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Liquid-liquid phase separation: A new perspective to understanding aging and pathogenesis

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SUMMARY Mounting evidence has suggested that phase separation, and especially liquid-liquid phase separation (LLPS), underlies the formation of membraneless organelles, which are supramolecular assemblies of proteins and RNA molecules in cells. These membraneless organelles are also called biomolecular condensates. Evidence is now growing that condensates, such as stress granules, P bodies, Cajal bodies, and nucleoli, play vital roles in biological processes, like RNA storage and processing, signaling regulation, transcription regulation, gene regulation, and transport. Conversely, condensates may cause diseases, such as neurodegenerative diseases and tumors, when they go wrong. Condensates initially have liquid-like properties, but accumulating biological and chemical mutations with age render them into a more solid-like state, like amyloids in Alzheimer's disease, Huntington's disease, and Parkinson's disease. Research into phase separation is still in its infancy, but this field is a promising avenue for treatment of aging-related diseases.

Keywords phase separation, condensate, aging, cancer, amyloid

Liquid-liquid phase separation (LLPS) describes a phenomenon whereby proteins and/or nucleic acids in a solution separate into a dense phase that resembles liquid droplets when their concentration rises above a certain level, just like oil drops forming in water. The dense phase will form biomolecular condensates such as membraneless organelles (MOs). MOs such as nucleoli were described as early as the 1830s (*1*). Recently, Keizer *et al*. found that, outside of cell division phases, chromosomes are actually almost liquid and that this structure may be formed through LLPS (*2*). Condensates have been reported to have several functions including the enhancement or inhibition of cellular reactions, sensing changes in the niche (the environment surrounding cells in aging tissue), and buffering biomolecule concentration (*3*). In a lot of biological processes, condensates play an important role and thus condensate assembly, a phase change, the quality control system (QCS), and the relationship between condensates and aging-related diseases need to be understood.

Condensate assembly

When a liquid phase is formed by LLPS, the liquid state is maintained by continuous interactions between biomolecules in the liquid phase. A common way that proteins form a liquid phase is through multivalence of phase-separating molecules (*4*). Multivalence is derived from folded interaction domains or intrinsically disordered regions (*5*). Besides proteins, RNAs have also been found to be key factors in the formation and composition of condensates (*6*). Cooperation between folded protein domains and nucleic acids may be necessary for most phase separation processes.

When condensates begin to assemble, two types of molecules are needed, scaffold molecules and client proteins. Scaffold molecules have numerous valences and play a role in promoting LLPS, and client proteins have lower valences and specifically bind to elements in the scaffolds (*7*). Cells employ various mechanisms to regulate the formation of condensates. Phosphorylation and/or methylation of proteins was reported to change the saturation concentration of proteins *via* post-translational modification (*8,9*). That said, LLPS is very sensitive to RNA concentrations and cells can control transcription to regulate condensates. In addition, other factors including temperature, the concentration of metabolites, pH, and ions also regulate the formation of condensates and cooperate to change them.

Phase change and aging

Although condensates are initially in a liquid phase, they will age and change to a more solid phase under certain conditions (*10*). The liquid phase can age into

a gel or glass state. In gelation, the physical crosslinks between condensate components reach a percolation threshold and the condensate changes to a gel. However, condensates are soft glasses themselves and can harden into a solid-like state as a result of changes in temperature or density. Both a gel and glass state can slow down protein dynamics and promote aggregation. There are various types of aggregated proteins, such as oligomers, amyloid fibrils, and disordered or amorphous protein aggregates. A leading villain, amyloid fibrils lead to various neurodegenerative diseases; once formed, they can become seeds and change other soluble proteins into an amyloid state (*11*). Aging is a two-edged sword: it can preserve the biological structure and suppress redundant biochemical reactions during stress adaption, but it is also linked to various diseases (*12*).

A few factors have been reported to promote condensate aging, such as high protein concentrations, loss of binding partners, and insufficient water. Conversely, increasing the heterogeneity of the protein composition in condensates could be a way to suppress aging; as an example, adding a few binding partners such as RNA and RNA-binding proteins (RBPs) will delay or prevent the aging of condensates (*13*).

