Further Development 3.16

Ribosomal Selectivity: Selective Activation of mRNA Translation

It has long been assumed that ribosomes do not show favoritism toward translating certain mRNAs. After all, eukaryotic messages can be translated even by *E. coli* ribosomes, and ribosomes from immature red blood cells have long been used to translate mRNAs from any source. However, evidence has shown that ribosomal proteins are not the same in all cells and that some ribosomal proteins are necessary for translating certain messages. When Kondrashov and colleagues (2011) mapped the gene that causes numerous axial skeleton deformities in mice, they found that the mutation was not in one of the well-known genes that control skeletal polarity. Rather, it was in ribosomal protein Rpl38. When this protein is mutated, the ribosomes can still translate most messages, but the ribosomes in the skeletal precursors cannot translate the mRNA from a specific subset of Hox genes. The Hox transcription factors, as we will see in Chapters 12 and 17, specify the type of vertebrae at each particular axial level (ribbed thoracic vertebrae, unribbed abdominal vertebrae, etc.). Without functioning Rpl38, vertebral cells are unable to form the initiation complex with mRNA from the appropriate Hox genes, and the skeleton is deformed (FIGURE 3.26). Mutations in other ribosomal proteins have also been found to produce deficient phenotypes (Terzian and Box 2013; Watkins-Chow et al. 2013).

Figure Legend:

Model of ribosomal heterogeneity in mice. (A) Ribosomes have slightly different proteins depending on the tissue in which they reside. Ribosomal protein Rpl38 (i.e., protein 38 of the large ribosomal subunit) is concentrated in those ribosomes found in the somites that give rise to the vertebrae. (B) A wild-type embryo (left) has normal vertebrae and normal Hox gene translation. Mice deficient in Rpl38 have an extra pair of vertebrae, tail deformities, and reduced Hox gene translation. (After N. Kondrashov et al. 2011. Cell 145: 383–397.)

Further Development 3.18

Control of RNA Expression by Cytoplasmic Localization

Not only is the timing of mRNA translation regulated, but so is the place of RNA expression. A majority of mRNAs (about 70% in *Drosophila* embryos) are localized to specific places in the cell (Lécuyer et al. 2007). Just like the selective repression of mRNA translation, the selective localization of messages is often accomplished through their 3′ UTRs. There are three major mechanisms for the localization of an mRNA (see Palacios 2007):

1. *Diffusion and local anchoring.* Messenger RNAs such as nanos diffuse freely in the cytoplasm. When they diffuse to the posterior pole of the Drosophila oocyte, however, they are trapped there by proteins that reside particularly in these regions (FIGURE A).

Localized protection. Messenger RNAs such as those encoding the Drosophila heat shock protein Hsp83 float freely in the cytoplasm, but like nanos mRNA, hsp83 mRNA accumulates at the posterior pole. In contrast to nanos mRNA, hsp83 mRNA is degraded everywhere except at the posterior pole, where localized proteins protect the hsp83 mRNA from being destroyed (FIGURE B).

Active transport along the cytoskeleton. Active transport is probably the most widely used mechanism for mRNA localization. Here, the 3′ UTR of the mRNA is recognized by proteins that can bind these messages to "motor proteins" that travel along the cytoskeleton to their final destination (FIGURE C). We will see in Chapter 9 that this mechanism is very important for localizing transcription factor mRNAs into different regions of the *Drosophila* oocyte.

(See Further Development 3.19, Stored Messenger RNA in Brain Cells, online.)

Further Development 3.21

In Situ Hybridization

Figure legend

In situ hybridization. (A) Whole mount in situ hybridization for *odd-skipped* mRNA (blue) in a stage-9 *Drosophila* embryo. (B) Antisense RNA probe with uridine triphosphate conjugated to digoxigenin (DIG). (C) Illustration of two cells at the border of the *odd-skipped* expression pattern seen in the box in (A). The cell on the left is not expressing *odd-skipped*, whereas the cell on the right is. The antisense DIG-labeled RNA probe with complementarity to the *odd-skipped* gene becomes hybridized to any cell expressing *odd-skipped* transcripts. Following probe hybridization, samples are treated with anti-DIG antibodies conjugated to the enzyme alkaline phosphatase. When nitroblue tetrazolium chloride (NBT) and 5-Bromo-4-chloro-3-indolyl-phosphate (BCIP) are then added to the sample, alkaline phosphatase converts them to a blue precipitate. Only those cells expressing *odd-skipped* turn blue.

