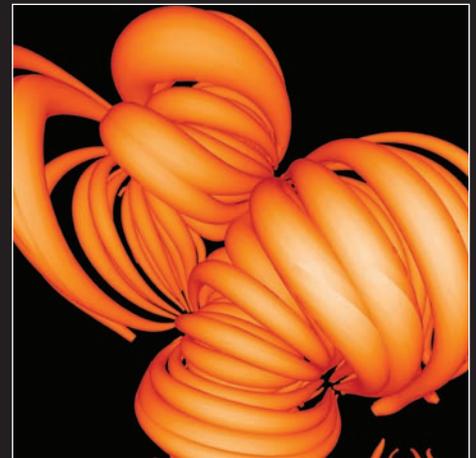
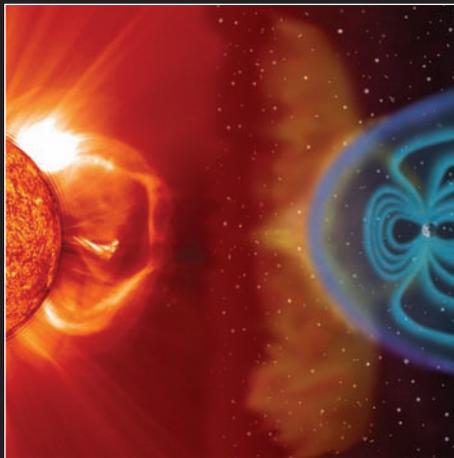




Space Faring

The Radiation Challenge

An Interdisciplinary Guide
on Radiation and Human
Space Flight



Module 2:

*Radiation Damage
in Living Organisms*

Educational Product

**Educators
and Students**

**Grades
9-12**

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Radiation Educator Guide

Module 2:

Radiation Damage in Living Organisms

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Module 2: Radiation Damage in Living Organisms

As we have discussed, space radiation can penetrate habitats, spacecraft, equipment, spacesuits, and even astronauts themselves. The interaction of ionizing radiation with living organisms can lead to harmful health consequences such as tissue damage, cancer, and cataracts in space and on Earth. The underlying cause of many of these effects is damage to deoxyribonucleic acid (DNA). The degree of biological damage caused by ionizing radiation depends on many factors such as radiation dose, dose rate, type of radiation, the part of the body exposed, age, and health. In this section, we will discuss the risks and symptoms of space radiation exposure including how and why this radiation causes damage, and how the body works to repair the damage. We will also discuss how scientists study the effects of radiation on living organisms, and why this research is important to NASA.

Why is NASA Studying the Biological Effects of Radiation?

NASA wants to keep astronauts safe and healthy during long duration space missions. To accomplish this challenging task, NASA has identified four significant health risks due to radiation that need to be well understood to enable the development of effective countermeasures. The risks are described in the NASA Bioastronautics Critical Path Roadmap, and include carcinogenesis, acute and late central nervous system risks, chronic and degenerative tissue risks, and acute radiation risks.¹ NASA scientists are working to understand the molecular, cellular, and tissue mechanisms of damage, which include DNA damage processing, oxidative damage, cell loss through apoptosis or necrosis, changes in the extra-cellular matrix, cytokine activation, inflammation, changes in plasticity, and micro-lesions (clusters of damaged cells along heavy ion tracks). Understanding that information will help researchers develop the appropriate countermeasures.

How Do Scientists Study Biological Change During Spaceflight?

Because the radiation environment in space is different than that on Earth, the biological responses will be different. As a result, NASA scientists must develop space biology experiments that are designed to carefully study model organisms in space. In this scenario, the organism is sent into space and allowed to grow and develop. This part of the experiment is called the flight experiment. The same experiment is also repeated on the Earth, and this is called a ground control. Careful analysis of both the flight experiment and ground controls are critical to understanding the biological changes that result from spaceflight.²

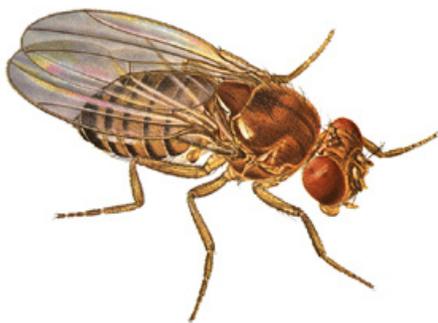
Many studies are also carried out in ground-based research. Opportunities to fly experiments can be rare, and experiments must be well planned. Ground-based research allows a variety of parameters to be tested so that the investigator can decide which will be the best to focus on in a spaceflight experiment. For radiation studies, ground-based research can also help in identifying the specific biological responses for a particular radiation source. This is because on Earth, biological experiments can be carried out using a source that simulates just one kind of radiation, rather than the complex mix of radiation types that make up the space radiation environment. With a better understanding of biological responses to space radiation, we will be able to better design our countermeasures.

NASA Ames researchers in the Drosophila lab.

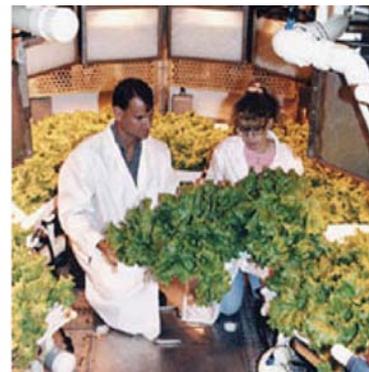


¹ <http://bioastroroadmap.nasa.gov/User/risk.jsp>

² <http://quest.nasa.gov/projects/flies/>



The fruit fly is a model organism.
Image Credit: insectslimited.com



NASA scientists are looking for better ways to grow plants both on Earth and in space.

Using Non-Human Organisms to Understand Radiation Damage

To fully understand the biological response of radiation in humans, NASA scientists begin the process by studying model organisms. In general, biological systems are similar across many species; studying one animal can lead to deeper understandings of other animals, even humans. Some animals are easier to study than others, and those with short life cycles make it quicker to study multigenerational genetic effects. Another reason these organisms are commonly used is because scientists know a great deal about them. For most model organisms, their entire genome, physiological, and behavioral characteristics are well understood. Model organisms are small in size, so large numbers of them can be grown and studied in a small volume, which is very important for the confined environment aboard spacecraft. Having a large population to study also reduces the statistical variation and makes the research more accurate. Much of our understanding of life and human disease is because of scientists' work with model organisms. This is also true for what is known about the biological effects of space radiation. Examples of model organisms include bacteria, yeast, worms, plants, fruit flies, and many others. Fruit flies, like humans, have reduced ability to learn when they are deprived of sleep. They can also sense the direction of gravity, and are affected by radiation. Moreover, they have many things in common with humans, including cellular processes, brain cell development, similar behaviors, and nearly identical disease genes. In fact, there is a great deal of similarity, or homology, between the DNA of these organisms and humans.

