

Should I Really Wear Sunscreen?

There seems to be a belief in our society that you have to have a tan to look right. This shows up in the notion that you look pallid and *really* uncool by the end of winter, and in the standard joke that you have to wear your dark glasses so *some* people's legs don't blind you when they first start wearing shorts. Some people think they beat this problem, though, by spending Spring Break in Florida—or, more easily, by going to a Tanning Salon. Then they develop a nice, “healthy-looking” tan.

This tanning reaction that humans undergo is obviously beneficial. The first time that anyone is exposed to the sun after a long period away from it usually results in a sunburn. We all know about *that*.. We also know that after a time, we become less sensitive and can stay in the sun for a longer time without burning. This usually correlates with the development of a tan—although there are wide variations in how much of a change people undergo, depending in part on their initial skin color, and on the extent to which ultraviolet light induces the synthesis of additional melanin. Even if it may be difficult to see the “tan,” it is usually easy to recognize its effect in the lessening of sensitivity to sunburn. It therefore appears to be the case that humans have developed a very effective method of producing one or more chemical compounds that help shield us from ultraviolet light.

Plants have a very similar reaction to UV light. Have you ever grown a plant in your room or apartment? If you have, you may have been tempted to put it outside in the summer. There are several good reasons to do this. For one, indoor light is often too dim for most plants. Unless your plant is one of the species we typically call “houseplants” (usually derived from the understory of tropical forests, where there has been strong selection for the ability to grow in dim light), it probably does best in a south-facing window, but is not at its best even so. A second reason to put your plant outside is that indoor light is not uniform; it is much brighter on the side of the plant facing the window. This makes the plant bend toward the window, and become asymmetric. A third reason is that the outdoors has wind. This jiggles and bumps the plant, stimulating a growth response called thigmomorphogenesis, which makes the plant more sturdy. All together, the plant is likely to grow better outside.

Just like us, however, plants can become sunburned. If you put your plant outside in a nice sunny spot and leave it there all day, you will probably find that its leaves become badly damaged. It would be much better to expose the plant to the sun gradually, increasing its exposure a little bit every day. If it's not easy to tell how long to leave it in the sun, you might put on your swimsuit and sit next to it...when you start to develop a sunburn, it's been long enough.

Also just like us, plants develop a tan—except for them, it's more of a yellow or purple. In response to UV light, plants produce anthocyanins, which are a family of related compounds whose exact structure determines whether they look yellow or purple to us. It is purple anthocyanins that make the leaves so dark on certain maples, plums, and other plants. The yellow anthocyanins are usually not apparent to us because of the darker chlorophyll. Independent of their color in the visible spectrum, the anthocyanins absorb UV. This prevents the UV from reaching other, more critical molecules in the cell (e.g., DNA), and thereby protects the leaves from damage.

If plants and humans develop sunburns when exposed to suddenly-increased doses of UV light, and if we've both evolved means of responding to UV by producing protective compounds, then there must be something pretty nasty about UV light. What does it do, and how?

Recall, for a moment, the work you have done with chlorophyll. What was its function? How did it work? How did you test these things experimentally?

If you think about your work with chlorophyll, you may see several general principles about light and how it interacts with biological systems. Light is energy—electromagnetic radiation. When a molecule absorbs light, it absorbs energy, and the energy has to go someplace. In the case of chlorophyll, the energy is passed on by means of a pre-arranged system of interacting proteins and membrane systems. The energy is used to do work, in the form of moving electrons (eventually to split water molecules into oxygen and hydrogen). Similarly, if light is absorbed by the visual pigments in our retinas, the energy is used to induce a change in the membrane of the photoreceptor cell, triggering a neural response. But, what if the light shines upon things that have no prearranged energy-transfer system and the absorbed energy has no place to go? Usually, the energy serves to agitate the molecules, and make them move about a little more—they heat up as the light energy is converted into kinetic energy. But if the wavelength of the light and the molecule are perfectly “tuned” to one another, the molecule can absorb the energy directly, and change its chemical properties.

