RNAs are versatile molecules capable of an array of functions. In addition to their role as information-carrying intermediaries in gene expression, they act as key catalytic, structural, and regulatory elements in the cell. As highlighted in this issue's Select, recent studies on RNA show how these molecules collaborate with proteins in essential biological processes such as pre-mRNA splicing, mRNA export, and translation control. Additionally, new findings reveal that RNAs can act as enzymes in an efficient, protein-free, self-sustained RNA replication system, providing further support for the notion that life could have originated from an RNA-based world.

### Oskar mRNA and PTB: "Trapped" in Translation



PTB (red) colocalizes with the asymmetrically distributed oskar mRNA (blue) in *Drosophila* oocytes. Image courtesy of F. Besse.

Spatial restriction of protein synthesis can affect cell fate outcomes during development and contribute to the formation of subcellular compartments in differentiated cells. A number of mRNA transcripts are known to be locally translated, including the *oskar* transcript of the fruit fly *Drosophila*. However, the mechanisms that ensure localized translation remain unclear. *Oskar* mRNAs are locally translated during *Drosophila* oogenesis. The mRNAs are transported to specific sites in the developing oocyte and maintained in a translationally repressed state until translation is activated. To gain insight into how *oskar* mRNAs are spatially and temporally regulated, Besse et al. performed a screen using a green fluorescent protein (GFP)-based "protein trap" to identify proteins that colocalize with *oskar* mRNA. The screen uses a transposable element to randomly tag proteins with GFP. Using this approach, they found

that the polypyrimidine tract-binding protein (PTB) colocalizes with *oskar*. Experiments with *ptb* mutants revealed that PTB is required for proper timing of *oskar* localization and translation. Furthermore, native gel electrophoresis and RNA affinity pull-down assays showed that PTB binds directly to the *oskar* 3' untranslated region (UTR). PTB binding mediates oligomerization of *oskar* mRNA into high-order complexes that could be observed by electron microscopy. This work reveals yet another role for the multifunctional PTB protein, which is also involved in pre-mRNA splicing and cap-independent translation initiation of messages containing internal ribosome entry sites. The work also suggests that multimerization of *oskar* mRNA into ribonucleoprotein (RNP) complexes may repress translation by reducing the accessibility of the mRNA to the translation machinery. *F. Besse et al. (2009). Genes Dev. 23, 195–207.* 

# The Yin and Yang of RNA Replication

If DNA is required to make proteins and proteins are required to make DNA, then how were the first proteins made and how was genetic information propagated in early life? RNA may provide answers to these questions because, like DNA, it can carry genetic information and, like proteins, it is capable of catalyzing chemical reactions. These aspects of RNA biology form the basis of the "RNA world" hypothesis, in which the current DNA-protein-dominated world may have been preceded by an RNA-dominated one—a world where RNAs acted both as the primary information carriers and as catalysts. In support of the RNA world hypothesis, RNA enzymes that catalyze RNA-templated RNA polymerization have previously been engineered using "in vitro evolution." Using a similar approach, Lincoln et al. recently described an efficient, self-sustained RNA replication system catalyzed by crossreacting RNA enzymes. In their system, an RNA enzyme, (E), catalyzes ligation of two RNA molecules, (A') and (B'), to produce a complementary enzyme (E'). The (E') enzyme, in turn, catalyzes ligation of (A) and (B) RNAs to produce the original enzyme, (E). Serial transfer experiments show that the system can replicate indefinitely when given a steady supply of RNA substrates. And interestingly, in systems containing complex mixtures of reactants (B1', B2', B3', etc.), some "recombinant" enzymes formed, out-competed the other enzymes, and grew to dominate the population. The selective advantage of the recombinant enzymes was found to be influenced by multiple factors, including their intrinsic catalytic capabilities and their relative resistance to inhibition by substrates in the reaction mixture. This work supports the notion of an ancient RNA world and provides an approach that may be useful for discovering enzymes with new catalytic functions, including functions that may have been important in an early RNA world.

T.A. Lincoln et al. (2009). Science. Published online January 8, 2009. 10.1126/science.1167856.