Other organisms like ordinary baker's yeast (*Saccharomyces cerevisiae*) also contain genes for DNA repair that are very similar to human genes with the same function. Therefore we can use yeast as a model system to explore the effects of radiation on cells. Like human cells, most yeast cells effectively repair DNA damage caused by UV radiation. However, some yeast strains have mutations that prevent them from performing certain types of DNA repair. Because they cannot repair all the damage to DNA, these cells usually die after exposure to UV radiation. In addition to sensitivity to UV radiation, yeast is also sensitive to space radiation. In a biological assessment of space radiation in low-Earth orbit, yeast inside special experiment hardware has been shown to have a decreased rate of survival following exposure to beta particles (electrons) and low-energy protons.³

Other findings suggest there are highly coordinated gene expression responses to gamma radiation. This knowledge is especially important when designing countermeasures for astronauts during long-term lunar surface operations or microgravity spacewalks.

Plants are also commonly used in radiation studies. It has been shown that plant growth is inhibited by radiation. Like mammals, the embryo of a plant is very sensitive to radiation damage as compared to the adult. The rate of seed germination is reduced, and the rate of growth is slowed.⁴

Excessive UV radiation will lead to an inhibition of plant growth processes in general. Such alterations in primary productivity (photosynthesis) can change entire ecosystems in the oceans, on land, and even in bioregenerative life support systems that would be aboard future spacecraft. Thus, NASA scientists need to understand how plants respond to radiation if future space explorers depend upon them for nutrient cycling and food. Experiments involving plants in space, like the Biomass Production System, have been a favorite of astronauts during long-duration stays onboard the International Space Station.⁵

3 <http://www.spaceflight.esa.int/users/index.cfm?act=default.page&level=11&page=2120>

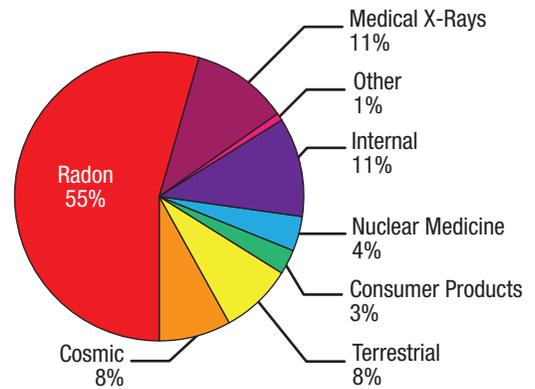
4 www.esd.ornl.gov/programs/ecorisk/documents/tm13141.pdf

5 <http://mediaarchive.ksc.nasa.gov/detail.cfm?mediaid=5186>

http://www.nasa.gov/images/content/58483main_Peggy_Whitson_Plants.jpg

What are the Risks and Symptoms of Radiation Exposure For Humans?

It is important to note that the biological effects of acute and chronic radiation exposure vary with the dose. An average background radiation dose received by an average person can be approximately 3 mSv/year (including radon) without causing detectable harm while an exposure of 1 Sv/hour can result in radiation poisoning (nausea, vomiting). A person exposed to 100 mSv has roughly a 1 in 200 chance of developing cancer later in life, while a 1,000 mSv dose will cause cancer in about 1 in 20 people. Receiving 3,000 to 5,000 mSv during a short period of time (minutes) will result in death in 50% of the cases. A person that experiences a massive 10,000 mSv dose will risk death in a matter of just a few days or weeks. Both acute and chronic exposure to such large doses can cause bleeding and inflammation due to lowered platelet counts. Suppressed immune system function and infections are possible due to lowered white blood cell counts. Reduced fertility or permanent sterility could also result. In addition to damage at the tissue, organ, and whole organism level, radiation has the ability to destroy molecules like DNA.



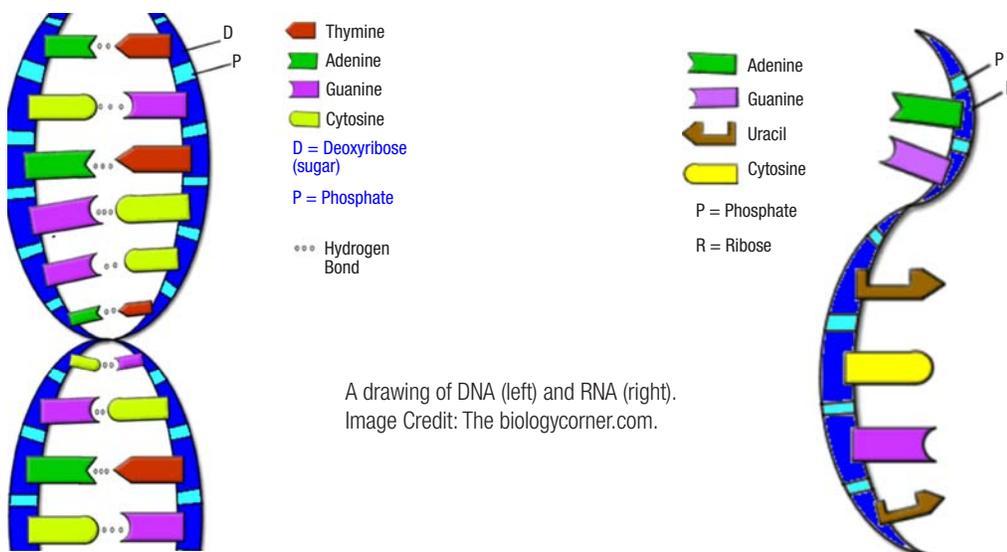
Radioactive radon gas produced from the breakdown of uranium in the Earth's crust accounts for over half of the radiation exposure to the general public. Image Credit: University of Illinois Extension

What is DNA?

DNA is the blueprint of life stored in the cells of every organism. DNA contains the code for all the information required for the synthesis of proteins, cell reproduction, and for organization of the tissues and organs. The information in the DNA is arranged in sections called genes. Gene codes are read by the cell's manufacturing system to make proteins. Proteins are the building blocks for biological structures, and also the functional machinery of the body. Therefore it is vital to our health for the structure of DNA to remain intact.

What is the Structure of DNA?

A DNA molecule has the shape of a double helix ladder that is only ~2 nm wide. DNA is made of individual units called nucleotides. The information in DNA is coded in paired pyrimidine and purine nucleotides along an incredibly long molecule. A nucleotide contains three different types of molecules: a phosphate, a ribose sugar, and a base. The backbone of the helix is made of alternating phosphate and ribose sugar molecules. The rungs of the ladder are base pairs. Each ribose of the backbone has a base attached, which pairs with a base that extends from the opposite backbone. There are four different types of bases in DNA: adenine, thymine, guanine, and cytosine. DNA is arranged into 23 chromosomes in human cells. If stretched out, the DNA of one chromosome, on average, would be about 5 cm. If all DNA in a cell were lined end to end, the molecule would reach about 3 m. If you took all the DNA in all the cells from one human and lined it end to end, it would reach from the Earth to the sun and back 70 times!





What is DNA's Role in Protein Production?