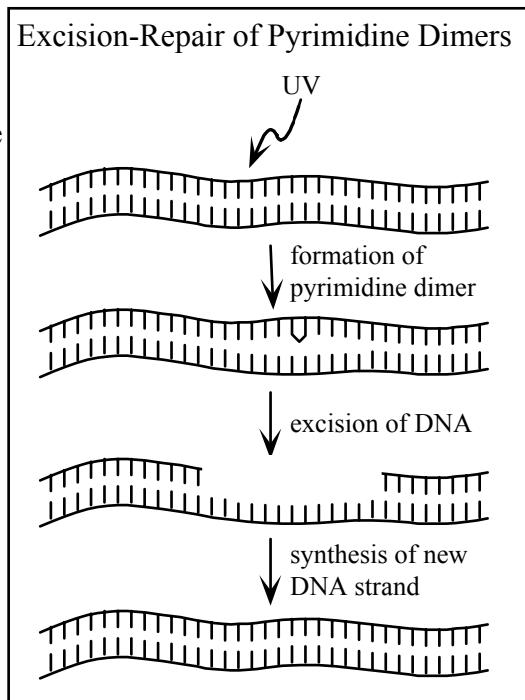
Just as the structure of chlorophyll makes it able to absorb red light directly, many organic molecules can absorb UV light directly. The major structural feature that makes this possible is the presence of several double bonds. This means lots of organic compounds absorb UV. Among them are proteins (check the structures of the amino acids phenylalanine, tyrosine, and tryptophan, for example) and nucleic acids (check the structures of the purine and pyrimidine bases, A, G, C, T, and U). There are some pretty important molecules in cells that can absorb UV light.

When an organic molecule absorbs UV light, the molecule is kicked into an “excited” state—just like chlorophyll is kicked into an “excited” state by red light. In the absence of an energy-transfer system, the excited molecule has two choices. First, it can release the energy and return to the ground state, giving off light in the process. That is, it can fluoresce. Remember our purified chlorophyll (purified away from the energy-transfer system), that exhibited red fluorescence when we shined light upon it. Similarly, organic molecules can fluoresce. Tryptophan and nucleic acids exhibit fluorescence when irradiated with UV, but the light that is given off is still in the ultraviolet part of the spectrum, so we can’t see it without appropriate instruments.

The second option available to an excited molecule is more damaging. The energy can make the covalent bonds less stable, and one may break. The broken bond is highly reactive, and rapidly forms a covalent bond with another unsuspecting molecule nearby. When this occurs, the UV has had the effect of altering the function of the irradiated molecule in a profound way. It usually inactivates it, usually irreversibly.

If a protein is damaged by UV light, there is usually little to worry about. The protein is simply one of many identical molecules in the cell, and can be replaced by a new protein molecule encoded by the appropriate gene. However, if the DNA is damaged by UV, then there are problems. Unless the damage can be repaired, the gene is dead.

The most common kind of UV-induced damage to DNA is the formation of “pyrimidine dimers.” This occurs where two pyrimidine residues (for example thymines) are side-by-side in the DNA, as in the sequence ACGTTGAT. UV could join the two T’s into a T[^]T dimer. When this happens, the DNA can neither be replicated nor read into mRNA, because a T residue involved in a T[^]T dimer cannot form a normal A:T base pair. The pyrimidine dimer thus creates a “roadblock” to the DNA- or RNA-polymerase, and prevents it from passing the dimer. In order to deal with this problem, all organisms have developed systems for repairing DNA damage. In



many species (but not, alas, us) there is an enzyme that binds to pyrimidine dimers and, when irradiated with visible light, reverses the pyrimidine dimer. In virtually all species (as far as we know) there is another system that repairs DNA damage by physically removing a portion of the damaged strand of DNA (leaving the undamaged strand intact), then copying the undamaged strand into a complementary copy, replacing the damaged region. Usually, this works pretty well. Unfortunately, the DNA repair system is not as careful as the system that normally replicates DNA in preparation for mitosis. Occasionally, mistakes are made—and we get UV-induced mutations.

Because we are diploid organisms, the occasional UV-induced mutation that inactivates a gene usually has no effect. This is because most mutations that inactivate a gene are recessive. Thus, most new UV-induced mutations are recessive, and are not observed. However, some mutations are dominant. Furthermore, it is possible to induce mutations in both copies of a gene. Although this is rare, we each have so many skin cells that it is possible for at least one cell to suffer such a fate. [You can do a rough calculation: if the probability of damaging one copy in a particular cell is one in a million (10^{-6}), then the probability of damaging both copies is $10^{-6} \times 10^{-6}$, or 10^{-12} . But, if you have roughly 10^5 skin cells per square centimeter of skin, and roughly 10^4 square centimeters of surface area, then you have a probability of one chance in a thousand of such an event in some skin cell. You can increase this probability by exposing yourself to higher doses of UV light.]

In general, UV-induced mutations in humans are not passed on to our offspring. To be exposed to UV light, cells must be near the surface. This includes our skin and eyes, and maybe our tongues if we open our mouths a lot while facing the sun, but does not include our gonads. These are always inside a sufficient thickness of tissue to be well shielded from UV. The UV-induced mutations are nonetheless official, real mutations. They are heritable *at the level of the cell* if not at the level of the whole organism. Any mutation induced in a skin cell will be passed on to the progeny of that skin cell. It is this fact that causes the problems.