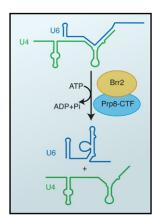
# Pcf11 Helps to Get the Message out

Messenger RNAs must be processed and exported to the cytoplasm, and the factors involved in these processes are connected to one another and to the transcription machinery via a network of interactions. These interactions coordinate mRNA maturation and are important for optimal gene expression. Previous work has established such connections between the transcription machinery and the export machinery, resulting in a model for how mRNAs make the transition between these two processes. In this model, the Yra1 export factor is recruited to actively transcribed genes by Sub2, a component of the Transcription/Export (TREX) complex. In recent work, Johnson et al. challenge this model by showing that Yra1 does

not require Sub2 to access transcription complexes. Rather, it binds directly to a 3′ end processing factor known as Pcf11. The Pcf11-Yra1 interaction is sufficient for Yra1 recruitment and it is conserved in humans, as cross-species interactions were observed. Interestingly, experiments with Yra1 truncation constructs revealed that Pcf11, Sub2, and the export factor Mex67 all bind the same domains of Yra1, suggesting that the interactions between Yra1 and these factors are mutually exclusive. Based on these observations, the authors propose that Yra1 binds 3′ processing complexes directly via Pcf11 and is then transferred to mRNA via its interaction with Sub2. Finally, Yra1 exchanges Sub2 for Mex67 as a prelude to mRNA export. Although the current work provides insight into 3′ processing factors in export, future studies may shed light on whether Yra1 affects transcription, mRNP assembly, and 3′ end processing. S.A. Johnson et al. (2009). Mol. Cell 33, 215–226.

#### Prp8 Brr-idges RNAs and Proteins in the Spliceosome

Pre-mRNA splicing is catalyzed by the spliceosome, a dynamic complex that assembles and disassembles with each round of splicing. Not only is this elaborate process required for substrate recognition and catalysis, but, according to the prevailing model, it is also important for ensuring the accuracy of splicing. During this process, the spliceosome must rearrange itself by forming and breaking numerous RNA-RNA interactions involving the spliceosomal small nuclear RNAs (U1, U2, U4, U5, and U6) and the pre-mRNA substrate. For example, before the first catalytic step of splicing can take place, the U4/U6 snRNA duplex must unwind to allow U6 to refold into its catalytically active form. These RNA rearrangements require the activity of several DExD/H family RNA-dependent ATPases, including Brr2, which has been implicated in U4/U6 unwinding. In recent work, Maeder et al. show that the C terminus of yeast Prp8, an essential splicing factor, is required for the ATP-dependent unwinding of a synthetic U4/ U6 duplex. They show that a C-terminal Prp8 fragment interacts with Brr2 in vitro and that disease-related Prp8 mutations inhibit this interaction. Notably, the Prp8 fragment stimulates Brr2's ability to unwind U4/U6. Surprisingly, however, the Prp8 fragment inhibits Brr2's ATPase activity, indicating that the Prp8 regulates these two functions of Brr2 somewhat independently and may couple these activities in vivo. These findings have implications for understanding splicing fidelity because they provide insight into how the activity of a spliceosomal ATPase might be limited to the correct stage of splicing. This temporal regulation of ATPases is a key feature of the "kinetic proofreading" model for how fidelity is maintained during splicing. In the context of this model, Prp8 would inhibit U4/U6 unwinding until the moment it is required for catalytic activation of the spliceosome. Further studies are needed

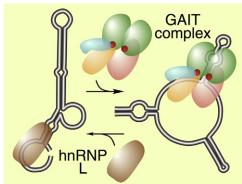


The Brr2 ATPase unwinds U4/ U6 only in the presence of a C-terminal fragment (CTF) of the U5 snRNP protein Prp8. Image courtesy of C. Maeder and C. Guthrie.

to more precisely define the roles of Prp8, Brr2, and other DExD/H ATPases in regulating RNA dynamics and fidelity in the spliceosome.

