Trends in Biochemical Sciences

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REVIEWS

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Review

There Is an Inclusion for That: Material Properties of Protein Granules Provide a Platform for Building Diverse Cellular Functions

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Proteins perform a staggering variety of functions in the cell. Traditionally, protein function was thought to be hard-wired into the folded structure and conformational dynamics of each protein molecule. Recent work describes a new mode of protein functionality driven by the collective behavior of many different proteins; most of which lack a defined structure. These proteins form clusters or granules in which unstructured polypeptides interact transiently. Nonspecific multivalent interactions drive the formation of phase-separated structures resembling aggregates. This type of functional aggregate granule can be thought of as a single supermolecular functional entity that derives function from its unique material properties. In this review we examine the emerging idea of protein granules as a new functional and structural unit of cellular organization.

Trends

Protein granules represent a diverse set of phenomena including clustering of enzymatic factors, liquid–liquid demixing or phase separation, formation of a solid-like aggregate, and amyloid fibril assembly.

Aggregation of proteins containing low-complexity domains and prion-like domains is a platform for building diverse cellular functionality by modulating the contents and stability of the aggregate.

The material properties of granules can be the basis for functions such as filtration and memory storage.

Granules can act as molecular switches and co-occurrence sensors, modulating a vast array of cellular functions.

Aggregation and Granules

Protein folding is driven by the hydrophobic effect, favoring the exposure of hydrophilic amino acids to the aqueous environment, and the burial of hydrophobic residues in the inner core of the protein [1–3]. Folding was historically regarded as the essential basis of function for any polypeptide chain. The phenomenon of protein self-association into aggregates, which becomes thermodynamically favored in the absence of folding, has therefore been thought to preclude protein function.

An alternate perspective on intracellular aggregates emerged when fluorescence imaging enabled the direct observation of protein localization in inclusion structures [4–7]. It thus became apparent that the subcellular organization of aggregates is not random, and that aggregation is therefore a regulated process. This new concept fueled the hypothesis that aggregated proteins might retain some element of identity and prompted the characterization of dozens of inclusion structures, cellular bodies, or granules to add to those that had been previously observed by electron microscopy and other techniques [4,6,8–16]. Several regulatory roles were proposed for the aggregates, including storage, sequestration, partitioning, and degradation of aberrant proteins and RNAs in order to protect the cell [9,17–21].

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Over the past decade, the list of granule structures has grown tremendously, complicating the phenomenology and etymology in the field. This is further compounded by the fact that some aggregate-resembling structures are more accurately described as **liquid droplets** (see Glossary), in that their constituents are highly dynamic and interact transiently with each other. In other words, the biophysical properties of cellular granules occupy a continuum from entirely liquid to completely insoluble. The common features of granule structures are: a lack of membrane surrounding them; a highly context-dependent occurrence; and a transient localization of protein markers to the granules. Attempts to consolidate these structures into a single category has given rise to the term **membraneless organelles**. The rapidly expanding phenomenology of such membraneless organelles suggests spatial **clustering** or granule formation may be the norm rather than the exception for many cellular proteins.

Alongside the expanding phenomenology of aggregate structures and granules, there is a new appreciation of the different ways in which these structures can form. Initially, cellular inclusions were thought of as out of equilibrium kinetically trapped precipitates in which protein aggregation is driven by swapping hydrophobic domains or Beta sheets. In accordance with *in vitro* observations, aggregates grow in size as a function of concentration after a long nucleating lag phase. More recently, it has been proposed, and in some cases demonstrated, that local heterogeneities in protein concentration can exist in a dynamic equilibrium that is consonant with the free energy differences between states [22]. It has been suggested that liquid–liquid phase separation is an example of dynamic equilibrium droplet structures formed in response to phosphorylation of droplet components, which changes their interaction properties. However, it is equally possible that droplet formation is an out of equilibrium steady state driven by the constant consumption of ATP that allows for maintenance of diffusive molecules in an unmixed state [22,23].

Aggregating Terminology

Differences of approach exist as to the terminology that is best suited for writing about protein self-association. First, many of the terms used to describe cell biological entities that look like granules [e.g., inclusion, inclusion body, inclusion structure, aggregate, dynamic droplet, membraneless organelle, (function)osome, and foci] are all to some extent etymologically contaminated by pre-existent notions of the biophysical and functional properties generally attributed to the term. Hence, the term aggregate often carries the connotation of a disordered, dysregulated, and pathological structure that has low internal mobility and excludes water. Second, it is infrequent that a distinction is made about the granule structure and the biophysical processes that may have contributed to its formation.

Since the field is far from settled on a unifying terminology, this review uses several of the above terms interchangeably. Cell biological structures are referred to as clusters, granules, or aggregates, without making assumptions about which biophysical model best describes their formation. The process of granule formation is referred to as aggregation in general, encompassing all known types of self-association. Where possible, a distinction is made between specific types of self-association (e.g., liquid–liquid phase separation or **amyloid** formation). It is important to note that a granule/aggregate may initially form as a result of a **phase transition**, and then mature into a crystal-like aggregate. Often, no single mechanism of formation can fully describe a cell biological granule. For example, synphilin 1 aggregates initially form through a first-order phase transition [24], and only later mature into insoluble, hydrophobically crosslinked aggregates resembling Lewy bodies. Conversely, stress granule (SG) components have been shown to undergo **liquid–liquid phase separation (LLPS)**, but can also form amyloid-like aggregates when overexpressed, mutated, or aged [25,26,89,90]. An important distinction seems to be between structures that are kinetically trapped and therefore stable (many aggregates and amyloids would fit into this category) and granules that

Glossary

Amyloid: ordered crystalline form of protein polymer that exhibits a regular structure usually held together by beta-sheet interactions. Amyloid structures exclude most water molecules and stain with dyes like thioflavin-T.

Amyotrophic lateral sclerosis (ALS): fatal degenerative motor neuron disease, also known as Lou Gehrig's disease. In addition to mutations in superoxide dismutase, mutations in the genes encoding RNA-binding proteins FUS, TDP-43, and several others are common in families with histories of ALS. Aggregates of these proteins are commonly found in the brains of affected individuals.

Clustering: stochastic local enrichment of proteins in a submicron cellular space. Clusters can be homotypic or heterotypic and have a short half life. No assumptions are made about what types of interactions (e.g., phase separation, hydrophobic interactions, and LCD interactions) drive clustering or keep the clustered components together. Typical cellular examples of clustering include Pol II clusters and receptor clusters.

Frontotemporal dementia (FTD): progressive degeneration in the frontotemporal lobe. ALS and FTD show overlapping features, and are now thought to be part of a spectrum disorder.

Hydrogel: Well-hydrated matrix of proteins that are crosslinked, minimizing diffusion. Hydrogels are nevertheless permeable to other molecules.

Intrinsically disordered proteins (IDP): proteins lacking a single defined folded structure. IDPs make up a large part of the proteome, and are especially prevalent among RNA- and DNA-binding proteins. An IDP typically has multiple ID regions.

Liquid crystal: structured fluid in which molecules are disordered (unlike in amyloids of solid crystals) but nevertheless can be induced to rearrange by switch-like mechanisms (e.g., phosphorylation).

Liquid granules or droplets: phase-separated structures that are held together by promiscuous interactions among the low LCDs of the constituents, and likely also by energy produced from continuous ATP hydrolysis. They are thought to