Name: _____ Period: _____ Date: _____

How environment, genes and behavior interact

When honey bee researchers began to investigate the role of genes in directing worker bees to perform different jobs, they focused on one gene in particular. They were interested in this gene because it was very similar to one found in fruit flies that was related to foraging; fruit fly larvae with different alleles of this gene behaved differently when searching for food. Because of this, fruit fly researcher named the gene *foraging* (abbreviated *for*). Honey bee researchers then named the related gene found in honey bees *Amfor*, for ***A**pis **m**ellifera* "foraging" gene. But what kinds of tests could honey bee researchers do to discover whether *Amfor* actually had something to do with foraging behavior in bees? What does a gene that influences behavior do inside a cell?

This data analysis activity will guide you through the experimental process honey bee researchers followed as they tried to understand the gene *Amfor* and what it might have to do with honey bee behavior. The background section will give you additional information about *Amfor*, and about the honey bee, that will help you understand this process. The following sections will outline some of the experiments that bee researchers have done. Look over the data presented in the figures and answer the questions that follow each passage presented in the following pages. Be sure to read the information surrounding the figures because it will help you answer the questions.

A. Background: The honey bee gene *Amfor*

Most genes are transcribed by the cell into messenger RNA (mRNA) that can then be translated into proteins. Researchers knew from past experiments that *Amfor* codes for an enzyme called protein kinase G or PKG. PKG contributes to many different signaling systems inside the cell. It does this by adding a phosphate group to other proteins. Attaching this small molecule to a protein changes its shape, and the change in shape makes the modified protein work differently. To make PKG able to catalyze this change in other proteins, another small molecule called cGMP must be present as well.

A1. If a cell began to produce more *Amfor* mRNA, what would you expect to happen to the amount of PKG being made in that cell?

I would expect that more PKG would be made in that cell.

A2. In a general way, what might happen to the proteins that PKG acts on if the amount of *Amfor* mRNA increased?

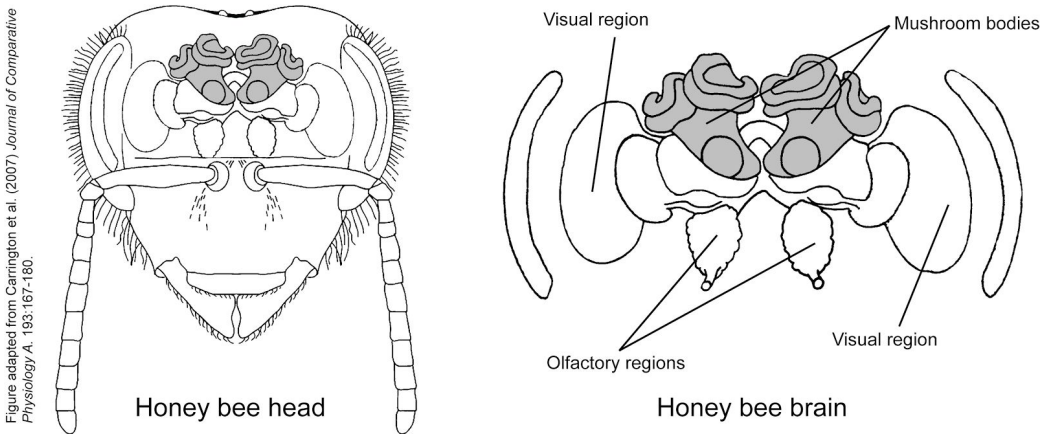
More of the proteins might have phosphate groups added, and/or proteins might have more phosphate groups added.

A3. Since *Amfor* is related to foraging behavior in fruit fly larvae, in what parts of the fly's body would you expect it to be present?

Any body part that could feasibly be related to foraging in larvae or adults—brain, eyes, wings, legs, etc.