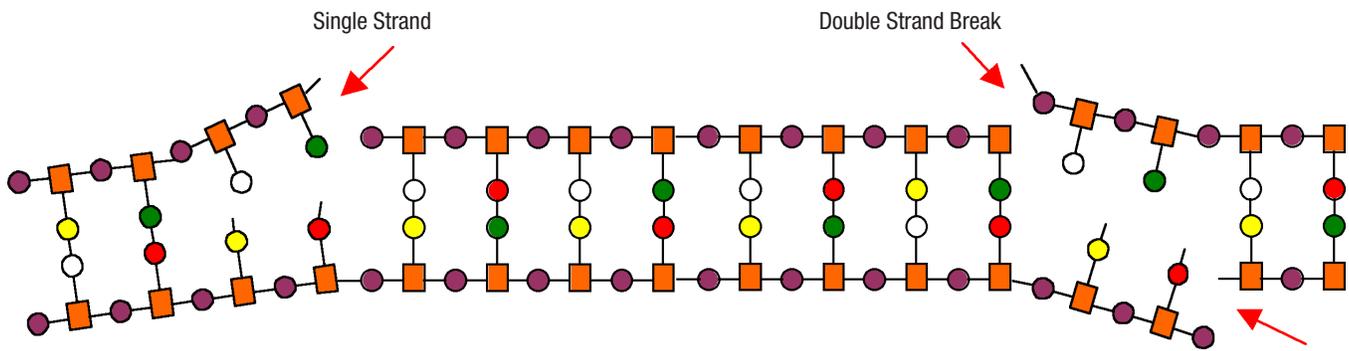
DNA is the storage unit for the information used to make proteins. Before any protein manufacturing begins, the cell must transcribe DNA into another molecule. This other “messenger” molecule will carry only the code for the specific gene to a ribosome, which is the site of protein production. This messenger molecule is called ribonucleic acid (RNA). The ribosome reads the gene code of a messenger RNA and manufactures proteins by assembling long chains of amino acid together, one after another, in a process called translation. Each amino acid is coded by a set of three nucleotides, or a triplet codon, during translation of the RNA message, the RNA molecule sequence is read (translated) three consecutive nucleotides at a time. A protein typically consists of hundreds of amino acids that have been joined together. For example, imagine an RNA molecule that is 300 nucleotides long. That RNA molecule will be decoded by a ribosome, and the ribosome will construct a protein that is a chain of exactly 100 amino acids. A simplified chart summarizing protein production is shown above.

What Kind of DNA Damage Occur Due to Radiation?

DNA is normally a long, continuous molecule that stores tremendous amounts of information vital for normal cell function. When a DNA molecule is broken, the long chain of information is fragmented and the original message to produce specific proteins is lost. When DNA is broken on one strand of the double helix, it is called a single strand break (SSB). If both strands of the DNA double helix are severed within 10 to 20 base pairs of each other, the break is called a double strand break (DSB). Other forms of damage that can occur include the loss of a base, and base modification such as oxidation. In many cases, cells are able to fix such breaks with repair systems that are specialized for different types of damage. The damage sites that remain can cause assembly of proteins to be stopped or started prematurely. If DNA replication occurs before the repair system finds the damage, there is a chance that a modified nucleotide is misread as a different nucleotide. In addition, sometimes the repair systems misread a damaged nucleotide and replace it with the wrong nucleotide. The result in both cases is a point mutation. A point mutation is a single change in the nucleotide sequence of a gene. This can alter the amino acid code, so that the protein produced from the gene has a different composition. Depending on where in the protein this occurs, the altered protein sequence can have no affect, or it can substantially alter the protein and protein function. The result may cause cellular or tissue abnormality. In more extreme cases, the presence of DNA damage that cannot be properly repaired can trigger apoptosis, or cell suicide (see the next section for information about apoptosis and radiation countermeasures). The individual cell is sacrificed to prevent greater damage to the whole organism.



NASA Space Radiation Laboratory, at Brookhaven National Laboratory in Upton, New York.



DNA model with two examples of DNA damage shown.

The parallel linked sequences of orange squares (□) and purple circles (○) represent the phosphate-deoxyribose sugar strands, or backbone, of a DNA molecule. The pairs of circles linking the two strands represent nucleotide base pairs. In the single strand break, note that although some nucleotide base pairs have been separated, only one strand of the two DNA strands has been broken (indicated by red arrow). In the more severely damaged double strand break example, nucleotide pairs have been split apart and *both* strands of the DNA molecule are broken (indicated by red arrows).

In some cases, the effects of radiation-induced DNA damage may not be readily or immediately observable. While some damage may not be severe enough to cause death to a cell or organism, its effects can become apparent several generations later. At right is a diagram of a normal DNA molecule before and after being hit by ionizing radiation.⁶

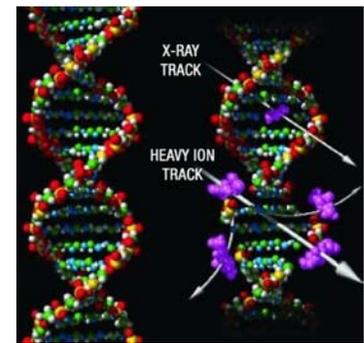


Image Credit: NASA

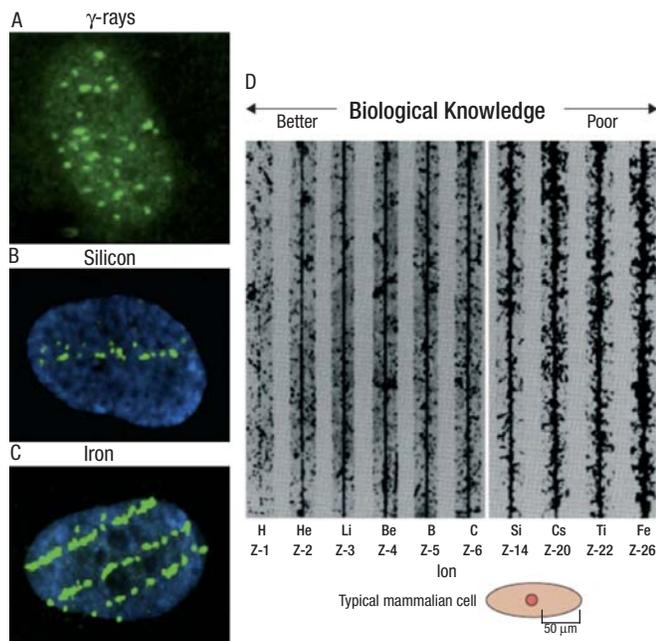
Damage to DNA can be caused directly or indirectly. As the ion travels through material, it will lose some of its energy to the molecules around it. Cosmic radiation contains heavy ions, which are the nuclei of atoms with atomic weights ranging from 14 (carbon) to 55 (iron) or greater. This means that the atom is missing anywhere from 14 to 55 or more electrons. As this particle moves through material, it will pull electrons from any source it can find. This ionizes the molecules along the path of the heavy ion. Protons, alpha particles, or larger fragments can be forcibly separated from the DNA. In addition, when the heavy ion moves through water, the hydroxide (OH⁻) ions in water can be ionized, losing an electron, to produce hydroxyl free radicals (\cdot OH). Such species have a strong propensity to restore the electron pair by pulling a hydrogen atom, complete with a single unpaired electron, from carbon-hydrogen bonds in sugars. One excellent source for this within cells is DNA. Nucleotide modifications or removal, SSBs, DSBs, or any combination of these can occur along or near the track of a heavy ion.

What Kind of Damage Can High Energy Ions Cause?

