Are aberrant phase transitions a driver of cellular aging?

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Why do cells age? Recent advances show that the cytoplasm is organized into many membrane-less compartments via a process known as phase separation, which ensures spatiotemporal control over diffusion-limited biochemical reactions. Although phase separation is a powerful mechanism to organize biochemical reactions, it comes with the trade-off that it is extremely sensitive to changes in physical-chemical parameters, such as protein concentration, pH, or cellular energy levels. Here, we highlight recent findings showing that age-related neurodegenerative diseases are linked to aberrant phase transitions in neurons. We discuss how these aberrant phase transitions could be tied to a failure to maintain physiological physical-chemical conditions. We generalize this idea to suggest that the process of cellular aging involves a progressive loss of the organization of phase-separated compartments in the cytoplasm.

Keywords:
aging; amyotrophic lateral sclerosis; chaperone; intrinsically disordered protein; mitochondria; neurodegeneration; phase separation; protein aggregation; protein quality control

Introduction

All somatic cells age and die. The process of aging is defined by a progressive decline in physiological function, and is associated with numerous hallmarks, among which are genomic instability, epigenetic alterations, telomere attrition, loss of proteostasis, deregulated signaling, defective intercellular communication, mitochondrial dysfunction, cellular senescence, and stem cell exhaustion [1]. These hallmarks of aging can be divided into three distinct categories: (i) the primary hallmarks or causes of cellular aging; (ii) the compensatory or antagonistic responses to cellular aging; and (iii) the integrative hallmarks of aging, which are ultimately responsible for the systemic functional decline [1]. Examples of the first category are proteostasis decline and telomere attrition; examples of the second category include cellular senescence and deregulated signaling; examples of the third category include altered intercellular communication and stem cell exhaustion. A major problem is to understand how all these different hallmarks of aging are connected and how they contribute to the overall process of aging.

Despite our limited understanding, it is generally accepted that the cause of aging is a time-dependent accumulation of cellular damage [2, 3]. In young healthy cells, this damage is largely repaired or removed. As cells age, damage increasingly accumulates. This is because in aging cells, homeostatic mechanisms are progressively weakened, and, as a consequence, the cell can no longer repair itself and maintain a functionally healthy state. Thus, at its core, aging is the decline of homeostatic capacity. Many concepts have been proposed to explain this deterioration of homoeostasis with increasing age. One proposal is that widespread damage to biomolecules such as proteins, lipids, or nucleic acids diminishes the homeostatic capability of cells [4]. Another idea is that aging involves a gradual decline in metabolic activity, through the loss of mitochondrial function [5–8]. A decline in mitochondrial functions would particularly affect homeostasis mechanisms, because they generally have a high demand for energy. Other concepts include the idea that aging is a consequence of an increase in macromolecular heterogeneity, driven by deregulated gene expression programs [9, 10]. However, what remains unclear is how all these diverse molecular defects are associated with aging. Are they driving the process of aging, or are they simple consequences of aging?

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Abbreviations:
ALS, amyotrophic lateral sclerosis; **FTD,** frontotemporal dementia; **FUS,** fused in sarcoma; **HSP70,** heat shock protein 70; **HSP90,** heat shock protein 90; **IDP,** intrinsically disordered protein; **RBP,** RNA-binding protein; **TDP-43,** TAR DNA-binding protein 43.

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not generally die of aging, but from the detrimental consequences of one or many age-related diseases, such as cancer, diabetes, or neurodegenerative disease. This has led to the proposal that aging itself may be a special form of disease or disease complex [11, 12]. Others instead see in aging a mostly benign progression of age that simply increases the risk of certain diseases [13]. Regardless of whether aging is a disease or not, what is clear is that aging goes along with a universal decline of function that will inevitably lead to the emergence of age-related diseases. Which of these diseases will eventually cause the death of an organism is highly variable and will depend on the genetic make-up of the individual and its life history. Although the question as to whether aging is a disease or not will remain controversial, what is clear is that aging and age-related diseases are intricately linked processes that cannot be separated from each other.

In this review, we explore the possibility that aging-induced fluctuations in physical-chemical parameters in cells, driven in part by metabolic decline and a failure of homeostatic systems, may trigger aberrant phase transitions with a consequent loss of control over intracellular organization. We propose that this concept may explain many of the multifaceted hallmarks of aging and the increasing risk of appearance of diseases with age.

Intracellular organization without membranes

The proper functioning of a cell requires the organization of macromolecules into compartments, which contain and enhance biochemical reactions. Many compartments have no membranes surrounding them. Prominent examples are P granules [14], Cajal bodies [15, 16], nucleoli [17, 18], or paraspeckles [19] in the nucleus, and stress granules [20, 21] and centrosomes [22] in the cytoplasm, and signaling complexes on the cytosolic face of membranes [23, 24].

