kin of the father (Haig, 1997). For example, one parental genome may see a benefit to higher expression of a certain gene product whereas the other may see it as a negative determinant. Within an embryo or an adult individual, the maternal and paternal alleles of a gene can therefore "disagree" over what is the optimal expression level. Increased dosage may favor the maternal kinship, and a decrease may favor the paternal kinship, or vice versa. The resulting "parental conflict" gradually leads to gene expression from one parental allele only. The parental conflict theory also predicts that genes expressed from only one parental genome may antagonize imprinted genes that are expressed from the other parental genome (Patten et al., 2016). The emerging data on the imprinted miRNAs fulfill these predictions and would therefore agree with the parental conflict theory.

In future research, it will be interesting to pinpoint the targets of the imprinted miRNAs in other relevant cell types besides neurons. Furthermore, antagonistic effects between maternally and paternally expressed genes can also be mediated by imprinted long ncRNAs (lncRNA), and several examples of this have been discovered (Patten et al., 2016). In neurons, one study reported that an imprinted, paternally expressed lncRNA (called IPW) derived from another chromosome downregulates *in trans* the maternally expressed *Meg3* polycistron (comprising miR-379/410) (Stelzer et al., 2014). In mammals, the relationship between the parental genomes is complicated and rather conflicted, and future research will likely reveal further levels of complexity.

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The Interlocking Lives of LARP7: Fine-Tuning Transcription, RNA Modification, and Splicing through Multiple Non-coding RNAs

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Elegant studies by Hasler et al. (2020) and Wang et al. (2020) uncover a novel role of LARP7 in facilitating the 2′-O-methylation of the spliceosomal U6 snRNA, which is functionally required for fidelity of pre-mRNA splicing and development of male germ cells.

Eukaryotic cells can fine-tune expression of protein-coding genes at any step along the way, from transcription and precursor mRNA (pre-mRNA) processing to nucleocytoplasmic export, degradation, and translation of mRNA. Not surprisingly, many human diseases and syndromes, including cancer, autoimmunity, and neurological disorders, stem from

