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Illuminating the interface: Protein aggregation at the condensate interface

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Biomolecular condensates are dynamic nonstoichiometric assemblies formed via phase separation of proteins, nucleic acids, and metabolites. These condensates, also known as membraneless organelles, are thought to facilitate efficient biomolecular interactions and spatiotemporally orchestrate the critical cellular biochemistry (1–3). The increase in the local concentrations of different biomolecules within the condensates accelerates vital physiological processes. For instance, RNA processing in stress granules, ribosome assembly in nucleoli, DNA repair in PML bodies, DNA packaging in nucleosomes, and mRNA regulation in P-bodies (2,3). However, under specific circumstances, this protein-rich concentrated milieu can promote aberrant phase transitions resulting in protein aggregation associated with debilitating neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases, amyotrophic lateral sclerosis, frontotemporal dementia, and so forth (4,5). The proteins that undergo biomolecular condensate formation often possess specific sequence and structural features that include oligomerization domains, intrinsically disordered regions, and folded domains (2,3). The presence of oligomerization domains facilitates protein clustering, while disordered regions serve as flexible linkers that mediate weak, transient, multivalent interactions. The folded domains provide stability to the condensate structure and mediate specific interactions with other proteins or nucleic acids. The interplay between these different domains within phase-separating proteins allows for the spatiotemporal regulation of condensate formation and dissolution (2). Under certain disease conditions, these domains experience a cascade of events due to misfolding, abnormal posttranslational modifications, genetic mutations, and so forth (4–5). These processes collectively drive the phase transition of liquid-like condensates into solid-like aggregates. Specifically, the oligomerization domains, which typically play pivotal roles in facilitating liquid-like condensation, can also promote the clustering of proteins into toxic oligomers and solid-like protein aggregates (3,6). This liquid-to-solid transition is believed to be promoted by the condensate surfaces (7–9). In the latest study published in *Biophysical Journal* (9), a team led by Clifford Brangwynne highlighted the crucial role played by the interfaces of p62 condensates in catalyzing the coarsening process of mutant polyglutamine (polyQ) aggregates prompting the formation of larger aggregates. However, the buildup of polyubiquitinated proteins within these enlarged aggregates recruit p62 and thus decreases the free pool, consequently impeding any further condensation of p62 (Fig. 1).

Using a light-inducible biomimetic condensate system, Choi et al. elucidated the phase behavior of p62 within the dynamic milieu of living cells (9). This study unfolds as an exquisitely orchestrated cellular process, closely entwined with oligomerization dynamics, ubiquitin-binding interactions, and the catalytic action of proteasomal machinery. In this study, the authors introduced mCherry-p62 constructs into living cells and observed a transition of soluble p62 into liquid-like condensates manifested prominently within the cytoplasm. Consistent with earlier studies, they found that the orchestration of p62 condensation required the concerted participation of two critical domains within the p62 molecule: the self-oligomerization domain (PB1) and the ubiquitinated substrate-binding domain. Furthermore, by inhibiting proteasomal degradation with MG-132, the authors observed an increase in cellular ubiquitinated protein levels leading to a