B. Background: The honey bee brain

The image on the left shows how the honey bee brain is positioned inside the head. The image on the right shows a cross-section of the brain. The gray shaded area is the mushroom bodies (this region comprises two symmetrical structures, one on each hemisphere of the brain, and is named for its shape!). The honey bee brain also has specific regions that process visual and olfactory information. These areas send information to the mushroom bodies, so this region may help assemble a complete picture of the sensory world that the bee experiences. This region is also involved in learning.



B1. Considering what you know about nursing and foraging behaviors, suggest a difference in what and/or how much information each of these brain regions might be receiving in nurses and in foragers?

- Visual region (optic lobes) (Variable: eg, might suggest that foragers use sight more, so have bigger optic lobes)
- Olfactory region (antennal lobes) (Variable: eg, might suggest that nurses rely only on scent, or that foragers need scent to find flowers, therefore either might have bigger olfactory regions)
- Mushroom bodies (Variable: eg, foragers might have bigger mushroom bodies because they rely more on memory of flowers and locations)

B2. Imagine a single neuron in the bee brain that has to transmit more information in when the bee is a forager than when she is a nurse.

a. Suggest a brain region that this neuron might be found in, and justify your answer.

Variable: reasoning should be similar to the examples listed above, ie, tied to behavioral differences.

b. Recalling what you know about Amfor protein's function, suggest how Amfor could be involved in the hypothetical neuron's changing role.

Amfor might help "activate" the neuron by changing the functioning of its proteins (by adding

phosphate groups).

C. Experiment 1: Is *Amfor* in the honey bee brain?

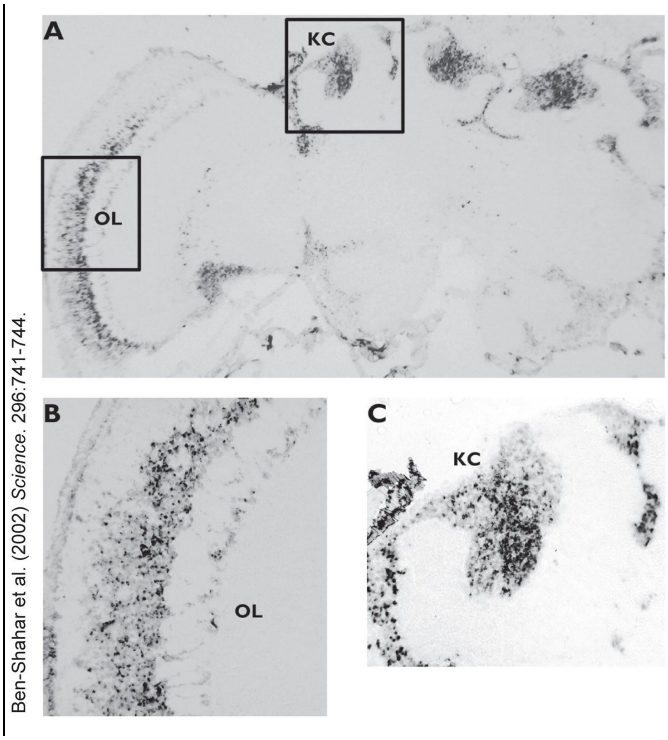
The first step in investigating the possible relationship between *Amfor* and honey bee behavior was to look at whether the gene is expressed (whether its mRNA and related protein are made) in the brain. To do so, researchers used a technique called *in situ* hybridization.

In situ hybridization works because of one of the chemical properties of nucleic acids—the strands of complementary DNA or RNA strands attract each other. When researchers want to find out whether a particular gene is being expressed in a particular tissue, they can create many copies of a single strand of DNA that is complementary to the mRNA that they want to find. These DNA strands also have a built-in dye that can be used to show the location of the mRNA . Researchers can then take a slice of preserved tissue and wash the labeled DNA strands (called "probes") over it; the probe will stick ("hybridize") to any copies of the matching mRNA within the tissue.

C1. *In situ* is a Latin phrase meaning "in position" or "in place". Explain how the name "*in situ* hybridization" describes the technique.

The technique shows where a gene is actually expressed (where mRNA actually is) inside a tissue sample.