Because of their high ionization density, heavy ions and high energy ions (or HZE particles) (discussed in the radiation background material) can cause clusters of damage where many molecular bonds are broken along their trajectory through the tissue. The cell's ability to repair DNA damage becomes impaired as the severity of clustering increases. These particles can also create damage along a long column of cells in tissue. In other words, cells will be damaged in streaks along the path of an HZE particle. Since HZE particles are rare on Earth, the prediction of biological risks to humans in space must rely on fundamental knowledge gathered from biological and medical research.⁷

⁶ http://science.nasa.gov/headlines/y2004/17feb_radiation.htm

⁷ <http://hrp.jsc.nasa.gov/?viewFile=program/srp>



Because spaceflight radiation biology experiments are extremely expensive and opportunities for flight are limited, NASA models spaceflight radiation exposure by studying organisms that have been exposed to radiation produced at special facilities here on Earth. Brookhaven National Labs (shown on the previous page)⁸ and Lawrence Livermore National Laboratory⁹ are two examples of facilities that are able to produce radiation that is similar to space radiation. This type of research greatly enhances our understanding of the biological response to space radiation, helps us to anticipate what may happen during future spaceflights, and to develop countermeasures to help protect astronauts from radiation. For example, scientists have learned that mutations, chromosomal aberrations in plant seeds, development disturbances and malformations in small animal embryos, and even cell death in bacteria have resulted from the traverse of a single HZE particle. Examples of cellular damage are shown in the figure above.

This diagram¹⁰ shows a comparison of radiation damage in three human cell nuclei (above left). The nuclei were exposed to (A) gamma rays, (B) silicon ions, and (C) iron ions. Following exposure, the cells were stained so the scientists could observe where DNA damage occurred. Every green spot is a DSB. Notice that the gamma ray (electromagnetic waves) exposure in (A) produced uniform damage, whereas cells exposed to high-energy heavy ions show DNA damage along the path traveled by the ion. In (B) there is one track and in (C) there are three tracks. The damage tracks of ions with differing masses are seen in (D). Note that heavier ions cause a wider path of destruction. Our understanding of biological damage caused by heavy ions is very limited. A cell has been drawn to scale for comparison purposes. Image Credit: crater.bu.edu.

What are the Consequences of DNA Damage?

If radiation changes the number or order of nucleotides (mutation) within a DNA molecule, the information that is stored within the DNA is altered. This can cause significant problems in cell structures and even their function. Even if a DNA molecule has had only one nucleotide deleted, that error could be perpetuated when translated into RNA. In other words, when the RNA is produced, it will be made as if that missing nucleotide had never existed in the first place. Interestingly, the ribosome will not know the difference, because the cell assembles the RNA based on what it reads in the DNA. As a result, the ribosome will assume that the information in the RNA is correct (although *we* know that the nucleotide order in the gene has been shifted by one nucleotide). Protein synthesis carries on, the triplet codons are read by the ribosome, and amino acids are gathered and assembled into a protein structure that the DNA had not coded for originally. In this example, a malformed protein will be constructed that could have significant negative consequences. In summary, when the genotype (genetic information) of a cell is changed, the phenotype (the outward observable expression of the genetic information) may also be changed. Radiation is an environmental stimuli that has the ability to influence whether or not a gene turns on and off, for example, some cancer genes.

⁸ http://www.bnl.gov/medical/NASA/NSRL_description.asp

⁹ <http://www.llnl.gov/>

¹⁰ Cucinotta, F., *Lancet Oncol* 2006; 7: 431–35.

What is the DNA Repair System?

The repair system is constantly monitoring our DNA to make sure it stays intact; proteins will congregate to sites of damage. So one way to measure DNA damage in tissues is by staining tissue samples to look for proteins involved in the repair system. This allows researchers to see where the damage occurred, and at how many sites. They can also monitor how fast the repair system takes to complete its job by staining the tissues at different times after radiation exposure. When possible, cells use the unmodified complementary strand of the DNA as a template to recover the original information. Without access to a template, cells use an error-prone recovery mechanism known as translesion synthesis as a last resort. Damage to DNA alters the three-dimensional configuration of the helix. These alterations can be detected by cellular repair mechanisms. Once damage is localized, specific DNA repair molecules move to the site. These molecules bind at or near the site of damage and induce other molecules to bind and form a complex that enables the actual repair to take place. The types of molecules involved and the mechanism of repair that assembles depend on the type of damage that has occurred and the phase of the cell cycle that the cell is in. Some examples of specific repair systems include: base excision repair (BER), which repairs damage due to a single nucleotide caused by oxidation, alkylation, hydrolysis, or deamination; nucleotide excision repair (NER), which repairs damage affecting longer strands of 2-30 bases. This process recognizes bulky, helix-distorting changes such as thymine dimers as well as single-strand breaks; and mismatch repair (MMR), which corrects errors of DNA replication and recombination that result in mispaired nucleotides following DNA replication.

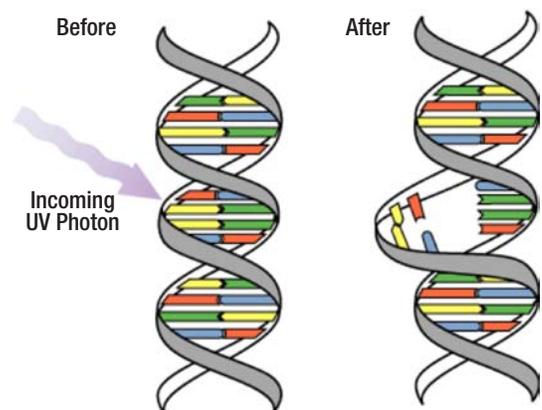
How Does UV Radiation Affect Us?

There are three kinds of UV radiation. UV-A radiation (wavelengths of 320-400 nm) plays a helpful and essential role in formation of Vitamin D by the skin. It is not absorbed by the ozone layer, and can cause sunburn and premature aging on human skin, immune system problems, and cataracts in our eyes. UV-B radiation (wavelengths of 290-320 nm), mostly impacts the surface of the skin. It is absorbed by DNA and the ozone layer, and is the primary cause of sunburn and skin cancer. After exposure to UV-B, the skin increases production of the pigment melanin. This darkens the skin and protects it by absorbing UV light. A dark tan is an indicator of extensive UV-related skin damage. The third is UV-C, which is completely absorbed by the ozone layer and oxygen in the atmosphere.

DNA readily absorbs UV-B radiation. In some cases, it causes the shape of the DNA to be changed. While cells are able to repair this damage through the use of specialized enzymes most of the time, sometimes damage is permanent and the irreparable damage has a cumulative effect that is perpetuated from then on as previously mentioned. UV damage can also cause a mutation, or change to the DNA of a gene. When this gene is transcribed and translated into a protein, the protein may contain an error that causes it to be misshapen, function improperly, lead to cancer, or even cause cells to kill themselves.¹¹

One in five Americans will develop skin cancer and one American dies from this disease every hour. People who have had several blistering sunburns before age 18 are at higher risk of developing melanoma, the most serious form of skin cancer. Individuals with fair skin and freckles have a higher risk of developing skin cancer, but dark-skinned individuals can also get this cancer. Regardless of your skin color, exposure to UV radiation can lead to premature aging of the skin, causing it to become thick, wrinkled, and leathery. Proteins in the lens of the eye can also be altered by radiation, leading to the formation of cataracts that can lead to partial or complete blindness. UV radiation may also suppress proper functioning of the body's immune system.

DNA readily absorbs UV-B radiation.



