Effects of pH alterations on stress- and aging-induced protein phase separation

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Abstract
Upon stress challenges, proteins/RNAs undergo liquid–liquid phase separation (LLPS) to fine-tune cell physiology and metabolism to help cells adapt to adverse environments. The formation of LLPS has been recently linked with intracellular pH, and maintaining proper intracellular pH homeostasis is known to be essential for the survival of organisms. However, organisms are constantly exposed to diverse stresses, which are accompanied by alterations in the intracellular pH. Aging processes and human diseases are also intimately linked with intracellular pH alterations. In this review, we summarize stress-, aging-, and cancer-associated pH changes together with the mechanisms by which cells regulate cytosolic pH homeostasis. How critical cell components undergo LLPS in response to pH alterations is also discussed, along with the functional roles of intracellular pH fluctuation in the regulation of LLPS. Further studies investigating the interplay of pH with other stressors in LLPS regulation and identifying protein responses to different pH levels will provide an in-depth understanding of the mechanisms underlying pH-driven LLPS in cell adaptation. Moreover, deciphering aging and disease-associated pH changes that influence LLPS condensate formation could lead to a deeper understanding of the functional roles of biomolecular condensates in aging and aging-related diseases.

Keywords Acidification · Membrane-less compartment · Neurodegenerative disease · Tumorigenesis · Protein aggregation

Introduction
Liquid–liquid phase separation (LLPS) refers to a demixing transition of an initially homogeneous solution that rearranges and separates into two phases that can coexist stably in solution: a dense and a dilute phase. During this physicochemical process, supersaturated macromolecules are separated from the solution to form a dense phase, while these supersaturated components in the dilute phase are depleted. The macromolecular dense phase has liquid-like properties and can exchange molecules rapidly with the dilute phase [1]. Studies in recent years have shown that LLPS is the driving force for the assembly of nonmembrane organelles and other functional biomolecular condensates, which can achieve spatiotemporal control of their internal complex biochemical reactions without physical barriers [2, 3].

Currently, the formation mechanisms of the resulting condensates formed by LLPS are preliminarily understood and are thought to rely on a network of weak and multivalent protein–protein interactions. Many proteins exhibit LLPS behaviors, and a common feature of such proteins is the presence of multivalent binding domains. Among these, intrinsically disordered regions (IDRs) are the main drivers that provide multivalent interactions [4]. Studies have shown that IDRs are rich in hydrophilic amino acids such as asparagine, glycine, proline, serine, arginine, and aspartate, whereas they are lacking in hydrophobic amino acids such as valine, threonine, leucine, cysteine, isoleucine, histidine,
and tryptophan [4, 5]. The enrichment of only a few amino acids in these domains results in low complexity that could mediate weak interactions. Other proteins that contain oligomerization domains and multiple-folded modular domains are also contribute to the multivalent interactions [5]. Furthermore, emerging roles of RNA molecules in the assembly of biomolecular condensates have been revealed. RNAs help establish the promiscuous interaction network through interactions with the RNA-binding domains of proteins, and their intermolecular interactions and self-assembly define the compositions of higher-order condensates [6, 7]. Accumulating evidence shows that protein post-translational modification (PTM) is another important mechanism that achieves cellular control of protein phase separation and condensate formation through processes such as phosphorylation, acetylation, SUMOylation, ubiquitination, methylation, and ADP-riboseylation [8, 9]. These modifications can alter the weak multivalent interactions by changing the charge, structure, hydrophobicity, and other properties of proteins, thus affecting phase separation behavior [8, 9]. In addition, not only protein PTM but also RNA PTM affects condensate dynamics. For instance, N\(^{6}\)-methyladenosine (m\(^{6}\)A) of RNA can modulate condensate formation and composition by regulating mRNA distribution into distinct condensates or changing the phase separation behaviors of its binding partners [10, 11].

There is mounting evidence that LLPS and condensate formation are widely present in the cells and play important roles in a wide range of physiological processes (Fig. 1), including chromatin organization, cytoskeletal assembly, signal transduction, transcriptional regulation, protein degradation, cell division and differentiation, and environmental response and adaptation [2]. The condensates can transition into different material states such as gel- or solid-like states [1, 12]. Therefore, maintenance of normal condensate material properties can ensure the assembly and disassembly of condensates in a tightly controlled manner to fulfill original functions, while aberrant phase transition is causatively associated with the onset and development of age-related neurodegenerative diseases and cancers (Fig. 1) [13]. In recent research, the development of methods to study LLPS has become an important objective. A series of tools have been developed to predict and analyze the phase separation capabilities of proteins [1]. Fluorescence microscopy observation techniques, including fluorescence recovery after photobleaching (FRAP) and superresolution imaging, can also provide more detailed information on the material properties, composition, and dynamics of biomolecular condensates. In addition, in vitro reconstitution using purified proteins is an accessory method used for studying LLPS [1]. These methods can help researchers to further elucidate the compositions of biological molecules and related biological reactions and explore the factors that drive or influence condensate formation, eventually providing new opportunities for the prevention and treatment of human diseases.

Phase separation of proteins is a multifactor dynamic process, and it occurs not only spontaneously under normal conditions, but also upon stimulation from an array of environmental factors, including changes in temperature, pH, ATP/energy, macromolecule concentration, and ionic strength [14]. These physiological parameters constitute a continuous phase boundary, and crossing this boundary by changing one or more parameters, such as by raising the temperature, depriving nutrients, lowering the pH, or changing other factors, can cause phase separation and the formation of condensates, which is an adaptive response of cells [15]. Homeostasis of pH is a prerequisite for the normal survival of organisms. Many proteins are very sensitive to pH alterations, and a very small change in pH can induce phase transition of proteins. Phase separation in most proteins is triggered at low pH; while in others, it is induced by alkaline pH [16]. In vivo, the mechanism by which pH regulates protein phase separation is not completely clear. Here, we review the literature on stress-associated pH fluctuation in cells, how cells maintain and regulate cytosolic pH, and the effects of pH changes on protein phase separation. The mechanisms by which pH can mediate phase separation are also discussed. Further research on these topics will not only advance our understanding of compartment formation affected by pH changes but will also provide important insight into the relationship between pH and a diverse set of human diseases.

**Diverse stresses induce intracellular pH fluctuation**

Cytosolic pH is a tightly controlled physiological parameter in all cellular systems, as almost all cellular processes depend on a constant pH for normal functions [17–21]. For instance, in yeast, pH is involved in replicative senescence of mother cells and rejuvenation of nascent daughter cells [22], and cytoplasmic acidification is critical for yeast cells to enter dormancy under stress conditions [23]. In plants, intracellular pH changes are components of a number of phytohormone signaling pathways, modulating gene expression and defence [21, 24]. In mammals, the maintenance of pH homeostasis is of key importance for the proper execution and regulation of neurotransmission [25]. Small changes in cytosolic pH can lead to major changes in metabolism, signal transduction, protein folding, and protein–lipid interactions [19, 20]. However, organisms are often exposed to diverse adverse conditions throughout their life cycles, and stress-induced cytosolic pH fluctuations are broadly present in the cells; these fluctuations are induced, for example, by osmotic stress, heat
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shock, and nutrient restriction [26–30]. Aging processes and human diseases, including neurodegenerative diseases and cancers, are also strongly linked with intracellular pH alterations [22, 31, 32]. Table 1 summarizes the stresses that can induce pH fluctuation in the cells of mammals, plants, and microorganisms. Here, we describe the relationships between pH changes and certain stresses, such as temperature perturbation, starvation, and osmotic challenges, as well as aging and aging-related diseases, including neurodegenerative diseases and cancers.

**Temperature perturbation**

Proper environmental temperature is a critical factor for cell survival. When the temperature becomes harsh, organisms must respond rapidly to adapt and thrive. The best-known stress response, the heat shock response (HSR), is a conserved transcriptional program mediated by heat shock factor 1, which is activated upon heat stress. It upregulates the transcription of a set of molecular chaperones to help the cell to manage the accumulation of heat-induced aberrantly
| Stress          | Species          | pH    | Effects of stress                                                                 | The reason of pH change                                                                 | References |
|-----------------|------------------|-------|-----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|------------|
| Heat shock      | Yeast            | ↓     | Membrane permeability increase                                                    | Protons in the environment influx                                                     | [19, 30, 33–35] |
|                 | *Drosophila*     |       | Slight cell swelling and altered metabolic activity                               | Intracellular protons increase                                                        | [28, 36, 37] |
|                 | Mammals          |       | Inhibit cell growth and change plasma membrane fluidity, permeability to small molecules, and membrane-bound enzyme activity | Inhibition of Na⁺–H⁺ exchange and metabolic pathways                                   | [38–42]    |
| Starvation      | Yeast            | ↓     | Decrease cytoplasmic mobility and volume, and cell enters dormancy                 | Energy shortage to pump protons out                                                  | [23, 43–47] |
|                 | *Plasmodium*     |       | Block mitosis                                                                      | Lack of energy to maintain the pH gradients inside and outside the cell               | [48]       |
| Osmotic stress  | Bacteria         | ↓     | Change cell volume and metabolic processes                                         | Cell loses water and the concentration of protons increases, and activate distinct OmpR-related pathways | [49, 50]   |
|                 | Protists          |       | Cells shrink, largely rearrange cellular proteins between compartments and decrease activity | Cell loses water and secretes protons                                              | [54–56]    |
| Weak acid       | Yeast Bacteria   | ↓     | Decrease cell growth rate and cell growth stasis                                  | Intracellular protons increase and inhibit the ability of cells to maintain normal pH | [45, 46, 57–59] |
| Hypoxia and anoxia | Mammals Plants | ↓     | Cytoplasmic acidosis or cell death                                                | Metabolites produced by anaerobic fermentation/respiration, such as lactic acid       | [26, 29, 60–62] |
| Alcohols        | Yeast            | ↓     | Interfere with membrane transport by changing the lipid composition of the plasma membrane | Proton permeability increase                                                          | [63, 64]   |
| Pathogen        | Plants           | ↓     | Cause pathological damage and activate defense responses                          | Oxidation burst                                                                      | [21, 65]   |
| Light intensity | Plants           | ↑ (light enhance), ↓ (light reduce) | Affect photosynthesis                                                             | Proton entering/leaving the thylakoids                                                | [21, 66]   |
| Aging           | Yeast (vacuole)  | ↑     | Cells lose their physical integrity, resulting in impaired function (such as mitochondria/lysosome dysfunction) and increased risk of death or diseases | Pma1 accumulates on the plasma membrane                                               | [22, 67]   |
|                 | Mammals (Rat hippocampus) | ↓     |                                                                                  | Na⁺–H⁺ exchange may be impaired                                                        | [32]       |
| Oxidative stress | *Plasmodium*     | ↓, ↑ (vacuole) | Lose pH control and decrease intracellular ATP level                              | Inhibition of V-ATPase activity                                                       | [68]       |
|                 | Mammals (bovine brain synaptic vesicle) | ↑      | Reduce neurotransmitter storage and release                                      |                                                                                      | [69]       |
| Cancer          | –                | ↑     |                                                                                    | Change the expression and/or activity of plasma membrane ion pumps and transporters that promote H⁺ efflux | [31]       |

*a*Changes in pH: increase (†), decrease (↓)
folded proteins and aggregates [70]. On the one hand, upregulated chaperones can efficiently refold nonnative proteins or promote the degradation of protein aggregates through autophagy or the ubiquitin–proteasome system (UPS). On the other hand, they can regulate the deposition of certain misfolded proteins into specialized cellular locations to shield them from degradation and to refold them after stress [71]. In addition to HSR, another adaptive mechanism called the unfolded protein response induced by endoplasmic reticulum stress is also activated upon heat exposure and helps to mitigate the damage caused by heat [72, 73]. Moreover, in different research models, ubiquitination-dependent [74–77] and autophagy-dependent degradation [78–82] have been observed to be induced after heat shock, and the activation of these degradation systems is essential for cell survival and recovery from thermally induced protein aggregation [74, 82].