Emerging evidence over the last decade now shows that many of these compartments are associated with age-related diseases. As an example, proteins that have been associated genetically with ALS, such as TDP-43 and FUS, are prominent members of stress granules, paraspeckles, RNA transport granules, and DNA damage repair sites [19, 21, 25–27]. In addition, there is increasing evidence that defects in nuclear compartments such as nucleoli and Cajal bodies are associated with cancer and protein misfolding diseases [28]. This suggests that membrane-less compartments are particularly vulnerable to diseases that are associated with aging.

Membrane-less compartments form through phase separation

Why are membrane-less compartments particularly sensitive to age-related disease? In the last 5 years cell, biologists have come to understand that formation of these membrane-less compartments is driven by a process of liquid–liquid demixing [29–31]. The compartments are thought to phase separate from the cytoplasm, leading to the formation of liquid droplets, which stably coexist with their surrounding environment. Prominent examples of compartments that have been shown to be liquids are P granules in *Caenorhabditis elegans* embryos [14], nucleoli [17], and stress granules [21, 32].

Work over the last 5 years has begun to attack the molecular mechanisms behind phase separation. These studies found that intrinsically disordered proteins, or IDPs, are an important class of proteins that drive phase separation [21, 33–42]. More than 30% of the human proteome contains extended regions of intrinsic disorder [43]. We do not know yet, but it seems possible that a large fraction of these IDPs are involved in phase separation and the organization of the intracellular environment.

There are different classes of IDPs. One prominent class is devoid of charges and contains polar amino acids (Q, N, S, G) with interspersed aromatic residues (F, Y). These regions of low sequence complexity have been termed prion-like domains, because of their similarity to proteins in budding yeast that form infectious proteins, or prions [36, 44, 45]. The flexibility of the chain, the absence of charges, and recurrent aromatic residues make prion-like domains very interactive. There are many such proteins in the genome; human cells, for example, contain over 100 prion-like proteins [36, 45], while in organisms such as the slime mold *Dictyostelium discoideum*, thousands of proteins carry such domains [46, 47]. Another class of IDPs is also characterized by low sequence complexity, but these proteins frequently contain amino acids with acidic or basic side chains. One example are RGG repeat containing IDPs such as Ddx4 [38]. Here, interactions between blocks of charges and aromatic residues play an important role in driving phase separation, which may additionally be regulated by arginine methylation.

Recent findings show that such low complexity IDPs may provide the link between compartment formation and disease. For instance, prion-like IDPs, such as FUS and TDP-43, are involved in forming RNA-containing compartments in the cytoplasm and nucleus, and they are associated with ALS [21, 48–52]. In these cases, the proteins form pathological aggregates in late stages of the disease. Pathological inclusions of FUS and TDP-43 contain additional RBPs, most notably stress granule proteins, such as the polyA-binding protein, eIF4G, and TIA-1 [53–55]. These observations suggest that compartments such as stress granules can change their physical properties and slowly mature into pathological aggregates. This raises an important question: what is the relationship between the role of proteins such as FUS and TDP-43 in compartment formation and their role in disease? Or, to put it another way, why would the liquid-like nature of membrane-less compartments make them so vulnerable to disease?

Liquid phase transition is very sensitive to changes in physical-chemical conditions

Liquid–liquid phase separation is a well-studied phenomenon in soft matter physics. An initially mixed solution of molecules separates into two (or more) phases after, for example, lowering the temperature (Fig. 1A). Typically,

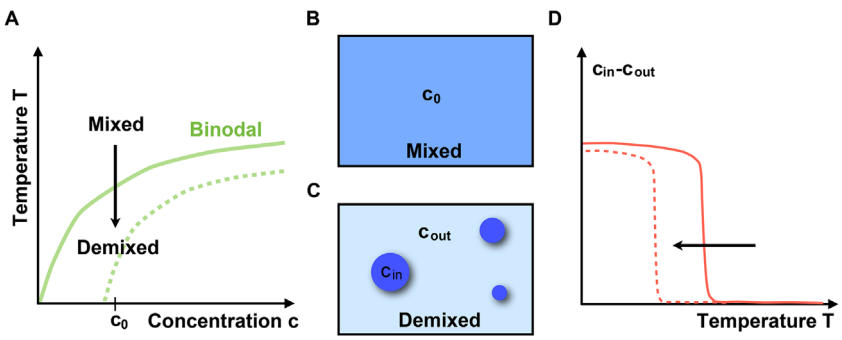

Figure 1. Sensitivity of phase separation to changing environmental conditions. **A:** Phase diagram of a binary mixture as a function of temperature *T* and concentration *c*. **B** and **C:** Upon decreasing the temperature an initially mixed state of mean concentration c_0 demixes, often leading to the formation of liquid droplets. **D:** The concentration of droplets inside minus outside, $c_{in}-c_{out}$, exhibits a jump-like response when crossing the binodal line in A). Reverting the temperature change or weakly perturbing for example the pH or the salt concentration (dashed line in A and D) can dissolve the drops again.