¹¹ <http://earthobservatory.nasa.gov/Library/UVB/>

How Do Sunscreens Work?

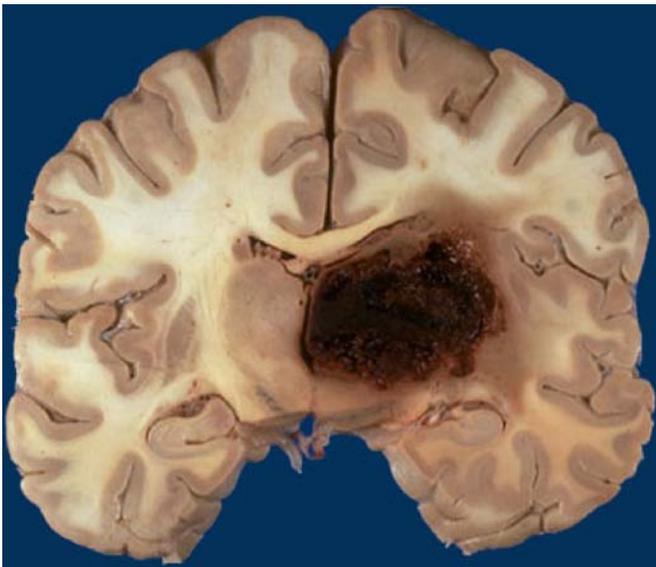
Sunscreens work as a very thin shield by stopping the UV radiation before it can enter the skin and cause damage. Some sunscreens contain organic molecules (such as oxybenzone, homosalate, and PABA) that absorb UV-B and/or UV-A radiation. Others use inorganic pigments (such as titanium dioxide and zinc oxide or both) that absorb, scatter, and reflect both UV-A and UV-B light. Sunscreens are labeled with a Sun Protection Factor (SPF) rating that could also be thought of as a sunburn protection factor. For example, suppose that your skin begins to redden after 10 minutes in the sun. If you protected it with an SPF 15 sunscreen, it would take 15 times as long, or 2.5 hours, to get a comparable burn. Remember, SPF relates only to UV-B protection; there is no standard measurement or rating for UV-A protection in the United States.

What is Degenerative Tissue Damage?

As we have discussed, ionizing radiation alters DNA such that cell repair processes, cell cycle, or cell division is affected. Low numbers of SSB or DSB may provide a trigger for the gradual loss of cycling cells. Loss of repair mechanisms, or loss or reduction of cell division results in tissue degeneration. This can occur in almost all tissues, including the nervous system.

There are also radiation-induced bystander effects. These are biological responses in cells that are not themselves directly in the path of ionizing radiation or in a field of radiation. In fact, new studies show that an even larger portion of bystander cells, sometimes at considerable distance from the irradiated cells, can be affected. The radiation effects can be transmitted directly from cell to cell through channels (gap junctions) connecting cells. Alternatively, a directly hit cell can secrete factors, or signals, which travel out of the hit cell to neighboring cells. Bystander effects have been clearly established in cell culture systems, and a few studies are starting to provide evidence that bystander effects occur *in vivo* (the natural setting). Bystander effects amplify or exaggerate the action of even low dose radiation, so they can significantly increase radiation risk and tissue damage. This may be particularly important when ionizing radiation hits the nervous system, where bystander effects could lead to loss of sensory, motor, and cognitive functions.¹²

Degenerative tissue damage and central nervous system damage could be particularly dangerous if it occurs in the brain of astronauts. The damage could cause altered behavior. Since damage to the nervous system is not repairable, it could reduce the ability of astronauts to work and respond to their environment, especially in an emergency. It could eventually lead to loss of control over their entire body, or death.



Cross-section through both hemispheres of a brain with a necrotic region (brown area to the right of center). Similar damage could result from prolonged or chronic exposure to radiation.

The image is an example of what prolonged or chronic exposure to radiation can cause. Necrosis is the term used to describe the process of uncontrolled cell death; there are mechanisms of controlled cell death also. Important cellular signaling does not take place during necrosis that makes it difficult for the immune system to identify, locate and recycle old dead cells. A cell that dies of necrosis might cause a chain reaction of nearby cell death as well. Necrosis in the brain can result in:

- Problems thinking clearly, or managing tasks you previously found easy
- Poor memory
- Confusion
- Personality changes

Depending on the extent and location of the necrosis, this damage can also lead to significant health problems and possibly death.

¹² Boyle, R., Radiation Biology Professional Development Course Charts, 2006.

Activity IIa: Biological Effects of Radiation Damage in Plants

Objectives:

- Analyze the genetic effects of radiation in plants.
- Describe changes in phenotype as a result of radiation damage.
- Discuss how radiation could cause the observed effects?

Research Question:

Will irradiation affect plant growth or morphology? How much does the level of irradiation affect the plants?

Discussion Questions:

1. What percentage of each irradiated group germinated for the plants you observed? What is the average germination rate for each irradiated group for the entire class? Is the difference in the average germination rate between each irradiated group and the control group statistically significant?
2. What was the average height of the plants in each irradiated group when the first flower was observed? Is the average the same for each irradiated group for the entire class? Is the difference in the average height between each irradiated group and the control group statistically significant?
3. What was the average number of seedpods that were present for each irradiated group for the plants you observed? Is the average the same for each irradiated group for the entire class? Is the difference in the number of seedpods between each irradiated group and the control group statistically significant?
4. What was the average number of seeds in each seedpod for each irradiated group for the plants you observed? Is the average the same for each irradiated group for the entire class? Is the difference in the average number of seeds within the seedpods between each irradiated group and the control group statistically significant?
5. How could you use the DNA of radiated and non-radiated plants to determine if there were genetic effects?
6. Were all effects negative? Why? Can you observe all effects? Why or why not? What do you think the effects on the next generation(s) will be?

Materials:

1. Irradiated Seed Set of desired type (*Brassica rapa*, *Arabidopsis*, radish, chrysanthemums, etc. You will need to obtain them from a vendor).
2. Record book to record data.
3. Camera to record data, if desired.
4. Light box
5. Grow light
6. Plant support sticks
7. Plant growth cubes
8. Bee sticks

Example: Plant Images:

The following images are the growth results of seeds that were planted after having been exposed to radiation (or no radiation, in the case of the control).



Control 16 days



50 Krad 16 days



150 Krad 16 days



500 Krad 16 days

Directions:

The exact protocol for this experiment will depend upon which vendor and specimen is chosen. For this experiment, plant the pre-irradiated seeds following the directions provided with the seed. Record the number of seeds planted and the date.

1. Observe and record each day the germination rate and rate of emergence and appearance of the seedlings of the control and irradiated types.
2. After the recommended time for germination of the plants (on day 5 for *Brassica*) record the total number of seedlings. Calculate the percentage of germination and the percentage of emergence. Record the appearance and height of the seedlings.
3. Each day record height, number of leaves, the general appearance of all plants, total height when the flower first opens, and date of the first flower. As necessary, stake up the plants. Tape 5" × 8" paper cards between the irradiated and control plants to provide a barrier and prevent accidental pollination between groups.
4. At the recommended time (day 14 to 18 for *Brassica*), use a bee-stick or a clean Q-tip to place pollen from one control plant onto another control plant's flowers. Record the date of pollination.
5. Use a bee-stick or a clean Q-tip to place pollen from one irradiated plant onto another irradiated plant's flowers. Record the date of pollination.
6. At intervals after pollination (up to about day 40 for *Brassica*), make frequent observations and record height, number of leaves, and number of seedpods.
7. At the recommended time (between day 41 to 45 for *Brassica*) harvest seedpods and count seeds found within the pods. Record number of seeds and calculate the average number of seeds per pod.