In addition to the activation of evolutionarily conserved systems that contribute to thermotolerance, temperature change is often coupled with fluctuations in cytoplasmic pH [28, 83]. Some studies have shown that heat shock acidifies the cytoplasm. For instance, in yeast, an intracellular pH drop can be induced by heat shock [35]. The same heat-associated pH changes have also been observed in Drosophila melanogaster [28] and rat hepatoma cells [41]. Stress-associated acidification is thought to be toxic to cells in some cases [29, 84]; whereas in other cases, it might be a cytoprotective strategy that promotes cellular fitness under stress [23, 33, 85]. For example, cytosolic acidification is required for HSR induction in translationally inhibited cells under heat shock, which allows the cells to adapt to high temperature by increasing the transcription of quality-control components [35, 86]. Furthermore, some stress granule (SG) resident proteins, such as DEAD-box RNA helicase Ded1 [87], poly(A)-binding protein 1 (Pab1) [34], and poly(U)-binding protein 1 (Pub1) [88] in yeast, and Ras-GTPase-activating protein SH3-domain-binding protein (G3BP1) in mammalian cells [89], have been reported to respond to elevated temperatures to undergo LLPS. Likewise, they can also respond to low pH that mimics the pH conditions during heat stress. Therefore, heat-induced acidification may play a key role in protein LLPS following heat exposure and then regulate SG dynamics and cell survival under or after stress.

The mechanism by which heat shock acidifies the cytoplasm is not fully understood. However, evidence has shown that the compositions and structures of the cell membrane are very sensitive to changes in temperature. In yeast, heat shock increases membrane permeability, resulting in proton influx and a rapid decrease in intracellular pH [90]. Studies have shown that intracellular pH disturbance is the triggering mechanism of thermotolerance in yeast [33], and changes in plasma membrane compositions contribute to the thermotolerance of cells, which may also be related to changes in membrane permeability [90]. In turn, heat-induced proton influx and pH decreases can activate plasma membrane ATPase, whose activity is necessary for cell survival under heat shock [33, 91]. The plasma membrane ATPase pumps intracellular protons out of the cell, partially offsetting the internal acidification resulting from the heat-induced increase in membrane permeability [33]. In mammalian cells, heat shock leads to a dramatic loss of plasma membrane Na⁺–K⁺ ATPase activity, which then results in loss of the inwardly directed electrochemical Na⁺ gradient across the membrane [39]. Therefore, it is speculated that Na⁺ gradient-dependent H⁺ export from the cytoplasm to the outside by Na⁺–K⁺ ATPase is affected and that the cytoplasm is acidified during heat stress.

**Starvation**

A decrease in cytosolic pH can also be caused by starvation. In yeast cells, numerous studies have shown that the cytoplasmic pH decreases from approximately 7.4 to approximately 6.0 under starvation conditions [43, 45, 47]. Pma1, the plasma membrane-localized P-type H⁺-ATPase in yeast, is involved in pumping protons out of the cells and is a primary contributor to the maintenance of cytosolic pH stability near neutrality [92, 93]. Importantly, its activation requires glucose-regulated phosphorylation [94]. In addition, other pumps, such as V-type H⁺-ATPases (V-ATPases), are also responsible for cytosolic pH regulation [95]. They work by pumping excess protons into the vacuole to regulate cytosolic pH homeostasis; they also maintain effective localization of Pma1 at the plasma membrane [95, 96]. Glucose is also required for V-ATPase activation because it mediates reversible associations between the V1 and V0 domains of V-ATPase [43, 97]. Under favorable conditions (with glucose), V-ATPase cooperates with Pma1 to pump protons out of the cytoplasm and help cells stabilize cytoplasmic pH in an ATP-dependent manner. However, upon glucose depletion, a drop in cytoplasmic pH is observed, as starved yeast cells lack efficient H⁺-ATPase assembly and activation to support the proton gradient across the membrane [46]. Intracellular protons cannot be discharged outside of the cell. Instead, they accumulate inside the cell; thus, cytosolic pH decreases. The increased concentrations of intracellular protons cause the phase transition of the cytoplasm from a fluid-like to a solid-like state, and such a dormant or quiescent state is a protective strategy for cell survival under conditions of starvation [23]. Likewise, evidence suggests that nutrient supply is also closely related to cytoplasmic pH in Physarum plasmodium. The cycle of intracellular pH corresponds to the period of the cell cycle of P. plasmodium. When P. plasmodium is growing in non-nutrient medium, the intracellular pH remains stable and then begins to decline gradually, which serves to block normal mitosis.
However, upon refeeding of starved *P. plasmodium* with the nutrient medium, intracellular pH can recover to normal values and the cell cycle resumes [48].

**Osmotic stress**

In addition to heat shock and starvation, osmotic stress is another important environmental factor affecting cell survival and growth. Organisms including microbes, plants, and mammals, are commonly confronted with hyperosmotic conditions, which trigger a series of actions resulting in downregulation of cellular activity and progression of disease [98–100]. When osmolarity changes, cells adjust their volumes accordingly in response to the changing environment. Cells mainly regulate volume changes by controlling substance influx and efflux, which is usually manifests as cell contraction or expansion, so that cells can return to a normal resting state [101–103]. A variety of membrane transporters are involved in this complex regulation process. For example, in a hypotonic environment, mammalian cells initially expand via water uptake and subsequently undergo compensatory shrinkage to partially regulate volume reduction, usually through efflux of KCl and organic osmolytes [104, 105]. In contrast, in hypertonic environments, cells undergo transient dehydration contraction by absorbing Na+, K+ and Cl− and then pumping out Na+ to regulate the increase in cell volume [104].

Intracellular osmotic homeostasis is necessary to maintain normal cell function and survival, and osmotic dysregulation is the basis of many diseases and their complications, including cataracts [106], epilepsy [107], inflammation [100, 108], and hypernatremia [109]. For instance, in hyperglycemia or hypergalactosemia, activated aldose reductase converts glucose and lactose to galactose and sorbitol, respectively, which accumulate in the lens and cause osmotic swelling, leading to diabetic cataracts [106]. In addition, cancer and aging processes are also closely related to intracellular osmotic regulation. Many studies have shown that ion channels and ion pumps are beneficial to the development and progression of cancer [110]. Given the importance of ion channels for osmotic homeostasis and the abnormal expression of transporters in many cancers [111], it is likely that the original homeostasis in cells will be disrupted, creating a more favorable internal environment for cancer development. Interestingly, Yes-associated protein (YAP), is a transcriptional coactivator that is widely activated in cancer cells [112], can sense the tumor microenvironment and modify the physicochemical properties of the surrounding environment by activating transcription, thereby promoting tumor development [113]. Moreover, YAP-activated transcription is mediated by the LLPS process, which also occurs under hypertonic conditions [114]. Aging does not directly cause disease, but in this process, the homeostasis of water in the human body is often disturbed [115]. Thus, normal osmotic regulation is impaired, and this impairment is followed by increases in the incidence and severity of diseases, such as hypoosmolality and hyperosmolality [116].

Interestingly, a growing body of evidence implicates hyperosmotic stress as a factor leading to internal pH alteration. In *Listeria monocytogenes*, a ubiquitous gram-positive food-borne pathogen, the initial response to osmotic stress caused by sorbitol or NaCl is a decrease in intracellular pH [50]. Hyperosmotic stress also leads to cytosolic acidification in *Dictyostelium discoideum*, which works as a novel signal mediator responsible for hyperosmotic stress responses [54]. Moreover, another study has indicated that hyperosmotic shock elicits a transient increase in *Escherichia coli* cytoplasmic pH, but the pH returns to normal values after osmotic adaptation [52]. However, whether and how osmotic dysregulation in mammalian cells alters pH and whether it is relevant to human diseases remain unclear; thus, these aspects require further investigation to advance our understanding of pH-related condensate formation and diseases.

Taken together, the evidence indicates that diverse environmental alterations contribute to intracellular pH fluctuations. Manipulating intracellular pH not only serves to maintain the morphology and function of cells to ensure normal growth and metabolic activities, but also is associated with the preservation of cellular equilibrium in response to several environmental factors, which could promote cellular fitness.

**Aging and neurodegenerative diseases**

Aging is usually an irreversible biological process and is considered to be a predominant risk factor for many neurodegenerative diseases [117]. Nine hallmarks of aging have been tentatively identified in different organisms, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. These hallmarks can be classified into three layers: primary hallmarks, antagonistic hallmarks, and integrative hallmarks, which co-occur during aging and are usually interconnected with each other; defining the exact relationships and causal network of these hallmarks may contribute to future studies on aging and aging-related diseases [118].

In addition to the hallmarks of aging discussed above, growing evidence shows that intracellular pH alterations are also intimately linked to aging processes and aging-related neurodegenerative diseases. In mammals, the intracellular pH of central neurons is tightly regulated, and its fluctuations are important for signaling and synaptic plasticity [119, 120]. Specifically, in cortical neurons, a mild intracellular
pH decrease occurs following an excitability increase, and this decrease acts as feedback to reduce local bioelectric activity and excitability. However, when the intracellular pH is outside a certain range and reaches its limits, there may be an increased risk of cell death [25, 119, 121, 122]. Importantly, a decrease in neural pH levels has been observed in a number of neurodegenerative disorders [123, 124] and even in the normal aging process [32, 125, 126]. Moreover, acute neuroinflammation has been observed to provoke intracellular acidification in the mouse hippocampus [127]. For example, in mammalian cortical neurons, intracellular pH is negatively correlated with aging, as evidenced by significantly lower pH in hippocampal slices from aged rats than in slices from young rats [32, 128]. Likewise, in human neurons, the intracellular pH has also been observed to decrease with aging [126, 129]. The mechanisms involved in decreased intracellular pH may be the disruption and overwhelemed of pH regulatory systems through processes including aging-related decreases in buffering capacity and disruption of diverse transmembrane acid/base-transporters [32, 128–130]. For instance, considering that Na⁺–H⁺ exchange is the dominant regulatory mechanism for proton extrusion in cultured hippocampal neurons, altered H⁺ homeostasis might be attributable to impaired Na⁺–H⁺ exchange (Fig. 2), which utilizes the inwardly directed electrochemical Na⁺ gradient generated by Na⁺–K⁺ ATPase to export H⁺ [32]. Moreover, limited ATP synthesis during aging might also affect ATP-driven ion pumping, including Na⁺ gradient generation by Na⁺–K⁺ ATPase [131]. The impacts of aging-related alterations on pH regulation are controversial. A slight decrease in intracellular pH may provide neuroprotection [132], while successively greater acidification may increase the vulnerability of brain tissue to stressful conditions [125, 133–135].

