Shh: Silencing the Hedgehog Pathway

"I'm so relieved," Ann said as she plopped down in the coffee shop booth where her friend Delores was reading e-mail on her laptop.

"Oh, Ann!? What did the doctor say?" Delores asked.

"Well, I do have skin cancer, but it's not melanoma. It's basal cell something. Anyway, it's very common and easy to treat," Ann reassured her.

"Is it genetic?" Delores asked, "or does it have something to do with that nice tan you showed off during your teens and twenties?"

"Well no one else in the family has had skin cancer." Grimacing, Ann added, "It's more likely I'm paying for my tan."

After Ann left, Delores searched for "basal cell cancer" on the Web. She wondered how her friend ended up with skin cancer. She found a 2004 paper by Athar and colleagues that explored BCC (basal cell carcinoma) and the effect of UV radiation. BCC, the most common kind of cancer, was linked to problems with the hedgehog signaling pathway. Exposure to UV radiation was one way to impact the pathway.

"More questions than answers," Delores sighed. She looked up "hedgehog signaling pathway" in Wikipedia. She found that this pathway controls cell division and is important in early development. The pathway was first discovered in fruit flies with a mutation that made them shorter and especially bristly. The researcher thought the fly larvae looked like hedgehogs.

Delores returned to the Athar article. The researchers divided mice into two groups and then exposed them to UV radiation. One group was given a drug called cyclopamine, a known antagonist to the hedgehog pathway, in their drinking water, and the other group got plain water. The mice that got the cyclopamine had many fewer BCCs at the end of the experiment.

"I wonder if they will give Ann cyclopamine for her BCC?" Delores thought as she closed her laptop.

Figure 10.1 Using a laptop at a coffee shop.

Core Investigations

I. Critical Reading: Cell Signaling Pathways

You should be familiar with the structure and function of proteins that have active sites, such as enzymes or antibodies. To complete this investigation, you should read Chapter 11: Cell Communication (specifically, Concepts 11.1 and 11.4) and Chapter 18: Regulation of Gene Expression (specifically, Concepts 18.4 and 18.5).

1. What is cancer? (Hint: Use of multiple sources for this definition, such as Cancerquest [http://www.cancerquest.org] in addition to the text, is recommended.)

2. What are some of the causes of cancer?

3. Interpret the graph in Figure 10.2 by answering the following questions.

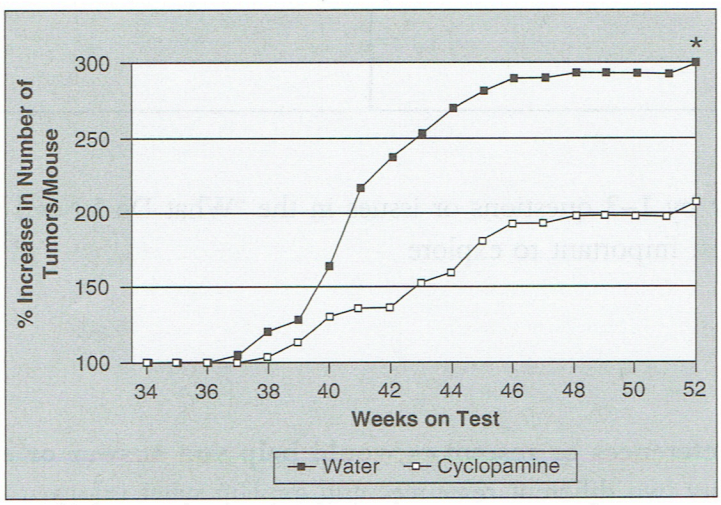

Figure 10.2 Effect of cyclopamine on BCC tumor formation in UVB-irradiated mice. (After Athar et al., 2004) (*Note:* The asterisk means the differences between the two treatments are statistically significant.)

a. On the basis of the shape of the curves, explain the patterns of tumor production in control and experimental mice in weeks 34–52.

b. What is the overall percentage increase in tumors for control versus experimental mice?

4. Use Figure 10.3 to answer the next two questions.

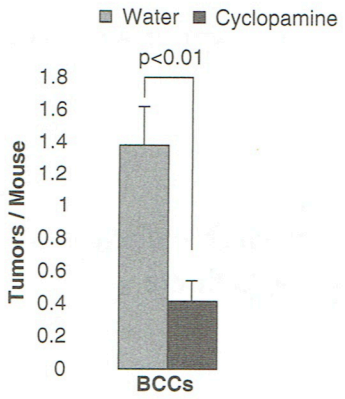

Figure 10.3 Average number of tumors per irradiated mouse with and without cyclopamine. (After Athar et al., 2004)

a. How effective was cyclopamine in treating BCC in the mice?

b. Which of these two data formats—the bar graph or the line graph—would be more effective in presenting the results of the experiment to the public? Which would be more effective for other scientists?

The hedgehog signaling pathway plays a crucial role in the development of many animal embryos. In addition, the hedgehog pathway controls regeneration of short-lived adult tissues, such as those in skin and blood. When the hedgehog pathway is active, transcription of proteins occurs in target cells followed by rapid division of those cells. The hedgehog pathway is also active in BCC and several other cancers.

The *hedgehog* gene family codes for signaling proteins that serve as ligands binding to receptors in nearby target cells. These proteins activate the hedgehog pathway in the target cells. The hedgehog pathway in the target cells has two membrane proteins named Patched (Ptch) and Smoothened (Smo), as well as several intracellular proteins.

When Shh (Sonic hedgehog) ligand binds to Ptch, then Smo is activated, the signal is transduced, and transcription and cell division result. In the absence of the hedgehog signaling protein, Ptch inhibits Smo, no signal is sent to the intracellular components of the hedgehog pathway, and thus transcription and cell division do not occur. Smo and the subsequent intracellular pathway may also be turned on by mutations that inactivate Ptch.

