Video Tutorial 13.1: The regulation of translation in developing Drosophila embryos

When you hear a term like “gene regulation” or “gene expression”, most often this is referring to the regulation of transcription, or sometimes to alternative splicing or regulation by microRNAs. But the protein-mediated regulation of **translation** should not be overlooked, and one of the best studied examples is found in the development of embryos of the fruit fly *Drosophila melanogaster*.

The fruit fly egg has distinct polarity. Along the length of the egg the anterior is at one end and the posterior at the other end. This polarity in the egg will give rise to the polarity of the body plan in embryos, larvae, and eventually flies. And this anterior-posterior polarity in Drosophila eggs depends on the anchoring of different RNA and protein molecules to each end of the egg and it also depends on the regulation of translation.

For the anterior, the gene bicoid is key. bicoid mutants have a somewhat unusual phenotype. [slide here] If a female fly is homozygous mutant for bicoid she herself appears normal, but all of her offspring will show a phenotype, regardless of their own genotype. The phenotype of these affected offspring from a bicoid mutant mother is that the embryos lack anterior structures, having instead a mirror image duplication of posterior structures. Thus, the normal role of the bicoid gene is to provide something in the mother that is necessary for development of the anterior end in the offspring embryos.

Recall that the cytoplasm in the early embryo basically all comes from the mother. Nearly all the cytoplasmic components (like ribosomes, metabolites, and macromolecules) that are present in the newly formed embryo have been contributed by the mother in the cytoplasm of the egg. If the mother **lacks** any of these components and fails to provide them in the cytoplasm of the egg, her offspring will suffer the consequences and exhibit a corresponding phenotype. Because the genotype of the mother affects the phenotype of the offspring, mutations with phenotypes like this are known as maternal effect mutants. Bicoid is an example of a maternal effect mutant—some macromolecule that the mother normally contributes to the embryo from the cytoplasm of her eggs is missing. In the case of bicoid, this macromolecule is needed for normal anterior development of the embryo.

For bicoid and for some other maternal effect genes, the macromolecule the mother contributes is the messenger RNA from the gene, the mRNA.

In the mother, the egg, or oocyte, develops within a structure referred to as an egg chamber. In the egg chamber the oocyte is connected to a special group of germline cells, called the nurse cells (outlined in purple here). The nurse cells provide nourishment and cytoplasmic components to the egg, the oocyte (outlined in yellow). The mother transcribes the bicoid gene in these nurse cells and this bicoid mRNA is contributed to the oocyte where it is present in the cytoplasm but not translated until after fertilization. [slide] As you can see in this image in which the bicoid RNA is stained blue, the bicoid mRNA is in fact localized and anchored at one end of the developing egg and newly fertilized egg. This localization and anchoring of the bcd mRNA involves the cytoskeleton, the microtubules in particular. The end of the oocyte at which the bicoid RNA is tethered will become the anterior of the embryo, and eventually the location of the head of the larva and the fly.

Upon fertilization, the nucleus in the Drosophila egg undergoes a series of very rapid divisions. In fact these are some of the most rapid nuclear mitotic divisions known in any organism, doubling the number of nuclei about every 9 minutes. After the nuclear divisions have produced several hundred nuclei these move to the periphery of the embryo where they undergo additional rounds of division forming several thousand nuclei around the periphery of the embryo. Although the nuclei divide very rapidly, there are no corresponding cell divisions during this early stage. It's only after the 13th round of nuclear division that plasma membrane then surrounds the nuclei to form individual cells around a central core of yolk. This stage is called the cellular blastoderm. Prior to cellularization the nuclei all reside in the same cytoplasm. Such an organization in which many nuclei reside in the same cytoplasm is known as a syncytium. Since there are no cell membranes between the nuclei in the syncytial stages, macromolecules can freely diffuse throughout this cytoplasm and affect all of the nuclei in the rapidly developing embryo.