In addition to cytosolic pH dysregulation, lysosomal/vacuolar pH dysregulation has also been implicated in aging and aging-related neurodegenerative diseases. Evidence is now emerging that defective lysosomal function is a major factor in the pathogenesis of different types of neurodegenerative diseases, specifically, a failure of the maintenance of a highly acidic lysosomal/vacuolar pH [136]. There is also increasing evidence for aging-related compromise of lysosomal function [22]. In yeast, vacuolar pH is a critical regulator of mitochondrial function and replicative lifespan. Vacuolar acidity declines with aging, and reduced vacuolar acidity disrupts pH-dependent amino acid homeostasis in the vacuolar lumen, resulting in age-related dysfunction of mitochondria and a shortened lifespan [67]. In addition,
lifespan extension via calorie restriction and methionine restriction requires vacuolar acidification [67, 137, 138]. The decrease in vacuolar acidification in yeast is due to excess accumulation of the major regulator of cytosolic pH, Pma1, in mother cells (Fig. 2). Vacuole acidity is thus antagonized by reduced cytosolic proton availability [22]. Importantly, V-ATPase is implicated in lysosomal acidification. Mutations in V-ATPase or proteins that regulate V-ATPase function are observed in aging-related neurodegeneration [136].

It is conceivable that during aging, oxidative stress might impair V-ATPase activity through increased oxidative modification of V-ATPase (Fig. 2), which is inspired by the observation that hydrogen peroxide inhibits bovine brain synaptic vesicle V-ATPase activity [69]. In fact, increased oxidative modification of V-ATPase subunits has been observed in aged rat brain tissue [139], and oxidative modification is known to impair the activity of certain enzymes [140, 141]. Alternatively, aging might alter lysosomal/vacuolar acidification via dynamic transcriptional regulation of V-ATPase subunits (Fig. 2), a mechanism that is supported by the observation of reduced V-ATPase subunit mRNA levels in hippocampal neurons in sporadic Alzheimer’s disease (AD) [142]. In conclusion, intracellular pH alterations, including cytosolic pH changes and lysosomal/vacuolar pH dysregulation, are also striking features that occur during the aging process and aging-related diseases onset.

The processes of aging and aging-related neurodegenerative diseases onset are typically accompanied by the formation of widespread intracellular protein aggregates [143]. Many RNA-binding proteins, such as fused in sarcoma (FUS), tau, alpha synuclein (α-Syn), and TAR DNA-binding protein 43 (TDP-43), are the main components of protein inclusions or aggregates in diverse neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) [144], frontotemporal dementia (FTD) [144, 145], Parkinson’s disease (PD) [146], and AD [147]. Furthermore, these disease-associated proteins are well known to undergo LLPS, and the failure to maintain their liquid-phase homeostasis may serve as a trigger of solid protein aggregate formation (Fig. 1) [148]. Diverse layers of regulation may affect their transition from a liquid-like state with physiological function to solid pathological aggregates. Therefore, it is reasonable to speculate that alterations in the intracellular microenvironment, such as pH changes during aging, provide these phase-separated neurological disorder-related proteins with the opportunity to change their phase separation behaviors and increase the risk of aggregation. In fact, evidence has already shown that LLPS of α-Syn and its subsequent maturation into protein aggregation are pH-mediated [149]. Therefore, further investigations on aging-induced pH dysregulation will not only advance our understanding of aberrant LLPS and compartment formation but will also provide important insight into the onset of aging-associated pathologies.

### Cancers

In recent years, increasing evidence has linked LLPS and condensates to tumorigenesis. A growing number of cancer-associated proteins have been reported to have the ability to undergo LLPS and form biomolecular condensates, such as speckle-type POZ protein, which is involved in oncogenic substrate degradation [150]; p53-binding protein 1 (53BP1) and FET proteins, which are involved in the DNA damage response and genomic stability [151, 152]; and EWS-FLI1, β-catenin, YAP, and PDZ-binding motif (TAZ), which are involved in transcriptional regulation [114, 153–155]. In all of the above cases, disrupting functional condensate assembly of tumor suppressors or promoting aberrant condensate assembly of oncoproteins contributes to the oncogenic process (Fig. 1a–d). In addition, aberrant assembly of other membrane-less compartments formed by LLPS, including SGs [156, 157], PML bodies [158], paraspeckles [159], and amyloid bodies [160], is also associated with cancer. The tumor suppressor p53 has been the “star molecule” of molecular biology and oncology since its discovery. It acts as a transcription factor, activating or inhibiting the transcription of various downstream target genes involved in cell cycle regulation, senescence, and apoptosis [161, 162]. p53 prevents tumor development through cell cycle arrest, DNA repair, and antioxidant protein production to maintain genome integrity and limit cell proliferation under adverse conditions such as DNA damage, hypoxia, oncogene expression, nutrient deprivation, and ribosomal dysfunction [162–164]. Moreover, its mutation, which tends to result in protein aggregation, is found in more than 50% of human cancers [165, 166]. Recent evidence has revealed that the p53 core domain can undergo LLPS and then undergo a phase transition to the solid-like state. Mutation of p53 can accelerate its solid-phase transition into amyloid aggregates (Fig. 1b) [167]. Therefore, it is a reasonable assumption that differences in the tumor microenvironment compared to the microenvironment of normal differentiated cells may trigger certain proteins to undergo LLPS and phase transition to solid aggregates, leading to further cancer progression.

As cancer cells grow at an uncontrolled high rate, they are usually challenged with an adverse macroenvironment characterized by hypoxia and nutrient starvation [168]. Apart from this, considerable evidence links cancer directly to pH alterations since a higher intracellular pH and a lower extracellular pH than those of normal differentiated cells are observed in most cancers, regardless of tissue origin and cell type [169, 170]. These differences may be attributable to changes in the expression and/or activity of plasma membrane ion pumps and transporters, as well as changes in metabolic activities [169, 170]. In turn, the increased intracellular pH and the decreased extracellular pH also synergistically enhance cancer progression. On the one
hand, the increased intracellular pH can increase cell proliferation, facilitate apoptosis evasion, and promote cytoskeletal remodeling for cell migration. On the other hand, the acidified extracellular environment can increase the activities of acid-activated proteases and promote extracellular matrix degradation, thereby accelerating tumor cell invasion and dissemination [31]. However, during these processes, whether and how pH alterations of cancer cells are related to the aberrant phase behavior of cancer-related proteins or aberrant formation of membrane-less compartments such as SGs, PML bodies, paraspeckles, and amyloid bodies, remains unclear and requires further in-depth investigation. Such research will provide more knowledge about the molecular basis of cancer and facilitate the development of new therapies.

**Cytosolic pH control by metabolism-based and transporter-based regulation**

Since pH control is a critical requirement for growth in all organisms, different organisms have adopted a number of common strategies to address the challenges of pH maintenance in the face of rapid metabolism and extracellular environment changes [171–173]. Cells separate metabolites, proteins, and biochemical processes in a manner dependent on compartmentalized membrane-bound organelles, each of which has distinct pH requirements and pH regulation mechanisms [172]. More importantly, cellular compartments have inherent pH buffering capacities. This buffering is achieved by the presence of various intracellular weak acids and bases, as well as the ionizable groups of macromolecules such as side chains of amino acids [174]. Moreover, cytosolic pH regulation also relies on metabolites produced by pH-dependent biological reactions [175]. Organic acids such as malate can produce or consume H⁺ via carboxylation and decarboxylation reactions. Therefore, correct synthesis, degradation, and transport of organic acids through the cytoplasm to other organelles are thought to be important strategies to regulate intracellular pH [176, 177]. In addition, the alternative pathways to glycolysis, the cyanide-resistant alternative respiration pathway and malate-derived lactic and alcoholic fermentation, which are unique to plants, jointly regulate pH homeostasis in plants [175]. In mammalian cells and fermenting yeast, CO₂ produced during metabolism can diffuse freely through biological membranes. It can react with water to form HCO₃⁻, which is an effective proton buffer and consumes protons to produce carbonic acid when the cells are confronted with an acute drop in intracellular pH [178, 179].

However, when cells are under long-term stress, the major regulatory mechanism to maintain cytosolic pH homeostasis is the membrane transport of H⁺, which involves a large array of distinct transport pathways. For example, P-type proton pumps are widely distributed on eukaryotic cell membranes, and they are the main determinants of proton efflux and cytoplasmic pH control in plants and yeast [19, 173]. As mentioned above, Pma1 is the most abundant protein in the plasma membrane of yeast and actively coordinates with V-ATPases to regulate cytosolic pH [19, 173]. V-ATPases can also acidify compartments in an ATP-dependent manner and are distributed in acidic organelles such as the Golgi apparatus, vacuole/lysosomes, and endosomes of all eukaryotic cells [180]. In yeast cells, V-ATPase activity is indispensable for vacuolar acidification during glucose metabolism and homeostasis of cytoplasmic pH in the short term. In the long term, V-ATPase is very important for the stability of Pma1 localization [95]. F-type proton pumps are mainly distributed in the bacterial plasma membrane, the mitochondrial membrane, and the plant endomembrane. In enterococci, when the cytoplasm is acidified, the level and activity of F-type H⁺-ATPase increase synchronously, leading to cytoplasmic alkalization [181]. When the pH value is restored to the initial value, the decrease in the amount and activity of the enzymes terminates proton extrusion. Thus, changes in the amount and activity of enzymes seem to be necessary for pH regulation [182].

Moreover, these proton pumps act in concert with a large array of other transporters. Increasing evidence indicates that a number of ion/H⁺ exchangers are also important for intracellular pH regulation in different organisms, including yeast, plants, and mammals [19, 172, 173]. These exchangers couple the transfer of H⁺ across biological membranes to counter-transport of other cations, such as Na⁺ or K⁺, to protect against excess acidification. Furthermore, Na⁺-coupled HCO₃⁻ transporters, which are involved in the uptake of extracellular HCO₃⁻, have also been reported to play key roles in the regulation of cytosolic pH. They contribute to the maintenance of CO₂–HCO₃⁻ equilibrium, the most important pH buffering system [183, 184]. Although the importance of proton extrusion in pH control has been revealed, acid-importing transporters such as Cl⁻–HCO₃⁻ exchangers, which allow HCO₃⁻ efflux, can efficiently prevent overalkalinization of the cells by working counter to CO₂–HCO₃⁻ transporters to enable the fine control of cytosolic pH [185].

In summary, cells exhibit a complicated pH regulation network dependent on the interplay among multiple transporters that import or export proton equivalents and metabolism-based regulatory mechanisms, and this network can accurately regulate and maintain cytosolic pH. More details can be found in recent reviews [19, 171–173].
pH-dependent phase separation condensate formation induced by stress

As discussed above, many types of stress cause a decrease in cytoplasmic pH, and these stress conditions are known to induce phase separation of proteins to form condensates. Here, we summarize the proteins that are known to form phase separation condensates in response to pH stress together with other stresses in which phase separations are mainly affected by pH alterations, such as heat shock and starvation (Table 2).

Many biomolecules undergo LLPS to form liquid-like condensates that mediate diverse cellular functions [222, 223]. For example, autophagosome formation is a process that is precisely regulated by protein phase separation. Atg1 complex formation is a prerequisite for preautophagosomal structure (PAS) assembly and autophagy initiation [202]. Recent research suggests that the PAS is a liquid-like condensate formed by phase separation of the Atg1 complex, which is critical for further dynamic recruitment of other proteins or factors during autophagosome formation. Notably, this process occurs under low pH and starvation conditions [200–202]. TORC1 is a modulator of PAS organization that targets Atg1 complex assembly by regulating the phosphorylation/dephosphorylation of Atg13, a component of the Atg1 complex [202, 224]. Its activity is also modulated by phase-separated compartments such as SGs. Under stressful conditions, including heat, starvation, and osmotic stress, TORC1 is recruited into SGs; as a result, TORC1 signaling is inhibited [225–227]. For example, in yeast, TORC1 is partitioned into heat shock-induced SGs, which then prevents an increase in the frequency of heat-induced DNA mutations [225]. Under osmotic stress, TORC1 in mammalian cells is similarly sequestered into SGs, thereby blocking its signal transduction to downstream effectors [227].