The figure below shows *in situ* hybridization of the gene *Amfor* in a slice of a honey bee brain.



Ben-Shahar et al. (2002) *Science*. 296:741-744.

Panel A shows part of a slice of honey bee forager brain that has been used for *Amfor in situ* hybridization. KC stands for Kenyon cells, the cells that make up the mushroom bodies. OL stands for optic lobe. (Refer to the information in Part B for help recognizing these structures.) Panel B shows a close-up of cells in the optic lobe. Panel C shows a close-up of cells in the mushroom bodies.

C2. Recall your answer to question number B1. Was *Amfor* mRNA found in any of the regions that you thought might have different jobs in nurses and foragers?

Will vary depending on original answers—should link to previous answer, eg, I thought optic lobes might be more active in foragers, and neurons in the optic lobes express *Amfor*.

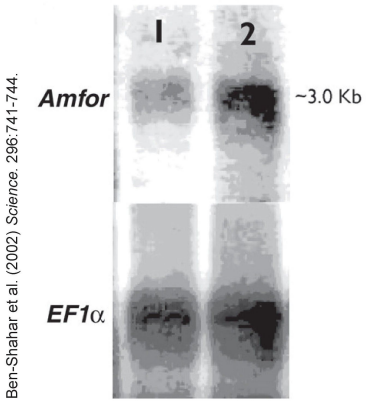
C3. When researchers compared in situ hybridizations of *Amfor* in nurse and forager brains, they could not detect any differences in the amount of mRNA—but *in situ* hybridization is not a good technique for precisely measuring the amount of mRNA in a tissue. What is this technique good for?

Discovering where a gene is expressed/whether or not mRNA of a gene is produced in a particular part of the organism.

D. Experiment 2A: Is *Amfor* expression different in nurse and forager honey bee brains?

The next step to investigating *Amfor*'s involvement in behavior was to identify whether there was more *Amfor* expression in either nurse or forager brains. One of the first ways developed to measure the amount of a specific gene's mRNA in a tissue sample was the Northern blot. Just like *in situ* hybridization, this technique uses a DNA probe. Tissue thought to contain the mRNA to be measured is ground up, and a chemical process is used to separate the RNA from the other cell parts. The RNA is run on a gel in order to separate it out by size. This gel is then transferred to a membrane that holds it in place, and the DNA probe is washed over the membrane. The more of the mRNA being measured exists in a tissue sample, the more probe will hybridize to that mRNA in the gel, and the more dye will show up. When comparing two different samples, researchers must make sure that the chemical process used to remove the RNA from the samples was not more successful in one sample than the other. To do this, they use a second probe to look at the amount of mRNA of a second gene, one whose quantity is expected to be about the same in all samples. This gene is called a "loading control".

In the figure below, researchers used Northern blot to look at the amount of *Amfor* mRNA, as well as the loading control gene *EF1 α*, in a nurse brain (sample 1) and a forager brain (sample 2).



D1. Which brain appears to have more *Amfor* mRNA?

The forager brain appears to have more *Amfor* mRNA.

D2. What does this result tell you about how much PKG protein you would expect to find in a nurse brain compared with a forager brain?

I would expect that there would be less PKG protein in the nurse brain compared with the forager brain.

D3. If the dye for *EF1 α* had been much darker in the forager brain sample than the nurse brain sample, how would that change your conclusions about the data? Explain your reasoning.