droplets of molecules form with an inside concentration that is much larger than the concentration outside of the droplet, in the surrounding milieu (Fig. 1B and C). In general, phase separation is very sensitive to changes in certain parameters, such as temperature, salt, pH, or molecular concentration [29]. Droplets form suddenly with a small shift of temperature or salt concentration, and upon reverting the temperature change or weakly perturbing the salt concentration (dashed lines in Fig. 1A and D), droplets can dissolve again. The sensitivity is connected to the steep response of the system, when crossing the binodal line in the phase diagram of Fig. 1A. While the concentration difference between inside and outside of the droplet changes only smoothly upon temperature variations deeply in the mixed and demixed region of the phase diagram, close to the binodal, it shows a steep jump-like response (Fig. 1D). Therefore, any liquid–liquid phase separation phenomenon will be extremely sensitive to changes in physical-chemical conditions.

Aberrant phase transitions are associated with age-related diseases

Because cellular compartments are formed by phase separation, they must also be sensitive to fluctuating intracellular conditions. Such conditions could, for example, be changes in temperature during heat shock or changes in macromolecular concentration of the phase-separating molecules. Cellular phase transitions would also be very sensitive to changes in the concentration of ions and small molecules. Indeed, it is now emerging that phase-separated compartments in living cells are exquisitely sensitive to changes in chemical or physical conditions, such as temperature or simple changes in the concentrations or affinities of macromolecules [18, 23, 24, 29, 38].

Is this sensitivity to changing conditions also associated with disease? Recent cell-free experiments with IDPs provide a clue about the relationship between liquid compartments and disease. In most cases liquid compartments quickly convert into more solid-like structures with different physical properties and structural organization, such as hydrogels and highly ordered fibrils. Proteins that show such behavior are FUS [21, 33, 34, 49] and hnRNPA1 [37, 56]. Initially, these proteins form liquid-like assemblies, which fuse, and recover quickly after photobleaching, suggesting rapid dynamics (Fig. 2A and B). However, with time the dynamic properties change, so that they stop fusing and show reduced turnover when examined with photobleaching techniques [21, 37, 49]. The material properties of this intermediate state are unclear, but a hydrogel-like state has been proposed [33, 34]. Finally, they can also convert into solid-like fibrillar aggregates that do not turn over anymore [21, 37] (Fig. 2C). Therefore, there appears to be a driving force for IDPs in liquid-like compartments to form solids with time. So far, the driving force for solidification is unknown and remains to be clarified. We speculate that it might be accompanied by an outflow of water or could be driven by polymer entanglement that leads to the formation of fibrillar aggregates.

IDPs can access different phase-separated material states

So far we have discussed the concept that many membrane-less compartments are associated with disease. Where they have been looked at, disease-related compartments form liquid-like structures in vivo. These diseases are often associated with IDPs, such as prion-like proteins. When expressed in vitro, these IDPs also form liquid-like droplets. However, unlike liquid-like compartments studied in vivo, these liquid-like droplets rapidly convert into more solid structures such as amyloid-like fibers.

Several features that enable IDPs to form liquid compartments also make them prone to form solid-like assemblies. First, IDPs have to be conformationally very flexible to form liquid compartments. Second, they have to be in a state of supersaturation to rapidly respond to changes in conditions. Supersaturation is a state of a solution that contains more dissolved molecules than could be dissolved by the solvent under normal circumstances. When a supersaturated solution demixes into a protein-poor and a protein-dense phase, the concentrations of the two phases change until they reach a new equilibrium concentration. Demixing of a supersaturated solution can be triggered through changes in binding affinities of the molecules or changes to the properties of the solvent. Supersaturation and the conformational flexibility of IDPs are necessary requirements for liquid compartment formation, but these two features also make IDPs prone to misfold and form more solid-like structures.