SGs are also dynamic membrane-less organelles, the formation of which is driven by LLPS [228, 229]. It has been reported that many proteins in SGs exhibit phase separation behavior under stress-associated pH changes. Pab1, an RNA-binding protein consisting of a short N-terminal sequence, four RNA recognition motifs (RRMs), a proline-rich low-complexity region (LCR) and a C-terminal peptide-binding domain. It plays a key role in controlling the polyadenylation, stability, and translation of mRNA in yeast cells [34, 190]. Pub1 is similar to Pab1 in that it is an RNA-binding protein with three RRM regions and one LCR [191]. Both Pub1 and Pab1 are core components of SGs and are prone to phase separation when temperature increases, pH decreases, or nutrients are lacking to help cells survive during stress [34, 88, 189]. In addition, G3BP1 is a central node and molecular switch in SG assembly. Its phase separation also occurs in an RNA-dependent manner under low pH and heat shock [89, 203]. Moreover, members of the Asp–Glu–Ala–Asp (DEAD)-box ATPase (DDX) family are widely present in both eukaryotes and prokaryotes [192, 194], and studies have suggested that many proteins in the DDX family undergo LLPS in vivo or in vitro, including Ded1, Dbp1, and Dbp2 in yeast; DDX3X, DDX4, and DDX6 in humans; and DeaD, SrmB, and RhlE in *E. coli* [192, 193]. Ded1p, an ATP-dependent DEAD-box RNA helicase in yeast, is an indispensable translation initiation factor and a component of SGs [188]. It can parse the secondary structure of mRNA 5′ untranslated regions for ribosomal scanning and recognition of the initiation codon [186, 187]. Studies have shown that Ded1p undergoes phase separation and forms condensates at elevated temperatures, or at lower temperatures when the pH is adjusted to that of the heat-stressed cytosol (heat-shocked cells experience a decrease in cytosolic pH). When in condensate form, Ded1p is translationally inactivated, which leads to a switch in translation from housekeeping transcripts to stress-responsive transcripts [87]. Therefore, heat shock-induced and temperature-associated pH change-induced Ded1p condensation in SGs is an adaptive response to survive heat shock. It promotes an evolutionarily conserved heat shock response that selectively translates housekeeping or heat shock transcripts [87]. Similarly, another DDX family member in yeast, Dhh1, is responsible for the assembly and disassembly of RNA-containing membrane-less organelles. Dhh1 also exhibits enhanced phase separation at low pH, which mimics the pH conditions during glucose starvation [192].

Moreover, evidence indicates that in changed growth conditions, enzyme activities can be acutely regulated through the formation of phase separation-induced enzyme condensates, which restrict or promote specific biochemical reactions in membrane-less organelles, suggesting the importance of phase separation in regulating the metabolism of cells [47, 195]. For instance, glutamine synthetase (Gln1) is an indispensable metabolic enzyme that catalyzes the synthesis of glutamate and ammonium into glutamine, a process that requires ATP. Gln1 forms filaments during a state of advanced cellular starvation, and filament formation leads to enzymatic inactivation [197]. Further evidence demonstrates that starvation-induced cytosolic acidification is the trigger for Gln1 condensate formation, and many metabolic enzymes follow this principle to help cells endure and recover from severe starvation conditions [47].

In addition to the above-mentioned findings, there are other proteins for which LLPS is directly or indirectly affected by pH changes, increasing cell fitness or inducing diseases. For instance, Sup35 is a translation termination factor in budding yeast [198]. It can form condensates upon energy depletion or at a low pH. This pH-dependent phase
Table 2  Proteins undergoing LLPS and response to stress-induced pH changes

| Protein   | Function                                                                 | Domain function in LLPS | RNAa         | Species                | Stress                           | Effects of pH                                                                 | References                  |
|-----------|--------------------------------------------------------------------------|--------------------------|--------------|------------------------|----------------------------------|--------------------------------------------------------------------------------|------------------------------|
| Ded1p     | Initiate translation                                                    | IDR, RRMs                | +            | Yeast                  | Acidic pH, heat shock            | −                                                                              | [87, 186–188]               |
| Pab1      | Control mRNA polyadenylation, stability, and translation                | LCR, RRM s              | −            | Yeast                  | Acidic pH, heat shock            | Act as signal messenger and affect electrostatic interaction                  | [34, 189, 190]             |
| Pub1      | Regulate translation                                                    | LCR, RRM s              | −            | Yeast                  | Acidic pH, heat shock, glucose starvation | Affect protein solubility and electrostatic interaction                       | [88, 191]                 |
| DDXs      | Coordinate mRNA de-capping and decay, regulate general translational repression | LCDs                  | +, − (if excess)| Bacteria, Yeast, Mammals | Acidic pH, glucose starvation   | −                                                                              | [192–194]                 |
| Gln1      | Promote the conversion of glutamate into glutamine                     | −                        | −            | Bacteria, Yeast         | Acidic pH, glucose starvation    | Act as signal messenger                                                       | [47, 195–197]             |
| Sup35     | Terminate translation                                                   | The N-terminal prion domain and the electrically neutral domain | −            | Yeast                  | Acidic pH, glucose starvation    | Act as signal messenger                                                       | [44, 198]                 |
| Atg1 complex | Participate in PAS assembly                       | IDR s                   | −            | Yeast                  | Acidic pH, glucose starvation    | Possible act as signal messenger                                               | [199–202]                 |
| G3BP1     | Promote SG assembly and inhibit RNA aggregation                        | IDR s, nuclear transport factor like domain, RBD | +            | Mammals                | Acidic pH, heat shock, osmotic stress | Affect protein solubility and electrostatic interaction                       | [89, 203]                 |
| SARS-CoV-2 N protein | Participate in viral RNA replication and virion packaging | IDR I                   | +            | Virus                  | Acidic pH                        | Affect electrostatic interaction                                               | [204–206]                 |
| α-Syn     | Act as a SNARE-complex chaperone and contribute to Parkinson's disease pathogenesis | The N-terminal region (most family disease mutations occur) and the “non-amyloid-β component” region | −            | Mammals                | Acidic pH                        | Affect electrostatic interaction                                               | [149, 207–209]           |
| 4R-Tau    | Induce tubulin assembly and stabilize microtubules                     | The microtubule-binding repeats | −            | Mammals                | Lower-critical solution transition | Affect electrostatic interaction                                               | [16, 210–212]            |
| FUS       | Participate in DNA repair, transcription, and RNA biogenesis           | LCDs                   | +, − (high ratios)| Mammals                | Acidic pH, DNA damage, heat shock | −                                                                              | [213, 214]                 |
| 53BP1     | Regulate the DNA damage response and p53 signaling                     | The oligomerization domain and BRCT domain | −            | Mammals                | Acidic pH, DNA damage, light     | −                                                                              | [151, 215, 216]            |
The nucleocapsid protein (N) of the severe acute respiratory syndrome coronavirus (SARS-CoV-2) is a multivalent RNA-binding protein that is essential for viral RNA replication and virion packaging [206]. The N protein can partition into SGs and interact with G3BP1/2 to block the assembly of SGs through RNA-dependent liquid phase separation and thus disrupt the immune response of host cells [204]. Notably, phase separation of the N protein occurs under physiological conditions and is enhanced at low pH [205]. α-Syn is an IDP for which aggregation into amyloid-like fibrils is associated with PD pathology [207, 208]. One study found that α-Syn initially undergoes phase separation and becomes rigid over time and eventually transforms into solid-like aggregates. Low pH can promote α-Syn LLPS and further increase the maturation and nucleation of α-Syn aggregates, which is relevant to PD pathogenesis [149]. Additionally, pathological inclusions of the microtubule-associated protein Tau have been reported to accumulate in patients with several neurodegenerative diseases [210–212]. Evidence indicates that the microtubule-binding repeats of the Tau protein have a strong propensity for liquid demixing, which occurs over a wide range of pH values. The phase separation of these four repeats at different pH values would concentrate the most aggregation-prone Tau residues and further promote amyloid formation [16]. Interestingly, in addition to natural proteins, artificially constructed polypeptides can also undergo phase separation. Elastin-like polypeptides (ELPs) are recombinant protein polymers composed of pentapeptide (Val–Pro–Gly–Xaa–Gly)_L repeat units, which are recurring motifs in tropoelastin in a wide range of species. ELPs are often used as new biomaterials for drug delivery and tissue engineering [218–221]. One study found that ELPs can exhibit reversible phase separation triggered by a wide range of pH values, and this pH responsiveness is controlled by the type and number of ionizable residues and the molecular weight of the ELPs. This property of specific pH-controlled ELP phase separation can be applied in drug delivery systems for local cancer therapy, as various tumor types usually have different pH values than healthy tissues [217].

Finally, as we discussed above, many cancer-associated proteins have been reported to undergo LLPS and to be involved in biomolecular condensate formation. 53BP1 is a binding partner of p53 [230] that can directly regulate p53 and affect p53 target gene expression [231]. It is also one of the main regulators of the DNA damage response, loss of which has been associated with apoptosis and cancer cell proliferation [232]. Studies have found that 53BP1 undergoes LLPS at DNA damage sites, forming DNA repair condensates that recruit and stabilize p53 [151]. If the expression of 53BP1 is changed or LLPS behavior is affected, the
disruption of condensate formation leads to destabilization of p53 and reduced induction of its target genes as well as cell cycle arrest [151]. Interestingly, it has been reported that 53BP1 can respond to low pH to form 53BP1 droplets [151]; thus, further studies on the relationships of pH regulation and 53BP1 LLPS will help enhance our understanding of tumorigenesis. However, besides 53BP1, research on the relationships between cancer-associated proteins and pH dysregulation are limited. Considering that the physicochemical properties and microenvironment of cancer cells are different from those of normal cells [168], two important research topics in the future are whether these proteins undergo pH-regulated LLPS and how pH-regulated LLPS is relevant to tumorigenesis. In addition, research on how microenvironmental changes in cancer cells affect the dynamics of intracellular membrane-less organelles such as SGs, PML bodies, paraspeckles, and amyloid bodies, whose aberrant assembly is associated with cancer, is also needed. Such research will provide further evidence regarding the links among pH, LLPS, and cancer.

**Mechanisms underlying pH-mediated phase separation**

**pH changes mediate protein–protein/RNA interactions**

Some proteins or their specific domains possess the ability to sense stresses directly and thus undergo phase separation in response to these stresses [34, 88]. It is known that LLPS is driven by multivalent weak macromolecular interactions (protein–protein, protein–RNA, and RNA–RNA interactions), disruption or alteration of which would affect protein phase separation behaviors [5, 233]. Therefore, pH changes can influence intramolecular or intermolecular protein–protein/RNA interactions by changing the net charges of components, thereby driving LLPS (Fig. 3). For example, G3BP1 is a multidomain protein composed of two folded domains and two IDRs. Under nonstress conditions, its central negatively charged, glutamate-rich IDR can interact with the C-terminal positively charged RG-rich region to allow G3BP1 to fold into a compact state. This compact state is an autoinhibitory conformation that disrupts G3BP1 phase separation. However, at a low pH, protonation of the clustered glutamates changes the net charge of the acidic IDR and disrupts its stable electrostatic interactions with the RG-rich region, allowing G3BP1 to expand from its original tightly self-inhibited state and release the RG-rich region. G3BP1 can then further facilitate intermolecular protein–RNA/protein interactions to drive LLPS, which is consistent with the observation that heterotypic interactions among G3BP1 and RNA molecules drive SG assembly [89, 203]. In addition, a low pH can directly trigger Pub1 assembly, and this pH-dependent assembly formation is sensitive to salt concentrations, suggesting that electrostatic interactions promote Pub1 assembly. Self-interactions among the RRM domains are the main drivers for Pub1 phase separation, and acidic pH may change the charge distribution in the RRM domains, thereby mediating the electrostatic interactions.

