How the fly got its neck

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1 Introduction

How does an embryo decide where along its body to grow legs? How can it tell its head from its tail? As we saw when looking at gene expression in bacteria, cells combine the information encoded in their DNA with cues from their neighboring cells and the environment in order to make decisions. In the case of embryonic development these decisions are related to determining where in the body cells will differentiate, proliferate and ultimately become morphological structures such as legs or wings or any other tissue type of a multicellular organism.

Here, we will explore how cells within a fruit fly read out a signal in order to make decisions about where the “neck” of the fly will be formed. We will propose a simple model for this decision and calculate the predictions the model makes for flies whose developmental program has been genetically altered. We will then test those predictions experimentally by imaging these genetically-modified flies and analyzing the results using Matlab and find some surprises!

2 The development of the fruit fly

The fruit fly *Drosophila melanogaster* begins its life as an egg of about 500 µm in length and a radius of about 100 µm. Throughout the first three hours of its development, the initial nucleus undergoes multiple rounds of division resulting in approximately 6000 nuclei located on the periphery of the embryo. This process is shown diagrammatically in Figure 1(A) while an images of a real embryo developing taken using fluorescence two-photon microscopy is presented in Figure 2(A).

As we see from the images in Figure 2(A) throughout the early development of the fruit fly all nuclei look exactly the same. Yet, a genetic program
Figure 1: Schematic of the development of the fruit fly *Drosophila melanogaster*. (A) The fly begins its life as an egg with a single nucleus. By three hours into its development this nucleus has gone through multiple rounds of division resulting in 6000 cells located all around the periphery of the embryo. (B) At this time cells already express various combinations of gene products determining their ultimate developmental fate.

has already taken place during these initial hours that has determine the ultimate developmental fate of each one of its cells. In Figure 1(B) we see how different cells express various combinations of gene products which specify which part of the adult fly they will become as morphology starts to emerge in the embryo during a process called gastrulation. One such morphological decision is the appearance of the cephalic furrow as seen in Figure 2(B). This cephalic furrow appears at about 35% embryo length and separates the head from the thorax.

How does the embryo figure out where to place the cephalic furrow? In this exercise we will explore a simple model that proposes a mechanism for the placement of morphological features along the embryo axis by testing its predictions experimentally.
Progressive nuclear divisions
Anterior end
Posterior end
Cephalic furrow formation
Cephalic furrow
35% embryo length
50 µm
(A)
(B)

Figure 2: The early development of the fruit fly using fluorescence microscopy. Two-photon microscopy movie of a fruit fly embryo whose nuclei are fluorescently labeled. Time stamps indicate the approximate time since fertilization. (A) As time since fertilization progresses (shown in each frame) the number of nuclei increases. By three hours into the development 6000 nuclei can be observed near the surface of the embryo. (B) At this point massive morphological changes occur throughout the embryo. One of these changes is the emergence of the cephalic furrow at about 35% embryo length. This furrow is a tissue fold that will determine the separation between the head and the thorax of the embryo.
3 The French flag model

A very popular model to explain how embryos can measure the position where morphological features will appear is called “The French flag model” and was proposed by Wolpert [1]. The basic idea of this model is shown in Figure 3. Here, a gradient of a molecule called a “morphogen” is present along the anterior-posterior axis of the embryo. Cells determine where they are along the embryo by measuring the local morphogen concentration. In the example of the Figure the cells that see a concentration above “threshold 1” carry out a specific developmental program indicated with blue. Cells that see a concentration between “threshold 1” and “threshold 2” adopt the white cellular fate while those that see a concentration below “threshold 2” carry out the red developmental program.

A classic morphogen in the fly is the activator Bicoid. This protein is produced at the anterior end of the embryo and diffuses throughout the its whole length [2]. The result is an exponential gradient as shown in Figure 3, which can be mathematically described as

\[
[Bcd](x) = [Bcd]_0 e^{-x/\lambda}. \tag{1}
\]

Here \([Bcd]_0\) is the Bicoid concentration at the source, \(x\) is the position along the embryo’s axis, and \(\lambda\) is the decay length of the exponential gradient.

Bicoid is one of the main determinants of positions along the anterior-posterior axis of the embryo. Now, let’s assume that it marks the position of the cephalic furrow at \(x_0\). The corresponding Bicoid concentration at that position is

\[
[Bcd](x_0) = [Bcd]_0 e^{-x_0/\lambda}. \tag{2}
\]

Let’s imagine that we rescale the gradient. This can be done, as we will see below, by changing the number of copies of the bicoid gene in the Drosophila. The resulting Bicoid profile would then be

\[
[Bcd]_{\text{rescaled}}(x) = \alpha [Bcd]_0 e^{-x/\lambda}, \tag{3}
\]

where \(\alpha\) is our rescaling constant.