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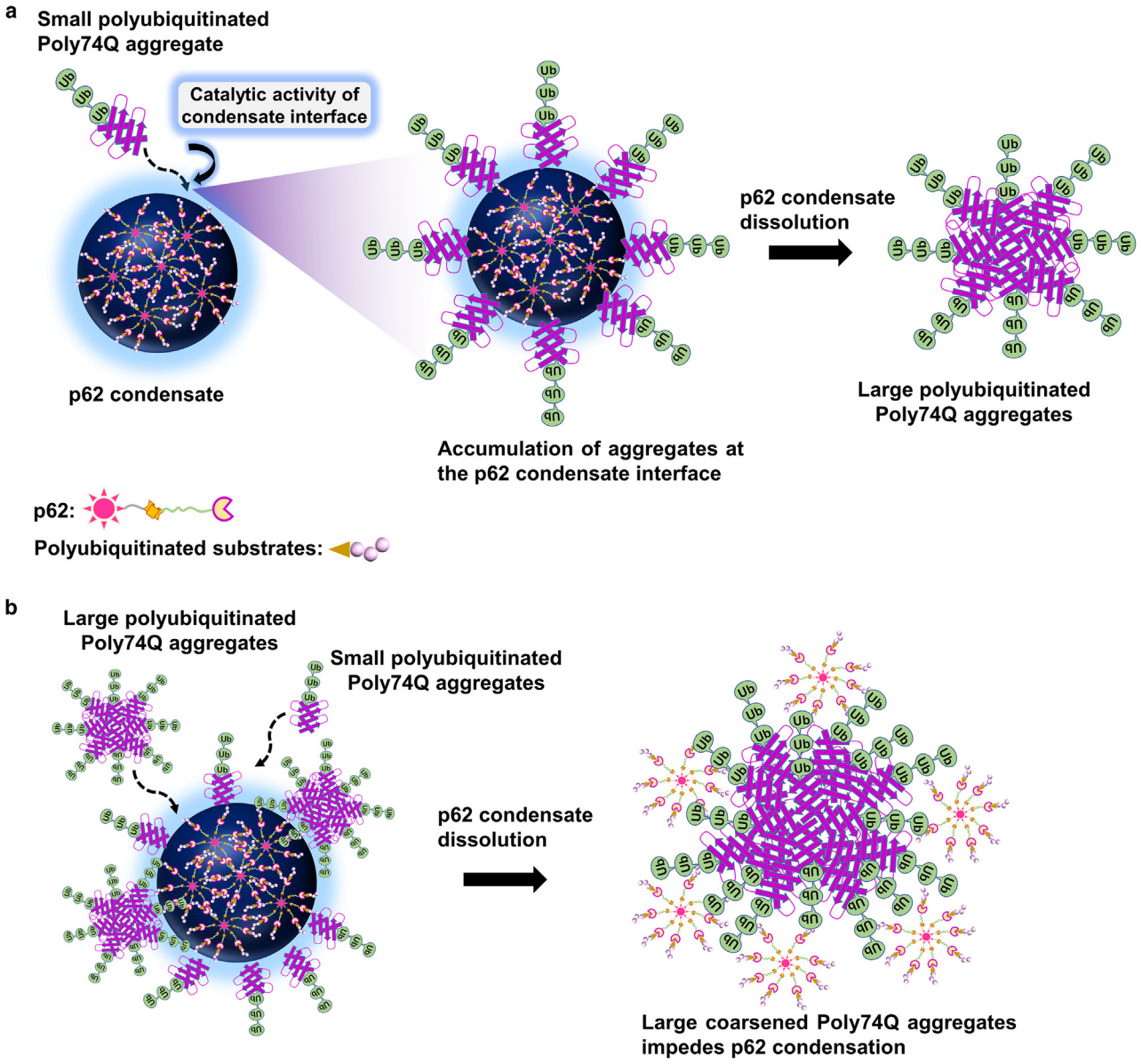

FIGURE 1 The surface of p62 condensates orchestrates polyubiquitinated Poly74Q aggregate coarsening. (*a*) Attachment of small polyubiquitinated Poly74Q aggregates at the interface of p62 condensates integrates and coarsens the aggregates. (*b*) Interfacial recruitment of large, coarsened aggregates reduces the pool of soluble p62 required for initiating new condensate formation. To see this figure in color, go online.