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**Fig. 3** Roles of pH in biomolecular condensate formation. Under nonstress conditions, proteins and RNAs are dispersed in the cytoplasm. When cells are exposed to stresses such as starvation, heat shock, or acid stress, the intracellular pH changes, and this change is accompanied by the formation of protein- and RNA-containing biomolecular liquid-like condensates. During this process, pH plays multiple functional roles in triggering liquid–liquid phase separation (LLPS)-driven condensate formation; for example, it affects protein–protein/RNA interactions, alters protein solubility, or acts as a messenger to transmit stress signals. pH changes can also enhance phase separation, which may gradually mature and result in transformation into an irreversible gel-/solid-like state.
interactions [88]. Moreover, the pH range that can induce artificially recombinant ELP phase separation is related to the pKa. This suggests to a certain extent that pH can affect protein–solution or protein–protein interactions by changing the number of ionizable residues of proteins, thus triggering phase separation [217]. Likewise, phase separation of Pab1 at low pH is also an electrostatically mediated process [34]; thus, the principle of pH-dependent protein condensate formation mediated by electrostatic interactions may be generalizable to many proteins.

Notably, pH changes not only initiate protein LLPS by facilitating intramolecular or intermolecular interactions but also enhance phase separation and its further maturation into a gel state or a pathological solid state (Fig. 3). Phase separation of α-Syn is mediated by an interplay of electrostatic interactions in the unstructured N-terminal domain and hydrophobic interactions in the central NAC domain, while the charge distribution in these domains is strongly dependent on the pH value. A lower pH serves to change the net charges and hydrophobicity of different domains as well as the interactions between these domains, leading to significant structural reorganization. Thus, pH-mediated diverse changes in α-Syn accelerate the maturation of phase separation and subsequent protein aggregation [149, 209]. Similarly, a reduction in pH can enhance the intramolecular interactions of the phase-separated SARS-CoV-2 N protein and lead to irregularly shaped assemblies with less liquidity, in vitro [205]. Indeed, phase separation proteins that contain flexible LCDs are highly prone to forming pathogenic aggregates. This could explain, to some extent, why hundreds of proteins are highly prone to forming aggregates during aging. During aging or chronic pH stress, these phase separation proteins can transition into irreversible aggregates, which could then lead to persistent condensate formation, such as persistent SG formation, even after the stress subsides. Persistent condensates typically exhibit solid-like properties; and as a consequence, other pathological changes and neurodegenerative disorders occur [233].

**pH changes affect protein solubility**

In addition to engaging in the promiscuous interactions that function in phase separation, macromolecules must reach a critical concentration threshold to start LLPS. Evidence indicates that not all LCRs and IDR s function as autonomous modules that drive phase separation; instead, they function as modifier sequences, regulating the solubility of phase-separating proteins and the material properties of condensates [234]. Long-term evolutionary pressure has tuned the solubility of Pub1 to be very close to the critical threshold for phase separation. This not only endows Pub1 with a solubility that is conducive to growth but also enables Pub1 to quickly sense and respond to stress. In fact, changes in pH can affect the solubility of Pub1 and lead to the formation of stress-responsive Pub1 condensates [88]. Changes in pH appear to be able to decrease the solubilities of many proteins. In yeast, a decrease in pH results in a phase transition of cytoplasm from a fluid-like to a solid-like state, which might be caused by decreased solubilities of a series of proteins and subsequent formation of intracellular solid-like assemblies, such as SGs [23, 235]. Moreover, the relationship between pH and protein isoelectric point is closely related to solubility. The closer the pH value is to the isoelectric point of the protein, the lower its solubility, and the more likely it is that phase separation occurs [236, 237]. This could explain to a certain degree why the microtubule-binding repeats of Tau are most prone to phase separation when the pH is close to the isoelectric point but less prone to demixing when the protein solubility is increased in response to pH that is substantially higher or lower than the isoelectric point [16]. Therefore, it is believed that one of the mechanisms by which pH triggers protein phase separation is the alteration of protein solubility (Fig. 3).

**pH changes act as messengers to transmit stress signals**

In the face of adverse conditions, intracellular pH might act as a messenger to signal changes in the environment, triggering phase separation of proteins to promote cell fitness. Upon heat shock, cells can integrate signals of different temperatures and temperature-induced pH changes into a unified response to provide a trigger for phase separation. For example, Pab1 undergoes LLPS autonomously through temperature-dependent structural changes under conditions of stressful temperatures [34].

However, how does a cell sense other stresses, such as starvation, to trigger LLPS to help cells survive diverse adverse conditions? Previous studies have indicated that proteins and protein-associated condensates that undergo LLPS under starvation conditions, such as Pub1, Gln1, Dhh1, and PAS, can also respond to low pH [47, 88, 192, 200, 201]. Considering that cytosolic pH is rapidly and reversibly regulated by glucose metabolism, the stress information perceived by these proteins is most likely transmitted through pH. Evidence has shown that cytosolic pH is a second messenger for glucose to mediate activation of the PKA pathway through V-ATPase [43]. Therefore, a change in pH might be an extremely sensitive readout of other changes in the environment, especially starvation, to induce protein LLPS and cellular adaptive responses (Fig. 3).

In this way, pH is capable of playing diverse functional roles in the regulation of LLPS, including by affecting protein–protein/RNA interactions, altering protein solubility, and acting as a messenger to transmit stress signals.
Conclusion and perspective

From viruses to prokaryotes and eukaryotes, the formation of macromolecular condensates by phase separation is emerging as a principle means for cells to regulate cellular functions and adapt to environmental changes. Cells encounter a variety of stresses, some of which can cause cytoplasmic pH fluctuations. In this review, we have summarized the relationships between pH changes and certain stresses, such as heat shock, nutrient stress, and osmotic stress, and described which proteins or physiological processes can respond to stress-associated pH changes through phase separation. We have also highlighted the diverse ways by which pH fluctuation can influence protein phase separation. For example, pH can act as a signal to transmit stress information, mediate protein–protein/RNA interactions, and affect protein solubility, thereby regulating protein/RNA phase separation.

Despite the research progress concerning the relationships between stress-associated pH changes and phase separation discussed in this review, further in-depth investigations are still needed. It is worth noting that pH might not be the sole determinant of stress-induced phase separation and condensate formation. For stresses such as heat shock, changes in both intracellular temperature and pH are involved, which can lead them to differences in protein phase separation behavior and condensate material properties [88]. The interplay of pH, temperature, ion strength, RNA concentration, protein concentration and other factors forms a sophisticated network that dynamically affects the phase behavior of proteins. However, some questions remain. How does pH interact with other factors in this process? What are the differences and similarities in the roles of pH among the different stress-induced phase separation processes? Preliminary evidence suggests that the properties of different condensate materials formed by different groups of proteins can be used by cells to build a hierarchical stress-adaptive system that is fine-tuned to different conditions [88]. In other words, when encountering different types of stresses or the same stress with different intensity or duration, a cell can regulate the activities of multiple proteins to achieve specific biological functions by concentrating specific cellular components in the condensates (or excluding them from the condensates) for a favorable period of time. The cells can then determine when to restart growth. In this way, control of condensates can be used by the cells as a method to promote adaptation to stress. Therefore, revealing the differences and similarities among the various roles of pH in addressing different types of stress will provide insights into the mechanisms underlying the protein separation involved in cellular adaptation. Moreover, pH values might be changed considerably by different stresses, and a given protein might display different phase separation behaviors under different pH values. Therefore, the identification of proteins that respond to different pH values or have behavior changes that accompany pH changes may provide vital clues for investigation of the machineries involved in the influences of pH on cellular functions.

Finally, intracellular pH changes and phase separation condensate formation are linked to aging, aging-related neurodegenerative diseases, and cancers. It would be interesting to further investigate how aging-induced pH changes affect protein phase separation. Importantly, innovative drug delivery strategies could be developed for specific local cancer therapy by exploiting the altered intracellular and extracellular pH in tumors. Attempts to modulate pH and SG formation could also spur the development of innovative approaches for cancer therapy.

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Declarations

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Data availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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References