Why are you the size you are? Why doesn't your skin continuously expand into big floppy bags that hang off and flap in the wind? Obviously, our cells know when to stop dividing. There are molecular cues that cells send to one another to control their division—when there are enough, they stop. The mechanisms that cells use for this control depend on specific proteins with well-defined biochemical functions. The proteins are, of course, encoded by genes.

When these growth-control genes are mutated in a cell, then that cell may no longer recognize the signals sent to it by its neighbors. Rather than arrest its cell division as it is supposed to do, it begins to divide in an unregulated manner. Depending on the cell type, unregulated growth may result in an outgrowth of the skin as the mutant phenotype is passed from parent cell to progeny cells. This forms a carcinoma—a tumor. In some cases, unregulated growth results in cells that are displaced from the skin, enter the circulatory system, and migrate to new locations—metastasis. The former can usually be removed surgically, often with no lasting harm to the individual; the latter is exceptionally difficult to deal with, and is commonly fatal.

We have talked here about the effects of UV light, about how it causes physical damage to protein and DNA molecules, and how this damage can have significant consequences for the genetic properties of single cells—cancers, carcinomas, melanomas, etc. Our tans help protect us from massive UV damage, but they are not 100% effective, and cannot always prevent single-cell mutations. Sunscreen helps more.

Unfortunately, it is difficult to obtain first-hand knowledge of the effects of UV light when studying humans (beyond the sunburn/tan phenomenon). To obtain convincing evidence of the link between UV and skin cancer, we need to turn to epidemiology and examine cancer rates and UV exposures among very large numbers of individuals. The difficulty of this kind of study and the time required to obtain the data make the initial findings seem tenuous. This is probably part of the reason that there remain Tanning Salons that advertise a “safe tan.”

In the laboratory, we should do an experiment that will investigate the effects of UV unambiguously. How would you design such an experiment? Let's go through a few of the steps.

First, we need to define the question we want to ask. What we really want to know is whether UV light really does induce mutations. This is probably the most important question, since it could influence our behavior and even survival (well beyond the issue of sunburn).

To ask this question, we need an experimental approach. Clearly, we need to shine UV light on something, and look for mutants in its progeny. (We need to look at the progeny, of course, because we need to learn whether any possible mutations really are *heritable*.) Even saying this much tells us something about the experiment: we need an organism that produces progeny quickly, and for which the progeny-producing cells can be exposed to light. This rules out humans, mice, and even flies—but a single-celled microorganism would work just fine. Let's use yeast.

If we use a microorganism, will our results apply to humans? Will our experiment really be relevant to us? The answer to this question is “yes.” By focussing on mutations, and inheritance of genetic information, we are studying something so basic to life on Earth that the results will be applicable to virtually all living things. Anything we find for yeast will be relevant for any cells on the surface of any other organism.

We can now describe the experimental design more clearly: we will spread some individual yeast cells on nutrient agar in a Petri dish, and then irradiate the cells with UV light. We will allow the cells to divide and form colonies on the surface of the agar. Each colony

will be the thousands of progeny of the original single cell that we put at that position on the plate and then treated with UV. If a colony exhibits a mutant phenotype, then the UV must have induced a mutation.

This is a pretty good design. It will not only tell us whether mutations can be induced, but, because many cells can fit on a Petri dish, it will allow us to determine the frequency of mutation—whether one cell in ten, or one cell in a million is mutated by UV light. We can even vary the time of exposure to UV (using a different time on each of several Petri dishes), and determine how the mutation frequency changes with the UV dose. The specific details of this experiment are described in the Lab Manual.

Before you go to your discussion session this week, think about (and develop answers to) the following questions:

Questions aimed at understanding the reading:

1. How does ultraviolet light cause damage to cells?
2. Describe a mechanism whereby UV light might cause skin cancer.

More interesting questions:

3. Some organisms exhibit “alternation of generations” in which a diploid form alternates with a haploid form. Which form would you expect to be more sensitive to UV light, and why?
4. At a party on Friday night, you become involved in a discussion of UV light. Someone suggests that mRNA is the molecule that is the most important “target” for damage by UV light (because it is single-stranded). Provide an argument that you would use either to support or refute this idea, according to your understanding of how UV light affects cells.
5. Is a thymine-dimer repair system likely to be evolutionarily ancient, or something that evolved only recently? You can support your answer with several different kinds of logic. See how many you can think of.
6. What’s all the fuss about Chlorofluorocarbons (CFC’s) and the ozone hole? [You’ll need to know a few things that are not covered in the discussion that is presented here. But, this has been in the news a lot, so you may be well-equipped to answer this question. If it’s not clear, ask. What does ozone do? Be careful—stratospheric ozone is not ecologically equivalent to automobile-exhaust ozone (smog).]