PERSPECTIVES

BIOPHYSICS

Can phase separation buffer cellular noise?

By suppressing concentration fluctuations, condensation may stabilize cellular processes

By **Joshua A. Riback**[1] and **Clifford P. Brangwynne**[1,2]

Scientists have long marveled at the ability of complex patterns to emerge from seemingly chaotic origins. In biology, each cell must circumvent the stochastic nature, or noise, inherent to chemical reactions and molecular diffusion. This noise introduces large fluctuations in messenger RNA (mRNA) and protein concentrations, which might be deleterious to processes such as biosynthesis, macroscale organization, and self-replication. Noise may be decreased through compartmentalization, including liquid-liquid phase separation (LLPS), a process underlying the formation of membraneless compartments (*1*, *2*). On page 464 of this issue, Klosin *et al.* (*3*) provide evidence that LLPS can buffer noise in cells and may play a role in stabilizing various biological circuits.

LLPS appears to be a fundamental mechanism driving the assembly of dozens of membraneless organelles, also known as biomolecular condensates (*1*, *2*). Condensates are the location of various processes, including transcription and RNA processing, and are thought to facilitate these processes by concentrating key biomolecular factors (*4*, *5*). Although some data suggest that protein clustering can buffer noise (*6*), evidence for buffering by LLPS has been largely absent.

Compartmentalization of the cellular interior, whether by LLPS or other means, could provide a passive filter—that is, an energy-independent suppression of noise (*7*). For example, segregating factors into different compartments may avoid undesirable interactions that would occur under concentration fluctuations. Additionally, compartmentalization imposes a rate-limiting step, such as nuclear export, which effectively buffers fluctuations (*8*, *9*). The prospect that LLPS can filter noise is particularly attractive because it is grounded in the underlying physics of phase transitions.

LLPS occurs when a system becomes supersaturated with "sticky" molecules, including proteins that contain weakly self-associating, intrinsically disordered regions (IDRs), such that they condense into liquid droplets (thus separating molecules from the surrounding liquid phase). This is represented by the "phase diagram": For a single solute component, its concentration outside of the droplet will be fixed at the saturation concentration (C_{sat}), the maximum quantity of a substance that can dissolve (see the figure). This implies that fluctuations in total concentration primarily change the size (volume fraction) of each of the phases, not the concentrations of the component in each phase. This provides a potential mechanism for passive filtering of fluctuations in the availability of molecules for reactions and signaling.

Klosin *et al.* analyzed a physical framework to understand the non-equilibrium fluctuations imparted by LLPS of a transiently expressed protein, and demonstrated that phase separation lowers the concentration fluctuations in the cytoplasm and nucleoplasm versus those of the total expressed protein. These data provide a strong proof of principle that LLPS can act as a passive filter. Future studies should deduce the importance of such buffering in the robustness of cellular phenomena. This also demonstrates a connection between noise buffering and quantitative determination of phase diagrams—particularly the value of C_{sat}—in living cells.

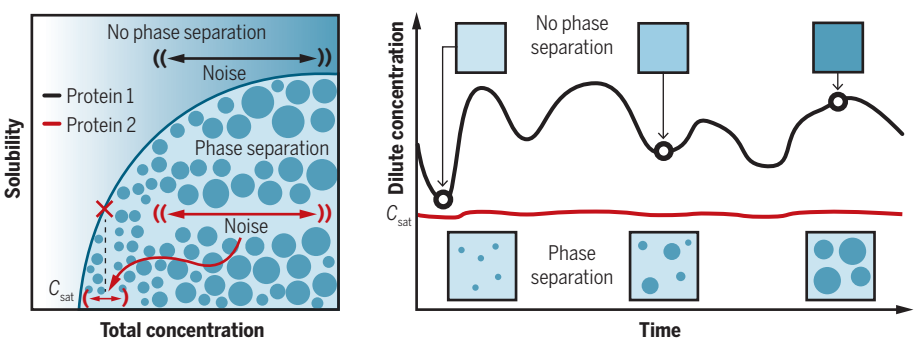
Although many assert that a hallmark of LLPS is a single fixed C_{sat} (*1*, *2*, *10*), quantifying such phase behavior has rarely been achieved for endogenous intracellular condensates. In the few cases where the phase diagrams of model IDR-containing proteins have been mapped in living cells (*11*), the idea of a fixed C_{sat} seems to be valid, implying that such condensates buffer concentration. However, in emerging results for endogenous systems, including for key components of the nucleolus, Cajal bodies, and processing (P) bodies, C_{sat} was found not to be fixed (*12*), consistent with theoretical considerations (*13*).

What is the key distinction between endogenous condensates and model IDR-driven droplets that may complicate the simple "fixed C_{sat}" picture? In endogenous condensates, multiple heterotypic interactions likely occur, often involving self-associating IDRs, as well as proteins with folded domains and nucleic acids. For such multicomponent systems, the phase behavior becomes more complex, and the concentration of a single component only represents part of a high-dimensional phase diagram (*14*).

Given that intracellular LLPS is not governed by fixed concentrations, can endogenous condensates buffer noise? Although Klosin *et al.* do not directly address multicomponent LLPS, they discuss fluctuations

A phase diagram constrains protein fluctuations

"Sticky" molecules (with low solubility) can undergo phase separation, which occurs only above the saturation concentration C_{sat} (left, defined by red X). Fluctuations in the total concentration of sticky proteins (protein 2) may be suppressed by phase separation (right, red). For proteins not undergoing phase separation (protein 1), less noise buffering occurs (right, black).