Why Does NASA Study Radiation Effect in Plants?

It has been shown that plant growth is inhibited by radiation. Like mammals, the embryo of a plant is very sensitive to radiation damage as compared to the adult. The rate of seed germination is reduced, and the rate of growth is slowed.¹³ Excessive UV radiation will lead to an inhibition of plant growth processes in general. Such alterations in primary productivity (photosynthesis) can change entire ecosystems in the oceans, on land, and even in bioregenerative life support systems that would be aboard future spacecraft. Thus, NASA scientists need to understand how plants respond to radiation if future space explorers depend upon them for nutrient cycling and food. Experiments involving plants in space, like the Biomass Production System, have been a favorite of astronauts during long-duration stays onboard the International Space Station.¹⁴

(Note: Equipment and materials, including irradiated seeds, for this activity are commercially available from various educational resources.)



NASA scientists are looking for better ways to grow plants both on Earth and in space.

¹³ www.esd.ornl.gov/programs/ecorisk/documents/tm13141.pdf

¹⁴ <http://liftoff.msfc.nasa.gov/news/2003/news-plants.asp>

Activity IIb: Biological Effects of Radiation Damage in Yeast

In this experiment, students will explore how well sun-screening materials protect live yeast cells from harmful UV radiation. Different sun protection factors, brands, or even sunglasses may be used to expand the range of items tested. Note: this activity will be referred to in Module 3 because it can also be used to demonstrate countermeasures against radiation.

Objectives:

- Discuss countermeasures for UV radiation.
- Describe phenotypic changes in yeast as a result of radiation damage.

Research Question:

What is the most effective method of preventing UV damage in yeast?

Discussion Questions:

1. What are the effects of different types of sunscreen on yeast?
2. How can your health be affected by exposure to ultraviolet radiation?
3. Why use yeast to study the effects of UV radiation?
4. Do you see any differences between areas of the Petri dish? If so, describe them.
5. Did some SPF's of sunscreen protect the yeast cells better than others? Why?
6. Does yeast grow less in some areas? Does it grow more than in others? Why?
7. Does UV pass through plastic wrap? Plastic Petri dish covers?
8. Why is it important to not expose an open yeast extract dextrose agar plate for very long? What is aseptic technique?
9. What can you conclude from the results of your experiment?
10. Describe another experiment you could perform to obtain more information about the effects of UV radiation on cells.

Materials:

1. Yeast-Extract Dextrose media plates, from kit or recipes for YPD (yeast peptone dextrose media) can be found online. Use the search term "yeast peptone dextrose media". See sample recipe below.
2. UV-sensitive yeast suspension in liquid media and wild type yeast suspension in liquid media (this needs to be prepared from a stock sample that is purchased from a vendor). Ensure there is enough for the number of plates that will be plated (1 ml of cells per plate is recommended).
3. A source of UV radiation such as direct sunlight. For this or future experiments, the radiation source could also be changed. (Depending upon the size and design of the experiment, you may want to include black lights, halogen, or fluorescent light bulbs to determine if they also produce damaging radiation.)
4. Several kinds of sunscreen (each with different SPF), black paper, cloth, metal foil, or other types of materials that can be used to experiment with UV shielding.
5. Sterile water, sterile pipets, and sterile toothpicks (Sterile toothpicks are toothpicks that have been autoclaved to kill any microbes that might be on them. You can buy them from a scientific vendor or you can buy off-the-shelf toothpicks and autoclave them in your lab).
6. Plastic wrap (to cover plates).

A sample recipe for Yeast-Extract Dextrose media is:

Ingredients:

1. Bacto-Yeast extract 10.0g
2. Bacto-Peptone 20.0g
3. Dextrose 20.0g
4. Bacto-Agar 20.0g
5. Distilled Water 1000ml

Directions:

Dissolve the ingredients (70g total) in 1000ml distilled water and sterilize by autoclaving at 121°C for 15 minutes. The media is ready for use after 42-48 hours at 25°C.

Tips:

Remember to store the prepared media below 8°C and protect it from direct light. Store the ingredients in a dry place within tightly sealed containers at 2-25°C.

Purpose:

Yeast extract and peptone provide carbon, nitrogen, minerals, vitamins, trace ingredients and other essential growth nutrients. Yeast extract supplies vitamin B-complexes, which stimulate the growth of yeasts and bacteria. Dextrose is the energy source.

Directions:

1. Ensure that your hands and the work area are clean. Use soap and water and wipe your hands and your work area with alcohol and a paper towel. Good aseptic technique will ensure that the plates do not get contaminated with other organisms.
2. In this step you will plate the yeast suspension. You may want to do this for each group or allow the students to perform the task. Swirl the container of UV-sensitive yeast. Using aseptic technique and a sterile pipet, add 1 ml of the yeast cell suspension uniformly on top of the agar in the Petri dish for every plate that will be used in the experiment. Close the lid. Gently tilt and rotate the dish to spread the liquid. If there are places the liquid does not cover, reopen the dish and use the rounded end of a sterile toothpick to move the liquid over them. Sterile glass beads could also be used to spread the cells across the plate. Let the liquid soak into the agar. Place the Petri dish in a dark place for 10-20 minutes until the liquid soaks into the media.
3. Label the dish (see the diagram at the end of this activity) by drawing lines on the top and bottom of the dish to divide it into 4 parts (you could divide it into more parts, depending upon the number of countermeasures you are investigating). Label one area "sun" as a control, and use the other three areas to test sunscreens or other items like cloth, foil, paper, or plastic. Ensure that one area on all plates do not get UV exposure (cover it with black paper during UV exposure) or make certain that at least one entire plate per group is designated as the control, which does not get UV exposure. Label each area on both the top and the bottom of the dish and tape the two halves of the Petri dish together along the side so that the lid does not rotate. For one group (or the entire class), have the students remove the lid and replace it with plastic wrap (tape it on tightly). This will test any possible shielding effects of the cover.
4. Spread sunscreen on the lid of the Petri dish (or on the plastic wrap) in the places you marked; use an equal amount in each section and spread the sunscreen evenly. You can also use plastic, foil, etc. instead of sunscreen. If you labeled an area "no sun," tape a square of dark paper over it. Make sure you know exactly where each sun screening material is used.
5. Expose the Petri dish to the sun or to a UV light. Vary the appropriate exposure times for the students from 20 minutes (in midday summer sun) to as much as 4 hours (in midmorning winter sun) per dish. If you are exposing the Petri dish to the sun, make sure that the surface of the agar is aimed directly at the sun (perpendicular to the incoming radiation). If students are careful, the lids could be removed and replaced with some clear plastic wrap during the exposure (to reduce any possible shielding effects of the lid). Consider allowing one group to remove the lid for a direct exposure.
6. After the exposure, wipe the sunscreen off the lid of the Petri dish. This will reduce the mess. Remove any other materials that were tested. If the students used the plastic wrap, just remove the wrap and replace it with the original lid. Place the Petri dish upside down in an incubator or in a dark place and let it grow for 1-2 days in an incubator at 30°C or 3-4 days at room temperature.
7. If desired, repeat these steps with a wild type strain as a control for comparison.
8. Compare the amount of yeast that has grown in different areas of the Petri dish and draw conclusions.

