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What can the fruit fly tell us about the human eye?

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Learning objectives

By the end of this lab students will be able to:

- Explain what is meant by the phrase "necessary and sufficient" and what types of experiments could be used to demonstrate these roles
- Explain the benefits of using model organisms in research
- Explain the GAL4/UAS system and transgenes
- Design an experiment to test a hypothesis
- Identify the hypothesis and prediction from a primary research article
- Use a database to identify a relevant primary article
- Dissect fly pupae

Background

The scientific method allows scientists to ask and answer questions about the world around them. The scientific method begins with an observation that leads to a question. From this question, a hypothesis is formed. A **hypothesis** is a statement that provides an explanation for an observation; a hypothesis must be testable, falsifiable, and based on prior knowledge of a phenomenon. To test a hypothesis, a prediction is generated, which is an "if, then" statement that includes the outcome of an experiment if the hypothesis is supported. The next step in the scientific method is to develop an experiment to test the hypothesis, and from the results of that experiment, conclusions can be drawn in order to determine the strength of the hypothesis.

When designing your own experiments or reading about experiments performed by others, it is important to think critically about the strength of the data that has been collected. For example, showing that there is a

correlation between two events is not as strong as showing that one event caused another event to occur. Scientists use different types of experiments to produce different types of data. Let's go through an example to illustrate this point. If you are a person that enjoys drinking coffee, you might **observe** that drinking coffee seems to help you wake up in the morning. You can **ask** whether the caffeine in your coffee causes you to feel awake. From that, you could **hypothesize** that the caffeine in the coffee causes you to feel awake, and you can design a set of experiments to test that hypothesis.

You can categorize the types of experiments and the data that they generate using a set of 3 different "bins" – Show It/Block It/Move It (Adams 2003). **Show It** experiments provide correlative data, which is the weakest type of data. For example, we could look at the ingredients of your coffee or perform a chemical analysis to determine the levels of caffeine present in the coffee that you drank this morning. The data that you collect from this analysis demonstrates a correlation between caffeine present in the coffee and feeling awake. **Block It** experiments use techniques to block the function or action of something and observe the effect. They can demonstrate that something is necessary for an effect or outcome. If you were to drink decaffeinated coffee in the morning and did not feel awake, you might conclude that the caffeine in your normal coffee was ***necessary*** for the feeling of awakeness that you usually feel. This is a stronger piece of evidence to support your hypothesis. **Move It** experiments involve moving something to a place where it is not normally found, or isolating a component from a mixture and testing only that component. For example, if you were to drink pure caffeine alone (*not recommended*) and felt awake, then that would suggest that the caffeine alone (and nothing else in the coffee) was ***sufficient*** to make you feel awake. This is a very strong piece of evidence to support your hypothesis. If you put all three pieces of data together, you have strong support for your experimental hypothesis.

In molecular terms, **Show It** experiments correlate the localization or presence of an mRNA or gene product (protein) with an event or effect. Again, these are the weakest types of experiments because they only show that a protein or mRNA is present at the proper time and/or location, but they do not show that the protein is involved in

the process or structure you are studying. Techniques that are utilized in Show It experiments include immunofluorescence and *in situ* hybridization. **Block It** or **Loss-of-function** experiments use techniques such as RNA interference (RNAi), mutation, or chemical inhibition to block the function of a protein or molecule and observe the effect. If the treatment alters the process that you are studying, then it suggests that the protein that was blocked normally participates in that process and is **necessary** for it to occur or for a structure to form. **Move It,** or **Gain-of-function** experiments provide the strongest type of evidence. These experiments often use micromanipulation or overexpression techniques to demonstrate that a given protein or molecule is **sufficient** to induce a process to occur or to generate a structure.

In this lab, you will be learning about the role of a protein called Eyeless (Ey) in the fruit fly, *Drosophila melanogaster*. *Drosophila* is a very popular model organism that is used to study many different processes in a number of fields of biology including evolution, genetics, development, and cell biology. A **model system** or a **model organism** allows researchers to study a specific gene, protein, pathway, structure, or process in a less complex organism. However, because gene structure and function is highly conserved through evolution, the information learned about that gene, protein, or pathway in the model organism can be applied to our knowledge of the similar pathway in more complex organisms (such as humans). Flies are one of the premier genetic model organisms because they are inexpensive and easy to maintain, and they have a short generation time. Because they have been studied for over 100 years, there are many genetic tools available that allow researchers to manipulate the expression of target genes. However, the genes and molecular pathways in the fly are very similar to the pathways in humans.

