Proteasome Cytoplasmic storage reservoirs that are mobilized upon exit from quiescent state	[21]
	Aggresomes		Associated with microtubule organizing center	Forms when proteasome's degradative capacity is exceeded	[17]
	Centrosome/ Spindle pole bodies (SPB)		Mitotic spindle poles	Microtubule organizing center	[16,36]
	U bodies			Sites for assembly & storage of uridine-rich snRNPs (spliceosome)	[23]
Nuclear bodies	Nucleoli (singular: nucleolus)		Forms around actively transcribing ribosomal gene clusters	Function in rRNA transcription, processing and ribosomal subunit assembly	[38]
	Histone locus bodies (HLB)		Associated with chromosomal locus of histone genes	Transcription and 3′-end processing of replication-dependent histone genes	[24,25]
	Cajal bodies (CBs)	Spheres, coiled bodies	Associate transiently with specific genomic loci	Involved in biogenesis of histone, snRNA (spliceosome) and small nucleolar (sno)RNA genes; function in trafficking of snRNPs and snoRNPs	[13,25]
	PML bodies	ALT-associated PML bodies, ND10-, PODs (PML oncogenic domains) or Kr bodies	Associate transiently with specific genomic loci	Induced by DNA damage; to maintain telomeres using an alternative recombination-mediated lengthening mechanism	[35]
	Speckles	Interchromatin granule cluster	Often associated with Cajal bodies	Involved in the storage, assembly and modification of pre-mRNA splicing factors	[33]
	Paraspeckles		Interchromatin space of the nucleoplasm	Regulate gene expression by retaining RNAs in the nucleus	[12]
	Nuclear stress bodies		Nucleoplasm of human cells; frequently adjacent to chromatin blocks	Form in response to heat shock; participate in rapid changes of gene expression through chromatin remodeling and trapping of transcription and splicing factors	[9]
	Clastosome		Nucleoplasm	Form when elevated levels of proteins targeted for proteasome-dependent degradation queue up for proteolysis, recruit additional proteins for the proteasome	[20]
	Cleavage bodies		Adjacent to Cajal bodies	Functions in RNA transcription, splicing, and/or processing; preferentially required during DNA replication; perhaps also for histone gene transcription	[22]
	OPT (Oct1/ PTF/ transcription) domains		Appear in G1 phase next to nucleoli	Sites where particular genes and transcription factors are concentrated	[29]
	Polycomb (PcG) bodies		Associated with heterochromatin; larger foci localized near centromeres	Transcriptional repression complex *e.g.* of Hox genes	[31]

examples. Even though these structures are morphologically very diverse, they can be broken down into two different functional groups: active biochemical reaction centers and inactive storage compartments.

To assemble such membrane-free compartments in the cellular environment, large numbers of macromolecules have to interact in a coordinated manner. Templates do not seem to be necessary for this process, as suggested by the finding that many structures form *de novo* by using up soluble pools of macromolecules [40–42]. Strikingly, once a self-organizing structure is established, it can be maintained against steep concentration gradients over long timescales. With modern live cell imaging techniques, such as fluorescence recovery after photo bleaching (FRAP), we can now study the dynamics of membrane-free compartments in living cells. The surprising finding is that the constituents of these compartments turn over on the order of seconds to minutes [43–47]. They can also rapidly undergo changes in number and size. Thus, as predicted by the theoretical model of self-organization, these structures undergo constant changes on the molecular level, while at the same time being able to retain their identity. This, of course, raises the question of how a structure can be preserved in the face of constant reorganization.

The purpose of this article is to highlight the important role of protein disorder for biological self-organization. We propose that self-organizing macromolecular collectives are critically dependent on intrinsic disorder to remain dynamic and mediate their cellular functions. Importantly, intrinsically disordered regions within such assemblies are often compositionally similar to prion proteins. Their prion-like nature, however, makes them prone to misfold and aggregate. As a consequence, many of these proteins are now emerging in connection with protein misfolding diseases.

Self-organizing macromolecular collectives often undergo dramatic physicochemical transformations. Therefore, as a first attempt to unravel the relationship between protein disorder, prion propensities and self-organization, we will discuss several pertinent concepts from physics and chemistry.

2. Phase transitions

2.1. The rich phase behavior of macromolecules

Phase transitions are a common occurrence in nature. A phase transition takes place when a thermodynamic system switches from one state of matter to another. Liquids, such as water, can transform into a gas upon heating to the boiling point, causing abrupt changes in their physical properties. Phase separation can also occur when two or more distinct macromolecular species are dissolved in an aqueous solution. Perhaps the most commonly used and best-understood example in polymer chemistry is an aqueous two-phase system of two neutral polymers: polyethylene glycol and dextran. When these two polymers are mixed, they undergo repulsive interactions that lead to the formation of two stable liquid phases [48,49]. More complex phase behavior is observed for strongly interacting polymers such as polyelectrolytes of opposite charge [50]. In this case, the phase separation is heavily influenced by other factors such as the ionic strength of the solution.

Phase systems containing biological molecules have received less attention than synthetic polymer systems, most likely because of their greater complexity. Despite this fact, limited thermodynamic compatibilities have also been observed for diverse biomolecules [51,52]. Because biological macromolecules are more structured and compact, higher concentrations are generally needed to induce phase behavior. Consistent with findings from polymer science, differences in hydrophilicity are the most important factors that determine whether two given macromolecules are compatible or not. A factor that strongly affects the compatibility of proteins is denaturation. It can even lead to self-incompatibilities in mixtures containing a native and a denatured form of the same protein, thus triggering a phenomenon known as aggregation [53]. This indicates that structural changes can significantly alter the phase behavior of biomolecular solutions.

The fraction of the cytoplasm that contains macromolecules is remarkably high, occupying up to 30% of the available volume. In this high concentration environment, crowding effects are likely to play important roles. This is because large parts of the cellular space are occupied by uninvolved macromolecules, reducing the amount of solvent that is available to a certain macromolecular species. This excluded volume effect can dramatically increase the thermodynamic activity or effective concentration of macromolecules. A crowded environment can therefore alter the rates and equilibrium constants of biomolecular reactions, affecting enzymatic reactions and protein complex formation [48,54]. For large macromolecular assemblies, the excluded volume effect becomes a significant force and has consequently been proposed to drive the formation of large cellular structures [54,55]. Consistent with this, RNP granule assembly is strongly affected by crowding reagents [56].

In the presence of an excluded volume effect, phase transitions are expected to occur at much lower macromolecular concentrations. Given the abundance of macromolecules in living cells, Walter and Brooks suggested that phase separations should be a frequent occurrence in biological liquids [57]. The large number of different macromolecular species even makes it conceivable that multiple liquid phases could locally coexist. In the next section, we will examine the evidence for phase separation in living cells.

2.2. Phase transitions in biological systems

Phase separation in biological systems remained a theoretical concept for a long time; only few clear cases in living cells were reported. Early studies suggested a role for phase transitions in the structuring of membranes. Mixtures of phospholipids and cholesterol can undergo phase transitions, thus providing a possible basis for membrane organization in living cells. Subsequent studies indeed confirmed that biological membranes show rich phase behavior. Many membranes in living cells can for example form lipid rafts, specialized membrane microdomains, which serve as organizing centers for signaling molecules [58].

Other examples of phase transitions were reported in association with stressful environmental conditions [59]. Stress causes protein misfolding, and the resulting non-native proteins can clump together to form particles of very high molecular weight. Because these protein aggregates have the tendency to become insoluble in aqueous solutions, stress is often accompanied by liquid–solid phase separations. Although protein misfolding and aggregation are barely detectable in cells under optimal conditions, they are frequently observed in association with disease or aging. Aberrant phase transitions in cells of the eye lens, for example, cause cataracts, a disease in which the normally clear cytoplasm of these cells becomes opaque. To allow the passage of light, healthy eye lens cells lack larger-sized organelles and contain a concentrated solution of crystalline proteins. Under normal physiological conditions, these proteins exist in a single transparent phase. However, because of aging, the crystalline proteins phase separate, forming liquid droplets and solid aggregates that prevent the passage of light.

