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Eric Wieschaus' Lecture Part 2:
The Role of Morphogen Gradients in Development

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1. Keywords and Terms

Embryonic development, pattern formation, morphogen, diffusion, cooperativity, Drosophila, GFP, degradation, confocal microscopy, 2-photon microscopy.

2. Review Questions

1. **W**hat is a control experiment?
2. What is GFP? What is its value? How does it work?
3. What is a reporter? How does GFP differ from Luciferase?
4. What are the advantages/disadvantages of live versus fixed embryo imaging?

5. How would you convince yourself and others that attaching GFP (~25kd) to Bicoid (~55kd) does not change its normal behaviour (in other words, that the fusion is functional)?
6. Scientific discovery is often driven by advances in technology and methodology. Can you identify tools, techniques and/or methods that were used by Eric Wieschaus and his colleagues that were not available 20 years ago? Can you explain how they enabled EW to address the questions he is asking?
7. If the embryo is homozygous mutant for the gene bicoid it develops normally. Can you explain that?

3. Answers to Review Questions

1. An experiment is only meaningful relative to something else. For example, over-expression of the mutant form of a transcription factor may yield a dominant negative effect. To know that this effect is due to the mutation and not due to the over-expression, the control one would perform would be to over-express the wild type form of this transcription factor (at identical conditions). Then any effect could be attributed to the mutation and not the fact that the levels were raised.

2. GFP is a protein that emits fluorescence upon excitation with blue light. GFP can be genetically encoded and therefore fused to any protein of interest making it relatively easy to detect the fusion protein.

3. A reporter, such as luciferase or GFP, allows one to follow the location of a specific enzymatic reaction or protein. Luciferase catalyses the oxidation of luciferin to produce light, however, GFP does not need a substrate to emit fluorescence.

4. Live imaging enables one to study the dynamics of biological processes. There are no fixation artifacts.

5. The fusion between GFP and Bicoid rescues the mutant. In other words it provides the embryo with the normal function.

6. A key tool was the development of GFP as a reporter. The first demonstration of the usefulness of GFP was the seminal work by Chalfie et al (1994) in which the authors fused the cDNA encoding GFP to a gene of interest and expressed it in E.coli

yielding a fluorescent protein product (see assignment below). Chalfie was awarded the Nobel prize for this work. Confocal and 2 photon microscopes were not widely used/available. Confocal and 2 photon microscopy allowed EW to watch the movement of Bicoid with time over the entire embryo. Powerful computers with which to process data were not commonly available.

7. This is not a spelling mistake – the embryo develops normally if it is mutant for the gene bicoid. This question is aimed at understanding the difference between maternal and zygotic control of development. An embryo that is mutant for bicoid (it does not contain the gene for bicoid or only defective copies of this gene) will develop normally into an adult (because it is not needed zygotically); however, this adult (if female) will not be able to produce and therefore deposit bicoid mRNA into the eggs she produces (because this fly does not contain the normal bicoid gene, so any mRNA made from the mutant gene will be mutant). The embryos from such individuals will develop abnormally (because it is the protein made from the mutant mRNA that was deposited by the mutant mother). Genes that behave in this way are called maternal effect genes because the genotype of the mother matters. Other examples of maternal effect genes, other than bicoid, include torso, oskar and dorsal.

4. Discussion Questions

1. ~20 years prior to the discovery of Bicoid, Wolpert and Crick proposed that gradients of biologically active molecules (such as proteins or retinoic acid (RA)) may arise by diffusion from a localized source with degradation balancing production such that the gradients reach steady state. What does this model predict? How does the discovery of Bicoid relate to this model? Which of the results presented are consistent with this model, which ones are not?

2. In the simple model, the Bicoid gradient arises by diffusion from a constantly producing source at the anterior pole with spatially uniform degradation. Mathematically, the concentration of Bicoid changes with time at any given position within the embryo according to: $dC(x,t)/dt = D d^2c/dx^2 - kc + Q$ (1) where c is the concentration of Bicoid, D the diffusion coefficient, Q the production term, and k the degradation rate. At steady state, the solution to (1) is given by: $C(x) = C(0)e^{k/l}$ with l = square root (D/k) What is a steady-state? Why do scientists prefer to work with the

steady-state solution rather than the full time-dependent solution? What is the mathematical and what the biological reason?

3. Both the length constant l and the diffusion coefficient D were measured. With l= square root (D/k) which life-time/degradation rate do you expect? Over which time does the Bicoid gradient form? Can the gradient be at steady state?

4. Two different experiments to measure the diffusion coefficient in the embryo are presented. What do they have in common, where do they differ? Why do the measured diffusion coefficients differ? Which of the 2 diffusion coefficients do you believe more, the one measured with Bicoid or the one measured with biologically inert Dextran molecules? Within the framework of the above-mentioned model, which diffusion coefficient makes more sense?

5. How can the stability of the nuclear concentration of Bicoid at a given position be maintained? Do we know for sure how it is stabilized?

6. How is the information provided by the Bicoid gradient read by cells? What is cooperativity? How can you test whether there is cooperativity in the Bicoid response? Is the available data consistent with cooperativity?