C. Maeder et al. (2009). Nat. Struct. Mol. Biol. 16, 42-48.

### **GAIT-ing VEGFA Expression with an RNA Switch**



Protein-dependent RNA switch in the VEGFA mRNA 3'UTR integrates discrete environmental signals to regulate gene expression. Image courtesy of P.L. Fox.

Some transcripts are regulated in cis by RNA structures known as riboswitches, which function by altering their conformation in response to a signal, leading to regulation of the host transcript. Most known riboswitches respond to a single input, such as the binding of a metabolite or small molecule. But in recent work, Ray et al. discover a different kind of RNA switch in the 3'UTR of the human VEGFA mRNA. The VEGFA switch is different in that it can respond to multiple inputs that differentially affect VEGFA expression. They show that when macrophages are simultaneously exposed to hypoxia and interferon  $\gamma$  (IFN- $\gamma$ )—two opposing signals that induce and inhibit VEGFA expression, respectively-VEGFA is expressed and the hypoxia signal dominates. It does so by overriding IFN-γ-induced VEGFA repression mediated by the IFN- $\gamma$  activated inhibitor of translation (GAIT) complex, which binds a specific structural element in the VEGFA 3'UTR and inhibits VEGFA translation. The authors show that hypoxia helps to stabilize hnRNP L, which also binds the VEGFA 3'UTR, just upstream of the GAIT-binding site. RNAase probing experiments revealed that the VEGFA 3'UTR exists in two conformations: a "translation-silent" conformation induced by GAIT binding and a "translation-permissive" conformation induced by hnRNP L

binding, which occludes the GAIT-binding site. Therefore, the mutually exclusive binding of hnRNP L and GAIT to the VEGFA mRNA causes a switch between two conformations to affect VEGFA translation. Unlike riboswitches, the VEGFA RNA switch

requires regulatory proteins to interpret upstream signals, adding a layer of regulatory control. Furthermore, given that the switch responds to multiple inputs, it allows the host message to respond to complex signaling environments such as the cores of solid tumors, where hypoxic stress and IFN-γ signaling coexist. Future work may uncover similar RNA switches in other transcripts and may identify others that respond to still more varied and complex signals. P.S. Ray et al. (2008). Nature. Published online December 21, 2008. 10.1038/nature07598.

#### FOX2 in Alternative Splicing: Location! Location! Location!

In higher eukaryotes, alternative splicing is an important posttranscriptional mechanism for regulating gene expression and for generating protein diversity. To a large extent, alternative splice patterns are determined by RNA sequences located in and around alternatively spliced exons and the regulatory proteins that bind these sequences. FOX2 is one such regulatory protein that is known to bind RNA and influence alternative splicing. In recent work, Yeo et al. show that the locations of FOX2-binding sites around alternatively spliced exons help to determine whether the exon will be included or excluded from the mature transcript. Using a crosslinking-based approach called CLIP-seg in human embryonic stem cells (hESCs), they found thousands of FOX2-binding sites distributed nonrandomly throughout the genome. The sites were enriched within protein-coding genes, consistent with the role of FOX2 in alternative splicing. Furthermore, the FOX2 sites clustered in introns near both the 5' and 3' splice sites of alternatively spliced exons. And, they were enriched near alternative exons compared to constitutively spliced ones. Gene ontology analysis revealed that FOX2 targets are enriched for RNA-binding proteins and other splicing regulators, including the FOX2 message itself, suggesting a potentially important role for FOX2 in maintaining splicing programs in hESCs. When they examined the effect of FOX2 depletion on the splice patterns of selected target genes, they found that FOX2 sites in introns upstream of alternatively spliced exons tended to cause exon exclusion. Conversely, FOX2 sites in introns downstream of exons tended to cause exon inclusion. Therefore, the relative locations of FOX2 sites influence the choice between exon skipping and inclusion. Although the mechanistic basis of these observations remains unclear, the study adds to the growing list of "rules" that govern splice site choice during mRNA processing. G.W. Yeo et al. (2009). Nat. Struct. Mol. Biol. Published online January 11, 2009. 10.1038/nsmb.1545.

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