1. Alberti S, Gladfelter A, Mittag T (2019) Considerations and challenges in studying liquid-liquid phase separation and...
bionemembraneless organelles. J Biol Chem 294(18):7115–7127. https://doi.org/10.1074/jbc.M118.001192
5. Van Treeck B, Parker R (2018) Emerging roles for intermolecular RNA-RNA interactions in RNP assemblies. Cell 174(4):791–802. https://doi.org/10.1016/j.cell.2018.07.023
6. Van Treeck B, Proiter DSW, Matheny T, Khong A, Link CD, Parker R (2018) RNA self-assembly contributes to stress granule formation and defining the stress granule transcriptome. Proc Natl Acad Sci USA 115(11):2734–2739. https://doi.org/10.1074/pnas.1800038115
7. Hofweber M, Dormann D (2019) Friend or foe-post-translational modifications as regulators of phase separation and RNP granule dynamics. J Mol Biol 430(23):4619–4635. https://doi.org/10.1016/j.jmb.2020.02.004
8. Oldfield CJ, Dunker AK (2014) Intrinsically disordered proteins and intrinsically disordered protein regions. Annu Rev Biochem 83:553–584. https://doi.org/10.1146/annurev-biochem-072711-164947
9. Coote PJ, Cole MB, Jones MV (1991) Induction of increased cellular pH changes induced by hypoxia and anoxia in isolated sheep heart Purkinje fibres. Exp Physiol 77(1):165–175. https://doi.org/10.1133/exphysiol.1992.sp003570
10. Gao Y, Pei G, Li D, Li R, Shao Y, Zhang QC, Li P (2019) Multivalent m² ¯A motifs promote phase separation of YTHD proteins. Cell Res 29(9):767–769. https://doi.org/10.1038/s41422-019-0210-3
11. Ries RJ, Zaccara S, Klein P, Olearerin-George A, Namkoong S, Pickering BF, Patil DP, Kwak H, Lee JH, Jaffrey SR (2019) m² ¯A enhances the phase separation potential of mRNAs. Nature 571(7765):424–428. https://doi.org/10.1038/s41586-019-1374-1
12. Alberti S (2017) The wisdom of crowds: regulating cell function through condensed states of living matter. J Cell Sci 130(17):2789–2796. https://doi.org/10.1242/jcs.20200295
13. Wang B, Zhang L, Dai T, Qin Z, Lu H, Zhang L, Zhou F (2021) Liquid-liquid phase separation in human health and diseases. Signal Transduct Target Ther 6(1):290. https://doi.org/10.1038/s41392-021-00678-1
14. Alberti S, Hyman AA (2016) Are aberrant phase transitions a driver of cellular aging? BioEssays 38(10):959–968. https://doi.org/10.1002/bies.201600042
15. Ruff KM, Roberts S, Chilkoti A, Pappu RV (2018) Advances in understanding stimulus-responsive phase behavior of intrinsically disordered protein polymers. J Mol Biol 430(23):4619–4635. https://doi.org/10.1016/j.jmb.2018.06.031
16. Ambadipudi S, Biennart J, Riedel D, Mandelkow E, Zweckstetter M (2017) Liquid-liquid phase separation of the microtubule-binding repeats of the Alzheimer-related protein Tau. Nat Commun 8(1):275. https://doi.org/10.1038/s41467-017-00480-0
17. Khan YM, East JM, Lee AG (1997) Effects of pH on phosphorylation of the Ca²⁺-ATPase of sarcoplasmic reticulum by inorganic phosphate. Biochem J 321( Pt 3):671–676. https://doi.org/10.1042/bj3210671
18. Allmellan I (1992) The importance of being acid: the role of acidification in intracellular membrane traffic. J Exp Biol 172:39–45. https://doi.org/10.1242/jeb.172.1.39
19. Orij R, Brul S, Smits GIJ (2011) Intracellular pH is a tightly controlled signal in yeast. Biochim Biophys Acta 1810(10):933–944. https://doi.org/10.1016/j.bbagen.2011.03.011
20. Isom DG, Sridharan V, Baker R, Clement ST, Smalley DM, Dohlman HG (2013) Protons as second messenger regulators of G protein signaling. Mol Cell 51(4):531–538. https://doi.org/10.1016/j.molcel.2013.07.012
21. Felle HH (2001) pH: signal and messenger in plant cells. Plant Biol 3(6):577–591. https://doi.org/10.1055/s-2001-19372
22. Henderson KA, Hughes AL, Gottschling DE (2014) Mother-daughter asymmetry of pH underlies aging and rejuvenation in yeast. Elife 3:e03504. https://doi.org/10.7554/eLife.03504
23. Gao Y, Pei G, Li D, Li R, Shao Y, Zhang QC, Li P (2019) Multivalent m² ¯A motifs promote phase separation of YTHD proteins. Cell Res 29(9):767–769. https://doi.org/10.1038/s41422-019-0210-3
24. Ismail A, Takeda S, Nick P (2014) Life and death under salt stress: same players, different timing? J Exp Bot 65(12):2963–2979. https://doi.org/10.1093/jxb/eru159
25. Bright CM, Ellis D (1992) Intracellular pH changes induced by hypoxia and anoxia in isolated sheep heart Purkinje fibres. Exp Physiol 77(1):165–175. https://doi.org/10.1113/exphysiol.1992.sp003570
26. Diaz FE, Dantas E, Cabrera M, Benitez CA, Delpino MV, Duette G, Rubione J, Sanjuan N, Trevani AS, Geffner J (2016) Fever-range hyperthermia improves the anti-apoptotic effect induced by low pH on human neutrophils promoting a proangiogenic profile. Cell Death Dis 7(10):e2437. https://doi.org/10.1038/cddis.2016.337
27. Drummond IA, McClure SA, Poenie M, Tsien RY, Steinhardt RA (1986) Large changes in intracellular pH and calcium observed during heat shock are not responsible for the induction of heat shock proteins in Drosophila melanogaster. Mol Cell Biol 6(5):1767–1775. https://doi.org/10.1128/mcb.6.5.1767-1775.1986
28. Ishizawa K (2014) Intracellular pH regulation of plant cells under anaerobic conditions. Plant Cell Monographs 21:59–74. https://doi.org/10.1007/978-3-7091-1254-0_4
29. Weitzel G, Pilatus U, Rensing L (1987) The cytoplasmic pH, ATP content and total protein synthesis rate during heat-shock proteins in Drosophila melanogaster. Mol Cell Biol 6(5):1767–1775. https://doi.org/10.1128/mcb.6.5.1767-1775.1986
30. Triandafillou CG, Katanski CD, Dinner AR, Allan Drummond SP (2017) Stress-triggered phase separation is an adaptive, evolutionarily tuned response. Cell 176(3):419–434. https://doi.org/10.1016/j.cell.2018.12.035
31. Coote PJ, Cole MB, Jones MV (1991) Induction of increased thermostolerance in Saccharomyces cerevisiae may be triggered by a mechanism involving intracellular pH. J Gen Microbiol 137(7):1701–1708. https://doi.org/10.1099/00221287-137-7-1701
32. Horiuchi T, Katanski CD, Dinner AR, Allan Drummond SP (2017) Stress-triggered phase separation is an adaptive, evolutionarily tuned response. Cell 176(3):419–434. https://doi.org/10.1016/j.cell.2018.12.035
33. Coote PJ, Cole MB, Jones MV (1991) Induction of increased thermostolerance in Saccharomyces cerevisiae may be triggered by a mechanism involving intracellular pH. J Gen Microbiol 137(7):1701–1708. https://doi.org/10.1099/00221287-137-7-1701
transcriptional heat shock response. Elife 9:e54880. https://doi.org/10.7554/eLife.54880
36. Pauli D, Arrigo A, Tissières A (1992) Heat shock response in Drosophila. Experimentia 48:623–629. https://doi.org/10.1007/BF02118306
37. Zhong M, Kim SJ, Wu C (1999) Sensitivity of Drosophila heat shock transcription factor to low pH. J Biol Chem 274(5):3135–3140. https://doi.org/10.1074/jbc.274.5.3135
38. Aickin CC, Thomas RC (1977) An investigation of the ionic mechanism of intracellular pH regulation in mouse soleus muscle fibres. J Physiol 279:295–316. https://doi.org/10.1113/jphysiol.1977.sp012095
39. Burdon RH, Cutmore CMM (1982) Human heat shock gene expression and the modulation of plasma membrane Na+, K+-ATPase activity. FEBS Lett 140(1):45–48. https://doi.org/10.1016/0014-5793(82)80517-6
40. Kiang JG, McKinney LC, Gallin EK (1990) Heat induces intra-
41. Orij R, Postmus J, Beek AT, Brul S, Smits GJ (2009) Mechanisms of cytoplasmic pH regulation in hypoxic maize root cells measured by 23Na and 31P nuclear magnetic resonance. J Biol Chem 261(17):7797–7806. https://doi.org/10.1016/s0021-9258(19)37471-3
42. Petrovska I, Chakraborty S, Winardhi RS, Morgan LK, Yan J, Kenney LJ (2017) Non-canonical activation of OmpR drives acid and osmotic stress responses in single bacterial cells. Nat Commun 8(1):1587. https://doi.org/10.1038/s41467-017-02030-0
43. Dechant R, Binda M, Lee SS, Pelet S, Winderickx J, Peter Holehouse AS, Nüske E, Richter D, Baumeister W, Grill (2018) Phase separation of a yeast prion protein promotes cellular fitness. Science 359(6371):eaao5654. https://doi.org/10.1126/science.aao5654
44. Orj R, Urbanus ML, Vizeacoumar FJ, Giaever G, Boone C, Nislow C, Brul S, Smits GJ (2012) Genome-wide analysis of intracellular pH reveals quantitative control of cell division rate by pH, in Saccharomyces cerevisiae. Genome Biol 13(9):R80. https://doi.org/10.1186/gb-2012-13-9-r80
45. Orj R, Postmus J, Beck AT, Brul S, Smits GJ (2009) In vivo measurement of cytosolic and mitochondrial pH using a pH-sensitive GFP derivative in Saccharomyces cerevisiae reveals a relation between intracellular pH and growth. Microbiology (Reading) 155(1):268–278. https://doi.org/10.1099/mic.0.022038-0
46. Petrovasa I, Nüse K, Ender MC, Kulasegaran G, Malinovska L, Kroschwald S, Richter D, Fahmy K, Gibson K, Verbanac JM, Alberti S (2014) Filament formation by metabolic enzymes is a relation between intracellular pH and growth. Microbiol Rev 53(1):121–147. https://doi.org/10.1128/mmbr.53.1.121-147.1989
47. Petrovska I, Nüske E, Munder MC, Kulasegaran G, Malinovska (2010) Cytosolic pH is a second messenger for glucose as determined by fluorescence ratio-imaging microscopy. J Biol Chem 285(16):12122–12130. https://doi.org/10.1074/jbc.M909385200
48. Petrushova I, Winardhi RS, Morgan LK, Yan J, Kenney LJ (2017) Non-canonical activation of OmpR drives acid and osmotic stress responses in single bacterial cells. Nat Commun 8(1):1587. https://doi.org/10.1038/s41467-017-02030-0
49. Fang W, Siegemundfeld H, Budde BB, Jakobsen M (2004) Osmotic stress leads to decreased intracellular pH of Listeria monocytogenes as determined by fluorescence ratio-imaging microscopy. Appl Environ Microbiol 70(5):3176–3179. https://doi.org/10.1128/AEM.70.5.3176-3179.2004
50. Cassonka LN (1989) Physiological and genetic responses of bacteria to osmotic stress. Microbiol Rev 53(1):121–147. https://doi.org/10.1128/mmbr.53.1.121-147.1989
imbalance of ATP and NAPDH/H⁺ production as measured by CO₂- and light-induced depolarisation of the plasma membrane. Plant Cell Physiol 34(5):681–695. https://doi.org/10.1093/oxfordjournals.pcp.a078471

67. Hughes AL, Gottschling DE (2012) An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. Nature 492(7426):261–265. https://doi.org/10.1038/nature11654

68. van Schalkwyk DA, Saliba KJ, Biagini GA, Bray PG, Kirk K (2013) Loss of pH control in Plasmodium falciparum parasites subjected to oxidative stress. PLoS ONE 8(3):e58933. https://doi.org/10.1371/journal.pone.0058933

69. Wang Y, Floor E (1998) Hydrogen peroxide inhibits the vacuolar H⁺-ATPase in brain synaptic vesicles at micromolar concentrations. J Neurochem 70(2):646–652. https://doi.org/10.1046/j.1471-4159.1998.70020646.x

70. Vihervaara A, Sistonen L (2014) HSF1 at a glance. J Cell Sci 127(Pt 2):261–266. https://doi.org/10.1242/jcs.132605

71. Cabrera M, Boronat S, Marte L, Vega M, Perez P, Ayte J, Hidalgo E (2020) Chaperone-facilitated aggregation of thermo-sensitive proteins shields them from degradation during heat stress. Cell Rep 30(7):2430–2443.e4. https://doi.org/10.1016/j.celrep.2020.01.077

72. Kim JH, Park SJ, Park HJ, Kim BK, Kim GR, Kim JM, Huang SM, Chae JI, Park CK, Lee DS (2013) Testicular hyperthermia induces unfolded protein response signal activation in spermatozoa. Biochem Biophys Res Commun 434(4):861–866. https://doi.org/10.1016/j.bbrc.2013.04.032

73. Mizusawa M, Sharmin MM, Yonekura S (2019) Mild heat stress induces transcription of the beta-casein gene via unfolded protein response-activated XBP1 signaling in undifferentiated mammary epithelial cells. Anim Sci J 90(8):1026–1032. https://doi.org/10.1111/aj.13246

74. Maxwell BA, Gwon Y, Mishra A, Peng J, Nakamura H, Zhang K, Kim HJ, Taylor JP (2021) Ubiquitination is essential for recovery of cellular activities after heat shock. Science 372(6549):eabc3593. https://doi.org/10.1126/science.abc3593

75. Carlson N, Rogers S, Rechsteiner M (1987) Microinjection of proteins stimulates degradation mainly of newly synthesized proteins. J Cell Biol 104(3):547–555. https://doi.org/10.1083/jcb.104.3.547

76. Medicherla B, Goldberg AL (2008) Heat shock and oxygen radicals stimulate ubiquitin-dependent degradation mainly of newly synthesized proteins. J Cell Biol 182(4):663–673. https://doi.org/10.1083/jcb.200803022