5. Is the hedgehog signaling pathway a local or long-distance type of signaling? Explain.

6. Examine Figure 10.4 and identify which molecules are involved in reception, transduction, and response in the hedgehog pathway.

7. The mechanism of the activation of Smo by the hedgehog ligand binding to Ptch is not completely understood. However, the model shown in Figure 11.12 in the text shows a pathway with two membrane proteins, similar to the arrangement of membrane proteins in the hedgehog pathway. In this model, cell signaling is involved when the ligand binds to the first receptor protein, activating the G protein. The G protein then activates the second membrane protein, which transduces the signal to the interior of the cell. Explain how this mechanism might be applied to the two membrane proteins in the hedgehog signaling pathway.

8. As scientists evaluate new data, they frequently have to revise their models. Because we know that Ptch is an inhibitor of Smo and G protein is not involved, revise the model in Figure 11.12 to incorporate this new information.

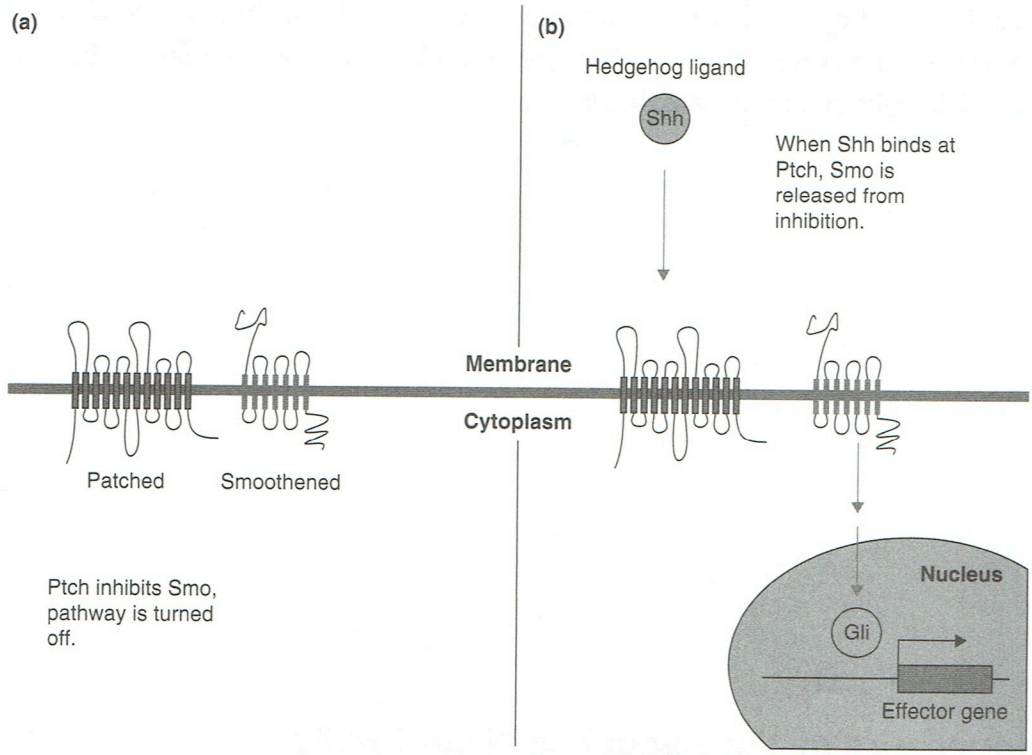

Figure 10.4 Schematic diagram of the hedgehog signaling pathway in vertebrates. (a) The target cell without the hedgehog ligand. Patched and Smoothened are transmembrane proteins embedded in the plasma membrane. Patched inhibits Smoothened and the pathway is turned off. (b) When the hedgehog ligand Shh joins with Patched, Smoothened is released from inhibition and the pathway is turned on. (Weitzman, 2002)

9. Cyclopamine is a known antagonist of Smo. Describe how cyclopamine reduces the number of BCCs in UVB-irradiated mice.

The hedgehog signaling pathway is active in the early embryo during development of the neural tube, motor neuron specification, left-right symmetry, body plan, limbs, and retinas (Matlack et al., 2006).

In the 1950s, sheep feeding on the corn lily (Veratrum spp.) in mountain pastures gave birth to a number of lambs with only one eye. The number of cyclopean lambs (named for the one-eyed Cyclops of Greek mythology) were explained when the compound later named cyclopamine was discovered in the corn lily. To see an image of cyclopia in sheep, go to http://teratology.org/jfs/NaturalTeratogens.html.

10. Explain how a failure to have cell division occur at a critical time during development could lead to lambs with one eye. See Concept 18.4 on critical events in the development of left-right symmetry and body plan.

II. Phylogenetics of the Hedgehog Gene Family

Nobel Prize researchers Christiane Nüsslein-Volhard and Eric Wieschaus investigated fruit fly mutations in order to make sense of the role of genes active in the development of fly embryos. They mutated one gene—later named *hedgehog*—that resulted in dense spines in shortened fly larvae.

Homologous *hedgehog* genes were later discovered in vertebrates. After these genes were sequenced in several different kinds of animals, they were compared and used to determine phylogenetic relatedness. If you have not yet studied phylogenetic classification, you may want to read Chapter 26 before completing this investigation. Consider the phylogram in Figure 26.12 in your text as you answer the following questions.

1. What species is used as the outgroup for the *hedgehog* gene in this phylogram? Provide a reason for using this species.

2. What does the phylogram tell us about the *hedgehog* gene in mammals and birds as compared to the *hedgehog* gene in mammals and amphibians?