Do cells normally avoid phase separation as in the cytoplasm of eye lens cells or can phase separation also be a means for spatiotemporal organization? Two recent studies suggest that liquid–liquid demixing phase transitions could indeed be functionally important [60,61]. In one report, the authors investigated the dynamics of P granules, RNA-rich bodies in embryos of *Caenorhabditis elegans* [62]. Components incorporated into P granules were in a dynamic equilibrium with a soluble pool in the cytoplasm. Remarkably, P granules showed liquid-like behavior, including fusion between P granule droplets, dripping in response to shear stress, and wetting of membranes. Using these macroscopic behaviors, the authors could determine values for viscosity

and surface tension, which were very similar to those reported for colloidal polymer systems.

Another study by Rosen et al. showed that mixtures of multivalent macromolecules could assemble into liquid droplets [63]. The authors focused on a system of three proteins: NCK, N-WASP and nephrin. In this three-component system, phase transitions were observed in the presence of three different protein–protein interaction sites: SH3 domain repeats in NCK, proline-rich motifs in N-WASP, and phosphorylated tyrosine residues in nephrin. Synthetic constructs that contained multiple repeats of these motifs readily formed liquid droplets *in vitro* and *in vivo*. Droplet formation was also observed for other multidomain proteins, including the RNA-binding protein PTB in association with short fragments of RNA. Thus, multiple weak interactions are sufficient to drive a multicomponent system into a phase-separated state. This is consistent with findings in polymer theory where the propensity to phase separate increases with the number of binding sites. The findings from Rosen et al. suggest that large phase-transitioning protein assemblies need to fulfill specific structural requirements. In the following sections, we argue that intrinsically disordered protein regions are particularly well adapted to meet these demands.

3. Intrinsic disorder in proteins

3.1. Intrinsically disordered proteins: abundant and versatile

Proteins with regions of little or no structure, so-called intrinsically disordered regions, frequently occur in eukaryotic proteomes. According to conservative estimates, about 30% of eukaryotic proteins contain regions of more than 30 amino acids that do not adopt a defined structure [64–66]. On the sequence level, IDRs are depleted in bulky hydrophobic and aromatic residues and are often enriched in polar or charged residues (arginine, glutamate, lysine, glutamine, and serine) or structure-breaking amino acids (glycine and proline) [67]. Consistent with this, a typical IDP features a low overall hydrophobicity, a high mean charge, and, in many cases, a low sequence complexity.

Despite being highly flexible and lacking a defined three-dimensional structure, intrinsically disordered proteins (IDPs) have important biological functions. IDPs play critical roles in gene regulation, signaling, and intracellular transport, and are often found at central positions in protein interaction networks [67–70]. Based on their molecular functions, IDRs can be grouped into four different classes: regions that (1) function as entropic chains; (2) are modified by posttranslational modifications; (3) are involved in molecular recognition; (4) facilitate molecular assembly. Thus, the functional diversity of IDRs is similar in extent to that of their foldable counterparts. Like compact globular proteins, IDPs also frequently interact with other macromolecules. When IDPs interact with their corresponding binding partners, they can undergo dramatic structural changes. However, disorder to order transitions have only been observed for a subset of IDPs. In fact, recent studies indicate that many IDRs remain disordered upon complex formation, a phenomenon, which was termed fuzziness.

3.2. Protein complex fuzziness and short linear motifs

Many protein complexes contain significant amounts of structural disorder in the bound state, a phenomenon that has largely been overlooked so far [71–73]. Four different conceptual models have been put forward to describe the functions of disordered regions within such fuzzy complexes: they may (1) adopt multiple alternative conformations (polymorphic model); (2) act as linkers that increase the conformational freedom and adaptability of two interacting regions (clamp model); (3) contain sites for binding partners or posttranslational modifications (flanking model); (4) remain completely disordered as part of their normal function (random model). Fuzziness is functionally important in a variety of settings, because it adds adaptability, versatility, and reversibility to protein binding and complex formation [71–73]. It is also important to point out that fuzzy complexes constitute independent functional states; they are not intermediates on the way to more compact structures. As we will see later, the maintenance of fuzziness is essential for cellular survival, because conversion of fuzzy complexes into aberrant static structures can trigger catastrophic protein misfolding and aggregation.

IDPs within fuzzy complexes often interact with their binding partners *via* short linear motifs (SLiMs). SLiMs are regions that consist of only 2–8 amino acids [74–77]. They interact with a diverse set of globular motif-recognition domains, including classic protein–protein interaction domains such as SH3, SH2, or WW. Upon binding to a motif-recognition domain, SLiMs often undergo a conformational change to adapt to the structure of the binding partner. Because of the small size of the target motifs, SLiM-mediated binding events are usually weak, transient, and have low specificity [75,76]. However, interactions may become stronger or more specific through cooperative binding events, involving multiple SLiMs and several globular protein–protein interaction domains. This multiplicity of binding was proposed to enable combinatorial decision-making processes and the formation of macromolecular complexes [77].

Despite the extreme evolutionary agility of IDRs, a recent comparative study succeeded in identifying a large number of putative SLiMs in intrinsically disordered domains [78,79]. The study was based on the assumption that functionally relevant motifs have a higher degree of conservation than their surrounding background regions. The findings suggest that at least 5% of amino acids in IDRs function as SLiMs. However, what is the function of the remaining 95%? SLiMs require malleable sequence environments to perform their function [80]. Therefore, the remaining sequence play important roles as adaptable carrier sequences that optimize and regulate the interaction between SLiMs and their binding partners. How sequence characteristics can modulate the conformation of IDRs on a more global level will be discussed in the next section.

3.3. Solubility and phase behavior of intrinsically disordered proteins

Many homogenous protein solutions become thermodynamically unstable when the protein concentration is above a few micromoles. This is due to the fact that the number of repulsive and attractive interactions increases linearly with protein concentration. Above a certain critical point, the system is driven into a thermodynamically more stable, phase-separated state. This effect is even more pronounced in crowded environments, in which a protein is confronted with a large number of additional heterotypic interactions. However, a protein's solubility is not only dependent on the concentration but also strongly influenced by its folding state. Early *in vitro* experiments for example showed that the critical concentrations are higher for pairs of globular proteins than for pairs where one of the components is a random coil [52]. This, and the observation that proteins readily form insoluble aggregates when the conditions become unfavorable, suggests that proteins are only marginally soluble in biological liquids [81]. Hence, protein folding may be viewed as a cellular strategy to keep a protein soluble.

Unlike globular proteins, IDPs cannot adopt a defined structure in the absence of their ligands. If we assume that all proteins have evolved to remain soluble throughout the lifetime of an organism, than IDPs must have developed alternative mechanisms to retain their solubility in the crowded environment of the cell. Recent computational and experimental studies found that isolated IDPs often form disordered globule states [82–87]. Importantly, these globules are not characterized by a defined structure but comprise an ensemble of conformations with similar compactness and stabilities. Studies on simple homopolymeric sequences such as polyglutamine or polyglycine have also revealed that water is a poor solvent for polar IDPs [82,88]. The collapsed state of such homopolymers is maintained through a dynamic network of internal hydrogen bonds, involving, for

the most part, backbone-to-backbone interactions. Given that many IDPs are compositionally and physicochemically similar to these archetypal IDPs, partially or fully collapsed states are likely to be a common occurrence. Consistent with this, a recent study found that the IDR of the translation termination factor and prion protein Sup35 adopts a collapsed state in aqueous solutions [84]. This suggests that these principles also hold for more complex, naturally evolved domains.

