expression of KDM4 family members, such as KDM4B and KDM4D (which demethylate H3K9me3), enhances the developmental potential of nuclear transfer-derived embryos, which may otherwise show defects in ZGA[15].

Along with the results from Sankar, Lerdrup, Manaf et al.[6], these studies suggest that the active removal of H3K9 methylation is broadly required for cellular reprogramming, including during the most important reprogramming event in development, the maternal-to-zygote transition. ❐

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Competing interests

The authors declare no competing interests.

SIGNALLING

Check for updates

YAP/TAZ phase separation for transcription

YAP and TAZ, paralogous mammalian genes, act as the key transcriptional effectors of the Hippo pathway. Two recent reports show that both YAP and TAZ form liquid–liquid phase-separated bodies that promote gene transcription by engaging in super-enhancers.

J. Matthew Franklin and Kun-Liang Guan

Although subcellular membraneless bodies were identified with the use of light microscopes, recognition that phase separation underlies the formation and function of such structures has been a relatively recent discovery that has expanded into a burgeoning field[1,2]. Two recent reports, including one in this issue of *Nature Cell Biology*, provide evidence that the Hippo pathway transcriptional co-activators YAP and TAZ can phase separate, through distinct modes, to regulate downstream transcription[3,4].

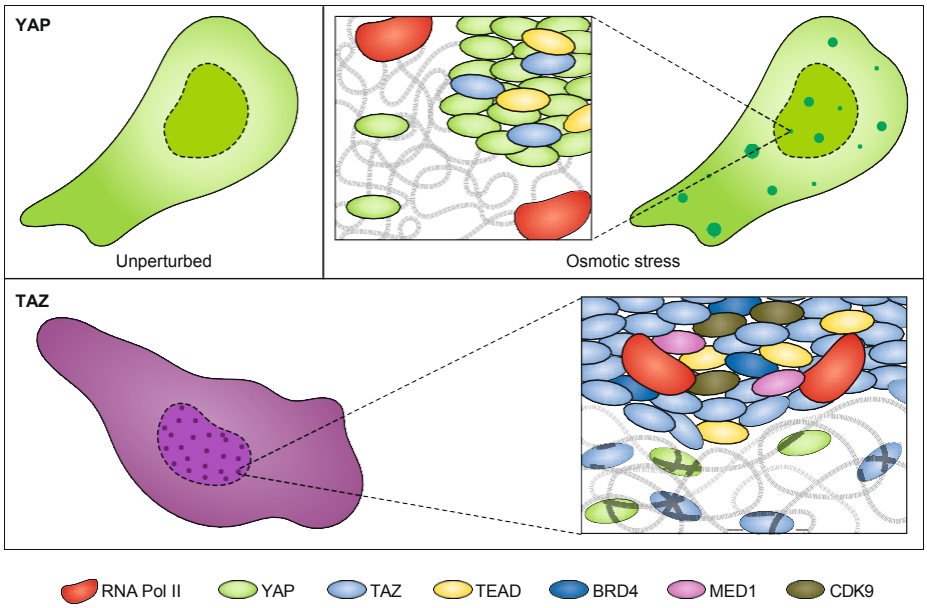
Phase separation is a thermodynamic phenomenon wherein a multicomponent mixture reaches a lower energy state by partitioning of components into physically distinct phases. The physical conditions that lead to macromolecular phase separation are sharply dependent on component concentration and composition, as well as buffer conditions. This allows biological systems to control phase separation through small changes in response to external cues[2]. However, this also creates many challenges for accurately defining if and when macromolecules are capable of phase separation in the cellular milieu.

Investigation of spatially defined transcription foci led to the recognition of liquid–liquid phase separation as a physical principle behind transcription regulation at super-enhancer regions[5]. Both MED1 and BRD4, two transcription co-activators, contain intrinsically disordered regions (IDRs) that allow the formation of phase-separated super-enhancer regions[5], which are thought to coordinate gene expression to achieve cell identity[5,6].

Interest in the Hippo pathway has surged as a result of the wide-reaching signal inputs that modulate the core pathway components in many biological contexts including development, tissue homeostasis, organ size, and oncogenesis[7,8]. Transcriptional regulation of the Hippo pathway is primarily through control of the translocation of YAP and TAZ into the nucleus, where they interact with the TEAD family of transcription factors[7]. A series of studies identified roles for YAP and TAZ in enhancer and super-enhancers[9–12]. MED1 co-occupies with 87% of YAP-bound regulatory elements, and MED1 recruitment to these sites is YAP dependent, indicating a possible interaction[10]. Similarly, YAP and TAZ recruit BRD4 to super-enhancer regions[11]. Given these relationships, is it possible that YAP and TAZ co-exist in phase-separated transcription factories along with other co-activators such as MED1 and BRD4? Two recent articles in *Nature Cell Biology* begin to answer this question[3,4].

Cai et al. report that YAP forms large, phase-separated condensates in the cytoplasm and the nucleus following osmotic stress and in vitro under crowding conditions[3] (Fig. 1). However, Lu et al. report that TAZ has a stronger ability to form phase-separated condensates in vitro without crowding agents and in cell culture, suggesting that TAZ constitutively partitions into condensates, driving target gene transcription[4] (Fig. 1).