This, therefore, suggests that a liquid drop is a metastable state, which will quickly convert into a gel or solid-like state if not regulated. Why would

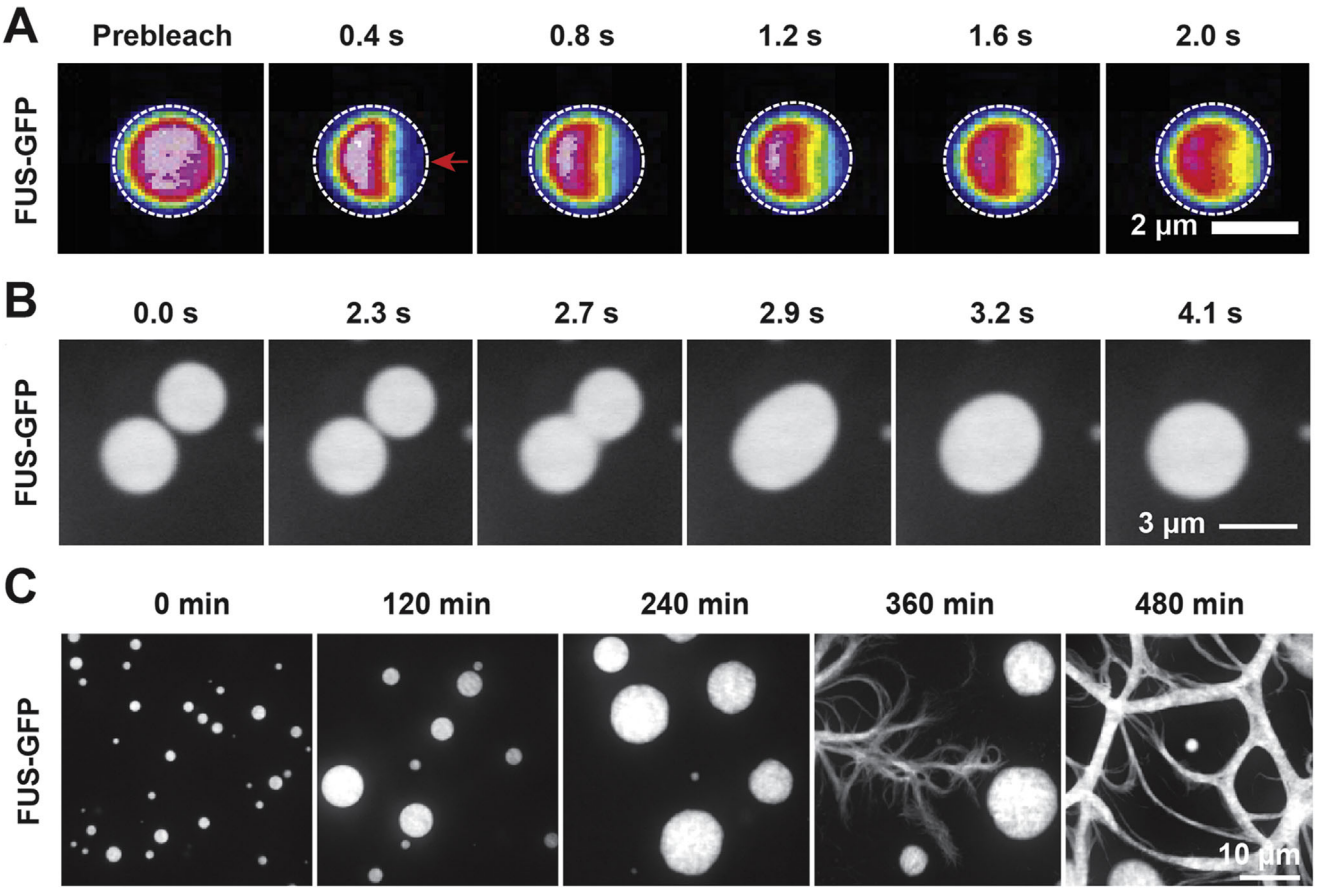

Figure 2. Dynamic behavior of FUS liquid droplets and droplet aging. **A:** Recovery of fluorescence intensity of an in vitro formed FUS–GFP droplet after half-bleach. The site of photobleach is marked by a red arrow. **B:** Montage of two in vitro reconstituted FUS–GFP droplets fusing under shear flow. **C:** Aging of in vitro reconstituted liquid droplets into fibrous aggregates.

the cell use proteins to form liquid-like compartments that can so easily transition into pathological solid-like forms?

One reason why IDPs may be prone to form aberrant solid structures is that cells have also learned to use IDPs to form solid-like structures with important physiological functions [57]. As one can easily imagine, if liquid-like compartments allow for concentrated biochemical reactions, then solid-like compartments could be used to inactivate or store macromolecules. Examples of solid-like structures formed by IDPs are the translational repressor Rim4 in yeast, where an amyloid-like state appears to regulate gametogenesis [58], and several RNA-binding proteins that function as molecular memories [59, 60]. In oocytes of *Xenopus*, organelles and molecules must be preserved for decades to be then passed on to the next generation. Indeed, *Xenopus* has evolved an amyloid-like mechanism to form a super-organelle called the Balbiani body, which stores mRNA and organelles such as mitochondria in quiescent oocytes [61]. These physiological amyloids are formed by a prion-like protein and must have some as yet unknown mechanism that regulates the formation and dissolution of these extremely stable structures.

