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Review

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Biomolecular condensates in Hippo pathway regulation

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Abstract: Hippo signaling is a highly conserved pathway central to diverse cellular processes. Dysregulation of this pathway not only leads to developmental abnormalities but is also closely related to the occurrence and progression of various cancers. Recent studies have uncovered that, in addition to the classical signaling cascade regulation, biomolecular condensates formed via phase separation play a key role in the spatiotemporal regulation of Hippo signaling. In this review, we provide a summary of the latest research progress on the regulation of the Hippo signaling pathway by phase separation, with a particular focus on transcriptional activation mediated by Yes-associated protein (YAP)/transcriptional coactivator with post-synaptic density-95, disks-large, and zonula occludens-1 (PDZ)-binding domain (TAZ) condensates. Furthermore, we discuss the utility of chemical crosslinking combined with mass spectrometry to analyze the TAZ condensate interactome and examine the role of the protein fused in sarcoma (FUS) in modulating the biophysical properties of TAZ condensates, which in turn influence their transcriptional activity and pro-tumorigenic functions. These insights not only advance our understanding of Hippo signaling but also offer new perspectives for therapeutic interventions targeting diseases linked to dysregulated YAP/TAZ activity.

Key words: Hippo signaling pathway; Phase separation; Transcription regulation

1 Introduction

The Hippo signaling pathway is a key regulator of tissue regeneration and organ size control, primarily via its modulation of cell proliferation and apoptosis (Harvey et al., 2003; Yu et al., 2015). Initially identified in *Drosophila melanogaster* via genetic screening, the Hippo pathway comprises a group of genes whose mutations all result in a similar phenotype of massive tissue overgrowth (Justice et al., 1995; Xu et al., 1995). Since their discovery, the Hippo pathway has been recognized as a highly conserved signaling network across multiple species, including mammals, highlighting its fundamental role in development and disease (Dong et al., 2007).

The core kinase cascade of Hippo signaling consists of mammalian Ste20-like kinase 1/2 (MST1/2) and Salvador 1 (SAV1), large tumor suppressor 1/2 (LATS1/2), and MOBKL1A/MOBKL1B (MOB1) (Fig. 1) (Dong et al., 2007). It has been well established that a wide range of intracellular and extracellular cues, such as cell density, mechanical stress, and stress-related conditions, activate the Hippo pathway (Yu and Guan, 2013). Upon sensing these upstream signals through different mechanisms (Ma et al., 2019; Fu et al., 2022), MST1/2 binds to the adaptor protein SAV1, facilitating the phosphorylation of the LATS1/2-MOB1 complex. Then, the activated LATS1/2 phosphorylates the downstream effectors Yes-associated protein (YAP) and transcriptional coactivator with post-synaptic density-95, disks-large, and zonula occludens-1 (PDZ)-binding domain (TAZ), leading to their cytoplasmic retention and subsequent degradation (Meng et al., 2016). Conversely, when the Hippo pathway is inhibited, YAP and TAZ translocate into the nucleus, where they interact with the transcriptional enhanced associate domain (TEAD) transcription factors and function as transcriptional co-activators to promote the transcription of target genes involved in cell growth and proliferation (Hong and Guan, 2012).

Given its pivotal role in regulating key cellular processes, the dysregulation of the Hippo pathway has been implicated in various pathological conditions, including cancer and developmental disorders (Yu

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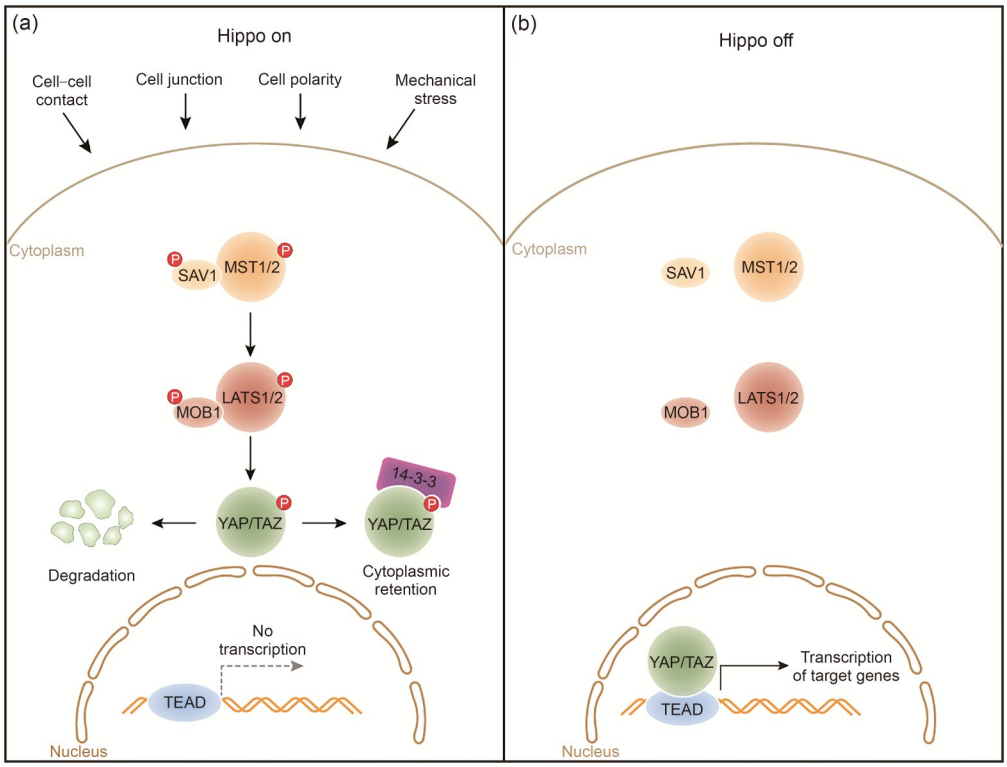