77. Parag HA, Raboy B, Kulka RG (1987) Effect of heat shock on protein degradation in mammalian cells: involvement of the ubiquitin system. EMBO J 6(1):55–61. https://doi.org/10.1002/j.1460-2075.1987.tb04718.x

78. Dokladny K, Zuhl MN, Mandell M, Bhattacharya D, Schneider S, Deretic V, Moseley PL (2013) Regulatory coordination between two major intracellular homeostatic systems: heat shock response and autophagy. J Biol Chem 288(21):14959–14972. https://doi.org/10.1074/jbc.M113.462408

79. Zhao Y, Gong S, Shunmei E, Zou J (2009) Induction of macroautophagy by heat. Mol Biol Rep 36(8):2323–2327. https://doi.org/10.1007/s12033-009-9451-4

80. Hsu SF, Chao CM, Huang WT, Lin MT, Cheng BC (2013) Attenuating heat-induced cellular autophagy, apoptosis and damage in H9c2 cardiomyocytes by pre-inducing HSP70 with heat shock preconditioning. Int J Hyperthermia 29(3):239–247. https://doi.org/10.3109/02656736.2013.777853

81. Zhang M, Jiang M, Bi Y, Zhu H, Zhou Z, Sha J (2012) Autophagy and apoptosis act as partners to induce germ cell death after heat stress in mice. PLoS ONE 7(7):e41412. https://doi.org/10.1371/journal.pone.0041412

82. Nivon M, Richet E, Codogno P, Arrigo AP, Kretz-Remy C (2009) Autophagy activation by NFKappaB is essential for cell survival after heat shock. Autophagy 5(6):766–783. https://doi.org/10.4161/auto.8788

83. Weitzel G, Pilatus U, Rensing L (1985) Similar dose response of heat shock protein synthesis and intracellular pH change in yeast. Exp Cell Res 159(1):252–256. https://doi.org/10.1016/s0014-4827(85)80054-9

84. Tombaugh GC, Sapolsky RM (1993) Evolving concepts about the role of acidosis in ischemic neuropathology. J Neurochem 61(3):793–803. https://doi.org/10.1111/j.1471-4159.1993.tb03589.x

85. Joyner RP, Tang JH, Helenius J, Dultz E, Brune C, Holt JJ, Huet S, Muller DI, Weis K (2016) A glucose-starvation response regulates the diffusion of macromolecules. Elife 5:e09376. https://doi.org/10.7554/eLife.09376

86. Jayaraj GG, Hipp MS, Hartl FU (2020) Functional modules of the proteostasis network. Cold Spring Harb Perspect Biol 12(1):a033951. https://doi.org/10.1101/cshperspect.a033951

87. Iserman C, Desroches Altamirano C, Jegers C, Friedrich U, Zarin T, Fritsch AW, Mittasch M, Domingues A, Hersemann L, Jahnel M, Richter D, Guenther UP, Hentze MW, Moses AM, Hyman AA, Kramer G, Kreysing M, Franzmann TM, Alberti S (2020) Condensation of Ded1p promotes a translational switch from housekeeping to stress protein production. Cell 181(4):818–831. e19. https://doi.org/10.1016/j.cell.2020.04.009

88. Kroschwald S, Munder MC, Maharana S, Franzmann TM, Richter D, Ruer M, Hyman AA, Alberti S (2018) Different material states of Ptb1 condensates define distinct modes of stress adaptation and recovery. Cell Rep 23(11):3327–3339. https://doi.org/10.1016/j.celrep.2018.05.041

89. Yang P, Mathieu C, Kolaitis RM, Zhang P, Messing J, Yurtsever U, Yang Z, Wu J, Li Y, Pan Q, Yu J, Martin EW, Mittag T, Kim HJ, Taylor JP (2020) G3BP1 is a tunable switch that triggers phase separation to assemble stress granules. Cell 181(2):325–345.e28. https://doi.org/10.1016/j.cell.2020.03.046

90. Piper PW (1993) Molecular events associated with acquisition of heat tolerance by the yeast Saccharomyces cerevisiae. FEMS Microbiol Rev 11(4):339–355. https://doi.org/10.1111/j.1574-6976.1993.tb00005.x

91. Panaretiou B, Piper PW (1990) Plasma-membrane ATPase action affects several stress tolerances of Saccharomyces cerevisiae and Schizosaccharomyces pombe as well as the extent and duration of the heat shock response. J Gen Microbiol 136:1763–1770. https://doi.org/10.1099/00221287-136-9-1763

92. Morosanne P, Slayman CW, Golfeau A (2000) Mutagenic study of the structure, function and biogenesis of the yeast plasma membrane H⁺-ATPase. Biochim Biophys Acta 1469(3):133–157. https://doi.org/10.1016/s0005-2760(00)00015-0

93. Perlin DS, San Francisco MJ, Slayman CW, Rosen BP (1986) H⁺/ ATP stoichiometry of proton pumps from Neurospora crassa and Escherichia coli. Arch Biochem Biophys 248(1):53–61. https://doi.org/10.1016/0003-9861(86)90400-5

94. Lecchi S, Nelson CJ, Allen KE, Swaney DL, Thompson KL, Coon JJ, Sussman MR, Slayman CW (2007) Tandem phosphorylation of Ser-911 and Thr-912 at the C terminus of yeast plasma membrane H⁺-ATPase leads to glucose-dependent activation. J Biol Chem 282(49):35471–35481. https://doi.org/10.1074/jbc.M706042200

95. Martinez-Munoz GA, Kane P (2008) Vacuolar and plasma membrane proton pumps collaborate to achieve cytosolic pH homeostasis in yeast. J Biol Chem 283(29):20309–20319. https://doi.org/10.1074/jbc.M710470200

96. Perzov N, Nelson H, Nelson N (2000) Altered distribution of the yeast plasma membrane H⁺-ATPase as a feature of vacuolar
H⁺-ATPase null mutants. J Biol Chem 275(51):40088–40095. https://doi.org/10.1074/jbc.M00701200

97. Parra KJ, Kane PM (1998) Reversible association between the V_i and V_o domains of yeast vacuolar H⁺-ATPase is an unconventional glucose-induced effect. Mol Cell Biol 18(12):7064–7074. https://doi.org/10.1128/MCB.18.12.7064

98. Alexandrov AI, Grosfeld EV, Dergalev AA, Kushnirov VV, Miermont A, Waharte F, Hu S, McClean MN, Bottani S, Leon S, Hersen P (2013) Severe osmotic compression triggers a slowdown of intracellular signaling, which can be explained by molecular crowding. Proc Natl Acad Sci USA 110(14):5725–5730. https://doi.org/10.1073/pnas.1215367110

99. Brocker C, Thompson DC, Vasiiliou V (2012) The role of hyper-osmotic stress in inflammation and disease. Biomed Concepts 3(4):345–364. https://doi.org/10.1515/bmc-2012-0001

100. Hohmann S (2002) Osmotic stress signaling and osmoadaptation in yeasts. Microbiol Mol Biol Rev 66(2):300–372. https://doi.org/10.1128/MMBR.66.2.300-372.2002

101. Shank BB, Rosenberg HM, Horowitz C (1973) Ionic basis of volume regulation in mammalian cells following osmotic shock. J Cell Physiol 82(2):257–265. https://doi.org/10.1002/jcp.1040820214

102. Wood JM (2015) Bacterial responses to osmotic challenges. J Gen Physiol 145(5):381–388. https://doi.org/10.1085/jgp.201411296

103. Eveloff JL, Warnock DG (1987) Activation of ion transport systems during cell volume regulation. Am J Physiol 252(1 Pt 2):F1-10. https://doi.org/10.1152/ajprenal.1987.252.1.F1

104. Christensen O, Hoffmann EK (1992) Cell swelling activates the Na⁺/H⁺ exchanger-1 activation in glutamate excitotoxicity. J Neurochem 59(10):1495–1501. https://doi.org/10.1002/nrb.20038

105. Cai D, Feliciano D, Dong P, Flores E, Gruebele M, Porat-Shliom I, Khotimchenko MY, Kleschevnikov AM (2016) Acute neuroinflammation provokes intracellular acidification in mouse hippocampus. J Neuroinflammation 13(1):283. https://doi.org/10.1186/s12974-016-0747-8

106. Prevarskaya N, Skryma R, Shuba Y (2010) Ion channels and the cytosol structure. Biol Open 8(7):bio044529. https://doi.org/10.1242/bio.044529

107. Andrew RD (1991) Seizure and acute osmotic change: clinical and neurophysiological aspects. J Neurol Sci 101(1):7–18. https://doi.org/10.1016/0022-510x(91)90013-w

108. Lee BK, Jung YS (2017) Sustained intracellular acidosis triggers the Na⁺/H⁺ exchanger-1 activation in glutamate excitotoxicity. Biomol Ther (Seoul) 25(6):593–598. https://doi.org/10.4062/biomolther.2017.018

109. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153(6):1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

110. Miermont A, Waharte F, Hu S, McClean MN, Bottani S, Leon S, Hersen P (2013) Severe osmotic compression triggers a slowdown of intracellular signaling, which can be explained by molecular crowding. Proc Natl Acad Sci USA 110(14):5725–5730. https://doi.org/10.1073/pnas.1215367110

111. Cai D, Feliciano D, Dong P, Flores E, Gruebele M, Porat-Shliom I, Khotimchenko MY, Kleschevnikov AM (2016) Acute neuroinflammation provokes intracellular acidification in mouse hippocampus. J Neuroinflammation 13(1):283. https://doi.org/10.1186/s12974-016-0747-8

112. Roberts EL Jr, Chih CP (1997) The influence of age of pH regulation in hippocampal slices before, during, and after anoxia. J Cereb Blood Flow Metab 17(5):560–566. https://doi.org/10.1097/00004679-199705000-00010

113. Lee BK, Jung YS (2017) Sustained intracellular acidosis triggers the Na⁺/H⁺ exchanger-1 activation in glutamate excitotoxicity. Biomol Ther (Seoul) 25(6):593–598. https://doi.org/10.4062/biomolther.2017.018

114. Ruffin VA, Salameh AI, Boron WF, Parker MD (2014) Intra- cellular pH regulation by acid-base transporters in mammalian
neurons. Front Physiol 5:43. https://doi.org/10.3389/fphys.2014.00043

131. Raffin CN, Sick TJ, Rosenthal M (1988) Inhibition of glycolysis alters potassium ion transport and mitochondrial redox activity in rat brain. J Cereb Blood Flow Metab 8(6):857–865. https://doi.org/10.1038/jcbfm.1988.143

132. Bonnet U, Bingmann D, Wildfang J, Scherbaum N, Wiemann M (2010) Modulatory effects of neuropsychoarmacopa on intra-cellular pH of hippocampal neurons in vitro. Br J Pharmacol 159(2):474–483. https://doi.org/10.1111/j.1476-5381.2009.00540.x

133. Baram TZ, Eghbal-Ahmadi M, Bender RA (2002) Is neuronal death required for seizure-induced epileptogenesis in the immature brain? Prog Brain Res 135:365–375. https://doi.org/10.1016/S0079-6123(02)35033-7

134. Roberts EL Jr, Rosenthal M, Sick TJ (1990) Age-related modifications of potassium homeostasis and synaptic transmission during and after anoxia in rat hippocampal slices. Brain Res 514(1):111–118. https://doi.org/10.1016/0006-8993(90)90441-d

135. Yao H, Sadoshima S, Ooboshi H, Uchimura H, Fujisawa Y, Kato K, Giulivi C (2009) Nitration of tyrosine residues 368 and 345 in the β-subunit elicits FoF1-ATPase activity loss. Biochem J 423(2):219–231. https://doi.org/10.1042/BJ20090594