Multidomain interactions between regulatory proteins require the simultaneous exposure of multiple SLiMs. However, if IDRs frequently adopt a collapsed state, than these functional motifs could be hidden in the interior of the globule. To prevent this from happening, the local sequence context is tuned to optimize the accessibility of SLiMs in space and time [72,80,89]. Importantly, proline residues, which would interfere with a conformational collapse because of their conformational rigidity, are highly enriched in SLiM-containing IDRs [80]. This suggests that proline residues could be used to locally regulate SLiM availability. Glycine residues could also play an important role, because they entropically prefer conformational disorder. The conformation of IDRs is also strongly dependent on the net charge per residue, with higher net charges promoting globule-to-coil transitions [90,91]. Long IDRs with complex charge distributions could even prefer extended structural ensembles in which multiple globules are connected by short flexible linkers. The preference for such elaborate geometries could be regulated by other factors such as the ionic strength or posttranslational modifications. Thus, a rich variety of conformational possibilities is emerging from simple design features such as the physicochemical characteristics of side chains or enzymatically introduced covalent modifications. Deciphering this conformational code will be one of the challenges that protein scientists need to tackle in the years to come.

4. Prion-like intrinsically disordered proteins

4.1. Prion-like IDRs have an intrinsic ability to assemble into amyloids

IDPs are a diverse group of proteins with various functions. Here, we want to limit ourselves to a subset of such IDPs containing long intrinsically disordered domains that are highly enriched for uncharged polar residues. We propose that these sequences are intimately involved in the formation of fuzzy macromolecular collectives. However, they have initially gained attention for their ability to self-assemble into one of the most highly ordered structures in biology: amyloids.

Amyloids form when large numbers of an amyloidogenic protein associate to form a fiber that is a single extended β-sheet. This cross-β structure imparts special features: a high affinity for dyes such as Thioflavin T and Congo Red and an extraordinary resistance to denaturants [92]. The crystal-like organization of amyloids also has important functional implications, endowing them with the ability to self-replicate. Remarkably, amyloids also have transmissible properties; Fragments of amyloid are passed between cells or organisms, and the self-templating ability of amyloid then replicates the structure, giving them an infectious property [93–96]. Proteins with such infectious properties are known as prions.

Prion mechanisms are causing several mammalian neurodegenerative diseases, such as Creutzfeldt–Jakob disease and mad cow disease. However, in single-celled organisms such as yeast, prions can act as epigenetic elements that impart specific heritable phenotypes. Depending on the genetic background and the environmental conditions, these phenotypes can be advantageous, benign, or detrimental [97–101]. This led to the proposal that prions are adaptive bet-hedging devices that enable survival in stressful environments by creating selectable phenotypic diversity [95,96,102,103]. However, this view is controversial and others have suggested that yeast prions are molecular degenerative diseases [104–106]. Recent evidence, however, shows that prions abundantly occur in wild yeast strains, suggesting that they may very well act as evolutionary capacitors that facilitate the survival of yeast in their natural habitats [107].

The prion properties of prion proteins reside in structurally independent prion-forming domains (PrDs). These domains are highly enriched for uncharged polar amino acids such as glutamine, asparagine, glycine, proline, serine, and tyrosine [97,108]. PrDs are at least 60 amino acids in length and predicted to be intrinsically disordered. A few experimental studies have explored the conformational properties of PrDs in their non-prion conformation and have indeed found that they are largely disordered [84,109]. Despite their general tendency toward disorder, they can spontaneously assemble into an amyloid-nucleating conformation. This transition involves a collapse of disordered monomers into various pre-molten and molten oligomers [84,110–112]. Disorder-to-order transitions of polypeptides within these oligomers are augmented by additional intermolecular interactions, eventually leading to the formation of an oligomer with characteristic cross-β structure. Once this structure is established, amyloid formation becomes self-sustaining and grows by depleting soluble conformers of the same protein.

4.2. Identifying prion-like IDRs on a genome-wide level

The distinctive compositional features of the founding yeast prions Sup35 and Ure2 stimulated the development of bioinformatics algorithms to detect prion-like proteins on a genome-wide scale. Initial attempts looked for an enrichment of asparagine and glutamine residues in a sequence stretch of defined size [113]. These studies revealed that prion-like domains are quite common in eukaryotic proteins but rare in prokaryotes. They also helped to uncover additional prion proteins, such as Rnq1 in yeast [114] and CPEB in *Aplysia* [115]. Although these simple approaches generated long lists of potential prion candidates, the number of experimentally verified prions remained low.

To more reliably predict prion proteins in large proteome datasets, a refined algorithm was developed based on the inherent similarity to known yeast prions. Using this algorithm, the yeast proteome was screened for prion-like proteins [97,116]. The algorithm returned about 200 candidates, which were ranked according to their compositional similarity to experimentally confirmed prions. A subsequent experimental analysis of the first 100 candidate PrDs – the analysis was limited to the prion-like portions because of the previously demonstrated transferability of PrDs – identified 19 domains with prion properties and many additional domains that were aggregation-prone.

What distinguishes prion-forming domains from domains that do not form prions? Domains with a propensity to form prions or amyloids were enriched for asparagine and depleted for glutamine, proline, and charged residues [97]. The prevalence of prolines and charged residues in non-amyloid-forming domains is in agreement with previous knowledge: prolines are inherently inflexible and can interfere with the formation of secondary structure; charged residues are disfavored because of potentially repulsive interactions and their general tendency for hydration. However, the uncovered distinction between asparagine and glutamine was unexpected, as these residues were considered to be equally potent in promoting prion formation [113,114,117].

An extensive mutational study confirmed opposing roles for these two chemically related amino acids [118]. Changing asparagines to glutamines in prion proteins decreased prion formation and increased the propensity to form proteotoxic, non-amyloid aggregates. In contrast, changing glutamines to asparagines enhanced prion formation and reduced toxicity. This finding could have important implications for distinguishing disease-causing aggregation-prone proteins from functional prions. Efforts to predict prions were also undertaken by other groups. Ross et al. for example have developed a different method that ranks candidate proteins using experimentally derived prion propensities [108,119]. This method was able to distinguish between prion and non-prion domains with high accuracy and it revealed a facilitating role for hydrophobic residues.

How conserved are the prion properties of these domains? Recent results from the Wickner lab suggest that domains with prion-like amino acid compositions can exist over long evolutionary time scales without being able to assume a prion conformation. A case in point is the PrD of the yeast prion Ure2. Even though the Ure2 proteins of *Saccharomyces castellii*, *Kluyveromyces lactis*, and *Candida glabrata* contain prion-like domains that are very similar to the PrD of *Saccharomyces cerevisiae* Ure2, these regions could not undergo a structural transformation to a prion state [120–122]. Conversely, the more distantly related PrD of *Candida albicans* was able to form a prion [120]. The continued presence of these compositional biases despite any discernible prion functionality suggests that other, non-prion functions are underlying their conservation. In agreement with this idea, alternative functions have occasionally been proposed [123–125]. Together, these data suggest that the prion-like amino acid composition is preserved for other reasons, thus hinting at the possibility that the prion propensities of some of these domains are an epiphenomenon.

4.3. Prion-like IDRs are aggregation-prone

Recent studies using the above-described algorithm have uncovered a number of proteins with prion-like domains in the human proteome [126,127]. In agreement with findings from yeast [97], many of these proteins contained RNA-binding domains. Disconcertingly, however, the same proteins are now emerging in connection with several neurodegenerative diseases [126]. This includes TDP-43, a protein that causes frontotemporal lobar degeneration and amyotrophic lateral sclerosis (ALS), and FUS, a protein mutated in certain familial forms of ALS. In all these cases, prion-like IDRs seem to play key roles in establishing the underlying protein misfolding pathologies. However, if these domains pose a major threat to cellular homeostasis and are intimately linked to proteinopathies, why are they so well conserved?