Fig. 1 Signaling cascade of the Hippo pathway. The Hippo pathway is regulated by various upstream signals such as cell–cell contact, cell junction, cell polarity, and mechanical stress. (a) When Hippo is activated, mammalian Ste20-like kinase 1/2 (MST1/2) phosphorylates Salvador 1 (SAV1), MOBKL1A/MOBKL1B (MOB1), and large tumor suppressor 1/2 (LATS1/2), leading to the phosphorylation of Yes-associated protein (YAP)/transcriptional coactivator with post-synaptic density-95, disks-large, and zonula occludens-1 (PDZ)-binding domain (TAZ) and their cytoplasmic retention by 14-3-3 or degradation. (b) When the Hippo pathway is inactivated, the unphosphorylated YAP/TAZ translocates into the nucleus and binds to transcription factors, such as transcriptional enhanced associate domain 1/2/3/4 (TEAD1/2/3/4), to promote the transcription of their target genes.

et al., 2015; Fu et al., 2022; Li and Guan, 2022; Zhong et al., 2024). Consequently, over the past two decades, tremendous efforts have been devoted to elucidating the molecular mechanisms underlying Hippo signaling regulation. Notably, while the conventional view holds that Hippo signaling operates in a linear cascade, emerging evidence suggests that critical regulators of this pathway can form phase-separated biomolecular condensates, serving as "signaling hubs" to modulate Hippo signaling.

In this review, we summarize the recent advances in understanding the regulation of Hippo signaling via biomolecular condensates. We explore how the fused in sarcoma (FUS) modulates the dynamicity of TAZ condensates through its chaperone-like function and discuss the impact of the biophysical properties of TAZ condensates on their transcriptional activity and oncogenic functions.

2 Biomolecular condensates and their roles in signal transduction

The concept of phase separation, originally used in physics to describe the partitioning of substances into distinct phases, has profoundly transformed our understanding of biomolecule organization within cells (Hyman et al., 2014). In biological systems, phase separation refers to the process by which biomolecules segregate from a homogeneous mixture into two distinct phases: a dilute phase with low biomolecule concentration and a condensed phase where biomolecules assemble into membrane-less structures, commonly referred to as biomolecular condensates (Banani et al., 2017; Che et al., 2023). These serve as dynamic, non-membrane-bound compartments that regulate a wide array of biochemical reactions and cellular processes both spatially and temporally. Notable examples

of biomolecular condensates in cells include stress granules, Cajal bodies, and P bodies, each of them playing the specialized roles in cellular homeostasis and function (Hyman et al., 2014; Boeynaems et al., 2018).

Although the detailed mechanisms of phase separation in biology remain elusive, it is generally believed that weak, multivalent interactions between biomolecules provide the driving force for the formation of these condensates in cells (Boija et al., 2021). A growing body of evidence suggests that macromolecules with multivalent interaction potential, such as proteins containing intrinsically disordered regions (IDRs) or repetitive motifs, are particularly prone to phase separation. These regions facilitate weak, transient interactions, including salt bridges, π–π stacking, π–cation interactions, and hydrophobic interactions, which collectively promote the assembly of condensates (Boija et al., 2021). Recently, the "sticker–spacer" model has emerged as a theoretical framework to explain the molecular principles underlying biomolecular condensation. In this model, "stickers" represent short, interaction-mediating motifs that drive the multivalent interactions, while "spacers" provide flexibility and modulate the physical properties of the condensates. For a more comprehensive discussion on this topic, readers are directed to several recent reviews (Martin et al., 2020; Abbas et al., 2021; Mittag and Pappu, 2022; Ginell and Holehouse, 2023).

While biomolecular condensates have been implicated in various biological processes (Alberti and Dormann, 2019; Zhang et al., 2020), their role as "signaling hubs" has become a topic of particular interest due to their precise control capacity for signal transduction. Firstly, biomolecular condensates are able to localize signaling molecules to specific subcellular compartments, ensuring high spatial specificity in signaling activation (Alberti et al., 2019). Through compartmentalization, signaling events are "licensed" to occur in the correct subcellular locations, minimizing off-target effects and enhancing the efficiency of signal propagation. Secondly, the components within condensates are highly dynamic, constantly exchanging with the surrounding environment (Banani et al., 2017). This unique property enables the rapid recruitment or release of signaling molecules, facilitating precise temporal control in response to external stimuli.