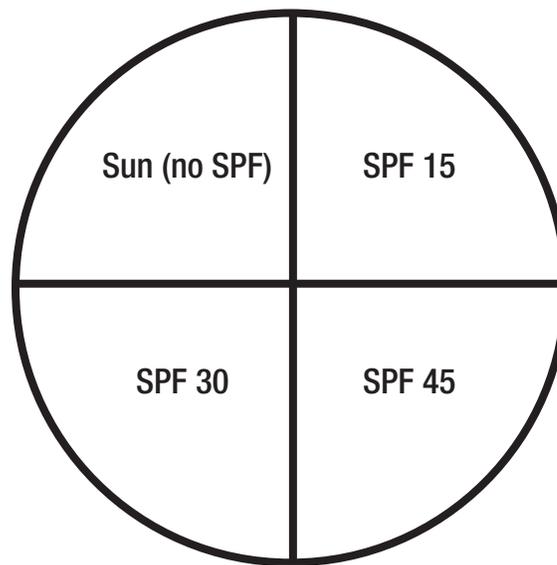
How Do Sunscreens Work?

Sunscreens work as a very thin shield by stopping the UV radiation before it can enter the skin and cause damage. Some sunscreens contain organic molecules (such as oxybenzone, homosalate and PABA) that absorb UV-B and/or UV-A radiation. Others use inorganic pigments (such as titanium dioxide and zinc oxide or both) that absorb, scatter, and reflect both UV-A and UV-B light. Sunscreens are labeled with a SPF rating that could also be thought of as a sunburn protection factor. For example, suppose that your skin begins to redden after 10 minutes in the sun. If you protected it with an SPF 15 sunscreen, it would take 15 times as long, or 2.5 hours, to get a comparable burn. Remember, SPF relates only to UV-B protection; there is no standard measurement or rating for UV-A protection in the United States.

Why Does NASA Study Yeast in Space?

Like the fruit fly, ordinary baker's yeast (*Saccharomyces cerevisiae*) also contains genes for DNA repair that are very similar to human genes with the same function. Therefore we can use yeast as a model system to explore the effects of radiation on cells. Like human cells, most yeast cells effectively repair DNA damage caused by UV radiation. However, some yeast strains have mutations that prevent them from performing certain types of DNA repair. Because they cannot repair all the damage to DNA, these cells usually die after exposure to UV radiation. In addition to sensitivity to UV radiation, yeast is also sensitive to space radiation. In a biological assessment of space radiation in low-Earth orbit, yeast inside special experiment hardware has been shown to have a decreased rate of survival following exposure to beta particles (electrons) and low-energy protons.¹⁵ Other findings suggest there are highly coordinated gene expression responses to gamma radiation.¹⁶ This knowledge is especially important when designing countermeasures for astronauts during long-term lunar surface operations or microgravity spacewalks.

(Note: Equipment and materials for this activity are commercially available from various educational resources.)



¹⁵ <http://www.spaceflight.esa.int/users/index.cfm?act=default.page&clevel=11&page=2120>

¹⁶ http://www.sanger.ac.uk/PostGenomics/S_pombe/docs/851.pdf

Activity IIc: Biological Effects of Radiation Damage in *Drosophila*

Objectives:

- Analyze the genetic effects of radiation *Drosophila melanogaster*.
- Describe phenotypic changes as a result of radiation damage.

Research Question:

What is your hypothesis for the phenotypic and genotypic results of crossing the selected traits? What are the visible effects (physical and behavioral) of radiation on *Drosophila*?

Discussion Questions:

1. What is your hypothesis for the results of crossbreeding the selected traits?
2. What phenotypes and phenotypic ratio do you predict for the F1 generation?
3. What genotypes and genotypic ratio do you predict for the F1 generation?
4. What are the expected results if the F1 generation were allowed to breed?
5. Model effects of radiation with crossbreeding of known strains of *Drosophila*.
6. Demonstrate or predict examples of genetic/phenotypic variations as a result of crossbreeding *Drosophila* with different traits.
7. Compare and contrast images and video of *Drosophila* that have been exposed to radiation with flies that are normal.

Materials:

1. *Drosophila melanogaster* of two different strains (e.g., wild-type and wingless).
2. Record book to record your findings.
3. Materials to cultivate specimens (cultivation refers to the raising/rearing/caring for of organisms including containers and food).
4. Magnifying glass or dissection microscope.
5. Fly sorting materials (sorting brushes and anesthetizing tools).

Directions:

1. Select the specific *Drosophila melanogaster* traits to be crossbred. This will require some research. Mate the flies obtained for the experiment. After five days, remove the adult flies from the container (only eggs and larva will remain in the container).
2. Observe and record the development of the larvae over the next ten days.
3. Observe the F1 generation and record the characteristics of the adults as they emerge. When enough adults have hatched to provide a good sample, remove the adults to a new container.
4. Anesthetize the adult flies (using a method that is safe for your classroom) and sort them according the visible phenotypic traits. Record the results of your observations. Analyze the results of crossing the two traits. (Anesthetizing flies means to give the flies an anesthetic that makes them go to sleep so you can handle them without them flying away. This can be done with a constant stream of Carbon Dioxide (CO₂). Another method is to use dry ice. Place dry ice with the flies in a sealed chamber and monitor the flies to ensure that they do not get an overdose).

Historical Context:

Unexpected events often determine the course of our scientific legacy. Around 1910, one such event was the decision of Thomas Hunt Morgan, a pioneer in genetics research. He decided to study the fruit fly, *Drosophila melanogaster*, instead of the costlier, preferred rabbit specimens because there was very little funding for basic research at universities. Fruit flies were chosen because they are small, found nearly everywhere, inexpensive to house, and reproduce in large numbers. An additional advantage was that fruit flies could be easily seen with only a simple hand lens. Much of Morgan's early work was done this way. Later, microscopes were used to study *Drosophila*.

Morgan bred wild-type (red-eyed) fruit flies by the thousands, and his team tried to create mutant flies with x-rays, acids, and other toxic substances. Finally, in one unaltered lineage of flies, the researchers found a surprise. Every single fly in that line had been born with red eyes, until one day a fly emerged from its pupa with white eyes. The image below shows the differences in fly eye color.¹⁷ Something had spontaneously changed in the white-eyed fly. Morgan realized that one of its genes had been altered and it had produced a new kind of eye. Morgan bred the white-eyed fly with a red-eyed fly and got a generation of red-eyed hybrids. And when he bred the hybrids together, some of the grandchildren were white-eyed. Their ratio was three red to one white. Here was a mutation, but one that didn't fit the current understanding. Scientists, at that time, thought that mutations created new species, but the fly that had acquired the white-eyed mutation remained a member of the same species. It could still mate with other fruit flies, and its gene could be passed down to later generations in proper Mendelian fashion.