One possible scenario is that the prion-like domains have been conserved to act as epigenetic switches during transcription or execute essential cellular functions in memory formation [95]. However, as discussed above, the prion functionality is limited to only a subset of these proteins, urging us to find alternative explanations. A possible solution is emerging from the finding that many prion-like domains are aggregation-prone. In fact, almost 70% of the prion-like domains identified in a recent systematic survey coalesced into microscopically visible aggregates when overexpressed in yeast cells [97]. Consistent with this observation, it was recently reported that the prion-like sequences of many RNA-binding proteins form fibrillar structures with amyloid-like properties [128,129]. Unexpectedly, these structures readily depolymerized in the presence of even low concentrations of denaturants, suggesting that they are much less stable – and more dynamic – than regular amyloid. Hence, it was proposed that prion-like sequence stretches have evolved to function as generic aggregation domains.

This discovery was based on the serendipitous finding that a chemical – biotinylated isoxazole (b-isox) – precipitated a distinct set of RNA-binding proteins from cell lysate. Many of these proteins contained low complexity regions with similarity to prion-like domains and were previously shown to localize to RNA granules. Among the identified proteins was the disease-associated protein FUS. Tyrosine-rich repeats (G/SYG/S) within the FUS prion-like domain were identified as key elements for the recruitment of FUS into b-isox precipitates. Further, *in vitro* experiments showed that the isolated prion-like domain of FUS could assemble into an elastic hydrogel. Ultrastructural studies revealed that this hydrogel was composed of a three-dimensional meshwork of well-defined filaments. Interestingly, the X-ray diffraction patterns of these filaments showed that they contained considerable amounts of cross-β structure. Similar findings were reported for other RNA-binding proteins. This led the authors to propose amyloid-like polymerization of prion-like domains as the underlying molecular mechanism of RNA granule formation.

This conclusion was inspired by earlier studies focusing on a group of related proteins, the FG repeat-containing nucleoporins [130–133]. FG nucleoporins are subunits of the nuclear pore complex, and, like many RNA-binding proteins, contain intrinsically disordered prion-like domains with periodically occurring hydrophobic repeats. In the case of the nucleoporins, however, these repeats consist of phenylalanines and glycines (therefore FG repeats). Interestingly, FG nucleoporins proteins were among the proteins that were precipitated by b-isox [128,129]. Moreover, earlier studies had shown that isolated FG-repeat domains assemble into hydrogels [130,131]. Because of their sieve-like properties, the authors proposed that hydrogels form the molecular basis for the size-exclusion barrier at the nuclear pore. Consistent with this, Frey et al. demonstrated that some FG domain hydrogels allowed highly selective access of nuclear transport receptors but rejected other proteins of similar size [130,131].

What are the structural features required for hydrogel formation by FG domain proteins? Initial results identified a critical role for phenylalanines [130,131,133]. However, a later report found that the cohesiveness of phenylalanines was not sufficient to explain the physical properties of hydrogels [132]. Using solid-state NMR spectroscopy, this study was able to pinpoint an additional type of intragel interaction: intermolecular β-sheets between the asparagine-rich spacer regions. This interaction was subsequently shown to be critical for the stability of the hydrogel. Hence, it was proposed that amyloid-like structures are the key elements that drive the formation of the size-exclusion barrier at the nuclear pore [132].

Interestingly, recent studies in *C. elegans* suggest a functional link between the nuclear pore and P granules [134]. P granules are prototypical RNA granules that have received much attention in recent years. They are found in all animals and play important roles in maintaining the undifferentiated state of the germ cell lineage. In *C. elegans*, P granules are often physically associated with the nuclear membrane. This led to the proposal that P granules are a functional extension of the nuclear pore complex [134]. Consistent with this idea, several constitutive P granule components carry long FG repeat-containing domains, which are compositionally very similar to the FG repeat domains of nucleoporins. Remarkably, multimerization of the FG repeat domain of one of these proteins induced the formation of cytoplasmic bodies that were reminiscent of P granules [134]. Together, these data provide compelling evidence that prion-like domains with periodically occurring aromatic residues could play key roles in the formation of membrane-free compartments. However, whether this involves their assembly into amyloid-like filaments and the formation of hydrogels is currently a matter of intense debates [135,136].

The resistance to the hydrogel concept stems from the fact that hydrogels have so far only been observed under extreme conditions. The formation of saturated FG-repeat hydrogels, for example, required very high protein concentrations and buffer conditions that were far-off from those in living cells [130–132]. In fact, under more physiological conditions, FG-repeat domains remained disordered and had a tendency to undergo weak, reversible inter-repeat interactions [137–139]. Thus, in the confined and highly dynamic environment of the nuclear pore, FG repeat domains are likely to be arranged into a network of reversibly interacting, disordered polypetide chains, and not into a rigid meshwork of amyloid-like filaments. Likewise, the formation of FUS hydrogels was only observed at very low temperatures and at extremely high protein concentrations [128,129]. Again these conditions are unlikely to exist in living cells. The extraordinary stability of *in vitro* formed hydrogels is also difficult to reconcile with previous findings about the liquid-like behavior of P granules in living cells (also see discussion by Weber and Brangwynne, [136]). Thus, it seems likely that hydrogels are just an abnormal conformational manifestation of a group of proteins with extraordinary structural plasticity. A literature survey reveals the extreme structural versatility of FG nucleoporins. These proteins have been reported to exist in an intrinsically disordered state [137], to form disordered amorphous aggregates [138], to adopt an

amyloid-like filamentous state [132], or to enter into a self-replicating prion conformation [140]. The fact that FG nucleoporins can adopt such a wide range of structures, suggests that they are extremely sensitive to the conditions under which they are studied.

4.4. A subset of prion-like proteins resembles elastomeric proteins

FG repeat-containing prion-like proteins are compositionally related to elastomeric proteins. These proteins assemble into fibrous structures that undergo elastic recoil when released from mechanical tension and have important roles in elastic tissues [141]. Like FG nucleoporins and RNA-binding proteins, many elastomeric proteins contain low complexity sequence stretches that are highly enriched for glycines, prolines, and hydrophobic amino acids.

What structure do elastomeric proteins adopt in their functional state? Research of elastomeric proteins is plagued by a long history of conflicting structural models. Studies provided evidence for a variety of conformational states, ranging from highly disordered fuzzy ensembles to rigid amyloid-like structures [141–146]. However, recent solid-state NMR studies and molecular simulations provided convincing evidence that elastomeric proteins such as elastin remain largely disordered in the assembled state [141,147,148]. According to this model, elastin is organized in random coils that are held together by scattered hydrophobic residues. In agreement with this, elastomeric proteins undergo phase transitions into liquid droplets in the cellular environment [149]. Given their hydrophobic nature, it is not surprising that elastomeric proteins are prone to form other structures, such as amyloids. To prevent this from happening, elastomeric proteins contain high amounts of prolines and glycines. These residues counteract the order-promoting tendencies of hydrophobic residues by keeping the backbone hydrated and conformationally disordered [141,150]. Given the extraordinary structural plasticity of elastomeric and prion-like proteins, results from *in vitro* studies need to be interpreted with caution. Even if experimental conditions are used that are close to those in living cells, one cannot be sure whether the identified conformations reflect the true natural state of these proteins. Therefore, whenever possible, attempts should be made to investigate these proteins *in situ*.