Therefore, compared to traditional membrane-bound organelles, "signaling hubs" formed through phase separation offer a more flexible and versatile mechanism for the spatial and temporal regulation of signal transduction. The capacity of condensates to rapidly assemble, disassemble, or remodel in response to actual cellular needs allows cells to fine-tune signaling pathways with remarkable precision, a crucial prerequisite for coordinating complex cellular responses to environmental changes and maintaining homeostasis (Alberti and Dormann, 2019; Zhang et al., 2020; Boija et al., 2021). In the next section, we summarize recent advances in deciphering the mechanism of how phase separation regulates the Hippo signaling pathway.

3 Regulation of the Hippo pathway by biomolecular condensates in the cytoplasm

Although it has long been known that most components of the Hippo signaling pathway are present in the cytoplasm, whether the signaling events occur in a compartmentalized manner in response to various upstream stimuli had remained largely unclear until recently. In recent years, a number of studies have provided compelling evidence that core kinases of the Hippo pathway can be regulated through phase-separated condensates, enabling high precision and versatility in signaling response (Fig. 2).

Among the various kinases in the Hippo signaling cascade, LATS1/2 plays a particularly prominent role in regulating Hippo signaling, as it directly phosphorylates and inhibits the transcriptional activities of YAP and TAZ (Yu and Guan, 2013). Thus, many biomolecular condensates exert their regulatory effects on Hippo signaling by converging on LATS1/2. Recent studies have shown that both LATS1 and LATS2 can form condensates in the cytoplasm; however, they appear to exert distinct effects on Hippo pathway activation (Li et al., 2021; Qin et al., 2024). For instance, LATS1 condensation, which is enhanced by the lipid-associated long non-coding RNA (lncRNA) *SNHG9*, inhibits the phosphorylation of YAP and promotes cancer progression (Fig. 2a) (Li et al., 2021; Lin et al., 2024). In contrast, LATS2 condensates protect the kinase from proteasome-mediated degradation and function as signalosomes for its activation, leading to YAP inactivation after actin cytoskeleton reorganization (Fig. 2b) (Qin et al., 2024). One possible explanation for these functional divergences is that the regions essential for LATS1 and LATS2 condensation share very