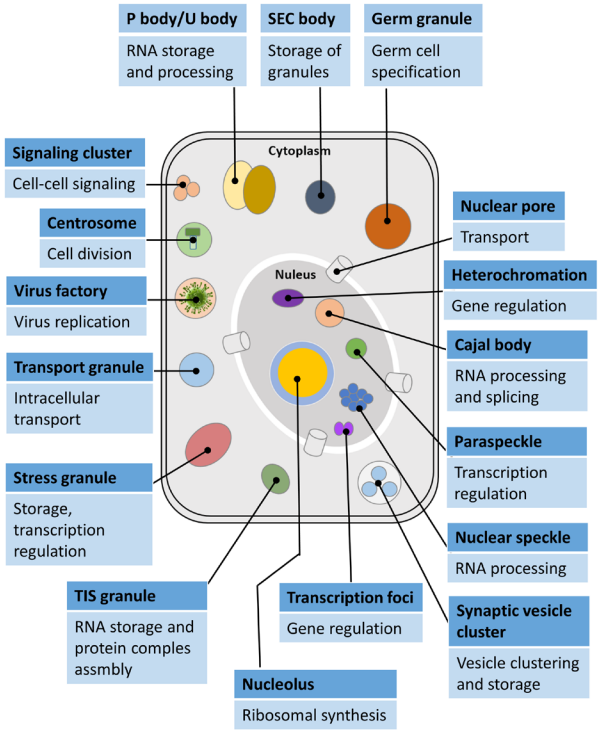

Figure 1. Functions of condensates. This graph provides an overview of the different condensates that have been studied in cells to date.

QCS for condensates

The QCS has gradually been recognized as an important regulator of condensate assembly, disassembly, and dynamic equilibrium. Recently, a study suggested that misfolding proteins accumulate in condensates and lead to aging (*14*). For example, stress granules combine with RNA and operate under stress, and they dissolve and release RNA once stress resolves. However, when misfolded proteins bind to stress granules, they are unable to release RNA (*15*). The same study found that the molecular chaperone 70-kDa heat shock protein (HSP70) can prevent the accumulation of misfolded protein in stress granules and even disassemble stress granules containing misfolded protein; those granules change from a liquid state to a solid-like state. In addition to chaperones, the ubiquitin-proteasome system has also been put forward as a component of the QCS. The ubiquitin-proteasome system is reported to play a key role in stress granule clearance, but the functions of most of the components in this system are still unclear (*16*). Another way to remove misfolded proteins is autophagy, which can selectively degrade condensates. For example, P62 was reported to be able to disassemble ubiquitin-positive stress granules. There are a few potential mechanisms that operate in the QCS, but in most instances they have not been studied in detail and more studies should be conducted to elucidate those mechanisms (Figure 1).

Mechanisms by which condensates are involved in aging-related diseases

Mounting evidence has indicated that abnormal condensate assembly or disassembly will lead to various diseases. Numerous diseases, such as tumors and neurodegenerative diseases, are driven by genetic or epigenetic mutations. A mutation in a LLPS protein will not only affect the protein itself but the interaction between it and other surrounding condensate proteins. Mutations in condensate-forming proteins could inhibit the formation of a condensate or affect condensate stability by changing the saturation concentration of scaffold proteins.

Abnormal condensate formation leads to various diseases. This happens in the following ways. Firstly, aberrant condensates disrupt proteostasis. Many neurological diseases are caused by mutations in QCS factors. For instance, a mutation in an autophagy protein led to an early onset neurodegenerative phenotype in an experiment with mice (*17*). Enhancing the QCS may be a viable option for preventing aging-related diseases. Secondly, aberrant condensates can disturb epigenetic gene regulation and heterochromatin formation. Abnormal LLPS could affect many epigenetic and chromatin regulatory factors and also promote oncogenic transformation-related enhancers and gene promoters (*18*). An aging-related abnormality in gene expression could be driven by aberrant condensates in the nucleus. Keeping condensates in good condition is crucial to maintaining tissues and organs, and especially in stem cells. Thirdly, aberrant condensates are involved in genome instability. Cancer cells frequently carry

point mutations, deletions, insertions, and fusions in their genome. To prevent these changes, cells employ a complicated system to repair the damaged DNA, and condensates are reported to be involved in the repair process. The aging process seems to weaken the ability of condensates to repair DNA (*19*). Fourthly, condensates lose their assembly sites on telomeres. As telomere attrition occurs with aging, condensate assembly is affected, and this may lead to cellular senescence and stem cell exhaustion. Moreover, tumor cells always form telomere clusters, which may be a backdoor for tumor cells to escape apoptosis (*20*). Fifthly, aberrant condensates disturb signaling in cells. There is increasing evidence that abnormal signaling events are frequently seen in cancers, and many of these may be linked to aberrant LLPS and condensate formation (*21*).