More generally, dormancy seems to be associated with the transition from a liquid to a solid-like state. In *C. elegans*, the fluidity of RNP granules in the germ line changes from solid- to liquid-like during the transition from quiescent oocytes to early embryos [62, 63]. In budding yeast, there is evidence that numerous proteins assemble into solid-like structures such as filaments, when yeast cells are challenged with extreme environmental conditions [64, 65], and in bacteria, there is an apparent transition to a glass-like state upon depletion of ATP [66]. Solidification of the cytoplasm has been hypothesized to allow cells to downregulate their metabolism and enter into a dormant state [64, 65].

A continuum model for phase separating IDPs

We propose that different IDPs adopt different material states and that the material properties of assemblies formed by these IDPs are tuned to purpose (Fig. 3). When biochemical reactions are favored, cells build liquid-like compartments that concentrate components that can execute diffusion-limited biochemical reactions. When inactivation or long-term storage is required, cells shut down biochemical reactions by inducing the formation of solid-like states that slow diffusion. This must in some way involve tuning of the binding affinities between interacting molecules.

Each phase-separating protein will have a preferred location on the

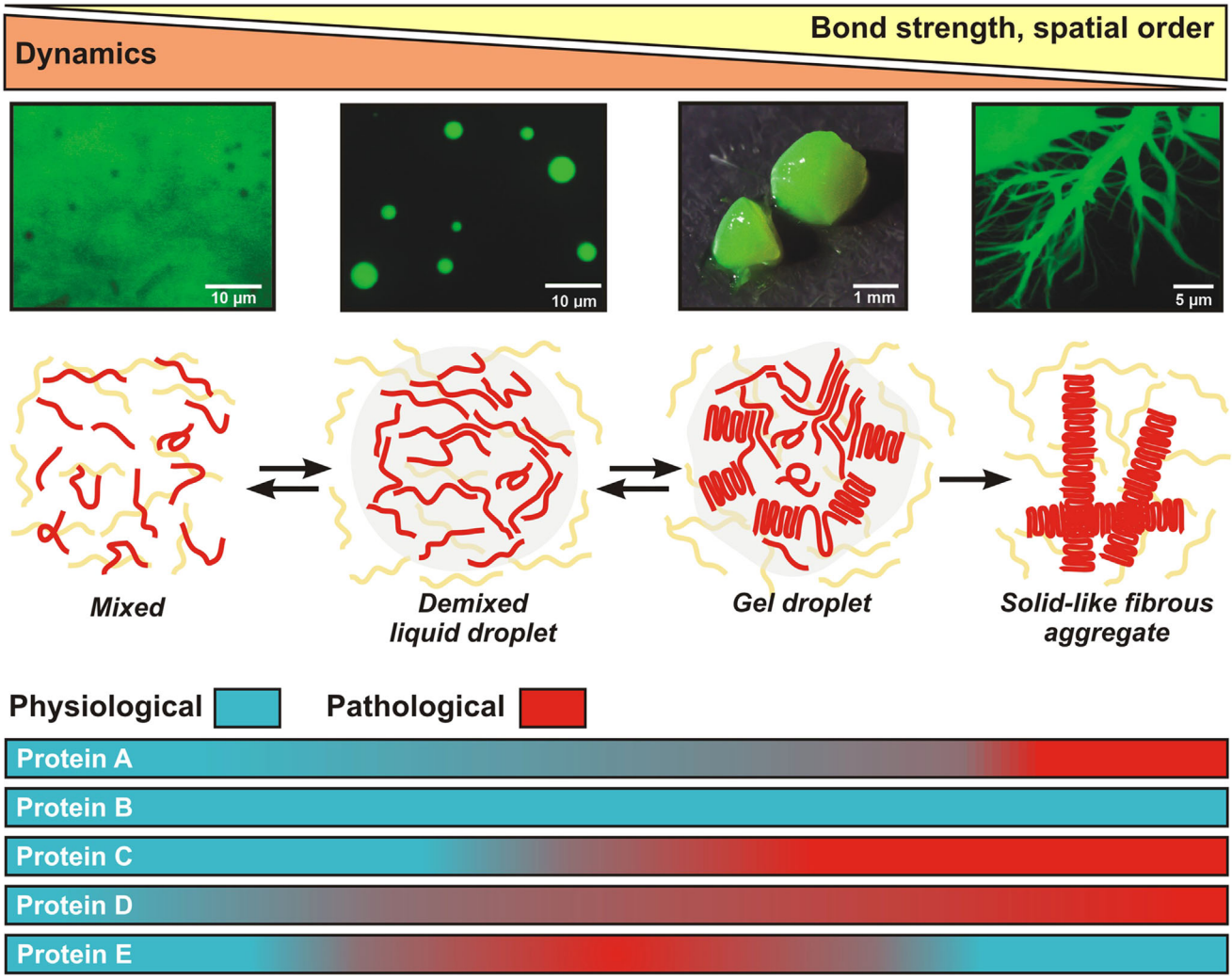

Figure 3. Continuum model of phase separation by intrinsically disordered proteins. An intrinsically disordered protein (shown in red) undergoes a phase transition into a liquid droplet by liquid–liquid demixing. The droplet "ages" with time and adopts different structures and material properties (gel, solid-like fibrous aggregates). The images on the top show purified FUS protein forming liquid droplets, a gel, and fibrous aggregates [21]. Bottom: The hypothetical proteins A–E span the whole range of the continuum. Pathological forms of these proteins are highlighted in red.

continuum of material states under physiological conditions, which has been under selection by evolution. However, because many IDPs are exquisitely sensitive to changes in the environment [67, 68], they may access other states along the continuum, when the physicochemical conditions change. Which range of different states a given protein covers will depend on the specific conditions and the evolved molecular properties of the protein. Movements along the continuum may be intended and could be used for sensing stress. This could be achieved by modifying the affinities of interacting IDPs through post-translational modifications. The fact that IDPs contain many residues such as serine, tyrosine, and arginine, which in principle are modifiable by posttranslational modifications, such as phosphorylation and methylation, suggests that cells make widespread use of such mechanisms.

Therefore, the reason that cells have evolved to use a mechanism that is so prone to error is that it allows tuning of the material properties of their compartments by modulating the binding affinities of IDPs. This comes with the trade off that subtle changes in binding affinities could shift the balance from physiological to pathological assemblies as seen in neurodegeneration. We next discuss mechanisms by which cells could stabilize and maintain metastable liquid-like states in cells.