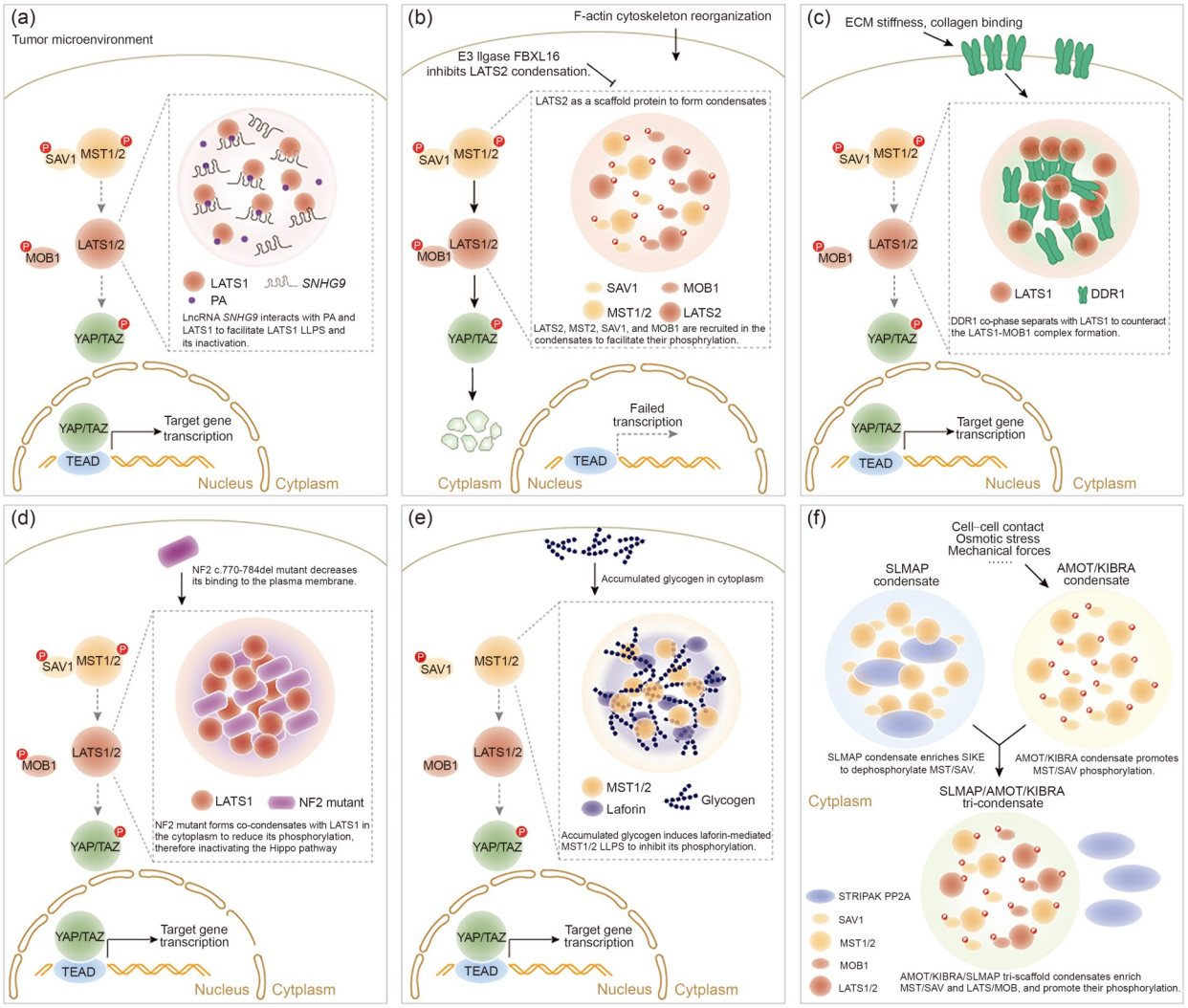

Fig. 2 Regulation of the Hippo pathway by biomolecular condensates in the cytoplasm. (a) In the tumor microenvironment, long non-coding RNA (lncRNA) *SNHG9* interacts with phosphatidic acid (PA) and large tumor suppressor 1 (LATS1) to facilitate LATS1 liquid–liquid phase separation (LLPS) and its dephosphorylation. (b) LATS2, mammalian Ste20-like kinase 1/2 (MST1/2), Salvador 1 protein (SAV1), and MOBKL1A/MOBKL1B (MOB1) are recruited into the condensates to facilitate their phosphorylation and Hippo activation. (c) Extracellular matrix (ECM) stiffness/collagen-induced discoidin domain receptor 1 (DDR1) co-phase separates with LATS1 to inhibit the formation of LATS1-MOB1 complex. (d) Neurofibromin 2 (NF2) mutant decreases its binding to the plasma membrane and forms co-condensates with LATS1 in the cytoplasm to restrict its phosphorylation, inactivating the Hippo pathway. (e) In liver tumors, accumulated glycogen induces laforin-mediated MST1/2 LLPS to constrain its phosphorylation and contribute to tumor initiation. (f) Sarcolemmal membrane-associated protein (SLMAP) forms biomolecular condensate at steady state that concentrates MST for inactivation. Upon cell–cell contact, osmotic stress, or mechanical forces, angiomotin (AMOT)/kidney and brain expressed protein (KIBRA) undergoes phase separation to promote MST/SAV phosphorylation. Long-term stimulation induces AMOT/KIBRA condensates to coalesce with SLMAP condensates, inhibiting striatin-interacting phosphatase and kinase (STRIPAK)-protein phosphatase 2A (PP2A) function. TEAD: transcriptional enhanced associate domain; FBXL16: F-box and leucine-rich protein 16; del: deletion; P: phosphorylation; YAP: Yes-associated protein; TAZ: transcriptional coactivator with post-synaptic density-95, disks-large, and zonula occludens-1 (PDZ)-binding domain.

low sequence similarity. Nonetheless, further investigations are needed to compare their condensation capabilities and elucidate the detailed mechanisms underlying their distinct roles in Hippo pathway activation.

In addition to functioning as scaffolding proteins that initiate condensate formation, LATS1/2 can also act as client molecules incorporated into condensates formed by other regulators. For example, discoidin

domain receptor 1 (DDR1), a collagen-binding receptor tyrosine kinase involved in mechanosensing in vascular smooth muscle cells, has recently been shown to regulate Hippo signaling through phase separation (Fig. 2c) (Liu et al., 2023). Upon stimulation by stiffness or collagen binding, DDR1 undergoes phase separation and forms coacervates with LATS1. Within these condensates, DDR1 interacts with LATS1 and prevents its binding to MOB1, a key regulator of LATS1 activation. These coordinated events inhibit LATS1 activity, leading to YAP activation and the subsequent expression of genes involved in cell proliferation and extracellular matrix (ECM) remodeling. Similarly, neurofibromin 2 (NF2), a tumor suppressor frequently mutated in neurofibromatosis type 2, has been found to sequester LATS1/2 within phase-separated condensates (Fig. 2d) (Jia et al., 2022). This sequestration restricts LATS1/2 localization to the plasma membrane, preventing their activation and contributing to Hippo pathway inactivation. The above findings provide a mechanistic link between NF2 dysfunction and the pathogenesis of neurofibromatosis type 2.