consequential shift in the p62 phase boundary. This observation underscores the pivotal role of ubiquitinated substrates in regulating p62 condensation. To delve deeper into the complex interplay between pathological protein aggregates and p62 condensates, Choi et al. chose mutant Huntingtin Exon1, a model system directly linked to Huntington's disease. Notably, the length of the polyQ tract within Huntingtin emerged as a key determinant of its propensity for pathological aggregation. Specifically, the cells expressing longer polyQ repeats (PolyQ74) triggered the formation of solid-like, ubiquitinated aggregates within cellular environments that exhibited an inexorable progression toward coarsening. Conversely, shorter polyQ repeats (PolyQ31) fail to culminate in aggregative assemblies elucidating a direct correlation between the polyQ length and aggregation propensity. Cells expressing PolyQ74 aggregates showed a significant colocalization of endogenous p62, K48-linked polyubiquitin chains, and PolyQ74 aggregates. Importantly, when exogenous p62 coexisted with PolyQ74 aggregates in cells, most colocalized p62 molecules were immobile, indicating a strong interaction, underscoring Huntingtin polyQ74's dual role in forming ubiquitinated aggregates and recruiting p62. It elevates this system to the status of an indispensable tool, to be employed synergistically with inducible p62 condensation, for unraveling the

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intricate complexity inherent to protein aggregation pathways. Upon the activation of p62 condensates via light stimulation within cells expressing PolyQ74, aggregates gather along the periphery of the condensates in distinctive ring-like structures (Fig. 1 *a*). This intriguing observation hints at a discernible preference for the interaction between PolyQ74 aggregates and the surfaces of p62 condensates. Interestingly, PolyQ74 aggregates quickly accumulated at condensate interfaces without increasing their total levels, suggesting a dynamic reorganization of pre-existing aggregates toward the condensate surface rather than creating new aggregates. Importantly, it emerges that smaller PolyQ74 aggregates, those measuring less than 5 μm^2, exhibit a pronounced propensity for accumulating at the interfaces of condensates in comparison with their larger counterparts. Further investigations revealed that successive cycles of p62 condensation and dissolution lead to a progressive coarsening and clustering of PolyQ74 aggregates at these interfaces (Fig. 1 *a*). Thus, p62 condensate formation emerges as an architectural force, spatially reconfiguring and coarsening pre-existing PolyQ74 aggregates at the condensate interface, with a pronounced affinity for smaller aggregates. This finding unveils a potent mechanism through which p62 condensates have the potential to exert influence over the dynamics of aggregation processes—a significant step toward comprehending their role in the realm of cellular protein homeostasis. Furthermore, the authors embarked on studies to unravel the impact of large, coarsened ubiquitinated aggregates on p62 condensation dynamics. Interestingly, when p62 condensation was induced in the presence of larger cytoplasmic PolyQ74 aggregates, these aggregates emerged as potent nucleation sites for the formation of new p62 condensates directly on their surfaces (Fig. 1 *b*). An intriguing correlation emerged between the size of the PolyQ74 aggregates and the extent of colocalization with p62. Larger PolyQ74 aggregates exhibited more robust recruitment of p62, culminating in a profound interplay between these aggregates and the cellular p62 reservoir. Furthermore, cells with sizable aggregates showed reduced de novo condensate formation, even under conditions favoring p62 phase separation, evident in phase diagram shifts. This shift indicates that the extensive coarsening of PolyQ74 aggregates interferes with further p62 phase separation. The enhanced p62 colocalization with larger aggregates is likely due to higher ubiquitinated substrate levels on the aggregates, which interact with p62's ubiquitinated substrate-binding domain and recruit soluble p62. Consequently, this recruitment of p62 by large aggregates diminishes the pool of free p62 available for initiating new condensate formation (Fig. 1 *b*). These findings elucidate a feedback mechanism that governs the interplay between p62 condensates and PolyQ74 aggregates, thereby probing the boundaries of cellular mechanisms aimed at mitigating the detrimental effects of proteins prone to aggregation.

In summary, these results presented by the Brangwynne lab open new avenues for exploring the role of condensate surfaces in the intracellular organization of membrane-less organelles in physiology and disease. This study predominantly illuminates the interaction between p62 condensates and mutant Huntingtin Exon1 in the context of Huntington's disease, providing valuable insights into this specific interaction. Future studies are required to elucidate the broader landscape of protein-protein interactions, phase transitions, and amyloid formation within biomolecular condensates. Current findings not only expand our knowledge of how liquid-like condensates interact with solid-like toxic aggregates but also carry implications in novel therapeutic strategies against fatal neurodegenerative diseases associated with aberrant phase transitions and protein aggregation.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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