More than eight hundred RBPs exhibit LLPS, and a few of them tend to accumulate as protein aggregates in diseases (*22*). The relationship between RBPs and neurodegenerative diseases was first unveiled with the discovery of TDP-43 as the key factor that accumulates in the spinal cords of patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (*23,24*). A nuclear protein, TDP-43 can phase separate; its fibrils are able to induce further TDP-43 aggregation, and the protein irreversibly accumulates in neurons over time (*25*). RBPs also contribute to the pathophysiology of tauopathies, including Alzheimer's disease (AD). Many proteins can assemble into highly ordered structures called amyloids, which are characterized by fibrillary arrangement. Amyloid fibrils are often very stable assemblies that exclude water and contain a large proportion of cross-ß-sheets. A study has suggested that cross-ß-sheets underlie the formation of condensates (*26*). However, the role that cross-ß-sheets playing in neurodegeneration is still unclear.

Cancer cells can activate oncogenes and inactivate tumor suppressor genes through genomic alterations. Both oncogene activation and suppressor inhibition involve phase separation. Dysregulated condensates have been found in various cancers (*27*). Stress granules, a type of condensate, promote cell survival under stress. There are usually microenvironment characteristics such as hypoxia and a high level of reactive oxygen species in cancer cells that induce stress granule assembly. For example, *KRAS* is one of the most frequently mutated oncogenes in human cancers, and it is linked to stress granule assembly in pancreatic ductal adenocarcinoma, colorectal adenocarcinoma, and lung adenocarcinoma (*28*). Paraspeckles are nuclear bodies that are able to regulate gene expression. Abnormal assembly of paraspeckles has often been observed in various tumors. In hepatocellular carcinoma, paraspeckle assembly increases as a result of inflammation, which induces *STAT3* activation. Over-activation of *STAT3* promotes cell survival, inflammation, epithelial to mesenchymal transition, and cancer cell maintenance (*29*) (Figure 2).

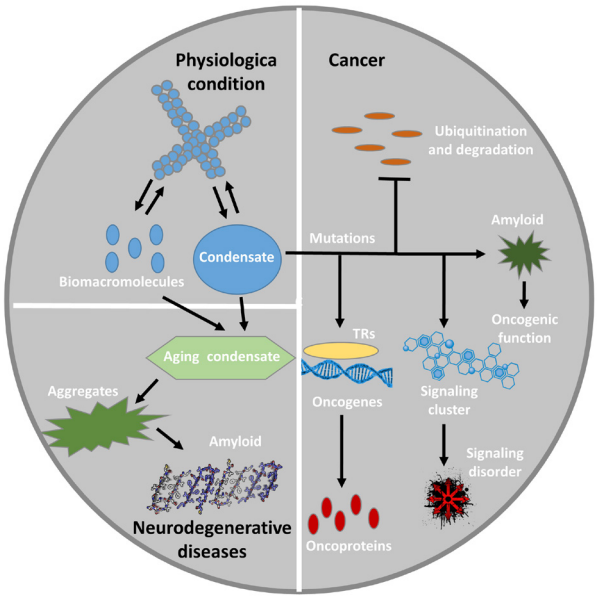

Figure 2. LLPS in cells and its relationship to neurodegenerative diseases and cancers.

Conclusions and perspectives for the future

Our understanding of phase separation and biomolecular condensates has increased greatly over the past decade, but it is still in its beginning stages. The role played by many factors and domains in condensate assembly and maturation is unclear. In the QCS, a few well-known proteins such as chaperones are also key components in regulating condensates. Further elucidation of the components of the QCS offers promise since it will reveal how cells cooperate with condensates and also uncover the link between out of control condensates and diseases. Numerous studies on aging-related diseases have indicated that condensates can promote neurodegenerative diseases and cancers by forming amyloids, affecting genetic and epigenetic regulation, losing the ability to repair DNA, and disturbing signaling in cells. Phase separation and condensates have given us new insights into aging-related diseases and unveiled new molecular mechanisms of those diseases. This could lead to the development of new diagnostic methods and therapies. Moreover, condensates and related factors could be new targets for drug development. This offers fresh hope for the treatment of intractable diseases such as cancers and neurodegenerative diseases.

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