4.5. A role for prion-like IDRs in macromolecular assembly?

The presence of prion-like domains in large dynamic structures, such as the nuclear pore complex or RNA granules, suggests a potential role for these domains in molecular assembly. In fact, disordered protein regions are well known to play important roles in the self-assembly of large biological structures such as viral capsids or bacterial flagella [151]. To test the universality of this concept, Peter Tompa et al. investigated the prevalence of protein disorder in protein complexes by interrogating protein–protein datasets from high-throughput studies [152]. This systematic study revealed a strong positive correlation between the size of a protein complex and its overall amount of predicted disorder. The observed relationship was even more pronounced for disordered regions that exceeded a size of 30 amino acids, suggesting that long flexible regions assume critical functions in protein complex assembly. Although the functional role of IDRs in large protein complexes has not been studied systematically, it seems to be emerging that most of these domains remain at least partially disordered when in the bound state. Thus, the concept of fuzzy protein complexes [71–73] will be essential for understanding the underlying molecular mechanisms of their formation. According to this model, disordered regions may directly be involved in the binding process, perhaps in the form of short linear motifs or sites that are modified by posttranslational modifications. Alternatively, they may provide the flexibility that is required to ensure productive interactions between proteins.

In agreement with a potential role of prion-like IDRs in the formation of large fuzzy complexes, prion-like domains have large sizes [97] and often contain short linear motifs [78]. To investigate the possibility that prion-like IDRs are involved in the formation of macromolecular assemblies, we performed a computational analysis to identify functional domains that are frequently associated with prion-like domains in yeast proteins. The domains that were most often found in combination with prion-like sequences were the RNA-binding domains RRM and Pumilio (Fig. 1). This is in agreement with previous studies in humans, which also reported a strong enrichment in RRM-containing proteins [126]. However, other domains are also intimately linked to prion-like domains. For example, prion-like domains are frequently associated with ENTH and SH3, which play important roles in the formation of dynamic macromolecular networks during endocytosis and actin cytoskeleton formation.

To get further insight into the functional role of prion-like IDRs, we grouped prion-like proteins from yeast according to their molecular function. Five different functional clusters were identified: transcription, DNA binding, RNA binding, RNA processing, and transport (Fig. 2). Clustering according to their association with cellular components also produced interesting results. It revealed that prion-like domains are most frequently found in proteins that are associated with the cytoskeleton, the nucleus, RNP complexes, or chromatin (Fig. 3). This also involves a strong enrichment in proteins that are associated with the cortical actin network, the nuclear pore, and RNA granules. Strikingly, all three of these cellular components were proposed to undergo liquid–liquid demixing phase transitions.

As discussed in one of the previous sections, phase transitions are dependent on multiple, weak interactions between multidomain proteins. Rosen et al. focused on the interaction network of the Arp2/3-regulating protein N-WASP to demonstrate that a system of multidomain proteins can transition into a liquid droplet state [63]. Consistent with an important role of prion-like proteins in this process, large portions of the yeast N-WASP homolog Las17 are composed of low complexity sequences, and one of these sequence stretches was even identified as prion-like (see Supplement for details). These protein regions are highly enriched for proline-rich SLiMs, and many of them have already been shown to be involved in transient protein–protein interactions [153–155]. However, our data suggest that Las17 is just one prion-like protein among many others involved in the formation

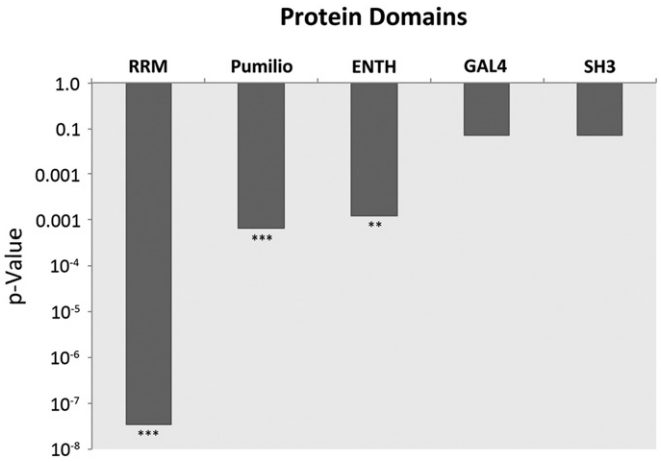

Fig. 1. Protein domains enriched in PrD-containing proteins from *Saccharomyces cerevisiae*. PrD-containing proteins in the yeast proteome were predicted based on their compositional similarity with known prion proteins (see Supplement for details). The algorithm identified 195 prion candidates, which were subsequently analyzed for their association with SMART domains using DAVID (see Supplement for details). Five types of protein domains were enriched in PrDs: RNA-binding domains (RRM, Pumilio), epsin homology membrane-interacting modules (ENTH), zinc finger-like DNA binding domains (GAL4), and protein interaction domains (SH3). Three of those were highly significant: RRM ($p = 3.5 \times 10^{-8}$), Pumilio ($p = 6.7 \times 10^{-4}$), ENTH domains ($p = 0.0013$).

of large phase-separating assemblies that regulate the actin cytoskeleton during endocytosis.

In yeast, endocytosis of extracellular cargo proteins requires the formation of actin patches that move inward through polymerization of actin. The formation of these actin patches is dependent on a complex molecular machinery [156,157]. The initial components that are recruited to actin patches include clathrin and multiple scaffold proteins and clathrin adaptors: Yap1801 and Yap1802 (AP180 homologs), Ent1 and Ent2 (epsin homologs), Ede1 (Eps15R homolog), Scd5, Sla1, and Sla2. These proteins recruit additional components, such as Pan1, End3, and Las17, to form a complex network of interacting factors. Within this macromolecular assembly, Pan1 and Las17 are involved in recruiting and activating the Arp2/3 complex to nucleate actin assembly. Strikingly, most of these proteins carry prion-like domains (Fig. 4).

To investigate whether this functional association is conserved, we searched the proteomes of fruit flies and humans for proteins with prion-like amino acid compositions (see Supplement for the results of the prediction). We discovered 656 proteins in fruit flies and 219 proteins in humans that contained a prion-like domain. The identified proteins showed a functional enrichment that was similar to yeast (Fig. 5 and Supplemental Figs. S1–S5). Together, these findings suggest that prion-like domains are intimately involved in transcription, chromatin remodeling, RNP complex formation, and cytoskeleton-dependent cellular processes. All these processes require the formation of dynamic assemblies, which, upon reaching a critical point, could demix into a distinct liquid state. Fuzziness emerges as a key ingredient for such macromolecular assemblies, because it endows these dynamical processes with adaptability, versatility, and reversibility.