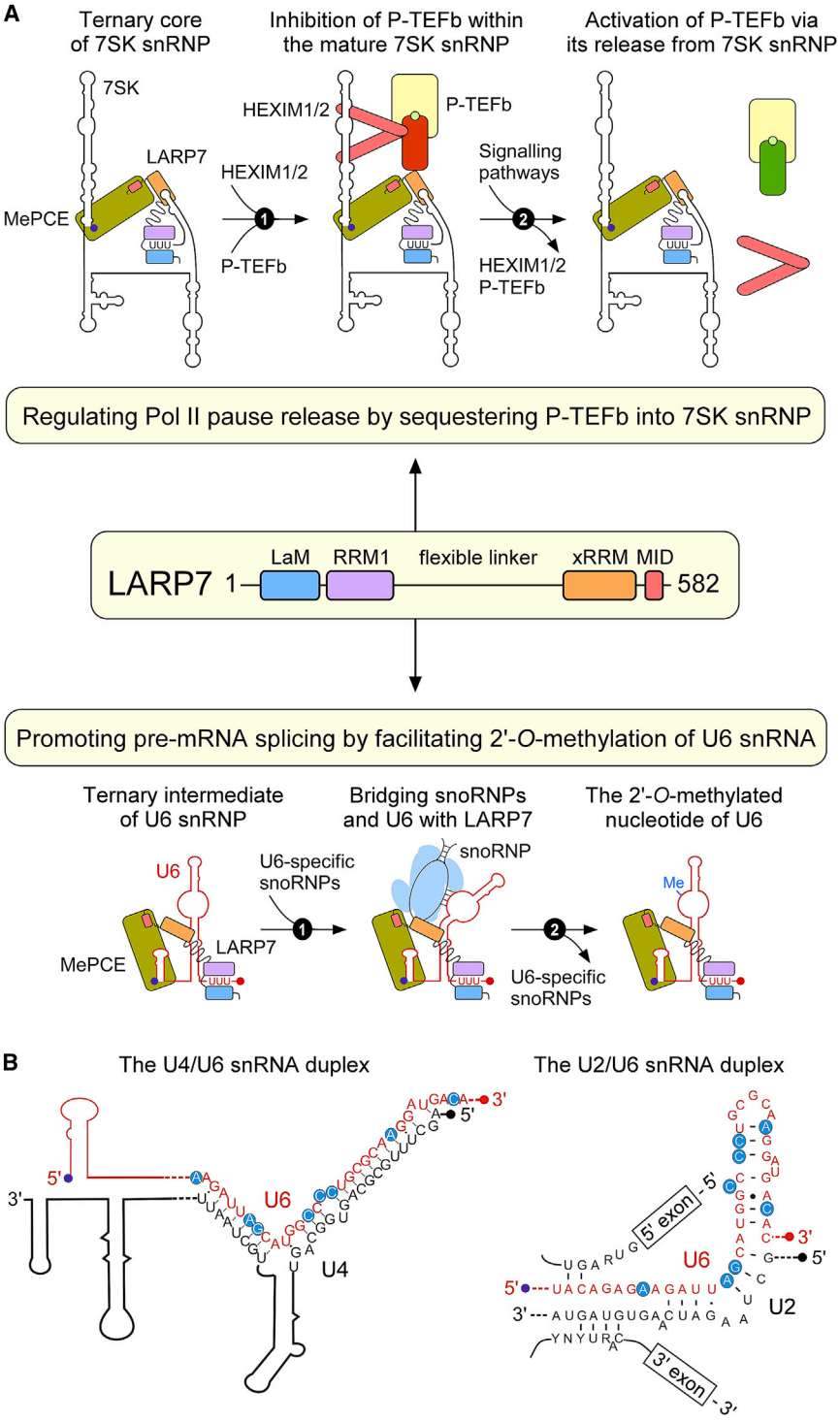

Figure 1. Dual Functions of LARP7

(A) Schematic representation of the role of LARP7 in regulating RNA Pol II transcription via 7SK snRNP and promoting pre-mRNA splicing via facilitating the 2′-*O*-methylation of U6 by U6-specific snoRNAs. The hypothetical interaction of LARP7 with MePCE within the intermediary U6 snRNP (bottom) is depicted.

(B) Locations of the U6 nucleotides requiring LARP7 for their 2′-*O*-methylation in the U4/U6 duplex (left) and the U2/U6 duplex within the pre-catalytic spliceosome (right). Magenta, red, and black circles at the snRNA termini represent the 5′ end γ-monomethylphosphate, the 3′ end cyclic phosphate, and the 5′ end 2,2,7-trimethylguanosine cap, respectively.

perturbations in gene expression programs, which can be caused by the inability of regulatory factors to execute their mission. Among them, members of a large family of RNA-binding proteins (RBPs) that interact with target RNAs via the dedicated RNA-binding domains can affect all steps of gene expression. In a recent issue of *Molecular Cell*, the two studies by Meister, Fischer, and Liu laboratories reported an exemplary case of the RBP-driven control that could go awry (Hasler et al., 2020; Wang et al., 2020). The authors focused on La-related protein 7 (LARP7), of which reduced levels and loss-of-function gene mutations have been linked to cancer and Alazami syndrome, a severe developmental disorder. Rather surprisingly, both works show that LARP7, which regulates transcription by RNA polymerase (Pol) II through targeting 7SK snRNA (7SK) (He et al., 2008; Krueger et al., 2008), also acts as an auxiliary factor promoting the ribose 2′ hydroxyl group methylation (2′-*O*-methylation) of the spliceosomal U6 snRNA (U6) at all known sites via the U6-specific small nucleolar RNAs (snoRNAs). This exciting finding reveals novel interconnections between transcription, RNA modification, and pre-mRNA splicing machineries that have an impact on pre-mRNA splicing and gene expression at large.

