APA regulator

The 3′ untranslated regions (3′ UTRs) of mRNAs function as regulatory platforms that determine mRNA stability, subcellular localization and translation efficiency. These functions are mediated mostly through binding of microRNAs and RNA-binding proteins to regulatory elements in 3′ UTRs. The proportion of human and mouse genes that use alternative cleavage and polyadenylation (APA) to generate mRNA isoforms with different 3′ UTR lengths is thought to be at least 50%, implicating APA as an important novel layer of gene regulation. By designing a reporter system that comprises a proximal alternative polyadenylation signal (PAS; which recruits the 3′-end processing machinery) upstream of a 3′ UTR fragment containing its canonical PAS, Agami and colleagues screened an RNA interference library for RNA-binding proteins required for APA and identified the nuclear poly(A)-binding protein PABPN1. Loss of PABPN1 resulted in proximal PAS usage, and genome-wide analysis of APA in human cells showed that loss of PABPN1 resulted in extensive 3′ UTR shortening, implicating PABPN1 as a repressor of APA. *In vitro* and *in vivo* data suggest that PABPN1 interacts directly with PAS regions and competes with the 3′-end processing machinery for binding to the weak, noncanonical, proximal PASs, thereby suppressing PAS-mediated cleavage at proximal sites. Using the 3′ UTR of cyclin D1—which is a target of the miR-17-19 cluster—as a test case, the authors showed that enhanced proximal PAS usage as a result of PABPN1 knockdown compromises miRNA-mediated repression. Finally, short triplet-repeat expansion mutations in the *PABPN1* gene, which cause an autosomal-dominant muscular dystrophy disorder in humans, have a similar effect on PAS selection as PABPN1 knockdown, suggesting that the disease phenotype is associated with misregulated APA. (*Cell* **149**, 538–553, 2012) *AH*

Translational control of viral infection

When mammalian cells are invaded by viruses, the first line of defense involves production of cytokines such as interferon-β (IFN-β). A central event in this response is the activation of transcription factor NF-κB. Under normal conditions, NF-κB localizes to the cytoplasm in a complex with its inhibitor, IκBα. Upon viral infection or under the influence of certain other stimuli, IκBα is phosphorylated, ubiquitinated and degraded by the proteasome, releasing NF-κB, which then translocates to the nucleus and promotes transcription of target genes, including IFN-β. Now Herdy and colleagues find that during viral infection IκBα levels are also regulated at the level of translation. eIF4E is a low-abundance translation initiation factor whose phosphorylation status at Ser209 is altered after infection with various viruses, but the consequences of this modification were not clear. The authors compared the responses to viral infection of mouse embryonic fibroblasts expressing either wild-type or S209A eIF4E. The mutation led to impaired replication of different RNA viruses, including those whose translation does not require eIF4E, suggesting that the effect was due to altered translation of host protein(s). In fact, the S209A-expressing cells produced more IFN-β in response to stimuli than did wild-type cells, and this was responsible for inhibiting viral replication. To dissect the mechanism responsible for the higher levels of IFN-β, the authors performed genome-wide polysome analysis and found that the mRNA for IκBα was less abundant in cells expressing eIF4E S209A than in wild type; immunoblot assays revealed that IκBα protein abundance was reduced by half. Consequently, NF-κB transcriptional activity was higher in the mutant cells. Finally, the authors showed that mice expressing S209A eIF4E had better survival than wild-type animals after infection with vesicular stomatitis virus. These data indicate that dephosphorylation of eIF4E might be a host response to limit viral infection, promoting increased NF-κB activity via a translational control mechanism. (*Nat. Immunol.* doi:10.1038/ni.2291, published online 29 April 2012) *IC*

Written by Inês Chen, Arianne Heinrichs & Michelle Montoya

Translation under hypoxia

Eukaryotic protein synthesis is initiated by binding of eIF4E to the 7-methylguanosine 5′ cap of mRNAs. Under hypoxic conditions, eIF4E is inhibited but protein synthesis continues to occur. Lee and colleagues sought to investigate whether an alternative pathway for translation initiation would account for the translation capacity of hypoxic cells. Previous work showed that hypoxia can activate EGFR mRNA translation through HIF-2α, a transcription factor that participates in the maintenance of oxygen homeostasis. The authors now find that HIF-2α associates with polysomes and with the 3′ UTR of EGFR mRNA in hypoxic cells, indicating that under these conditions HIF-2α's role goes beyond transcription. HIF-2α knockdown affected association of EGFR mRNA with polysomes and also decreased the global rates of hypoxic translation. RBM4, an RNA-binding protein involved in the control of translation, was found to interact with HIF-2α during hypoxia and is required for its recruitment to the EGFR 3′ UTR. RBM4 depletion affected hypoxic translation similarly to HIF-2α knockdown. Using PAR-CLIP, the authors identify a CGG motif that is important for the secondary structure of the EGFR 3′ UTR and essential for hypoxia-inducible translation. Further analysis of the PAR-CLIP data reveals similar CGG sites in most target reads, and the authors suggest that RBM4 recognizes RNA hypoxia-response elements (rHREs) in the 3′ UTR of mRNAs to recruit HIF-2α and initiate hypoxic translation. As the 3′ hHRE is able to induce hypoxia-dependent translation of transcripts with different 5′ UTRs that are otherwise not hypoxia inducible, the authors examined involvement of the 5′ cap. They found that HIF-2α–RBM4 assembles with eIF4E2, a cap-binding protein and eIF4E homolog, and that the complex binds to m7-GTP beads in an eIF4E2-dependent manner. In addition, eIF4E2 knockdown affected translation of multiple protein targets identified by PAR-CLIP. Finally, the authors find that cap-dependent translation machinery switches between eIF4E and eIF4E2 as a function of oxygen availability: eIF4E polysome association was observed under normal oxygen levels, whereas eIF4E2 was incorporated under hypoxia. The findings suggest an essential role for the HIF-2α–RBM4–eIF4E2 complex in oxygen homeostasis. (*Nature* doi:10.1038/nature11055, published online 6 March 2012) *MM*