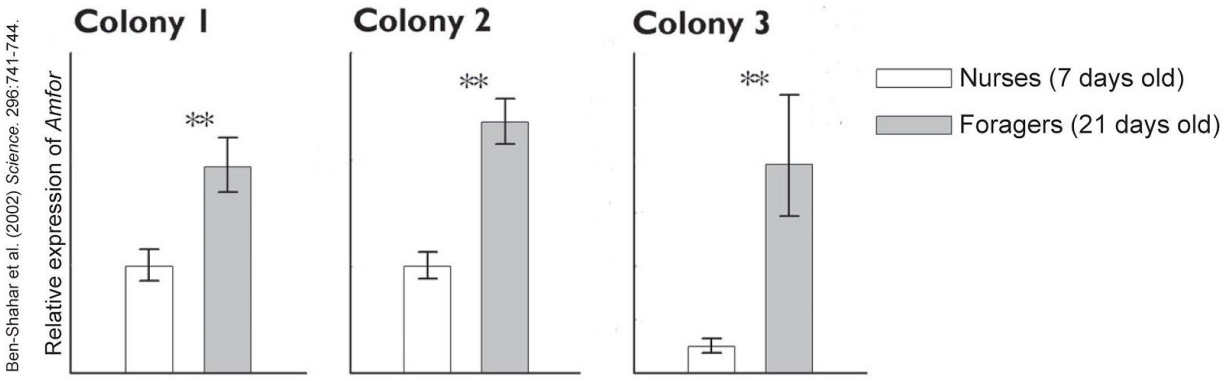
That would indicate a technical error (much more mRNA or much more staining in the forager sample than the nurse sample overall) so that nothing could be concluded about the true difference in *Amfor* expression.

E. Experiment 2B: Is *Amfor* expression different in nurse and forager honey bee brains?

Although Northern blots can be used to compare the quantity of mRNA in two samples, many factors, including the temperature of the room, the amount of time the probe is washed over the gel, and which part of the gel the sample is inserted into can affect how well the probe hybridizes to mRNA in the sample. The invention of the polymerase chain reaction (PCR), a chemical process that allows researchers to make many copies of a small starting amount of DNA, led to a new technique that measures quantities of mRNA that have been separated from a tissue sample. This technique is called Quantitative Reverse Transcriptase Polymerase Chain Reaction, or qRT-PCR.

To perform qRT-PCR, researchers first use an enzyme, originally discovered in viruses, that can read mRNA and convert the message back into DNA. This process is called reverse transcription. Researchers convert mRNA in their samples into DNA. Then, they use the PCR process to copy that DNA many times. PCR works by using heat to separate the double strands of DNA, then allowing enzymes to copy both strands; this process is repeated for many cycles, and the amount of DNA doubles each time. Along with the chemical ingredients needed for PCR, researchers performing qRT-PCR add a special fluorescent dye that is only visible when it is bound to intact DNA. After the copying stage of each cycle, the amount of fluorescence is read by a machine. As the amount of DNA corresponding to the mRNA being measured increases, the amount of fluorescence also increases. If one sample has more mRNA than another, the amount of fluorescence in that sample will be consistently greater. Researchers can quantify the amount of mRNA in one sample compared with another by counting the number of cycles it takes for each sample to reach a predetermined amount of fluorescence.

The figure below shows the results of an experiment that used qRT-PCR to compare the amount of *Amfor* mRNA in nurse and forager brains.



Note that the values on the y-axis in this graph are not absolute units of measurement. They are relative units, meaning that the average expression quantity of the nurses is defined as 1, and the expression in other groups is expressed in terms of how many times larger it is than the average nurse.

** Indicates that there is a significant difference between the average expression levels of the two

groups.

E1. Do the results of the qRT-PCR experiment agree with the Northern blot experiment?

Yes, they agree with the Northern blot.

E2. Why might a researcher measure the same thing using two different techniques?

To have more evidence, and be more certain of the conclusions drawn (not necessary but for reference: if certain types of errors can occur in one type of technique, they probably will not occur in another type of technique, so there will be different types of biases in each; therefore if two different techniques produce the same result, the conclusions drawn from that result are better supported.)

E3. What hypotheses might the researchers suggest for why nurse and forager *Amfor* levels are different? List at least two biological differences between nurse and forager bees.

Variable, eg:

Nurses are younger than foragers (on average.)

Foragers are more physically active than nurses.