One of the most useful tools developed for the fly is the **GAL4/UAS system**. The GAL4/UAS system is a gene expression system that was adopted from yeast. GAL4 is a transcriptional activator that binds to and promotes the transcription of genes that are downstream of an Upstream Activating Sequence (UAS) (Elliot 2008). Genetic information in the form of genes is found on chromosomes in the nucleus. That genetic information must be copied

or transcribed into messenger RNA (mRNA), which is transported from the nucleus to the cytoplasm. The mRNA is then translated into protein in the cytoplasm. Once translated, the protein can perform its assigned functions in the cell – signaling, structural, transport, etc. However, not all of the genes in the genome are expressed in every cell. There are sets of "housekeeping" genes that are necessary for all cells to function, and these are transcribed in every cell. However, there are also cell-type specific genes that are only transcribed in certain cell types and not in others. For example, cells of the immune system, B cells, will transcribe antibody genes. (Antibodies allow immune cells to locate and neutralize foreign invaders in the body). Muscle cells, or myocytes, do not transcribe the antibody genes because they are not involved in the immune response; instead, muscle cells transcribe the genes required for muscle function such as actin and myosin. There are a number of different factors that control which genes are going to be transcribed or expressed in a given cell. One type of regulator of gene expression is a **transcription factor**. Transcription factors are proteins that bind to regulatory regions of DNA and can either activate or repress transcription of nearby genes. GAL4 is a transcription factor that will bind to a specific DNA sequence called the UAS sequence. Scientists can engineer flies that have the UAS sequence near the coding sequence of any gene they are interested in studying. They can then cross flies that have a UAS-gene of interest to a fly that is expressing GAL4. This will lead to transcription of the gene of interest in any cell that

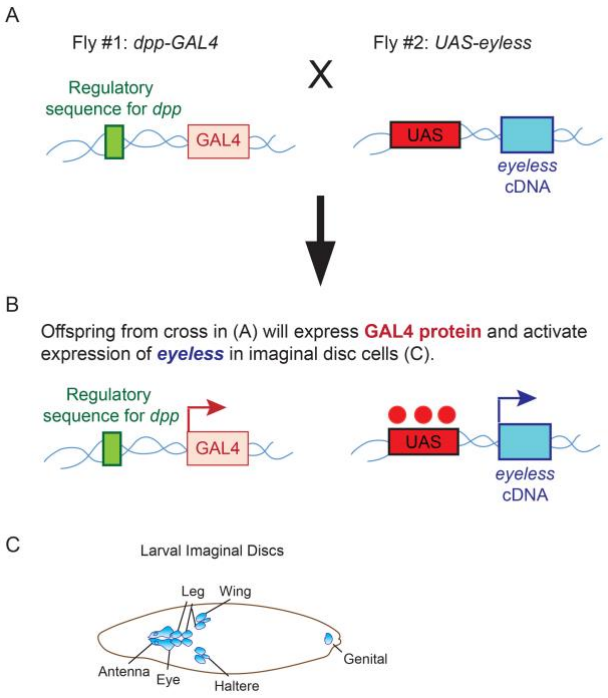


Figure 1: Schematic of the use of the GAL4/UAS system to over-express Eyeless protein in the imaginal discs. (A) *dpp-GAL4* flies are crossed to *UAS-ey* flies. (B) Offspring will express GAL4 in the imaginal disc cells, which will activate expression of the *eyeless* gene in those tissues. (C) Approximate location of imaginal discs in the larval body. Blue shading indicates ectopic expression of Eyeless protein.

contains the GAL4 protein (Fig. 1A, B; Elliot 2008). Often times, scientists want to study specific tissues or organs, and to do this, they will use flies that express GAL4 only in those tissues. For example, the Eyeless protein is normally expressed in the eye imaginal discs because its transcription is controlled by an eye-specific promoter sequence. By using this eye-specific promoter sequence to control the expression of the GAL4 gene, flies can be generated that only express GAL4 in the eye tissue and not in any other cells in the body.

Adult structures in the fly are made from pockets of tissue called **imaginal discs**. There are 15 imaginal discs in the larvae that will generate the mouthparts, antennae, eyes, legs, halteres (balancing organs), wings, and genitalia in adult flies (Fig. 1C). In this lab, you are going to be looking at flies that are expressing the GAL4 transcription factor in all 15 imaginal discs. The GAL4 is controlled by the promoter for the *decapentaplegic (dpp)* gene (Fig. 1). One important thing to keep in mind is that GAL4 is not a protein normally found in the fly. It is a yeast protein that has been genetically engineered to be expressed in these fly tissues. Because of this, you could consider these flies to be **genetically modified organisms (GMOs)**. You are going to look at what happens if you over-express the Eyeless (Ey) protein in all 15 imaginal discs in the fly. Eyeless is a transcription factor that is normally expressed in the eye tissue of the fly.

Prelab Questions:

1. Find one example of something that demonstrates the concept of "necessary" and/or "sufficient." This could be an example from the news, from biology, from another field of study, or from your daily life (such as the caffeine/coffee example).

    a. If your example is from a news article, explain the evidence that is provided and discuss the strength of those lines of evidence. If the data are not very strong, suggest an additional piece of evidence or experiment that could strengthen it.

    b. Alternatively, if you develop a question based on an observation, briefly describe an experiment that could be performed to establish the connection (as with the coffee example from the introduction).

Lab Procedure:

1. First, you are going to look at some control fly pupae, so that you can practice your dissection technique and become familiar with the structure of the fly pupae.

2. Use a pair of forceps to remove one of the pupae that is on the side of the vial. You want to look for a pupa that has darkened in color (Fig. 2; right), and is further along in development.