Why are IDPs in self-organizing macromolecular collectives prion-like? SLiMs and other functional motifs need to be embedded into a flexible sequence environment in order to function [80]. However, this critical design feature – scattered order-promoting residues in a disordered carrier sequence – has a downside; it also makes these proteins prone to misfold and aggregate. The order promoting regions can drive the spontaneous collapse of prion-like IDPs into β-sheet-rich oligomers; the absence of order in the flanking regions further accelerates this conformational transformation. Once a nucleus is formed, the aggregation reaction is difficult to stop, because IDPs are largely devoid of structural features that would prevent their incorporation into growing amyloid fibrils. Thus,

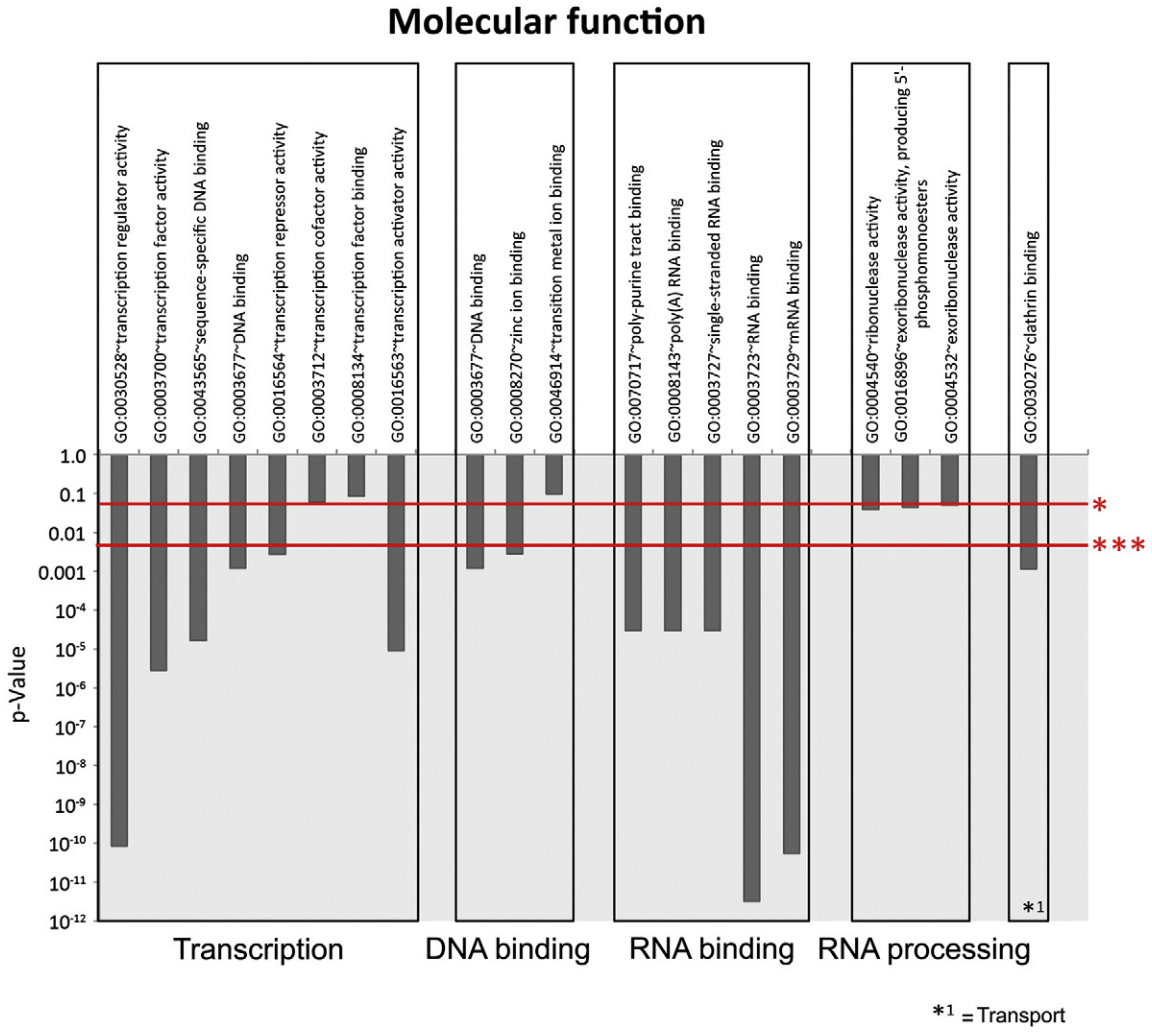

Fig. 2. Clustering of PrD-containing proteins from *Saccharomyces cerevisiae* according to their molecular function. PrD-containing yeast proteins were clustered according to their molecular function using predefined GO terms (Gene Ontology). The analysis was performed with DAVID (see Supplement for details). All proteins with an EASE score ≤ 0.1 were taken into account. Clusters were generated manually based on initially proposed clusters by the functional annotation tool of DAVID. We obtained 5 different clusters connected to transcription, DNA-binding, RNA-binding, RNA-processing, and transport. Red lines demarcate GO terms with highly significant enrichment ($p \leq 0.005$, ***) or significant enrichment ($p \leq 0.05$, *). All other terms had a p value ≥ 0.05.

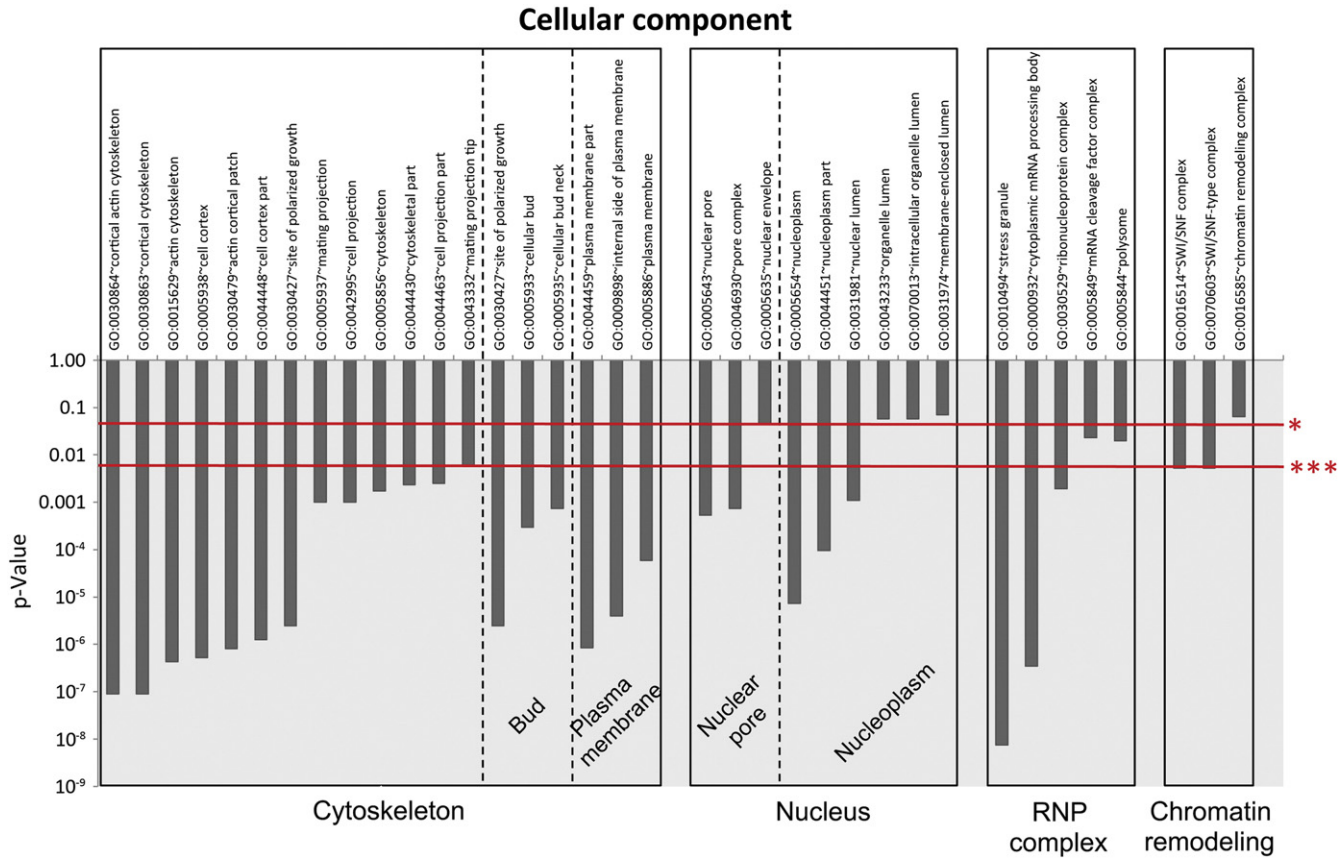

Fig. 3. Clustering of PrD-containing proteins from *Saccharomyces cerevisiae* according to their association with cellular components. PrD-containing yeast proteins were clustered according to their association with cellular components using predefined GO terms (Gene Ontology). The analysis was performed with DAVID. All proteins with an EASE score≤0.1 were taken into account. Clusters were generated manually based on initially proposed clusters by the functional annotation tool of DAVID. We observed an enrichment of the PrD-containing proteins in cytoskeletal compartments, in the nucleus (where we can distinguish accumulation between the nuclear pore and the nucleoplasm), in ribonucleoprotein (RNP) complexes, and chromatin-containing structures. Red lines demarcate GO terms with highly significant enrichment ($p \leq 0.005$, ***) or significant enrichment ($p \leq 0.05$, *). All other terms had a p value≥0.05.

we propose that the prion-like properties of many IDPs are a direct consequence of structural and functional constraints that enable these proteins to assemble into large fuzzy collectives.