Further Development 3.22

Chromatin Immunoprecipitation Sequencing (ChIP-Seq)

Figure legend

Chromatin immunoprecipitation-sequencing (ChIP-Seq). Chromatin is isolated from the cell nuclei. The chromatin proteins are crosslinked to their DNA-binding sites, and the DNA, bound to its proteins, is fragmented into small pieces. Antibodies bind to specific chromatin proteins, and the antibodies—with whatever is bound to them—are precipitated out of solution. The DNA fragments associated with the precipitated complexes are purified from the proteins and sequenced. These sequences can be compared with the genome maps to discover the precise locations of the genes these proteins may be regulating. (After A. M. Szalkowski and C. D. Schmid 2011. *Brief Bioinform* 12: 626–633 and Chris Taplin/CC BY-SA 2.0.)

Further Development 3.23

Deep Sequencing: RNA-Seq

Figure legend

Deep sequencing: RNA-Seq. (Top) Researchers begin with specific sorts of tissues, often comparing different conditions, such as embryos of different ages (chick embryos, as shown here), isolated tissues (such as the eye; boxed regions) or even single cells, and samples from different genotypes or experimental manipulations. (1) RNA is isolated to obtain only those genes that are actively expressed. (2) These transcripts are then fragmented into smaller stretches and used to create cDNA with reverse transcriptase. (3) Specialized adaptors are ligated to the cDNA ends to enable PCR amplification and immobilization for (4) subsequent sequencing. (After J. H. Malone and B. Oliver. 2011. *BMC Biol* 9: 34.)

FURTHER DEVELOPMENT 3.24

CRISPR/CAS9 Genome Editing

Figure legend

CRISPR/Cas9-mediated gene editing. The CRISPR/Cas9 system is used to cause targeted indel formation or insertional mutagenesis within a gene of interest. A gene-specific guide RNA (gRNA) is designed and introduced into cells together with the nuclease Cas9, for instance by co-injection into a newly fertilized zygote. The gRNA will bind to the genome with complementarity and will recruit Cas9 to this same location to induce a double-stranded break. Non-homologous end joining (NHEJ) is the cell's DNA repair mechanism that often results in small insertions or deletions (approximately 2–30 base pairs; a 2 base-pair insertion is illustrated here), which can cause the establishment of a premature stop codon and potential loss of the protein's function. In addition, plasmids carrying insertions with homology to regions surrounding the gRNA target sites are used to insert known sequences at the double-stranded break. Such methods are being explored as a way to repair mutations. PAM, protospacer adjacent motif.

FURTHER DEVELOPMENT 3.25

The GAL4-UAS System

Figure legend

Targeted expression of the *Pax6* gene in a *Drosophila* non-eye imaginal disc. (A) A strain of *Drosophila* was constructed wherein the gene for the yeast GAL4 transcription factor was placed downstream from an enhancer sequence that normally stimulates gene expression in the imaginal discs for mouthparts. If the embryo also contains a transgene that places GAL4-binding sites upstream of the *Pax6* gene, the *Pax6* gene will be expressed in whichever imaginal disc the GAL4 protein is made. (B) *Drosophila* ommatidia (compound eyes) emerging from the mouthparts of a fruit fly in which the *Pax6* gene was expressed in the labial (jaw) discs.

FURTHER DEVELOPMENT 3.26

The CRE-LOX System

Figure legend

The Cre-lox technique for conditional mutagenesis, by which gene mutations can be generated in specific cells only. Mice are made wherein wild-type alleles (in this case, the genes encoding the Hnf4α transcription factor) have been replaced by alleles in which the second exon is flanked by *loxP* sequences. These mice are mated with mice having the gene for Cre-recombinase fused to a promoter that is active only in particular cells. In this case, the promoter is that of an albumin gene that functions early in liver development. In mice with both of these altered alleles, Cre-recombinase is made only in the cells where that promoter is activated (i.e., in the cells synthesizing albumin). The Cre-recombinase binds to the *loxP* sequences flanking exon 2 and removes that exon. Thus, in the case depicted here, only the developing liver cells lack a functional *Hnf4α* gene.