Cai et al. suggest that the transcriptional activation domain (TAD) is an IDR responsible for the reversible phase separation of YAP under hyperosmotic stress in cell culture[3]. In vivo, fixed tissue samples from the mouse kidney showed significantly more nuclear YAP punctae in medulla cells than in cortical cells, consistent with the fact that the kidney medulla experiences higher osmolarity than the cortex region. Interestingly, using exogenous GFP-YAP expression, Cai et al. found that these large condensates (as large as ~1 μm) formed not only in the nucleus, but also throughout the cytoplasm, co-segregating with YAP kinases LATS and NLK[7,13]. Because phase separation is highly dependent on the physical environment, osmotic stress might induce this condensation, driving a specific stress response. However, the physical characteristics of the condensates created by GFP-YAP exogenous expression are distinct from the smaller and less uniform puncta of native YAP imaged by immunofluorescence and HaloTag genomic knock-in[3]. It should also be noted that previously published work of YAP under equivalent hyperosmotic stress conditions did not report such condensate formation[13]. Thus, further study is needed



Depiction of the findings from Cai et al.[3] and lu et al.[4]. Top, YAP forms phase-separated condensates after osmotic stress, and the YAP condensates co-localise with TAZ and TEAD and show adjacent localisation to rNA Pol ii clusters. Bottom, TAZ constitutively exists as phase-separated condensates with TEAD, BrD4, MED1, CDK9, and rNA Pol ii.

fusions[14] as done with MED1 and BRD4 (ref. [5]). Although both works suggest that the GFP fusion does not cause formation of condensates, native YAP/TAZ and FLAG-tag experiments showed smaller condensates. Further, assessing phase separation in vitro does not require the use of GFP fusion as done with both of these works, as standard DIC optics allow detection of phase-separated condensates without the need for fluorescence.

Both works address the functional link between specific gene transcription and YAP/TAZ condensates in a correlative manner using either qPCR of a few specific targets[3,4] or differential RNA-seq profiling[4]. Although YAP/TAZ gene activation correlated with the ability to form condensates[3,4], and previous data show YAP/TAZ gene targets have enhancer elements[9–12], it remains unknown whether

fluorescence in situ hybridisation survey of target gene localisation with respect to

to validate the claims. It is worth noting that YAP is active in promoting transcription under many physiological conditions without osmotic stress. Therefore, formation of phase-separated condensates is unlikely to be universally required for the co-activator function of YAP.

Cai et al. found that the transcription response to osmotic stress was dynamic[3]. Initially, YAP condensates did not co-localize with transcription machinery. However, after 2 h of stress treatment, YAP co-localized with nascent RNA and target gene expression was increased. The YAP condensates did not completely co-localize with RNA Pol II signals, rather they were found to be adjoining. These GFP-YAP condensates dynamically fused into micron-scale regions over time, leading to questions about which, if any, genomic regions are associated with these condensates. Lastly, the TAD domain of YAP is tied to downstream transcription, making it difficult to separate the ability to form condensates and transcriptional activity.

In this issue of *Nature Cell Biology*, Lu et al. provide evidence that TAZ constitutively exists as nuclear phase-separated condensates[4]. GFP-TAZ partitioned into phase-separated condensates in a concentration- and salt-dependent manner, whereas GFP-YAP did not under the same conditions. Only under molecular crowding did Lu et al. see GFP-YAP phase separate, consistent with the observation by Cai et al.[3]. In living cells, low-level exogenous expression of GFP-TAZ showed condensates in the nucleus. Lu et al. found that immuno- fluorescence of native TAZ showed puncta, but on a smaller size scale. Using protein domain mapping, surprisingly, Lu et al. showed that the coiled-coil domain of TAZ is essential for condensate formation, whereas the WW domain has a partial role. Thus, YAP and TAZ appear to use different domains for phase separation.

In terms of the transcriptional function of TAZ condensates, Lu et al. found that TAZ condensates co-localized with TEAD4 and many of the recognized components of phase-separated transcription hubs (BRD4, MED1, CDK9, RNA Pol II, and H3K4me3), providing support for a role of TAZ in phase-separated super-enhancers. However, TEAD4 did not form nuclear puncta in control cells, but only in GFP-TAZ transfected cells[4]. This suggests that exogenous expression of GFP-TAZ, albeit at low levels, may alter the phase equilibrium of TAZ and other proteins.

Notably, both works make the majority of their claims based on enhanced GFP (eGFP) fusion to YAP and TAZ[3,4]. Given the sharp sensitivity of phase separation to chemical composition and the non-negligible dimerisation of eGFP, proper assessment of protein condensation should be carried out using the monomeric form of fluorophore YAP/TAZ condensates would be useful to link functionality to transcription.

The potential role of YAP/TAZ in regulating transcription through phase-separated condensates offers a previously unappreciated biophysical view and molecular insight into how the Hippo pathway controls gene output. Further experimentation will be needed to both validate these findings and explore the functionality of YAP and TAZ condensates. ❐

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Competing interests

K.L.G. is a cofounder of and has equity interest in Vivace Therapeutics.