The feature that appeared at position \(x_0\) due to the Bicoid concentration \([Bcd](x_0)\) will now be shifted. In order to calculate its new position we equate equations 2 and 3 and obtain

\[
[Bcd]_0 e^{-x_0/\lambda} = \alpha [Bcd]_0 e^{-x_{\text{new}}/\lambda} \tag{4}
\]

which leads to

\[
x_{\text{new}} = \lambda \ln(\alpha) + x_0. \tag{5}
\]
Figure 3: French flag model of positional information. A morphogen gradient is present along the long (anterior-posterior) axis of the embryo. When the concentration of the morphogen exceeds a first threshold, cells adopt the “blue” developmental fate, while when they are below the first threshold but above a second threshold, they adopt the “white” fate.

This tells us that the new position of the feature should scale linearly with $\ln(\alpha)$. This prediction is shown in Figure 4 where we can see that the scaling of the position of the cephalic furrow with Bicoid dosage. Here, we have made use of the fact that $\lambda = 16.5\%$ [3]. In order to test the prediction put forth in Figure 4 we will have to change the dosage of Bicoid in the embryo and measure the position of the cephalic furrow using image analysis in Matlab. In the next section we discuss how to engineer this mutant fly, something that has been going on behind the scenes for the last few days.

4 Creating flies with different Bicoid dosages

There are multiple ways of varying the amount of Bicoid in an embryo. The easiest way it to reduce the Bicoid amount by a factor of two. Remember that flies are diploid, which means that they carry two copies of each chromosome and, as a result, they carry two copies of the bicoid gene. Halving
Figure 4: Prediction of the French flag model. Position of the cephalic furrow as a function of Bicoid dosage as predicted by Equation 5 using \( \lambda = 16.5\% \).

the Bicoid dosage can then be done by having one copy of the chromosome with a functional \textit{bicoid} gene and one copy with a mutant, non-functional gene. In this section we will walk you through the fly crosses that took place a few days before this experiment in order to create these mutant flies.

Before we begin, let’s look at some nomenclature. Female virgin flies are denoted by the symbol ♀, while males are represented by ♂. A wild-type chromosome is denoted by the symbol +, while a mutant \textit{bicoid} gene is denoted by the symbol \textit{bcd}. Since we’re dealing with a diploid organism we need to indicate what each chromosome is. For example, two wild-type chromosomes are indicated by + +. If we have a mutant \textit{bicoid} gene in one chromosome and wild-type one in another this is indicated by \textit{bcd} +.

\textit{Bicoid} is a protein that is provided by the mother. This means that if we do the mating

\[
♀ \textit{bcd} + \times ♂ + +
\]

the embryos coming out of this cross will have only half the amount of Bicoid protein they would have had if their mothers were \( \frac{+}{+} \). The question is then, how do we create flies with the genotype \( \frac{bcd}{+} \)?
In order to create these flies we had to do the previous mating

\[ \text{♀} + \text{♂}^{bcd/bcd} \]

so all offspring are \( bcd/+ \). We can then pick the females from this cross and use them as the mothers of our experiment. To get the wild-type Bicoid dosage we will just use wild-type flies.

5 Experimental protocol

We will take images of fly lines with different Bicoid dosages using light microscopy. The trick will be getting the embryos at the right developmental time point so that the cephalic furrow is easy to image. Our images will look similar to those shown in Figure 5. We will then use Matlab to analyze these images and determine the position of the cephalic furrow for the different Bicoid dosages in order to test the prediction of the French flag model shown in Figure 4.

![Figure 5: Images of a wild type Drosophila embryo acquired in bright field. (a) The embryo immediately after completing 13 rounds of nuclear division. Note that the periphery of the embryo, where the nuclei are, is lighter in color. The anterior of the embryo is marked by the small protrusion called the micropyle. The dorsal side of the embryo has less curvature than the ventral side. (b) The embryo immediately after cellularization. The formation of the cephalic furrow will occur within minutes. (c) Approximately one minute after cephalic furrow ingestion begins. The furrow is clearly visible. The arrows mark the position of the furrow on the dorsal and ventral sides of the embryo. This is the best time to see the cephalic furrow, as the cells at the periphery are still light in color. (d) A fully formed cephalic furrow. The furrow has moved slightly from its position at its initial ingression, and is more difficult to see.](image-url)

To measure the cephalic furrow, we need to isolate live embryos at the right stage of development, mount, and image them. There are two crucial
points here. First, we need to make sure the embryos are at the correct stage of development for formation of the cephalic furrow. Second, we need to remove the **chorion**, the protective shell of the embryo, before imaging. The chorion is reflective, and will greatly compromise image quality if not removed. Below, we describe the protocol we will use in lab. While it differs in some ways from our protocol, you may find it useful to watch the video description of acquisition and imaging of eggs found in this JoVE article: Figard, L., Sokac, A. M., *J. Vis. Exp.* **49**, e2503, doi:10.3791/2503 (2011).

We will provide you with slides where the embryos have already been mounted. Find a few embryos and take a time lapse movie with a frame every 30 seconds. Later on, you may want to bin the images so that the saved images are lower resolution to save on storage. From the time lapse movies, we will find the frame where the cephalic furrow first forms and determine its position. Remember that you can use Figure 5 as a guide.

**References**