Protein quality control mechanisms regulate phase transitions

Because of this propensity of IDPs to undergo aberrant phase transitions, cells must invest enormous effort to control phase transitions and prevent a conversion into an aberrant state. This is especially true for the liquid-like compartments, which, as we have discussed above, are likely to be metastable. Indeed, cells have developed

numerous quality control or proteostasis systems that regulate and prevent aberrant conformational transitions of IDPs [69]. One example are chaperones. These proteins are well known to play a critical role in recognizing and reversing aberrant protein conformational states [70]. Chaperones of the HSP70 family alone constitute up to 3% of the protein mass in a cell. These ATP-driven machines bind aberrantly folded proteins and cooperate with other chaperone systems, such as HSP90, to promote their refolding into a normal state. There also are ATP-independent chaperones, such as small heat shock proteins, which neutralize aberrant conformations through binding and sequestration. If chaperone systems fail, cells have disposal systems such as the proteasome or autophagy machinery in place, which remove aberrant structures by degradation [71, 72]. In fact, there are numerous examples where these systems fail in age-related diseases. For example, failure of the autophagy machinery is now considered one of the key pathomechanisms in ALS and FTD [71, 73–78]. There are many additional examples of how defects in the proteostasis machinery affect protein conformations and associated diseases, in particular in aging organisms.

Changes in metabolism have a strong impact on phase transitions

The sensitivity of phase transitions to different metabolic conditions means that a cell must also go to great lengths to control the metabolic state of the cytoplasm and to keep its energy levels constant. Less is known about how defects in this basic parameter is linked to disease. Simple experiments affecting the energy levels of the cell have been shown to alter the viscosity of nucleoli [17] and stress granules [79]. Severe changes in energy levels even affect the overall state of the cytoplasm in bacteria, yeast, and *D. discoideum*, by inducing a transition from a fluid to a solid-like state [65, 66]. This indicates that, in living cells, membraneless compartments are out of equilibrium systems that require constant energy input to maintain their dynamic properties. The continuous activity of ATP-driven machines may, for example, be employed to rearrange molecules or loosen up tight interactions that form with time. Indeed, evidence is now emerging that ATP-driven machines such as helicases and chaperones are intimately involved in regulating the material properties of RNP granules. In the case of the helicase DDX6, inactivation led to the conversion of RNP granules from a disordered liquid-like state into a well-ordered crystal-like assembly [62]. In addition, in yeast cells, the recovery from a solid-like stress granule state depends on an ATP-driven chaperone, without which cells cannot recover from a solid-like state of the cytoplasm [32, 80].

