RESEARCH HIGHLIGHT

Crank up the volume: Osmotic stress induces WNK1 phase separation

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Cell shrinkage, which occurs in a hypertonic environment, results in a rapid rebound response to restore cell volume and ion concentrations. In a recent study published in *Cell*, Boyd-Shiwarski et al. identify WNK1, a known homeostatic regulator of cell volume, as a molecular crowding sensor, and demonstrate how WNK1 contributes to cell volume recovery through cell shrinkage-dependent phase separation.

Cells quickly adapt to changing osmotic conditions to maintain homeostasis and prevent cell death. Some of this demand is handled by the WNK (With No Lysine (K)) protein kinases which respond to osmotic stress to normalize cell volume and ionic composition. WNKs (WNKs 1–4 in humans) are large, primarily unstructured proteins, aside from a kinase domain near the N-termini and several small domains and coiled-coil regions. WNK1 (> 2000 residues) is ubiquitously expressed in mammalian tissues and is an essential gene in mice and in cells (see depmap.org). Because certain gain-of-function mutations cause hypertension, it is studied as a human disease gene. WNKs 2–4 exhibit more tissue-restricted expression and have both overlapping and unique functions. OSR1 (oxidative stress responsive 1) and SPAK (STE20/SPS1-related proline/alanine-rich kinase) are direct downstream effector kinases of WNKs. Osmotic stress activates WNK1 via trans-autophosphorylation.[1] OSR1 and SPAK interact with and are activated by WNKs, and in turn, regulate downstream targets such as ion cotransporters and channels to maintain ion homeostasis and cell volume.[2]

A long-standing conundrum has been the paradoxical nature of WNK kinase regulation. It has been known for nearly 20 years that WNK1 is activated by both hypotonic and hypertonic stresses.[3] The paradox became more pronounced when WNK1 kinase activity was found to be inhibited by chloride binding to its kinase domain.[4] Hypotonicity gives rise to low intracellular chloride. This yields a straightforward mechanism whereby WNK1 is activated as chloride vacates the kinase active site. However, hypertonic conditions cause water to leave the cell, and intracellular chloride concentrations rise as a result of decreased cell volume. Elevated chloride should inhibit WNK1 kinase activity, but instead WNK1 is activated to facilitate a regulatory volume increase. These and other findings suggested that WNK1 has two distinct activation mechanisms. One possible explanation for how WNK1 is activated under these conditions came from a study of the chloride channel LRRC8A. p38/MSK1 pathway-dependent phosphorylation of LRRC8A facilitates cellular chloride efflux under hypertonic conditions. The authors suggest that LRRC8A may only prevent excessive increases in chloride to optimize WNK1 activation, pointing out that intracellular chloride due to hypertonic stress is still elevated.[5]

In a recent paper in *Cell*, Boyd-Shiwarski et al. uncover a mechanism that reconciles WNK1 activation under hypertonic conditions.[6] They find that WNK1 is a sensor of molecular crowding caused by cell shrinkage (Fig. 1). Crowding, induced by water efflux from the cell, induces WNK1 phase separation. Phase-separated molecular condensates are membraneless organelles containing proteins and other molecules within cells. Cells utilize phase separation to sort or sequester specific molecules into intracellular droplets, yielding concentrates of molecules within the condensates for quick adaptive responses to environmental changes.[7] These phase-separated condensates may serve as physical platforms for kinase and substrate connections through induced proximity to increase phosphorylation and control of intracellular signaling.[8] For this reason, over the last decade, phase separation is increasingly viewed as a molecular basis of intracellular spatial organization dynamics and signaling. Condensates are engaged in a range of fundamental biological processes that govern the organization and functional regulation of cells. Although condensate formation within the cells remains incompletely understood, intrinsically disordered regions (IDRs) of proteins contribute to the establishment of phase separation. WNKs contain kinase domains near their N-termini followed by extended segments of low-complexity sequence, normally defined as unable to fold into secondary or tertiary structures and frequently found within IDRs. These disordered regions participate in WNK1 phase separation.

The *Cell* study builds on the authors' previous work to show decisively that with increasing amounts of hypertonic stress applied to cells, WNK1 forms phase-separated droplets within seconds. Until recently it was unclear whether these punctate structures were membraneless.[9] The puncta numbers increase rapidly then begin to drop while sizes remain stable, individual puncta merge, fluorescence recovery after photobleaching occurs rapidly, and at high concentrations of WNK1 spinodal decomposition (demixing into web-like networks) occurs. All these observations are consistent with phase separation. SPAK, OSR1, and WNK3 all colocalized with phase-separated WNK1 suggesting that the droplets might concentrate WNK pathway components to enhance signaling.