Beyond LATS1/2, other signaling kinases and regulators of the Hippo pathway have also been shown to exert their functions via phase separation mechanisms. During liver malignant transformation, accumulated glycogen forms liquid-like condensates that sequester MST1/2, thereby preventing YAP phosphorylation (Fig. 2e) (Liu et al., 2021). This aberrant YAP activation promotes liver enlargement and tumorigenesis, revealing the previously unrecognized role of glycogen accumulation in this process. In addition, the Hippo pathway-positive regulators angiomotin (AMOT) and kidney and brain expressed protein (KIBRA) can independently or cooperatively form condensates under conditions of high cell density and osmotic stress in cultured mammalian cells (Fig. 2f). These condensates recruit core upstream kinases of the Hippo pathway, creating a signaling hub that facilitates Hippo activation (Wang et al., 2022). Furthermore, AMOT/KIBRA condensates can coalesce with Hippo-inactivating sarcolemmal membrane-associated protein (SLMAP) condensates into a common phase, mitigating the inhibitory effects of striatin-interacting phosphatase and kinase (STRIPAK)-protein phosphatase 2A (PP2A) on Hippo signaling. Thus, unlike other cases where condensate formation and dissolution regulate activity, condensate function can also be modulated through a multiphase coalescence mechanism without dissolution (Wang et al., 2022). Notably, the phase separation behavior by AMOT and KIBRA has also been observed in *Drosophila* and organoid systems, highlighting its evolutionary conservation across species (Bonello et al., 2023).

Finally, the tumor suppressor Merlin has been shown to form solid-like condensates that are essential for Hippo pathway activation. The formation of Merlin condensates is regulated by phosphatidylinositol-4-phosphate (PI4P)-mediated plasma membrane targeting, and their solid-like material properties are critical for exhibiting their function (Guo et al., 2024). These intriguing findings link the material properties of condensates to the regulation of signal transduction, providing novel insights into how the physical characteristics of biomolecular assemblies influence cellular signaling.

4 Regulation of the Hippo pathway by biomolecular condensates in the nucleus

Like many other signaling pathways, the Hippo signaling cascade integrates various upstream stimuli to ultimately regulate the transcriptional coactivators YAP and TAZ, which function to stimulate the expression of target genes in the nucleus. While the previous section discussed the roles of biomolecular condensates in regulating Hippo signaling in the cytoplasm, now we turn our focus on how phase separation impacts the transcriptional activation of YAP and TAZ in the nucleus (Fig. 3).

It has been reported that YAP rapidly forms condensates in both the cytoplasm and nucleus in response to hyperosmotic stress (Fig. 3a) (Cai et al., 2019). This phase separation ability of YAP is driven by its C-terminal low-complexity domain (LCD) that contains a transcription activation domain (TAD). While the function of cytoplasmic YAP condensates remains unclear, it is known that nuclear YAP condensates recruit TEAD1, RNA polymerase II (Pol II), and other transcriptional co-activators to promote the transcription of target genes. Apart from hyperosmotic stress, YAP formed nuclear condensates in a mouse lung adenocarcinoma model following anti-programmed cell death protein-1 (anti-PD-1) immunotherapy (Yu M et al., 2021). Mechanistically, the