LARP7 belongs to the La and La-related protein (LARP) superfamily of RBPs that orchestrate major phases of RNA metabolism, from synthesis and pre-mRNA maturation to translation. It comprises an N-terminal La module, containing a Lupus antigen motif (LaM) and a canonical RNA recognition motif (RRM1), a 256-aa flexible linker, a C-terminal domain with an atypical RRM (xRRM; also referred to as RRM2), and the final 31-aa long α-helical extension containing the MePCE interaction domain (MID) (Figure 1A, middle). Previous studies have established a definitive role of LARP7 in regulating transcription by RNA Pol II through facilitating inhibition of the positive transcription elongation factor b (P-TEFb; also referred to as CDK9/CycT heterodimer) (reviewed in Quaresma et al., 2016). To do so, LARP7 interacts with the scaffolding RNA Pol III-transcribed 7SK and its 5′ γ-methylphosphate capping enzyme MePCE to

form the core of the 7SK small nuclear ribonucleoprotein (7SK snRNP). Subsequently, this ternary core enables the 7SK-interacting HEXIM1/2 proteins to bind and inhibit P-TEFb, the critical transcriptional kinase that, once released from 7SK snRNP, stimulates the transition of RNA Pol II from promoter-proximal pausing to productive elongation at most genes (Figure 1A, top). Within the core and the fully assembled 7SK snRNP, MePCE and LARP7 protect 7SK from nucleolytic degradation. Whereas MePCE interacts with the 5′ hairpin of the nascent 7SK to catalyze its capping, LARP7 utilizes LaM and RRM1 motifs to bind the UUU-3′OH tail of 7SK. In addition, xRRM of LARP7 interacts with the 3′ hairpin of 7SK. This in turn triggers the interaction of the C-terminal MID of LARP7 with the 7SK cap-bound MePCE, resulting in MePCE inhibition, which effectively prevents 7SK de-capping and concludes formation of the core of 7SK snRNP. Finally, binding of LARP7 with the 3′ hairpin of 7SK facilitates the sequestration of P-TEFb into 7SK snRNP. Illustrating the significance of LARP7 in transcription and splicing, decrease in its levels, which triggers disintegration of 7SK snRNP and the subsequent activation of P-TEFb, stimulates transcription of key epithelial-mesenchymal transition regulators in breast cancer and modulates alternative pre-mRNA splicing. Furthermore, transient depletion of 7SK, which recapitulates the activation of P-TEFb upon the loss of LARP7, augments nascent transcription at promoter-proximal pause sites and enhancers globally in embryonic stem cells.

The two studies are now redefining the function of LARP7 by adding a novel layer of interactions that expand its role beyond transcription. They present compelling evidence that, in addition to 7SK, LARP7 binds the RNA Pol II-transcribed C/D box snoRNAs, which function as guides directing the 2′-*O*-methylation of dedicated RNA targets. Excitingly, LARP7 interacts with only a subset of snoRNAs guiding the modification of U6 (Tycowski et al., 1998), the central catalytic snRNA of the spliceosome. Gratifyingly, and in agreement with an earlier study (Krueger et al., 2008), the RNA Pol III-transcribed U6, but no other spliceosomal snRNAs, interacts with LARP7. In more detail, LARP7, U6, and the specific snoRNAs form ternary complexes, wherein LARP7 employs its La module to interact with the newly formed U6 and its xRRM to bind the snoRNAs containing an evolutionarily conserved LARP7 binding box. Moreover, the study by Wang et al. (2020) shows that LARP7 associates with the protein subunits of the mature C/D box snoRNP. Consequently, the loss of LARP7 leads to deficient 2′-*O*-methylation of U6 at all known sites without affecting the levels of U6 and snoRNAs or formation of their RNPs. Thus, the novel function of LARP7 emerging from these studies is that of a molecular bridge connecting the U6-specific methylation guide snoRNAs and their target U6, facilitating the loading of U6 onto the snoRNPs, of which methyltransferase fibrillarin catalyzes the U6 2′-*O*-methylation (Figure 1A, bottom). Given that 7SK is required for the high-affinity interaction of LARP7 with MePCE, which in turn ensures stability of LARP7 (Barboric et al., 2009), and that MePCE binds and caps U6 (Yang et al., 2019), it would be interesting to examine if the efficacious 2′-*O*-methylation of U6 requires MePCE as well.