We can also envision that the cytoplasm contains many small molecules that tune the phase behavior of proteins and RNA. Trehalose, for example, is produced in great amounts in stressed cells and keeps proteins in a more soluble state [81–83]. Phase-separating proteins have evolved in the complex environment of the cytoplasm, and therefore depend on the chemical heterogeneity and the specific physical and chemical conditions that characterize the cytoplasm (neutral pH, high potassium and low sodium concentrations inside, high background concentration of macromolecules, small molecules, etc.). Any changes to these parameters will therefore have sweeping consequences for cellular organization and homeostasis. Conversely, cells could use changes in physicochemical conditions such as pH to regulate cellular functions through rapid phase transitions, as has recently been shown for yeast entering into a dormant state [64, 65].

How aging and age-related diseases affect RNP granules

In the following, we will take the example of RNA compartments to illustrate how the numerous changes in aging cells could lead to aberrant phase transitions that cause disease and accelerate aging.

Protein concentration

One trigger of aberrant phase transitions could be changes in macromolecular concentrations or heterogeneity. As discussed before, RNP granules are formed in a concentration-dependent manner, through phase separation. This process has advantages, but it is also dangerous, because it requires a cell to keep the concentrations of phase-separating proteins in a defined range. Aging is associated with a loss of control over gene expression and a resulting increase in molecular heterogeneity and changes in macromolecular concentrations [84]. This could elevate or reduce the cellular concentration of phase-separating proteins, with immediate consequences for compartment formation. Increases in heterogeneity could also change the well-balanced composition of RNP granules, which may be required for maintaining RNP dynamics and functionality. The conformations of IDPs within RNP granules may be controlled through complex formation with other proteins or RNAs, and changes in the concentrations of binding partners may increase the incidence of aberrant conformational states that arise within RNP granules. Aging-induced changes in molecular heterogeneity could, thus, dramatically shift the phase behavior and physical properties of intracellular compartments. Indeed, when investigated in vitro, the RNA-binding protein FUS takes up different material properties depending on the concentration of the protein [21]. At physiological concentration, it forms droplets that remain liquid for many hours. However, when the protein concentration is raised further or one just waits for a longer time, a gel-like state develops.

Nuclear and cytoplasmic localization of proteins

There now is evidence that normal aging as well as neurodegenerative diseases affect the shuttling of many RBPs between the nucleus and the cytoplasm [85–89]. However, maintaining a correct nucleo-cytoplasmic distribution of RBPs is important, because the nucleus is a very different environment than the cytoplasm. Altered

intracellular distributions of RBPs could significantly affect the compositions and properties of RNP granules. In fact, mutations in disease proteins often increase the cytoplasmic and decrease the nuclear concentration of the affected protein [86]. A prominent example is FUS, in which most of the ALS-associated mutations map to the nuclear localization sequence. Direct consequences of these mutations are loss-of-function defects in the nucleus, which cause additional problems with transcription, RNA processing, or DNA damage repair [48, 50, 90]. However, gain-of-function defects have also been observed [49]. These gain-of-function defects could be related to aberrant phase transitions, which alter the material properties of FUS compartments. Thus, one can imagine that changing the composition and the dynamics of RNP granules through an increased cytoplasmic FUS concentration could cause gain-of-function defects through aberrant phase transitions.

Binding affinities and mutations

Aberrant phase separation could also be induced by changing the binding affinities of interacting IDPs. For example, mutations that increase the propensity of phase-separating proteins to spontaneously convert into a solid state have been identified in ALS patients. A prominent example is the prion-like protein TDP-43, where most of the mutations map to an intrinsically disordered C-terminal domain that drives the phase behavior of this protein [26]. Further examples are FUS and hnRNPA1, which also form solid-like aggregated states more rapidly when mutated in the prion-like domain [21, 37, 91]. It is possible that many mutations exist in a given proteome that slightly change the phase behavior of affected proteins, but under normal conditions are phenotypically silent. However, when these mutations are placed in the right genetic background, they may unfold devastating effects. This was illustrated by the finding that expansions of a low complexity Q-rich sequence in ataxin-2 are usually without effect, but when combined with mutations in the protein TDP-43 dramatically accelerate the development of a disease [92]. We do not know how many such subtle changes exist in a given proteome. In particular, in the late stages of aging, when cells start to lose control over homeostasis, these polymorphisms may increasingly impact compartment formation, with severe effects on cellular function. Changes in the general physical or chemical conditions of the cytoplasm would also strongly affect the phase behavior of RNP granule-forming proteins. In vitro experiments with RBPs have shown a strong dependence of phase separation on pH, ions and temperatures [21, 33, 34, 37, 38, 91, 93]. Thus, any failure of homeostatic mechanisms normally balancing these parameters will have catastrophic effects for RNP granule dynamics and function. Especially in the late stages of aging, cells may struggle to maintain homeostasis, and basic parameters such as ion concentrations and pH would increasingly get out of control.