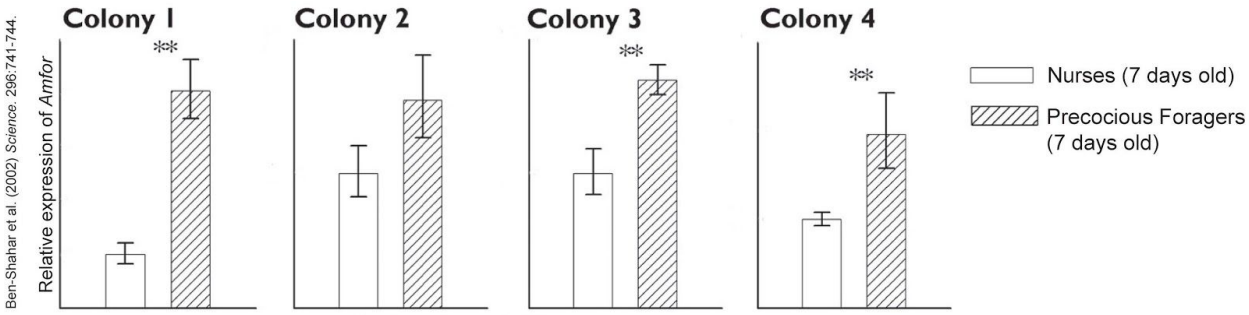
Foragers must use their memory more than nurses, to navigate and learn what flowers are profitable.

Nurses live in a more controlled environment than foragers.

F. Experiment 3: What makes forager *Amfor* expression different from nurse expression?

Honey bee researchers can create special honey bee colonies, called "single-cohort colonies", in which all the bees are young adult bees that have just emerged after pupation. In these young colonies, some bees will quickly mature and begin to forage when they are just a week old, much younger than the average forager age of 21 days. These bees are called "precocious" foragers.

Honey bee researchers compared *Amfor* expression in nurses from single-cohort colonies to precocious foragers from these same colonies using qRT-PCR.



F1. In which group was *Amfor* expression higher?

Amfor expression was higher in the precocious foragers.

F2. What difference between nurses and foragers did this experiment rule out as a possible cause of the *Amfor* expression difference between these groups?

This experiment demonstrated that age does not necessarily explain the increase in *Amfor* expression; even foragers the same age as the nurses they are compared with have higher expression.

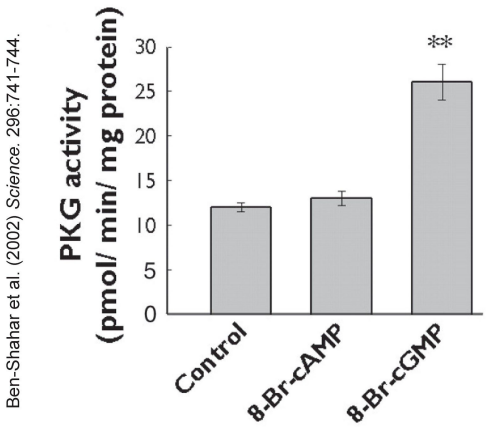
F3. After the preceding experiment, researchers had shown that forager brains typically have a different amount of *Amfor* mRNA than nurse brains, but they had not shown that *Amfor* mRNA, or PKG protein, causes foraging behavior. What type of experiment could they do to test this?

G. Experiment 4A: How does a change in PKG activity affect foraging behavior?

For this section, recall the background you read in the beginning of this worksheet about what the protein encoded by *Amfor*, called PKG, does inside the cell. Review it if you need to.

When researchers study enzymes, they can use an activity assay to test how much of an enzyme there is, or how well the enzyme is working. A sample of enzyme is added to a chemical reaction that enzyme promotes, and the researcher can quantify how well the enzyme is functioning by measuring the amount of product that is created.

Below is a figure that shows what happens to PKG activity when researchers test the effect of different chemicals added to the reaction. 8-Br-cGMP is a molecule that has very similar structure to cGMP; recall that cGMP is a small molecule that binds to PKG to help PKG perform its functions inside the cell. 8-Br-cAMP is a molecule that has a similar size to 8-Br-cGMP, but is a little different in structure, so that it cannot bind to PKG in the same way.