4.6. A role for prion-like IDRs in stress-induced phase transitions?

Our analysis is pointing toward an important function for prion-like domains in the assembly of fuzzy macromolecular complexes. The dynamic, self-organized state of these assemblies affords high structural flexibility and multivalent binding. This makes it very unlikely that amyloid-like conformational states are involved. Therefore, we predict that most prion-like domains have been conserved to assist weak, dynamic interactions within fuzzy macromolecular collectives. Other functional roles, such as the ability to adopt a self-propagating prion state, may sporadically arise because of the extraordinary evolutionary agility of these domains. Such self-propagating conformational states may be an important source of heritable phenotypic diversity in yeast and other single-celled organisms [102,107]. In this section, we discuss the possibility that the structural plasticity of prion-like domains could also play an important role in facilitating protein aggregation in response to stress.

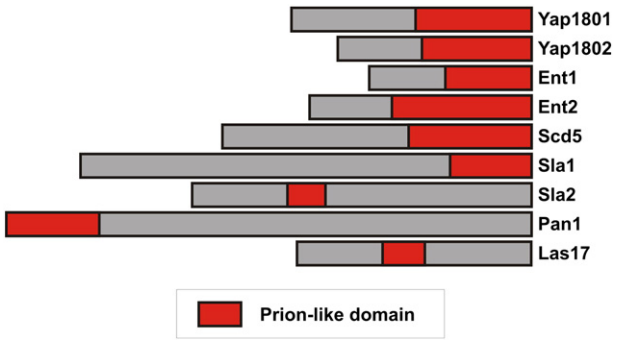

Fig. 4. Predicted PrDs in yeast proteins that are involved in endocytosis. Protein regions with prion-like amino acid composition are highlighted in red.

Many organisms operate at temperatures where small changes can have very dramatic effects on the solubility of their proteomes. Consistent with this, stress or aging can lead to widespread protein aggregation [158–161]. However, even mild environmental changes, such as the removal of a carbon source, can cause protein aggregation on a massive scale [39]. This raises an important question: are cells generally trying to prevent protein aggregation or can aggregation also be the desired consequence of a cellular program?

In agreement with the latter scenario, mounting evidence suggests that cells can actively promote protein aggregation, specifically under conditions of mild stress. A recent study identified two stress-inducible compartments, termed IPOD (insoluble protein deposit) and JUNQ (juxtanuclear quality control), in eukaryotic cells [37,162]. The JUNQ contained highly mobile misfolded proteins and was proposed to provide a specialized environment for chaperone-mediated refolding or protein degradation. The IPOD on the other hand contained misfolded proteins that were largely immobile. Sequestration in the IPOD may reduce aberrant interactions between misfolded proteins and

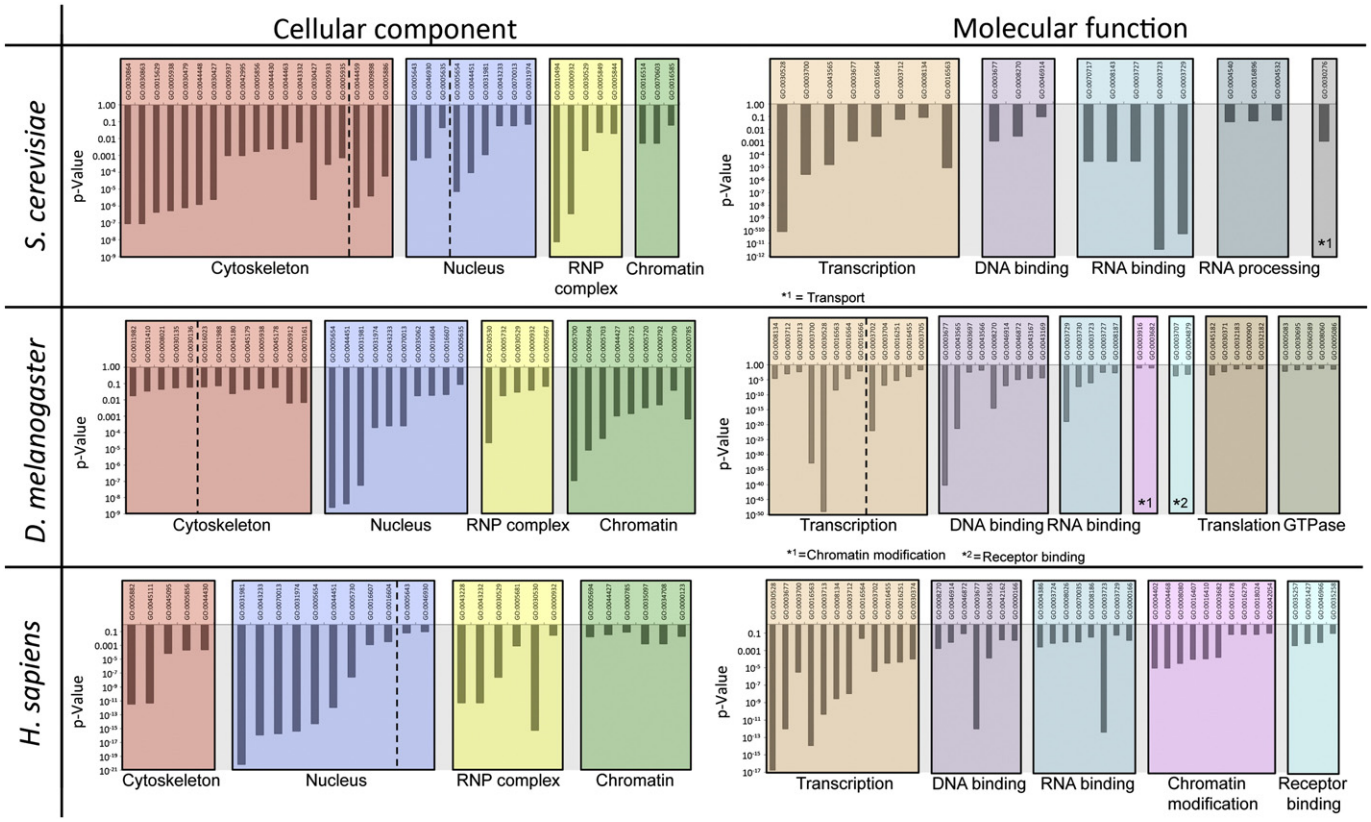

Fig. 5. Comparative functional analysis of PrD-containing proteins from *Saccharomyces cerevisiae*, *Drosophila melanogaster* and *Homo sapiens*. PrD-containing protein were clustered according to their molecular function or their association with cellular components using the GO term annotation of DAVID (EASE score ≤ 0.1). Clustering according to their association with cellular components returned four groups that were conserved across all three species: cytoskeleton, nucleus, RNP complex, and chromatin. Clustering based on molecular function produced three conserved groups: transcription, DNA binding, and RNA binding. Note that additional clusters were identified that were specific to only one or two of the species. See Supplemental Figs. S2–S5 for more details on the GO terms.

essential cellular components, which led to the proposal that the IPOD could serve a cytoprotective function. Both, IPOD and JUNQ are asymmetrically inherited in dividing yeast cells [163,164], suggesting that they may also be involved in lineage-specific aging.