5. Answers to Discussion Questions

1. For a mathematical model see the next question. The model predicts that the gradient has an exponential profile (if degradation is spatially uniform) or a linear profile (if there is a localized sink). Further, if the life-time of the morphogen is short with respect to the time over which the gradient forms the gradient is at steady state. This means that both the spatial distribution (profile) and the total number of morphogen molecules within the system do not change. Bicoid was the first gene shown to have the characteristics of a morphogen. Consistent with the proposed model are the findings that i) Bicoid has an exponentially decaying concentration profile, ii) the concentration of Bicoid within a nucleus at a given position is stable suggesting that the Bicoid gradient is at steady state. On the other hand, the low diffusivity of Bicoid (~0.3 µm^2/sec) is inconsistent with this model because it fails to explain the actual distribution (length scale) of the Bicoid gradient.

2. The steady state is the time independent solution of a differential equation (the solution when the time derivative is set to 0: $dC/dt = 0$). Many chemical systems relax exponentially fast to the steady state. Mathematically, the solution to equation

(1) is much simpler at steady state (note, not all differential equations have a solution when the time derivative is set to 0). Biologically, the morphogen gradient does not change after reaching its steady state and target genes therefore would have to read a stable (and not a changing) morphogen concentration.

3. The Bicoid gradient is read by its target genes about 2-3 hours after fertilization. The observed length constant is ~100 µm and the measured diffusion coefficient is 0.3µm2/sec. Then, k = Dl2 or with T = l/k the life-time would be ~10 hours. For the gradient to be at steady state the life-time would have to be much smaller than the time at which it is used, T<<t, but in fact we find that T>t. So the system can't be at steady state.

4. One experiment uses Bicoid itself (with a GFP tag adding a mass of ~27kd) the other uses a biologically inert molecule, Dextran, of roughly the same size as Bicoid. Presumably Dextran interacts differently with the environment in which it diffuses than Bicoid. For example, Bicoid carries a nuclear localization signal and during interphase is predominantly localized to nuclei whereas Dextran is not. Differences in the experiments are the time and length scales over which the diffusion is measured. For Bicoid a tiny, 16x16µm spot is bleached and its recovery measured at 1 sec intervals (complete after less than 2 min), whereas for Dextran, fluorescent molecules are injected at one end of the embryo and the appearance of fluorescence at a distance (at the posterior end several hundred µm away) after much longer time interval measured. One is tempted to believe the experiments with Bicoid more than with Dextran simply because of the presumed differential interaction with its medium. However, the diffusion coefficient measured for Dextran makes more sense (as can be seen by fitting the numbers into the equations above).

5. We have seen above that, given the measured diffusion coefficient and length scale, the Bicoid gradient cannot be at steady state (within the frame work of this simple model). This predicts that the levels of Bicoid must keep rising and consequently the concentration of Bicoid at a given position must keep changing with time. And yet, the nuclear concentration of Bicoid at a given position remains stable (within ~10%) over the last 5 cell cycles. This could be achieved if the number of nuclei (the total nuclear volume) increases such that it would balance the increase in Bicoid. For example, if the amount of Bicoid increases by a factor of 1.4 from cycle to cycle then one would predict that the total nuclear volume shows a corresponding increase thus maintaining a stable nuclear concentration.

6. The transcription factor Bicoid binds directly to regulatory sites upstream of the hunchback gene. Bicoid has high affinity for some of the sites and low affinity for the

other sites, meaning that at low concentrations of Bicoid only the high affinity sites are occupied. Binding of Bicoid facilitates the binding of other Bicoid molecules to DNA. To test whether there is a cooperative effect in the response to Bicoid, one can dissect the gene regulatory site. For example, one can use a single Bicoid binding site and see how this element responds to Bicoid; alternatively one can increase the distance or change the orientation of the Bicoid binding sites. It might be worth reading Monod's paper on cooperativity and the work by Struhl on the Bicoid response (see below for references).

6. Explain or Teach These Concepts to a Friend

Gradients of morphogens provide the embryo with positional information. How is this positional information translated into patterns of gene expression? How can cells/nuclei reliably distinguish small (~10%) concentration differences of the morphogen? What does cooperitivity mean?

7. Research the Literature on Your Own

Wolpert, L. Positional information and the spatial pattern of cellular differentiation. J Theor Biol 25, 1-47 (1969)

What is the French flag model? How does this relate to Bicoid?

Crick, F. Diffusion in embryogenesis. Nature 225, 420-422 (1970).

Do you know of any gene that behaves like the hypothetical substance in Crick's paper?

Monod, J., Changeux, J. P. & Jacob, F. Allosteric proteins and cellular control systems. J Mol Biol 6, 306-329 (1963)

How does this work relate to the interpretation of the Bicoid gradient by target genes?

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W. & Prasher, D. C. Green fluorescent protein as a marker for gene expression. Science 263, 802-805 (1994).

What did Chalfie do for the first time? How did this change the way scientists look at proteins of interest?