The work of scientists such as Morgan established the science of genetics. His work resulted in the discovery of sex-linked and autosomal genes. Autosomal genes are those carried by any chromosome except the sex chromosome. Morgan's work with *Drosophila* went on to explain linkage, two different genes being on the same chromosome and not randomly assorted as had been understood, and the concept of crossing-over, the transference of genes from one chromosome to another. Morgan's work with *Drosophila* eventually earned him the Nobel Prize in Physiology or Medicine in 1933.

(Note: Equipment and materials for this activity are commercially available from various educational resources.)

References:

Flies In Space: <http://quest.nasa.gov/projects/flies/>

FlyBase: <http://flybase.bio.indiana.edu/>

Morgan's experiments involved red- and white-eyed fruit flies like these.



¹⁷ Bhattacharya, S, presentation entitled "Basic Biology for Engineers Short Course, Unit: Nematodes and Insects, Title of Lesson: D. melanogaster" August, 2003.

Activity IId: Three-Dimensional Modeling of DNA Damage

In this activity, you will model DNA and DNA damage.

Objectives:

- Visualize the three-dimensional structure of DNA.
- Simulate the random damage to DNA caused by radiation.
- Describe molecular changes as a result of radiation damage.

Research Question:

What types of damage can arise from high-energy particles hitting DNA?

Discussion Questions:

1. What bonds are the easiest to break?
2. How many breaks do you get with a single hit?
3. How easy is it to repair the damage?
4. How does your choice of model affect the type of damage observed?
5. What type of damage is most difficult to repair?
6. How does this relate to the types of damage that are more difficult for biological repair systems in cells to recognize and repair?

Materials:

1. DNA Building kit (DNA models can be constructed using a few common kitchen items; plastic kits can also be purchased, see references below).
2. Two to three partners (this activity is best done as a team).

Methods:

1. Construct a DNA model. Have one team member remove a bond while the others are not looking. Ask the students to identify which bond has been “broken.” Record the type of damage that occurred.
2. Have the students repair the damage. Repeat the exercise by removing one or more bonds, bases, or nucleotides. This represents the various types of damage that can occur.

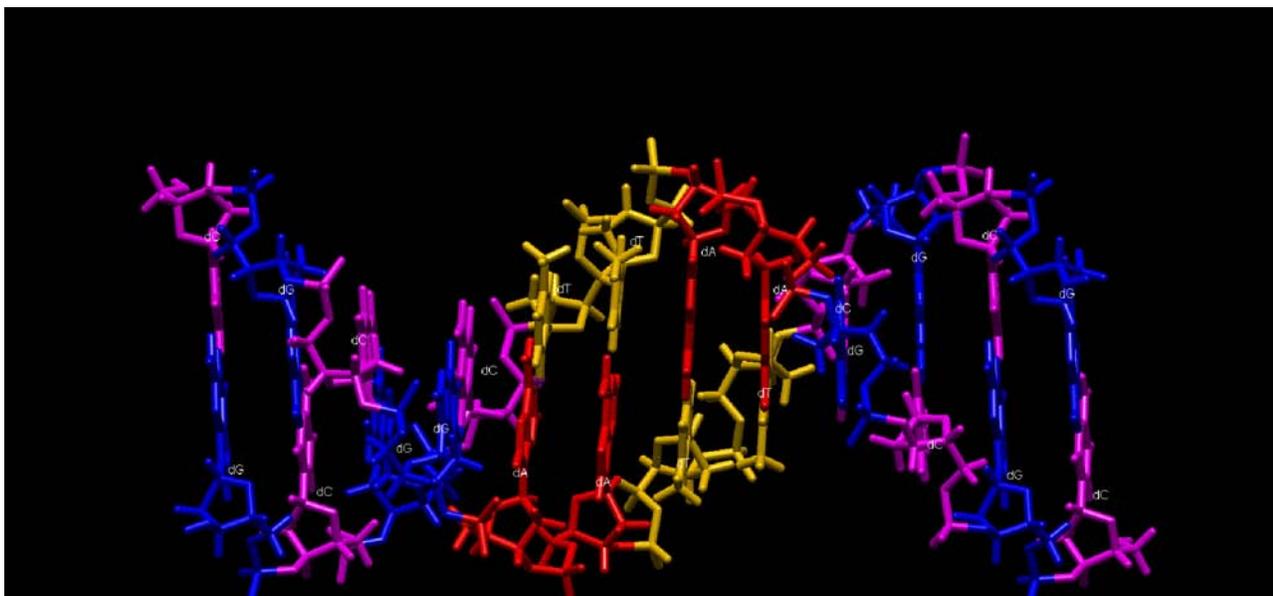


Image Credit: Department of Biochemistry & Molecular Biophysics at the Washington University School of Medicine.

Historical Context of the Discovery of the Shape of DNA

Since DNA itself is so small, there are no methods to directly image DNA or the damage caused by radiation. All measurements must be gathered indirectly. The first method used to determine the structure of DNA was x-ray crystallography. This was first done in the late 1940's, when the structure of DNA was still a mystery. At the time, x-ray crystallography was commonly used to determine the crystalline structure for molecules much smaller than DNA.

Just like a glass crystal will refract sunlight to produce a rainbow, x-rays directed at a crystal formed from molecules will bounce off the repeated angles and scatter in a specific pattern. This information can be used to calculate the repeating angles in the crystal, which tell us about the arrangement of molecules in the crystal. Rosalind Franklin and her colleagues were the first to learn how to prepare DNA as a crystal, and took the first x-ray pictures of DNA using the technique of x-ray crystallography. In 1953, James Watson and Francis Crick then used these results and clues from other measurements to painstakingly construct a three-dimensional model of the DNA molecule. They proposed that DNA was a double helix, with the base pairs A-T and G-C on the inside, like rungs on a ladder.¹⁸ Today, scientists continue to study the three-dimensional structure of DNA, and use computers for complex calculations and graphical analysis to speed up the process of modeling and visualizing DNA¹⁹ (above) and DNA damage.

¹⁸ Watson, JD, Crick, FH, Nature vol. 171:737-738 (1953).

¹⁹ <http://dasher.wustl.edu/ffe/images/DNA%20B-Form%20Dodecamer.png>

Appendix 1:

Module 2: Additional Websites

NASA Quest

<http://quest.nasa.gov/>

NASA Science website

<http://science.nasa.gov/>

Lawrence Livermore National Laboratory

<http://www.llnl.gov/>

Genetics Science Learning Center: University of Utah

<http://gslc.genetics.utah.edu>

Appendix 2: National Education Standards²⁰ by Module

Module 2: Radiation Damage in Living Organisms

Content Standards: 9-12

Content Standard A:

Science As Inquiry

Understanding about scientific inquiry

Abilities to do scientific inquiry

Content Standard B:

Physical Science

Interaction of energy and matter

Content Standard C:

Life Science

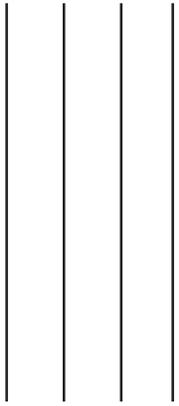
Molecular basis of heredity

Matter, energy, and organization in living systems

²⁰ <http://lab.nap.edu/html/nses/6a.html>



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