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SPLICING

Phasing alternative exons

> Tyr residues… mediate higher-order assembly of RBFOX1 in the nucleoplasm

RNA-binding proteins, including many splicing factors, often contain intrinsically disordered regions (IDRs) and sequences of low amino acid complexity. These regions can undergo liquid–liquid phase separation, leading to the formation of membrane-less organelles, and can foster protein aggregation and the formation of toxic amyloid-like inclusions. Two papers in *Cell* now show that low complexity sequences and IDRs in splicing factors induce the formation of higher-order protein assemblies that can regulate alternative splicing.

Ying *et al.* studied the RNA binding protein fox-1 (RBFOX) family of splicing factors, which contain IDRs and low complexity sequences of unknown function, and which regulate alternative splicing by interacting with the complex large assembly of splicing regulators (LASR). Density gradient sedimentation experiments indicated that RBFOX proteins and LASR not only interact, but that they form organelle-like, higher-order protein assemblies. Further analysis revealed that 13 Tyr residues in the carboxy-terminal domain (CTD) central region (C2) were necessary for the formation of these higher-order assemblies.

The CTD and even the C2 fragment alone formed fibrous, amyloid-like assemblies *in vitro*, but mutating ten of the Tyr residues to Ser abolished aggregation. In cells, wild-type RBFOX1 forms unevenly distributed nuclear speckles, but the Tyr-mutated RBFOX1 or RBFOX1 lacking the CTD exhibited diffused distribution. This suggested that the Tyr residues in the CTD of RBFOX1 mediate higher-order assembly of RBFOX1 in the nucleoplasm.

Next, the effect of higher-order assembly on splicing was tested. In cells lacking endogenous RBFOX, expression of RBFOX1 lacking the CTD was unable to restore alternative-exon exclusion or inclusion in RBFOX target genes, whereas expression of the Tyr-mutated RBFOX1 restored alternative-exon exclusion but not inclusion. This indicated that the formation of higher-order assemblies by RBFOX proteins is required for the inclusion of alternatively spliced exons in RBFOX target genes.

In another study, Gueroussov *et al.* found that multiple C-terminal exons in heterogeneous nuclear ribonucleoprotein (hnRNP) genes undergo mammalian-specific alternative splicing; these exons overlap with IDRs that contain low complexity Gly and Tyr (GY)-rich motifs. The interactions between any of the proteins HNRNPD, HNRNPAB and HNRNPA1 was dependent on both partners containing their GY-rich alternative exons. Furthermore, although wild-type HNRNPD can localize to nuclear foci, HNRNPD lacking its GY-rich alternative exon, or HNRNPD in which the Tyr residues in this exon were mutated to Ser, did not localize to foci (similar observations were made with HNRNPA1). Moreover, inclusion of the GY-rich exon enhanced liquid–liquid phase separation by HNRNPA1 *in vitro*. Thus, GY-rich alternative exons of hnRNPs control the formation of large protein assemblies in a Tyr-dependent manner.

Depletion of different hnRNPs showed that they have combinatorial as well as overlapping functions in controlling alternative splicing programmes, which reflect the similarities in their RNA-binding specificities and in their C-terminal IDR sequences. Importantly, the outcome of alternative splicing was dependent on the presence of hnRNP GY-rich exons and thus on their capacity to form higher-order protein assemblies.

Interestingly, as three of the LASR subunits are hnRNPs (albeit not those studied by Gueroussov *et al.*), it would be interesting to test whether and how they might influence the formation of higher-order assemblies of RBFOX–LASR.

Together, these data reveal a new mechanism of controlling alternative splicing through phase separation-mediated formation of higher-order assemblies of splicing factors. This could potentially concentrate and bring together different splicing components.

Eytan Zlotorynski

DIGITAL VISION

ORIGINAL ARTICLES Ying, Y. *et al.* Splicing activation by Rbfox requires self-aggregation through its tyrosine-rich domain. *Cell* **170**, 312–323.e10 (2017) | Gueroussov, S. *et al.* Regulatory expansion in mammals of multivalent hnRNP assemblies that globally control alternative splicing. *Cell* **170**, 324–339.e23 (2017)
FURTHER READING Banani, S. F., Lee, H. O., Hyman, A. A. & Rosen, M. K. Bimolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* **18**, 285-298 (2017)