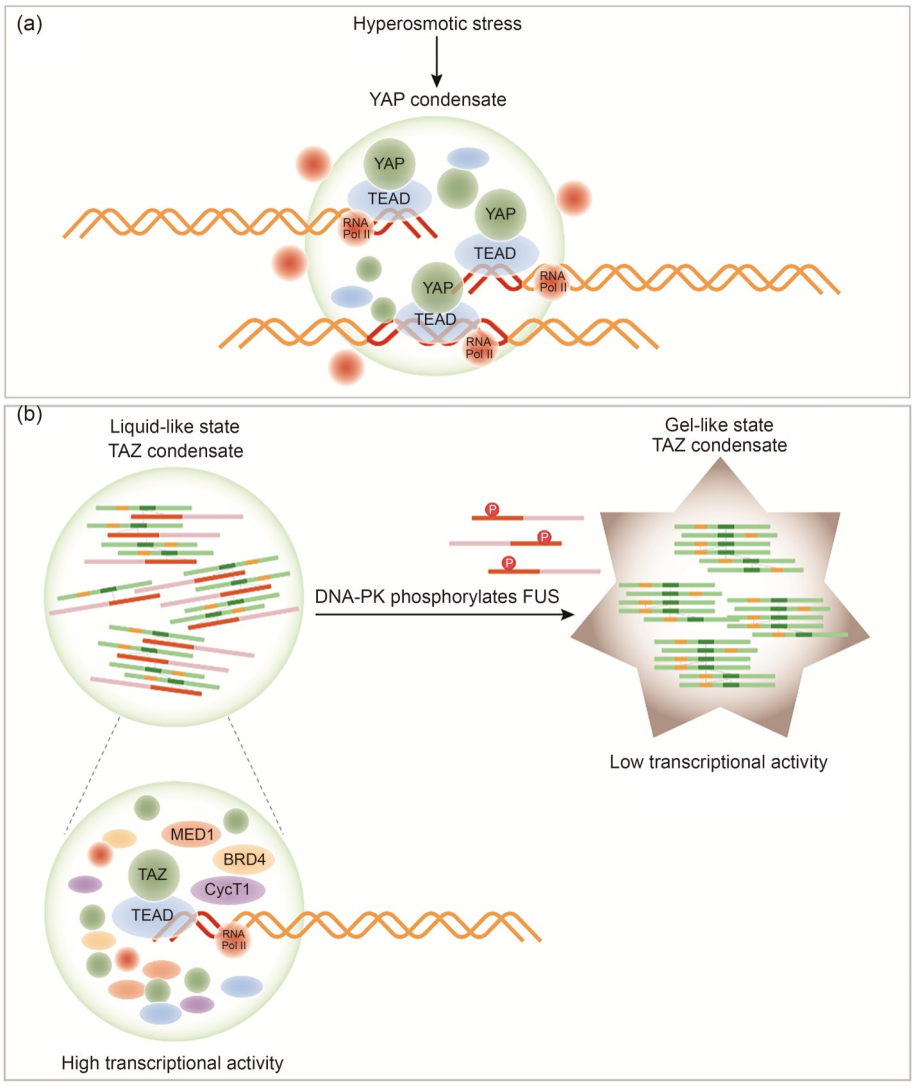

Fig. 3 Regulation of the Hippo pathway by biomolecular condensates in the nucleus. (a) Upon hyperosmotic stress or interferon-γ (IFN-γ) stimulation, Yes-associated protein (YAP) forms condensates in the nucleus. These condensates enrich RNA polymerase II (Pol II), transcriptional co-factors such as transcriptional enhanced associate domain (TEAD), and accessible chromatin regions to promote target gene transcription. The red segment of DNA represents the enhancer region. (b) Liquid-like transcriptional coactivator with post-synaptic density-95, disks-large, and zonula occludens-1 (PDZ)-binding domain (TAZ) condensates are highly dynamic, recruiting transcription factors such as TEAD, cyclin-dependent kinase 9 (CDK9), and CDK9-cyclin T1 (CycT1) to cooperatively enhance target gene transcription. Protein fused in sarcoma (FUS) sustains the transcriptional activity of TAZ condensates by maintaining their liquidity. However, under the conditions of FUS depletion or DNA-dependent protein kinase (DNA-PK)-mediated FUS phosphorylation, which disrupts the interaction of FUS with TAZ, these condensates transition into a gel-like state, leading to reduced transcriptional activity. BRD4: bromodomain-containing protein 4; MED1: mediator subunit 1.

upregulation of interferon-γ (IFN-γ) within the tumor microenvironment during immunotherapy stimulates YAP nuclear condensation. These YAP condensates act as transcriptional hubs for target genes, contributing to IFN-γ-dependent adaptive resistance in tumor immunotherapy. Altogether, these findings demonstrate that YAP condensation is a mechanism adopted by cells to regulate gene expression under stress conditions. However, whether a common stress-sensing mechanism exists to drive YAP phase separation in response to different types of stimuli remains an open question.

Recent studies have also underscored the underlying role of phase separation in tumorigenesis in YAP-fusion-induced cancers. For instance, YAP1-mastermind-like domain containing 1 (MAMLD1) and C11ORF95-YAP1, two recurrent YAP fusions identified in patients

with supratentorial hemispheric ependymomas, have been shown to contribute to ependymoma tumorigenesis through a phase separation mechanism. Within these YAP fusion condensates, transcription factors and co-activators such as bromodomain-containing protein 4 (BRD4), mediator subunit 1 (MED1), and TEAD are enriched, whereas repressive regulators such as polycomb repressive complex 2 (PRC2) are excluded, leading to the induction of long-range enhancer–promoter interactions that promote transcription and oncogenic programs (Hu et al., 2023; Chung et al., 2024).

Interestingly, although YAP and TAZ share extensive sequence similarities, recent studies have demonstrated their distinct phase separation behaviors. Compared to YAP, TAZ has a much higher propensity to undergo phase separation and constitutively form condensates in the nucleus (Lu et al., 2020). Domain mapping analysis revealed that both the WW domain and the coiled-coil (CC) domain are required for TAZ phase separation, with the latter likely accounting for its differential phase separation properties when compared to YAP.

Moreover, the formation of TAZ condensates is tightly regulated by upstream Hippo signaling. The phosphorylation of TAZ by LATS1/2 inhibits its phase separation, preventing the formation of nuclear condensates. However, in its unphosphorylated form, TAZ enters the nucleus, where it undergoes phase separation and forms condensates that act as transcriptional hubs. Similar to YAP condensates, TAZ condensates compartmentalize various transcriptional co-factors and facilitate the activation of target gene expression, further illustrating the role of phase separation in fine-tuning the transcriptional outputs of the Hippo pathway (Lu et al., 2020; Wei et al., 2021).

5 Biophysical property regulation of TAZ condensates by FUS links to transcriptional activation

While significant progress has been made recently in uncovering the role of YAP/TAZ condensates in transcriptional activation, several outstanding questions remain unanswered: What is the full composition of YAP/TAZ condensates beyond the known transcription factors and coactivators? How are YAP/TAZ condensates dynamically regulated in response to cellular stresses? Are these yet-to-be-identified factors essential for the dynamicity and function of YAP/TAZ condensates? Our recent research provides some important clues on these questions (Shao et al., 2024).