However, the bicoid mRNA is tethered to *one* end (shown here, stained in blue), so it cannot diffuse freely throughout the syncytium. When the egg is fertilized and the nuclear divisions begin, bicoid mRNA is translated into the Bicoid protein. [image] The Bicoid protein (stained here in brown) is free to diffuse throughout the cytoplasm, and it forms a concentration gradient—the highest concentration at the end where the mRNA was tethered, and decreasing concentration further away over about a third of the length of the embryo, as shown here.

This gradient of Bicoid protein affects the development of the embryo in two different ways to guide formation of the anterior-posterior axis.

Firstly, Bicoid protein is a transcriptional activator. All of the nuclei in the developing embryo have binding sites for the Bicoid protein in the regulatory regions of important, Bicoid-regulated genes. But since the Bicoid protein forms a concentration gradient, nuclei are exposed to different levels of the Bicoid protein in different regions of the embryo. In the most anterior end nuclei are exposed to the highest levels of Bicoid protein and the target genes that are expressed here bind Bicoid only weakly so require high levels of Bicoid protein to drive their expression, whereas target genes expressed further down the gradient are responsive to lower levels of Bicoid protein. Substances, like Bicoid protein, that are found in a graded distribution and can direct different responses at different concentration levels are called morphogens.

One gene whose expression is activated by Bicoid is the gene, Hunchback. In a promoter region of the Hb gene there are six binding sites for the Bicoid protein. Three bind Bicoid weakly and three bind strongly. It appears there is some co-operative binding of Bicoid to the Hb promoter (that is, once Bicoid is bound at one site it makes binding more likely at the other sites). This co-operative binding, along with some feedback mechanisms, results in expression of the Hb gene throughout the anterior of the embryo, with a pretty abrupt cut off where the levels of Bicoid drop below the threshold level needed for activation.

In addition to this Bicoid driven Hb expression the mother fly also deposits some Hb mRNA in the egg. But translation of Hb RNA is inhibited in the posterior of the embryo through the action of the posteriorly restricted nanos protein as described in the text and in Box 13-2. This posterior repression of Hb translation further restricts Hb protein to the anterior region of the embryo. It turns out that this Hb protein in the anterior regions acts **with** Bcd in regulating the Bcd target genes.

So, regulating transcription of target genes along the Bicoid gradient is the first way in which the Bcd protein acts.

The *second* function of Bicoid protein is to regulate *translation*. mRNA of the *caudal* gene is the target of *this* regulation. Caudal mRNA is one of the other maternally supplied mRNAs that is deposited into the egg by the mother. But unlike bicoid mRNA the caudal mRNA is not localized and is present throughout the embryo. However, the Caudal **protein** is only produced in the **posterior** of the embryo where it plays a role in directing development of posterior structures. This *posterior* production of Caudal protein is achieved because the Bicoid protein in the anterior inhibits translation of caudal mRNA there.

The Bcd protein binds directly to specific sequences located in the 3' untranslated region, the 3'UTR, of the caudal mRNA. One known mechanism for how this bound Bcd protein inhibits translation is shown here. The bound Bcd protein interacts with other proteins that prevent the 5' Cap structure of the caudal mRNA from interacting with the initiation factors needed for recruitment to the small ribosomal subunit and the onset of translation. Furthermore, recent studies have shown that Bicoid mediated repression of caudal translation also involves microRNAS that bind alongside Bcd protein to the 3' UTR of caudal.

The role of bicoid described here, as well as the role of nanos described in the text, are just two examples that illustrate the importance of the regulation of translation in the very earliest stages of embryonic development. Translational control isn't the only mechanism used for anterior-posterior patterning. We saw that localization and anchoring of RNAs and the control of transcription are important too. And transcriptional control is particularly important in later stages of embryonic development as we describe more in Chapters 13 and 14.

Over the course of evolution there has been a finely tuned integration and combination of all these regulatory mechanisms in directing embryonic development. But in the very initial steps of establishing the anterior-posterior axis of the early embryo **translational regulation** of maternally contributed mRNAs plays a central, critical role.

References and further reading:

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