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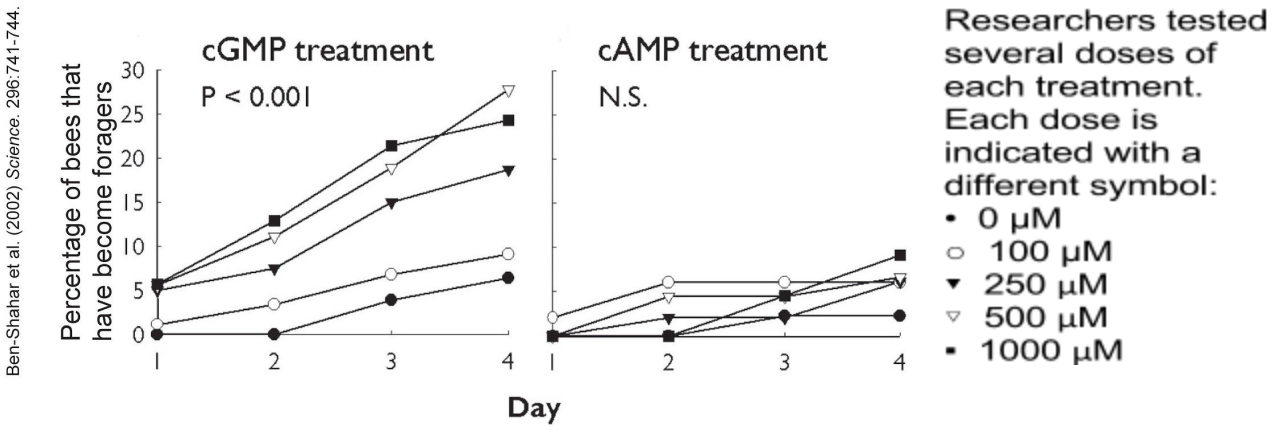
G1. How does PKG activity change when 8-Br-cGMP is added, compared with when 8-Br-cAMP or a control with nothing but a control solution is added?

G2. What might happen to the activity of PKG inside the brain of a bee that is injected with 8-Br-cGMP?

G3. Suggest a way that 8-Br-cGMP could be used in an experiment that tests whether the product of *Amfor* expression causes increased foraging behavior.

H. Experiment 4B: How does a change in PKG activity affect foraging behavior?

After testing the effect of 8-Br-cGMP on PKG activity, bee researchers treated 4-day old adult bees with a brain injection of either 8-Br-cGMP or 8-Br-cAMP, added them to a colony, and then observed how many of the treated bees began to forage over the next 4 days, when the bees were 5-8 days old.



H1. Describe any differences you see between the behavior of the bees treated with 8-Br-cGMP, and those treated with 8-Br-cAMP.

H2. What effect do you think increased PKG activity has on foraging behavior in honey bees? Support your answer with the data above.

H3. How might your answer to question H2 be different if the effects of these two chemicals on foraging behavior were identical, even though one affects PKG activity and the other does not?

H4. What additional control is missing from this experiment that could help you interpret the hypothetical results described in H3? In other words, what do the two treatments (8-Br-cGMP and 8-Br-cAMP) have in common, and how could you test for that experimental factor's effects?

Summary questions:

1. Present evidence from Experiment 4 that not every bee with increased PKG activity immediately become a forager.

2. Some experiments that change conditions in the environment that a bee encounters (like the amount of food in the hive, or the number of larvae that need food) make bees more or less likely to become foragers. Would you expect the treatments in these experiments to change brain *Amfor* levels? Explain your reasoning in your answer.

3. Some subspecies of honey bees produce workers that mature into foragers more quickly or more slowly than others. Would you expect these subspecies to have differences in the sequence of the *Amfor* gene? Explain your reasoning in your answer.

4. Would you expect nurse bees in a faster-maturing subspecies to have different brain expression of the *Amfor* gene than nurse bees of a slower-maturing subspecies? Explain your reasoning in your answer.