Since protein–protein interactions within biomolecular condensates are typically dynamic and transient, we began our investigation by developing a novel strategy based on cross-linking mass spectrometry (XL-MS) to capture and analyze TAZ condensates. The new cross-linker used in our study, named long-*N*-hydroxysulfosuccinimide and aryl sulfonyl fluoride cross-linker (L-NHSF), features a flexible and extended linker between its functional cross-linking modules. This design enhances the capture of intermolecular interactions, making it ideal for detecting weak and transient interactions under phase separation conditions. To determine the TAZ condensate interactome, we reconstituted TAZ condensates in the presence of cell extracts, fixed them with L-NHSF, and then affinity-purified the condensates for subsequent mass spectrometry analysis. Using this innovative "coacervate-and-lock" strategy, we were able to identify several previously unrecognized components of TAZ condensates. Among these, we focused on FUS for further functional characterization due to its established role in modulating phase separation (Murthy et al., 2019).

Our analysis of FUS in TAZ condensates revealed several intriguing findings. Firstly, FUS associates with TAZ condensates via its N-terminal LCD, which directly interacts with the TAZ CC domain. Given that the CC domain is critical for TAZ phase separation, FUS appears to inhibit TAZ condensation, likely by reducing the multivalency required for TAZ phase separation. Secondly, the interaction between FUS and TAZ is highly dynamic and is regulated by phosphorylation. When the S/G-Y-S/G motifs in the FUS LCD are phosphorylated by DNA-dependent protein kinase (DNA-PK), FUS loses its ability to associate with TAZ and modulate TAZ phase separation. Finally, by measuring the biophysical properties of TAZ condensates in the presence or absence of FUS, we surprisingly discovered the chaperone-like role of FUS in regulating the dynamicity of TAZ condensates. The disruption of this function caused TAZ condensates to transition into a gel/solid-like state, accompanied by reduced transcriptional activity and diminished pro-oncogenic functions.

In summary, our work combines an improved cross-linker (L-NHSF) with mass spectrometry to identify interacting proteins within biomolecular condensates, a strategy with broad potential for studying other liquid–liquid phase separation (LLPS)-driven condensates. Importantly, we uncovered a chaperone-like function of FUS in maintaining TAZ condensate dynamics, which promotes its transcriptional activity (Fig. 3b). Overall, these findings advance our understanding of the link between the biophysical properties of condensates and their functional regulation.

6 Biophysical properties—a new twist in condensate regulation

Like TAZ, accumulating evidence points to the tight connection between the material properties of condensates and their functional activities. For example, estrogen-induced estrogen receptor α (ERα) transcriptional condensates initially activate target genes through LLPS at the enhancers. However, prolonged estrogen stimulation triggers a pathological liquid-to-gel transition of ERα condensates, a process associated with transcriptional suppression (Nair et al., 2019). Similarly, stress-responsive heat shock factor 1 (HSF1) forms reversible condensates to enable rapid cytoprotective gene activation, whereas their solidification abrogates this adaptive response (Gaglia et al., 2020). During oncogenic transformation, A-kinase anchoring protein 95 (AKAP95) assembles transcriptionally active condensates that coordinate gene activation and RNA splicing. However, these functions are markedly compromised when AKAP95 condensates lose their dynamics (Li et al., 2020). Moreover, the abnormal phase separation of RNA-binding proteins such as FUS, transactive response (TAR) DNA-binding protein 43 (TDP-43), and heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1)—often caused by mutations or aberrant post-translational modifications—leads to pathologically irreversible aggregates, a hallmark of neurodegenerative diseases (Kim et al., 2013; Murakami et al., 2015; Conicella et al., 2016).

While changes in the biophysical properties of condensates are linked directly to functional alterations, inherent differences in these properties may also underlie functional distinctions between protein homologs. A recent study has demonstrated that ubiquitously transcribed tetratricopeptide repeat on chromosome X (UTX, encoded by lysine-specific demethylase 6A (KDM6A)) and its Y chromosome homologue, UTY, form condensates with distinct material states and functional outcomes (Shi et al., 2021). While UTX forms liquid-like condensates that support its tumor-suppressive functions, UTY condensates adopt a more gel-like, less dynamic state, potentially contributing to its lower tumor-suppressive activity. Notably, cancer-associated UTX mutations do not necessarily inhibit the ability of UTX to form condensates but instead alter its material properties by reducing condensate dynamics and diffusion rates, ultimately impairing its tumor-suppressive activity.