Posttranslational modifications and binding partners

Another source of aberrant phase transitions could be a failure of post-translational mechanisms that normally control the assembly and fluidity of compartments, in particular methylation and phosphorylation [20, 33–35, 38, 94]. Moreover, RBP proteins such as FUS usually function in a complex with polyvalent molecules, such as RNA or poly(ADP) ribose (PAR) [21, 95–98]. Thus, global or local changes in RNA or PAR concentrations could lead to downstream effects, which negatively impact compartment dynamics and function. That such changes have a strong effect on compartments is illustrated by the finding that the nucleolus hardens when transcription is inhibited [99]. Once a liquid compartment has turned into a more solid state, additional problems may occur. An increase in the amount of cross-β sheets may lead to the trapping of proteins, as shown for aberrant assemblies of FUS [49]. IDPs often show promiscuous binding behavior and some IDPs exist in very low numbers, suggesting that aberrant trapping of IDPs could be a significant problem, especially for those IDPs that exist in low concentrations and have essential functions. This has been demonstrated recently for TDP-43 aggregates in the cytoplasm, which trap nucleo-cytoplasmic shuttling factors, thus causing a breakdown of nuclear transport and potential secondary defects in splicing and transcription [100].

Misfolded proteins and stress

Chronic stress is another aspect that could accelerate aging through aberrant phase transitions. There is evidence that in stressed cells, RNP granules specifically recruit misfolded proteins such as defective ribosomal products (DRiPs), especially when the protein quality control machinery is overwhelmed [101]. IDPs in RNP granules seem to have a tendency to bind misfolding-prone proteins [32]. Trapping of misfolded proteins in RNP granules could promote intra-compartment cross-seeding events and subsequent spreading of cross-β sheet conformations to compartment-forming proteins, followed by compartment hardening. Failure of protein quality control mechanisms such as ATP-driven chaperones or disposal mechanisms could further accelerate this hardening reaction and cause additional functional defects.

Metabolic state and mitochondria

Decline of mitochondrial activity has been commonly linked to aging and age-related diseases, but it has been hard to pinpoint why. We suggest that, as mitochondrial function declines, changes in the properties of the cytoplasm that are governed by mitochondria, such as regulation of ATP levels or lipid metabolism, will make it harder and harder to properly regulate the numerous homeostasis mechanisms that regulate phase transitions as described above. This will further accelerate the defects commonly seen as problems of aging. Furthermore, there is now substantial evidence of a link between mitochondrial failure and age-related neurodegeneration [5, 102, 103]. Regardless of whether or not mitochondrial defects are a cause or a consequence of these diseases, defective mitochondria will likely accelerate the

pathology as phase transitions start to fail.

We can now see why patients with mutations in proteins such as FUS and TDP43 only develop disease later in life. When cells are young, they have an active metabolism that allows them to control the tendency of these proteins to aggregate. However, one can imagine that when mitochondrial activity begins to decline, for instance through the accumulation of somatic mutations, the metabolic potential of the cells will also decrease, thus triggering a loss of homeostasis and aberrant phase transitions. Indeed, it is known that experimentally increasing the rate of mitochondrial mutations in mice will also increase the rate of aging [6]. The stochastic nature of age-related diseases could therefore be due in large parts to the stochastic nature of mitochondrial decline.

As discussed in this review, there are multiple different systems that prevent aberrant phase transitions in young cells. However, they will all decline with increasing age, causing an increasing loss of control over cellular organization. Thus, aberrant phase transitions could be an important driver that accelerates the aging process and causes the onset of age-related diseases.

Conclusions

Current research into aging and age-related diseases is biased toward "omics" approaches. This has generated tremendous insight, but often the underlying molecular causes of aging and age-related diseases have remained unclear. At its heart, aging is a process of cellular functional decline. In this review, we examined the idea that one aspect of this problem could be the difficulty of cells to properly regulate phase separation as the metabolic activity and the homeostatic capacity of the cells decreases. Because phase separation is involved in numerous different processes in a cell, this would help to explain why cellular aging is such a multifactorial process. Understanding aging and its associated diseases will require us to look at a cell as an out of equilibrium system that maintains its self-organized, dynamic state through continuous energy consumption, and through incredibly tight control over the concentrations and consistency of its intracellular components.

We have mostly concentrated on neurodegeneration, and in particular on ALS and related aging-associated diseases, because most experimental evidence on aberrant phase transitions has been generated for these diseases. However, other neurodegenerative disorders have also been tied to IDPs, such Alzheimer's and Parkinson's disease. Moreover, many forms of cancer are triggered by somatic mutations or aggregation of IDPs, one prominent example being the "guardian of the genome" p53 [104]. It is not hard to imagine that a failure of phase separation and compartment formation may also play important roles in the progression of these and other diseases such as diabetes.

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