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in other factors that reduce but do not eliminate the buffering by LLPS. Critically, they provide evidence for noise buffering of an endogenously tagged protein, nucleophosmin (NPM1), which forms condensates in nucleoli. Previous in vitro experiments showed that NPM1 LLPS is stabilized by heterotypic interactions with numerous components, including RNA (*15*), consistent with emerging in vivo findings of a nonfixed C_{sat} (*12*).

A simple argument illustrates how the picture for multicomponent phase separation becomes more complex, even when there is a fixed C_{sat}. Consider the simplest system, which comprises N identical yet independent components. Without phase separation, expression noise manifests through each of these N degrees of freedom. However, upon phase separation, the system loses one degree of freedom, such that the added noise for each component would be [1 – (1/N)] of their expression noise. This implies an interesting balance for multicomponent LLPS, as more components can be buffered, yet with each one being buffered to a lesser extent.

The study of Klosin *et al.* represents an important set of findings that open the door for further studies to delineate potential locations where LLPS may play a role in noise buffering. For example, could feedback through transcriptional condensation (*4*, *5*) be lowering the noise from stochastic mRNA production? Additionally, Cajal bodies and nuclear speckles, condensates relevant for mRNA processing, might have mechanisms to buffer processed mRNA availability. Cytoplasmic bodies—many of which contain mRNAs under various conditions, various stages of development, and in specific tissues—may contribute to cellular robustness by removing expression noise in translation. It is increasingly clear that LLPS must be considered to establish a complete description of noise buffering in living systems. ■

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AGING

Support cells in the brain promote longevity

Glial cells in the brain use neuropeptides to communicate stress responses and longevity

By **Jason Wayne Miklas**[1] *and* **Anne Brunet**[1,2]

Aging is a multifaceted process that results in organismal decay. At the cellular level, protein homeostasis is a key system that becomes dysregulated with age, causing the accumulation of aberrant or unfolded proteins. In a youthful individual, unfolded proteins normally trigger the unfolded protein response (UPR), which upregulates the protein clearance machinery and returns cells to a homeostatic state. The UPR is typically induced in a cell-autonomous manner. But some cells communicate protein folding stress to distal cells. For example, neurons communicate activation of the UPR to peripheral tissues to promote longevity in the worm *Caenorhabditis elegans* (*1*). On page 436 of this issue, Frakes *et al.* (*2*) show that support cells in the brain called glial cells (*3*) can also initiate long-range activation of the endoplasmic reticulum UPR (UPR^{ER}) in distal cells to coordinate stress resistance and longevity in *C. elegans* and that this occurs through neuropeptide secretion.

"...it will be interesting to determine whether similar neuropeptides are produced... in the human brain...."

A key component of the UPR^{ER} is the conserved transcription factor X-box–binding protein 1 (XBP-1), which coordinates a stress response program. Frakes *et al.* show that overexpressing a constitutively active form of XBP-1, *xbp-1s*, in glia is sufficient to extend life span in *C. elegans*. The authors identify four astrocyte-like cephalic sheath (CEPsh) glial cells as the specific subpopulation of glia that controls UPR^{ER} activation in distal intestinal cells, promoting life-span extension. XBP-1 expression in glia selectively triggers the UPR^{ER} but not other stress responses (such as mitochondrial UPR) in intestinal cells.

How do glial cells communicate with distal intestinal cells? In a previous study, neurons expressing *xbp-1s* induce the UPR^{ER} in a non–cell-autonomous manner by releasing small clear synaptic vesicles containing neurotransmitters that could in turn, directly or indirectly, affect intestinal cells (*1*). Frakes *et al.* show that unlike neurons, glia do not use the machinery involved in the release of small clear synaptic vesicles to regulate signaling with distal intestinal cells. The authors reasoned that the distance a signal from CEPsh glial cells would need to travel to intestinal cells (~300 μm in *C. elegans*) might require long-range-acting neuropeptides, which are secreted from neurons, neuroendocrine cells, and glia. There are 119 neuropeptide precursor genes in *C. elegans*, and their peptide products regulate key physiological processes, including cell-to-cell communication (*4*). Neuropeptides go through a series of processing steps before they are packaged in dense-core vesicles and transported out of the cell. Frakes *et al.* show that disruption of dense-core vesicle export and neuropeptide processing in glial cells suppresses UPR^{ER} activation in intestinal cells. Thus, neuropeptide secretion mediates the effect of glial cells on the periphery (see the figure).

Many interesting questions remain. The specific neuropeptide(s) being secreted are not known, nor are their downstream targets and mode of action. In mammals, several neuropeptides and neurohormones (such as growth hormone–releasing hormone) are secreted by neurons or neuroendocrine cells in the hypothalamo-pituitary axis and exert effects on energy metabolism in peripheral tissues (*5*). Conversely, other peptide hormones are produced by peripheral tissues—such as leptin (adipose), ghrelin (stomach), and insulin (pancreas)—and act in various regions of the brain and other organs (*6*). Some neuropeptides are conserved between *C. elegans* and humans (*7*). In *C. elegans*, the 119 neu-

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