Beyond extrinsic factors, such as temperature and pH, and intrinsic determinants, such as post-translational modifications and mutations, research increasingly suggests that condensate biophysical properties are also shaped by context-dependent protein interactors. As anticipated, heat shock proteins (HSPs) have been implicated in modulating condensate properties (Liu et al., 2020; Gu et al., 2021; Yu et al., 2021; Lu et al., 2022). For example, HSP27 prevents FUS condensates from aggregating into amyloid structures, while HSP70 and HSP27 regulate the phase behavior of TDP-43. In addition to molecular chaperones, other biomolecules may also exhibit chaperone-like functions in condensate regulation. To this end, our recent work identified FUS as an essential regulator that maintains the liquid-like properties of TAZ condensates, ensuring optimal transcriptional activation (Shao et al., 2024). This regulatory paradigm extends beyond proteins; for instance, a recent study has uncovered that the lncRNA *DNAJC3-AS1* prevents the excessive aggregation of fibrillarin (FBL) condensates, thereby maintaining their pre-ribosomal RNA (pre-rRNA) processing function (Sun et al., 2024).

Importantly, the impact of condensate material states on the functional outcome is context-dependent. In other words, not all condensates require a liquid-like state for optimal function. This concept is well illustrated by condensates involved in Hippo signaling. While many Hippo-related condensates, including those of TAZ, rely on liquid-like properties for proper function, Merlin regulates Hippo signaling by forming solid-like condensates (Guo et al., 2024). This unique material state is not only crucial for Merlin's medial apical localization and physiological function,

but also provides a protective mechanism against external perturbations, ensuring signaling stability in response to environmental fluctuations.

7 Concluding remarks

Over the past few years, significant progress has been made in understanding how the Hippo signaling pathway is regulated by phase separation. In the cytoplasm, core upstream kinases either assemble or are recruited into condensates to spatially organize Hippo activity in response to various stimuli. In the nucleus, YAP/TAZ condensates enrich transcription machinery and cofactors in spatially defined compartments, contributing to robust transcriptional activation. Despite these advances, studies on the regulatory mechanisms governing these condensates and their implications under physiological and pathological conditions are still in their infancy.

While the list of condensates involved in Hippo signaling regulation is rapidly expanding, their detailed compositional characterization is lagging, limiting our comprehension of their regulatory mechanisms. This is largely due to the weak and dynamic nature of protein–protein interactions within biomolecular condensates, posing challenges for identification using conventional biochemical purification methods. Our recent work addressed this challenge by employing XL-MS to map the interactome of TAZ condensates, which yielded the successful identification of FUS as a key regulator of TAZ condensate dynamics essential for transcriptional activation. In addition to XL-MS, proximity-dependent biotinylation techniques have recently been employed to analyze the composition of membrane-less organelles under in situ conditions (Markmiller et al., 2018; Frattini et al., 2021). Future studies integrating these approaches will be crucial for further elucidating condensate composition and regulation across diverse contexts.

Notably, it has been well established that TAZ activity is tightly regulated at the protein level. Under active Hippo signaling, TAZ undergoes continuous degradation via the ubiquitin-proteasome pathway, maintaining low protein levels; only when Hippo signaling is inactivated does TAZ accumulate in the nucleus and initiate gene transcription. Considering this, FUS may serve in a dual regulatory role, acting as a "rheostat" to fine-tune TAZ-dependent transcriptional activity. When nuclear TAZ levels are low, caused by either transient Hippo inactivation or accidental evasion from degradation, the FUS-mediated inhibition of TAZ condensation may suppress its target gene expression, keeping TAZ in a "poised" state that does not immediately trigger transcription upon nuclear entry. Conversely, when Hippo signaling is persistently inactivated and nuclear TAZ accumulates, FUS may function as a chaperone, preserving condensate dynamics and supporting robust transcriptional activation. While this proposed dual role of FUS in regulating TAZ condensation and transcriptional activity was not experimentally validated in our recent work, it remains an intriguing direction for future investigation.

Finally, given the strong link between TAZ dysregulation and disease, our study provides insights into potential therapeutic strategies targeting TAZ condensation. Firstly, we established that the CC domain exerts a dominant-negative effect on TAZ phase separation by disrupting multivalent interactions. Thus, a "dissolving strategy" utilizing a CC domain-mimicking peptide could effectively block TAZ condensation and suppress target gene expression. Secondly, since TAZ condensate dynamics are crucial for its function, a "hardening strategy" that selectively inhibits FUS chaperone activity within TAZ condensates could represent an alternative approach to inactivate TAZ function. Future research can focus on perturbing the biophysical properties of TAZ condensates. This will enhance our understanding of biomolecular condensates from a biophysical perspective and hold great promise for translational applications in combating TAZ-driven diseases.

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Author contributions

Huasong LU supervised the overall framework design, critically revised the manuscript, and provided guidance on figure visualization. Yangqing SHAO conducted comprehensive literature research, created all illustrations, and drafted the primary version of the manuscript. Yitong ZHANG specifically wrote the section on "Regulation of the Hippo pathway

by biomolecular condensates in the cytoplasm." Wenxuan ZHU contributed to the systematic review of phase separation mechanisms and authored the section on "Regulation of the Hippo pathway by biomolecular condensates in the nucleus." All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Yangqing SHAO, Yitong ZHANG, Wenxuan ZHU, and Huasong LU declare that they have no conflicts of interest.

This review does not involve any studies conducted on human or animal subjects by any of the authors.

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