In agreement with the notion that protein aggregation can be a cellular strategy, protein misfolding and aggregation were not sufficient to trigger the formation of JUNQ and IPOD under mild stress conditions. Compartment assembly also required the concerted action of molecular chaperones, protein-sorting factors, and protein-sequestration factors [163,165]. Expression of this machinery was restricted to times of acute stress through rapid changes in mRNA abundance and a proteasomal feedback mechanism. This indicates that environmentally challenged cells can undergo controlled phase transitions to promote the sequestration of misfolded proteins in specialized compartments. Curiously, the two molecular chaperones that were implicated in this process in yeast – Hsp42 and Sis1 – were predicted to contain long prion-like domains [97].

Molecular chaperones are a diverse group of proteins that bind to misfolded proteins to promote their re-folding and prevent the formation of non-specific protein aggregates. Interestingly, recent studies have shown that chaperones contain many regions that are predicted to be disordered [166,167]. The specific function of most of these sequence stretches, however, has so far remained undetermined. Despite this fact, several proposals have been made [166–168].

A closer look at the group of small heat shock proteins (sHSPs) illuminates the potential functional roles of IDRs in chaperones. sHSPs, such as Hsp42, are a conserved group of chaperones that can associate with a wide range of substrate proteins [169]. The major function of these promiscuous chaperones is to keep misfolded proteins from undergoing non-productive and potentially toxic structural transitions. Both the N-terminal and C-terminal extensions of sHSPs are highly flexible and at least partially disordered [169–172]. These regions have been implicated as binding sites for misfolded proteins and have also been shown to regulate the oligomeric state of sHSPs. In fact, many sHSPs exist as polydisperse oligomers that can change their size and organization based on the exposure to stress or upon interaction with substrate proteins [169]. In agreement with this, sHSPs often coalesce into large cytoplasmic structures in stressed cells [163,169,173]. These structures are co-aggregates of sHSPs and misfolded proteins where the sHSPs intercalate into the aggregate to prevent the formation of terminally misfolded states. Thus, it is conceivable that the disordered regions within sHSPs act as molecular spacers that keep misfolded proteins in a state that is accessible to reactivation by ATP-dependent chaperones systems.

Collectively, these findings and considerations suggest that certain types of protein aggregates are not a simple consequence of random protein misfolding events, but rather intended effects of a controlled cellular program, which eventually culminates in the formation of granules for protein storage (the IPOD) or specialized reaction centers for protein folding or degradation (the JUNQ). In fact, the dynamic network of misfolded proteins, chaperones, and chaperone cofactors provides all the ingredients required for such phase transitions: high conformational plasticity and weak, multivalent interactions between its components. Intrinsically disordered domains are likely to play key roles in this process, by functioning as polymorphic client recognition sites and enabling the formation of phase-separated, fuzzy assemblies that remain accessible to reactivation. A recent report provided further evidence for the involvement of prion-like domains in controlled phase transitions. The authors of this study identified a region of the acetyltransferase p300 that is highly

disordered and displays similarities to prion-like domains [174]. Interestingly, this prion-like domain provided an interaction interface for various misfolded proteins, promoting their aggregation.

Controlled phase transitions are not limited to protein systems; other phase-separated granules, such as stress granules and P bodies, contain large amounts of RNA [175,176]. P bodies and stress granules are found in all eukaryotes, suggesting that they perform an important cellular function. Current evidence indicates that P bodies mediate the repression and degradation of mRNAs. They contain a conserved set of proteins, which, in yeast, include the decapping enzymes Dcp1/Dcp2, the enhancer of decapping Edc3, and the exonuclease Xrn1. Stress granules on the other hand contain mRNAs stalled in translation initiation, together with translational silencers (Ngr1) and polyadenylation regulators (Pab1 and Pbp1), but no components of the mRNA decay pathway. Consistent with their distinct functions, stress granules and P bodies show differences in regulation, morphology, and their kinetics of assembly [175,177–179].

Several recent studies have provided evidence that prion-like domains play crucial roles in RNP granule assembly [128,129,180–182]. How these domains function on the molecular level, however, has so far remained elusive. One possible scenario is that prion-like domains self-associate to form a meshwork of interacting proteins. This could indeed be the case for prion-like IDRs with periodically occurring hydrophobic residues, because of their strong cohesive properties. The rapid dynamics of RNP granule assembly [43–45,47,183], however, are difficult to reconcile with amyloid-like modes of assembly, as previously proposed [128,129]. Another explanation is suggested by the finding that prion-like IDRs are highly overrepresented in proteins that bind to nucleic acids (Fig. 2) and often found in close proximity to RNA-binding domains [97]. This argues against an independent role for prion-like IDRs in RNP granule assembly but rather suggests that these domains could synergize with RNA-binding domains to bind to RNA. In agreement with such a function, the mammalian prion protein PrP harbors a low complexity region that shows significant RNA-binding activity [184]. Thus, weak multivalent interactions between RNAs and RNA-binding proteins might be all that is required for RNP granule formation. Consistent with this, RNAs have an important structural function in RNP granule assembly [185]. The structural role of RNA is further underscored by two recent studies [186,187]. These studies demonstrated that RNA molecules are sufficient to seed the assembly of nuclear bodies, suggesting that RNAs could also adopt a scaffolding function in cytoplasmic RNP granules.

While interactions between prion-like IDRs and RNAs are probably important, it is conceivable that prion-like domains perform functions that are similar to those in phase-separating chaperone networks. This mode of action may be particularly important for stress granule assembly, because their formation is often accompanied by general protein aggregation. Prion-like domains may promote polymorphic interactions with other stress granule proteins and could even use co-aggregating misfolded proteins as molecular scaffolds to enter a phase-separated state. In the recovery phase, the same domains may permit resolubilization by disaggregating chaperone systems.

Future studies that attempt to determine the conformational states and binding partners of prion-like IDRs in RNP granules are now necessary to make more definitive claims about their molecular functions. However, given the sequence diversity and structural flexibility of prion-like domains, it is quite likely that different molecular strategies are used. Regardless of their mode of action, what seems to be emerging is that prion-like sequences are intimately linked to self-organizing macromolecular assemblies in living cells.

5. Concluding remarks

Reductionist approaches have generated a wealth of information about biological systems. We now have detailed parts lists of the constituting molecules of organelles, cells, and tissues, and extensive information on the biochemistry of metabolic and signaling pathways. Invariably, however, we lack a good understanding of the integrated behavior of these parts. This is particularly obvious for the large group of intrinsically disordered proteins. These proteins are intimately linked to dynamic processes in living cells, but, despite their importance for biological self-organization, we have a very limited understanding of how they function on the molecular level. To study the molecular functions of these proteins, we need better molecular and computational tools, more powerful non-invasive single molecule techniques, and methods that allow us to reconstruct the full complexity of self-organizing biological systems in the test tube. New conceptual advances may also be required to bridge the gap between the nano-world of molecules and the world of macroscopic objects. All of these are crucial steps that we need to take to understand how the dynamical organization of living cells emerges from the collective properties of interacting macromolecules. We predict that this combined effort will not only change the way we conceive of living systems, but will also give important insight into the catastrophic changes that occur when dynamic biological processes malfunction.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbapap.2013.01.003.

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