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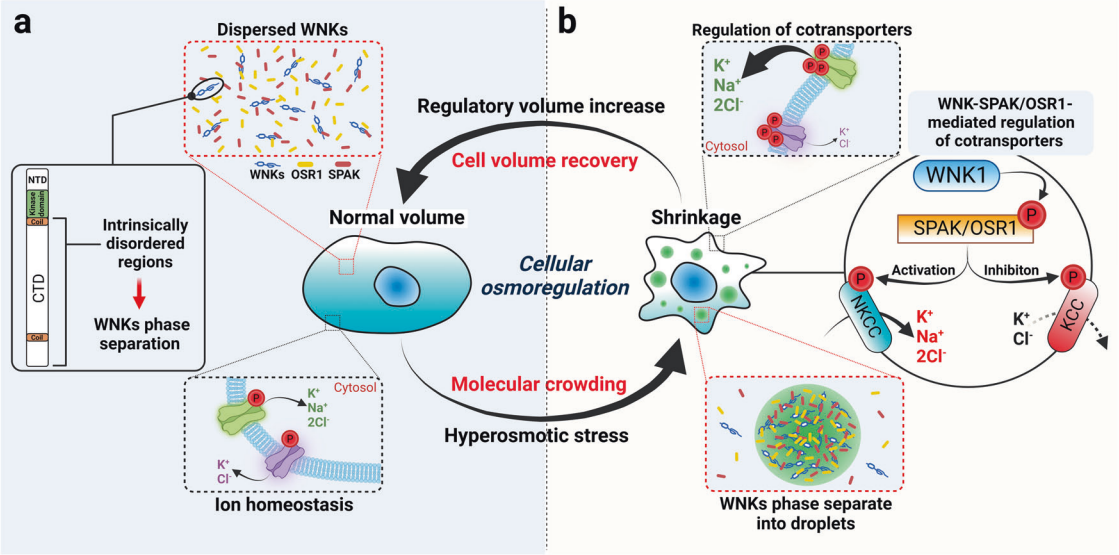

Fig. 1 Crowding-induced phase separation of WNK1 facilitates regulatory volume increase. a Under normal conditions, WNK1 is diffusely localized, often with punctate appearance, throughout the cytosol. **b** Hypertonic stress induces cell shrinkage, and thus increases molecular crowding. The mechanism put forth by Boyd-Shiwarski et al. is that the increased concentration of WNK1 within the cell leads to its phase separation. WNK1 activation is increased, and its downstream effector kinases, OSR1 and SPAK, also colocalize with WNK1 thereby enhancing WNK signaling to cotransporters, which leads to a regulatory volume increase. As volume recovery proceeds, the concentration of WNK1 decreases, less is present in droplets, and signaling decreases. Created with BioRender.com.

Boyd-Shiwarski et al. find that two regions located C-terminally to the kinase domain of WNK1 contribute to the phase separation behavior, and both are found primarily in IDRs of the protein. Two coiled-coils found within these regions also appear to contribute to phase separation, although to a lesser extent. A fragment of WNK1 containing only the N-terminus and kinase domain failed to phase separate. A fragment containing only one condensation-prone region and one coiled-coil phase separated, but not nearly as well as the full-length protein, implying that efficient phase separation requires multivalent interactions. The condensation behavior of these three fragments tracked well with the extent of SPAK/OSR1 and cotransporter NKCC1 activation, evidence that phase separation enhanced WNK pathway signaling. They also find that crowding-induced phase separation of WNKs may be an ancient conserved mechanism. *Drosophila* WNK behaves similarly and WNKs present in organisms as distant as *C. elegans* all have large C-terminal IDR regions even though the sequences of the C-terminal low-complexity regions diverge.

The mechanisms put forth thus far to explain WNK1 activation under hypertonic conditions (LRCC8A-mediated chloride efflux and WNK1 phase separation) are not mutually exclusive. In fact, both may contribute synergistically to facilitate regulatory volume increase.

The propensity of WNK1 to phase separate, which has been noted at low levels under isotonic conditions, opens the door to many questions and areas of investigation. How do SPAK/OSR1 activated in condensates communicate with plasma membrane cotransporters? Could additional biological macromolecules or post-translational modifications be required, augment, or inhibit WNK1 phase separation? SPAK/OSR1 dimerize, and WNK1 contains four binding sites for the kinases raising ideas about scaffolding both between WNKs and between the downstream kinases and their other substrates.[10] Could phase separation more generally impact WNK scaffolded complexes? What other processes might be affected by sequestration by WNK molecular condensates? The work by Boyd-Shiwarski et al. represents an elegant step forward in our understanding of WNK signaling.

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ADDITIONAL INFORMATION

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