136. Colacurcio DJ, Nixon RA (2016) Disorders of lysosomal acidification—the emerging role of v-ATPase in aging and neurodegenerative disease. Ageing Res Rev 15(5):e100437. https://doi.org/10.1016/j.aged.2016.05.004

137. Motil M, Demir AB (2014) Linking peroxiredoxin and vacuolar-ATPase functions in calorie restriction-mediated life span extension. Int J Cell Biol 2014:913071. https://doi.org/10.1155/2014/913071

138. Di Domenico F, Perluigi M, Butterfield DA, Cornelius C, Calabrese V (2010) Oxidative damage in rat brain during aging: interplay between energy and metabolic key target proteins. Neurochem Res 35(12):2184–2192. https://doi.org/10.1007/s11064-010-0295-z

139. Fujsawa Y, Kato K, Giulivi C (2009) Nitration of tyrosine residues 368 and 345 in the β-subunit elicits FoF1-ATPase activity loss. Biochem J 423(2):219–231. https://doi.org/10.1042/B20090594

140. Haynes V, Traaseth NJ, Elfering S, Fujisawa Y, Giulivi C (2010) Nitration of specific tyrosines in FoF1, ATP synthase and activity loss in aging. Am J Physiol Endocrinol Metab 298(5):E978–E987. https://doi.org/10.1152/ajpendo.00739.2009

141. Ginsberg SD, Alldred MJ, Counts SE, Cataldo AM, Neve RL, Jiang Y, Wuu J, Chao MV, Mufson EJ, Nixon RA, Che S, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ, Lee VM (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314(5796):130–133. https://doi.org/10.1126/science.1134108

142. Neumann M, Bensmann E, Dormann D, Jawaid A, DeJesus-Hernandez M, Ansorge O, Roebel S, Kretzschmar HA, Munoz DG, Kusaka Y, Yokota O, Ang LC, Bilbao J, Ramaders R, Haass C, Mackenzie IR (2011) FET proteins TAF15 and EWS are selective markers that distinguish FTLD with FUS pathology from amyotrophic lateral sclerosis with FUS mutations. Brain 134(Pt 9):2595–2609. https://doi.org/10.1093/brain/awr201

143. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388(6645):839–840. https://doi.org/10.1038/42166

144. Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM (1986) Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem 261(13):6084–6089. https://doi.org/10.1016/S0021-9258(17)38495-8

145. Zbinden A, Perez-Berlanga M, De Rossi P, Polymenidou M (2020) Phase separation and neurodegenerative diseases: a disturbance in the force. Dev Cell 55(1):45–68. https://doi.org/10.1016/j.devcel.2020.09.014

146. Raw YY, Singh N, Kumar R, Patil K, Pandey S, Datta D, Mahato J, Panigrahi R, Nalvarak A, Mehrsa S, Gadhe L, Chatterjee D, Sawyer AS, Maiti S, Bhatah S, Gerez JA, Chowdhury A, Kumar A, Padintheare R, Riek R, Krishnamoorthi G, Maji SK (2020) α-Synuclein aggregation nucleates through liquid–liquid phase separation. Nat Chem 12(8):705–716. https://doi.org/10.1038/s41557-020-0465-9

147. Bouchaud JJ, Otero JH, Scott DC, Szulc E, Martin EW, Sabri N, Granata D, Marzahn MR, Lindorff-Larsen K, Salvatella X, Schulman BA, Mittag T (2018) Cancer mutations of the tumor suppressor SPOP disrupt the formation of active, phase-separated compartments. Mol Cell 72(1):19–36.e8. https://doi.org/10.1016/j.molcel.2018.08.027

148. Kilic S, Lezaja A, Gatti M, Bianco E, Michelen J, Imhof R, Altmeyer M (2019) Phase separation of 3SBP1 determines liquid-like behavior of DNA repair compartments. EMBO J 38(16):e101379. https://doi.org/10.15252/embj.2018101379

149. Altmeyer M, Neelsen KJ, Teloni F, Pozdnyakova I, Pellegrino S, Grofter M, Rask MD, Strichler W, Jungmichel S, Nielsen ML, Lukas J (2015) Liquid demixing of intrinsically disordered proteins is seeded by poly(ADP-ribose). Nat Commun 6:8088. https://doi.org/10.1038/ncomms9088

150. Boulay G, Sandoval JG, Riggi N, Iyer S, Buisson R, Naigles B, Awad ME, Rengarajan S, Volorio A, McBride MJ, Brode LC, Zhou L, Stamenkovic I, Kadoch C, Rivera MN (2017) Cancer-specific targeting of BAF complexes by a prion-like domain. Cell 171(1):163–178.e19. https://doi.org/10.1016/j.cell.2017.07.036

151. Zamudio AV, Dall’Agnese A, Hanenre JG, Manteja JC, Afeyan KY, Hannef NT, Coffey EL, Li CH, Oksuz O, Sabari BR, Boija A, Kleen IA, Hawken SW, Spille IH, Decker TM, Cisse II, Abraham BJ, Lee TI, Taatjes DJ, Schuiers J, Young RA (2019) Mediator condensates localize signaling factors to key cell identity genes. Mol Cell 76(5):753-766.e6. https://doi.org/10.1016/j.molcel.2019.08.016

152. Lu Y, Wu T, Gutman O, Lu H, Zhou Q, Henis YI, Luo K (2020) Phase separation of TAZ compartmentalizes the transcription machinery to promote gene expression. Nat Cell Biol 22(4):453–464. https://doi.org/10.1038/s41422-020-0485-0

153. Kimoto F, Fukuda H, Imamochi-Oshi S, Saito H, Takekawa M (2008) Formation of stress granules inhibits apoptosis by suppressing stress-responsive MAPK pathways. Nat Cell Biol 22(24):453–464. https://doi.org/10.1038/s41422-020-0485-0

154. Thiediek K, Holzwarth B, Prentzell MT, Boelheke C, Klasener R, Ruf S, Sonntag AG, Maerz L, Grellscheid SN, Kremmer E.
Effects of pH alterations on stress- and aging-induced protein phase separation

218. Liu W, MacKay JA, Dreher MR, Chen M, McDaniel JR, Simnick AJ, Callahan DJ, Zalutsky MR, Chilkoti A (2010) Injectable intratumoral depot of thermally responsive polypeptide-radionuclide conjugates delays tumor progression in a mouse model. J Control Release 144(1):2–9. https://doi.org/10.1016/j.jconrel.2010.01.032

219. Lim DW, Nettles DL, Setton LA, Chilkoti A (2008) In situ cross-linking of elastin-like polypeptide block copolymers for tissue repair. Biomacromol 9(1):222–230. https://doi.org/10.1021/bm7007982

220. Betre H, Ong SR, Guilak F, Chilkoti A, Fermor B, Setton LA (2006) Chondrocytic differentiation of human adipose-derived adult stem cells in elastin-like polypeptide. Biomaterials 27(1):91–99. https://doi.org/10.1016/j.biomaterials.2005.05.071

221. McHale MK, Setton LA, Chilkoti A (2005) Synthesis and in vitro evaluation of enzymatically cross-linked elastin-like polypeptide gels for cartilaginous tissue repair. Tissue Eng 11(11–12):1768–1779. https://doi.org/10.1089/ten.2005.11.1768

222. Banani SF, Lee HO, Hyman AA, Rosen MK (2017) Biomolecular condensates: organizers of cellular biochemistry. Nat Rev Mol Cell Biol 18(5):285–298. https://doi.org/10.1038/nrm.2017.7

223. Shin Y, Brangwynne CP (2017) Liquid phase condensation in cell physiology and disease. Science 357(6357):eaaf4382. https://doi.org/10.1126/science.aaf4382

224. Memisoglu G, Eapen VV, Yang Y, Klionsky DJ, Haber JE (2019) PP2C phosphatases promote autophagy by dephosphorylation of the Atg1 complex. Proc Natl Acad Sci U S A 116(5):1613–1620. https://doi.org/10.1073/pnas.1817078116

225. Takahara T, Maeda T (2012) Transient sequestration of TORC1 into stress granules during heat stress. Mol Cell 47(2):242–252. https://doi.org/10.1016/j.molcel.2012.05.019

226. Yang YS, Kato M, Wu X, Lisitsos A, Sutter BM, Wang Y, Hsu CH, Wood NE, Lemoff A, Mirzaei H, Heinemann M, Tu BP (2019) Yeast Ataxin-2 Forms an Intracellular Condensate Required for the Inhibition of TORC1 Signaling during Respiratory Growth. Cell 177(3):697–710.e17. https://doi.org/10.1016/j.cell.2019.02.043

227. Wippich F, Bodenmiller B, Trajkovska MG, Wanka S, Aebersold R, Pelkmans L (2013) Dual specificity kinase DYRK3 couples stress granule condensation/dissolution to mTORC1 signaling. Cell 152(4):791–805. https://doi.org/10.1016/j.cell.2013.01.033

228. Wheeler JR, Matheny T, Jain S, Abrishc R, Parker R (2016) Distinct stages in stress granule assembly and disassembly. Elife 5:e18413. https://doi.org/10.7554/eLife.18413

229. Molliex A, Temirov J, Lee J, Coughlin M, Kanagaraj AP, Kim HJ, Mittag T, Taylor JP (2015) Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. Cell 163(1):123–133. https://doi.org/10.1016/j.cell.2015.09.015

230. Iwabuchi K, Bartel PL, Li B, Marraccino R, Fields S (1994) Two cellular proteins that bind to wild-type but not mutant p53. Proc Natl Acad Sci USA 91(13):6098–6102. https://doi.org/10.1073/pnas.91.13.6098

231. Cuelia-Martin R, Oliveira C, Lockstone HE, Snellenberg S, Gromusova N, Chapman JR (2016) 53BP1 integrates DNA repair and p53-dependent cell fate decisions via distinct mechanisms. Mol Cell 64(1):51–64. https://doi.org/10.1016/j.molcel.2016.08.002

232. Bi J, Huang A, Liu T, Zhang T, Ma H (2015) Expression of DNA damage checkpoint 53BP1 is correlated with prognosis, cell proliferation and apoptosis in colorectal cancer. Int J Clin Exp Pathol 8(6):6070–6082

233. Cao X, Jin X, Liu B (2020) The involvement of stress granules in aging and aging-associated diseases. Aging Cell 19(4):e13136. https://doi.org/10.1111/acel.13136

234. Franzmann TM, Alberti S (2019) Prion-like low-complexity sequences: key regulators of protein solubility and phase behavior. J Biol Chem 294(18):7128–7136. https://doi.org/10.1074/jbc.TM118.001190

235. Kroschwald S, Maharana S, Mateju D, Malinovska L, Nuske E, Poser I, Richter D, Alberti S (2015) Promiscuous interactions and protein disaggregates determine the material state of stress-inducible RNP granules. Elife 4:e06807. https://doi.org/10.7554/eLife.06807

236. Lobaskin V, Qamhieh K (2003) Effective macroion charge and stability of highly asymmetric electrolytes at various salt conditions. J Phys Chem B 107(32):8022–8029. https://doi.org/10.1021/jp027608+.

237. Zhang FJ, Roosen-Runge F, Sauter A, Wolf M, Jacobs RMJ, Schreiber F (2014) Reentrant condensation, liquid-liquid phase separation and crystallization in protein solutions induced by multivalent metal ions. Pure Appl Chem 86(2):191–202. https://doi.org/10.1515/pac-2014-5002

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