3. Under the dissection scope, use the two pairs of forceps to peel the fly out of the pupal case. Be careful not to tear the fly apart too much.

4. What do you see? Can you pick out the parts of the developing fly? (Include a drawing here with labels for the wing pads and eyes)

5. Now, repeat the same procedure with the "Eyeless Over-expression" flies, which are progeny from a cross between the *UAS-eyeless* flies and *dpp-GAL4* flies. Only half of the pupae will be over-expressing the *eyeless* gene, and half of them will not be. **Be sure that you choose the pupae that are longer because these are the Eyeless over-expressing flies.**

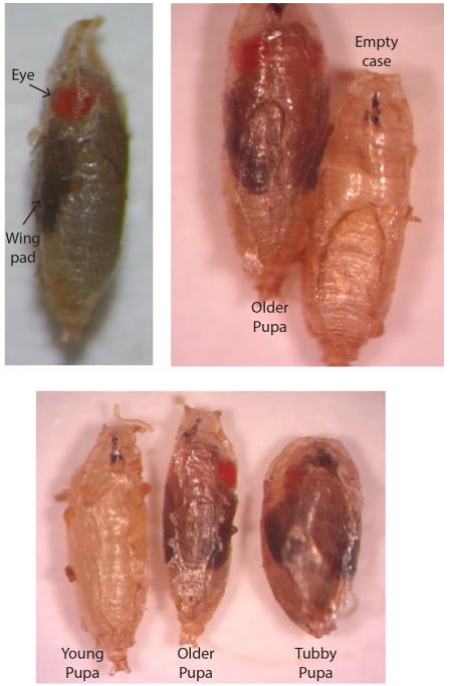


Figure 2: Images of pupae at different stages. For your dissections, you want to choose the "older pupae" where the eyespots (red/orange) and wing pads (blue/purple) are visible. The tubby pupae do not contain the *dpp-GAL4* transgene, so they will not be over-expressing Eyeless.

6. Describe/draw what you observe below.

Lab Questions

1. What type of experiment have you performed today (Show It/Block It/Move It)? Explain your answer.

2. In flies, genes are often named based on the **loss-of-function** phenotypes. What would you predict would happen if you blocked the function of the *eyeless* gene?

3. From this experiment and your prediction in the question above, what could you hypothesize about the role of the *eyeless* gene in the developing fly?

4. Develop a hypothesis about the role for the human homolog of *eyeless, PAX6*.

5. Now, read the accompanying Halder (1995) paper. What is the hypothesis and prediction for this paper?

6. In the paper, the authors state, "Our experiments …identify the gene that is necessary and sufficient to induce ectopic eyes at least in imaginal discs."
    a. In general terms, what does it mean if something is necessary and sufficient?

    b. Which specific pieces of data support each part of this statement? (Hint: they might not all be shown in the paper)

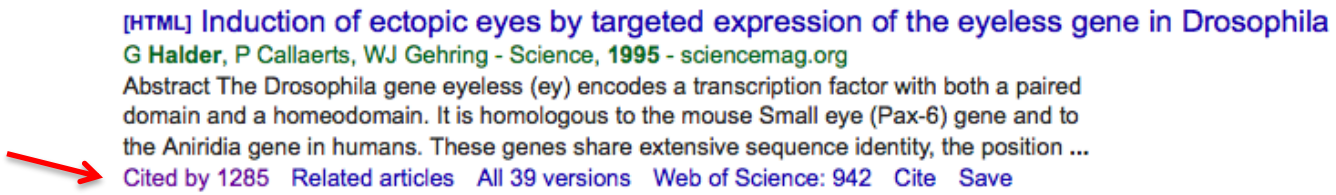
7. What is one additional question you have after reading this paper? Use this question to develop an additional experiment that you would like to do if this were your thesis project. You can do this in very general terms. For example, *can* eyeless *induce eye formation in other tissues, not just imaginal discs?*

Extension

1. Write a brief, 1-paragraph persuasive piece to explain to your parents, roommates, or other non-science students why studying fruit flies is important, using this experiment as part of your rationale.

2. Once a scientist has performed an experiment, analyzed the data collected, and made conclusions about whether that data supports or does not support the hypothesis, additional questions can be asked. These additional questions initiate the scientific method, leading to a new experimental hypothesis, prediction, and experiment. For example, the results from Halder (1995) led researchers (in this lab or in other labs) to follow up on these results.
    a. Find one primary article that followed up on the results from the Halder (1995) paper. Include a citation and/or copy of that paper to your instructor.
    b. Identify the hypothesis and prediction of the paper.
    c. Explain one figure that supports the hypothesis.

Hint: There are a number of ways to find related articles, but one place to start is to look up other papers that have cited that paper. You can do this by going to **Google Scholar**, entering the author's name and title of the article. Then, within the results, select **Cited By** link.



Then, on the left side, you can narrow the search results down by "articles."

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Halder G, Callaerts P, and WJ Gehring. (1995) Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila*. *Science*, 267(5205), 1788-1792.