Functionally, the reduction in 2′-*O*-methylation of U6 in the absence of LARP7 leads to a moderate number of changes in the splicing of a subset of introns, which could underlie Alazami syndrome (Hasler et al., 2020) and failed development of male germ cells (Wang et al., 2020). An important question arising from both studies is the mechanistic basis underlying these defects. Although studies on 2′-*O*-methylations of the spliceosomal U2 snRNA (U2) have revealed their contribution to the assembly of U2 snRNP and splicing activity itself (reviewed in Bohnsack and Sloan, 2018), the precise function of the U6 modifications have remained elusive. However, given the unusually high level of intron retention cases detected in both studies, it is plausible that these result from defects in splicing rather than misregulated alternative splicing events. Consistently, of the five snRNA components of the spliceosome, U6 is not only the most conserved and plays a central role in the catalysis of splicing, but the 2′-*O*-methylations guided by the snoRNAs map to the region of U6 that forms the catalytic structures together with its interaction partner, U2 (Figure 1B, right). The small number of affected introns indicates that the 2′-*O*-methylations are not essential for the splicing chemistry. Rather, they might confer fidelity or robustness to splicing by stabilizing the snRNA structures and interactions in the catalytic core of the spliceosome, consistent with the observation by Hasler et al. (2020) that depletion of LARP7 exacerbates the heat shock-induced splicing changes. Alternatively, the loss of 2′-*O*-methylations might affect unwinding kinetics of the U4/U6 duplex during spliceosome activation as many of the modifications map to the U4/U6 stem II (Figure 1B, left). Consistently with present studies, loss of an individual U6 2′-*O*-methylation has been linked to defects or alterations in splicing in a subset of introns (Ogren et al., 2019). The nature of how the possible structural perturbations translate into the observed changes in splicing needs further investigation. Finally, the core of 7SK snRNP serves as a scaffold for the little elongation complex to promote transcription of spliceosomal snRNAs by RNA Pol II (Egloff et al., 2017), expanding the regulatory potential of LARP7 in pre-mRNA splicing.

Somewhat surprisingly, neither study reports any obvious alterations in mRNA levels in stable *LARP7* knockout models, suggesting that the reduced 2′-*O*-methylation of U6 is the sole culprit for the observed functional defects. The lack of transcriptional effects could stem from compensatory mechanisms offsetting the increase of active P-TEFb upon the loss of LARP7, such as the observed decrease of the protein levels of CDK9 (Hasler et al., 2020). Thus, the employed systems, which mimic the nonfunctional *LARP7* in disease, may indeed be valuable for uncovering transcription-independent roles of LARP7. However, they evidently obfuscate expected transcriptional alterations following sudden activation of P-TEFb, which could in turn affect cotranscriptional pre-mRNA processing including alternative splicing. Future studies employing an acute and immediate depletion of LARP7 linked to nascent RNA-sequencing technologies shall clarify this issue.

In summary, these reinvigorating studies have widened our knowledge of LARP7. That specific signaling cascades activated

during genotoxic stress response and megakaryopoiesis impinge upon subunits of the ternary core of 7SK snRNP, including LARP7 raises an intriguing possibility that cells coordinate multiple levels of gene expression through this biomedically relevant RBP (Borisova et al., 2018; Elagib et al., 2013). What is more, it would be fascinating to explore whether perturbed 2′-*O*-methylations in U6 contribute to the genesis of cancers exhibiting diminished expression or function of LARP7. From the RNA-centric vantage point, the full repertoire of RNAs engaged by and/or modified via LARP7 has yet to be identified, which may in turn unearth even broader functional reach of LARP7. Thus, by incorporating into distinct RNPs, the evolutionarily conserved LARP7 may exert a multifaceted regulatory role in gene expression in health and disease.

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