

Biol 423L**Sept. 22/23****Mutation in Yeast**

Report due Oct. 6/7.

Readings: Hartwell Chapter 7 pp. 208-225.
See web site for the following PDFs:
Bakers Yeast and its Life Cycle
A Closer Look at Adenine-requiring Mutants in Yeast-
The Response of Yeast to Radiation
A Closer Look at Biological Consequences of UV exposure
A Closer Look at Repair of DNA

Objectives: The first step in discovery of the genes involved in a biochemical function or a developmental process is to identify mutations that change that function or process. In this exercise you will see how UV light can be used to increase the spontaneous mutation frequency in yeast. We will also attempt to reverse the effects of mutagenesis by photoreactivation, a light-dependent DNA repair mechanism.

Introduction: The yeast we are using is haploid which will make it easy to see new mutations if they occur. It has a defect in adenine biosynthesis. The gene encoding the A2 enzyme is non-functional because the *a2* allele is at the locus. This strain of yeast cannot grow on medium that is not supplemented with adenine. We are growing it on a complex media based on yeast extract that provides all essential nutrients. Although the *a2* yeast can grow on this media, it turns red because it accumulates a red colored intermediate in adenine biosynthesis. Wild type yeast metabolize this intermediate to adenine which is colorless. You can learn more about the biochemistry in the adenine-requiring mutants PDF on the web page. This strain of yeast can be mutated so that it no longer accumulates the red intermediate. We are going to use this easy-to-score phenotype to measure the effects of UV light on mutation frequency.

Materials needed per group:

10⁶ cfu/ml or "just turbid" suspension of GBS2730 (*rad2*, *ura3*, *ade2*) (strain made by Gwen Sancar).

Use GBS2730, at 15 cm from the lamp.

20 YED plates per group

UV-C germicidal lamp From Fisher #11-988

UV protective goggles

Sterile distilled water (1x 100 ml bottle per group)

L-shaped glass spreader

Alcohol lamp

Alcohol dish

P200 eppendorf pipetmen with sterile tips

Sterile 1.5 ml eppendorf tubes (1 beaker per group)
Aluminum foil
Marking pens
Glass plates for photoreactivation
Lab cart

Each student must provide their own linear graph paper and log-linear graph paper. Alternatively, graphs can be made in Excel or a similar computer program.

Method:

You will work in groups of two in order to make the following determinations:

- a. Determine the lethality caused by irradiation.
- b. Determine the increase in mutation frequency.
- c. Monitor the effects of photoreactivation.

1. The instructor will make a “just turbid” yeast suspension from cells grown on a plate following instructions in A classroom Guide to Yeast Genetics C11-14. Each group will be given a suspension of each strain in a sterile tube. The suspension has close to 10^6 cells per ml.

You will need to prepare two sets of YED plates with yeast spread on them. One set will have yeast at a 10^{-2} dilution and the other will have a 10^{-3} dilution of yeast. To make plates at a 10^{-2} dilution: make at least 1.4 ml of a 10^{-1} dilution of your original culture diluting the yeast into sterile water. Plate 100 μ l of this on a plate to achieve a 10^{-2} dilution. These should have approximately 10^4 cells per plate. Make another set of plates at 10^{-3} dilution. These should have approximately 10^3 cells per plate. Use sterile water, sterile eppendorf tubes and pipetmen with sterile tips to make the dilutions.

You will need 10 plates at each dilution.

All your samples will be exposed to UV for a chosen time period as outlined in the table below. Prepare the plates in foil covered packages of 2 plates, one with the yeast diluted to 10^{-2} cells per plate and one with the 10^{-3} dilution. You should have 1 package for each treatment (UV dose and sunlight dose). Label the packages with the time of UV irradiation and whether or not you will expose them to sunlight.

Then expose the plates to the following treatments.

Yeast	GBS 2370	GBS 2370
Dilution plated	10^{-2}	10^{-3}
1	UV 0 sec	UV 0 sec
2	UV 0 sec 2min sunlight	UV 0 sec 2 min sunlight
3	UV 1 sec	UV 1 sec
4	UV 1 sec 2min sunlight	UV 1 sec 2 min sunlight
5	UV 2 secs	UV 2 secs
6	UV 2 secs 2 min sunlight	UV 2 secs 2 min sunlight
7	UV 5 secs	UV 5 secs
8	UV 5 secs 2min sunlight	UV 5 secs 2 min sunlight
9	UV 10 secs	UV 10 secs
10	UV 10 secs 2min sunlight	UV 10 secs 2 min sunlight

To irradiate the samples: We have only one UVC lamp. The irradiation cannot be done in a bright room so we will have the light in our prep room, which will be dimly lit. When your plates are all labeled and your yeast has been appropriately diluted and spread on the plates, your group will bring them to the prep room. We will illuminate the plates 4 at a time to the correct dosage. They must be open for illumination because the plastic covers block out UV light. You will cover the illuminated plates and wrap them in foil.

Photoreactivation: Take the set of replicates for photoreactivation outside. Open the foil packages and spread the plates out on a clean surface. Leave the lids on. Put a flat glass pane over the plates to give them extra protection against UV rays from the sun. Expose the yeast to sunlight for 2 minutes. Wrap the packages up in foil again.

All plates will then be incubated at 30 degrees.

The yeast will need to grow for 2 days in order to determine which are red and which are white. Thursday and Friday, we will open the lab and leave the yeast plates available for counting. You have two dilutions for each treatment. One will give you either too many colonies to count easily or too few to give you a reliable

number depending on how many survive the treatment. Use the appropriate dilution to count the number of colonies for each treatment. Determine the number of cells that survived each UVC exposure time. In addition count the number of red colonies and the number of white colonies.

Report:

Write your report according to the normal style for this class.

Answer the following questions in the methods section of your report:

What are the negative controls for the effects of UV irradiation in this experiment?

What are the negative controls for the effects of sunlight?

For the results section:

1. Determine the survival rate for the cells exposed to UV only and for those exposed to UV and sunlight. You can make two curves on the same plot. Use semi-log graph paper to plot the surviving fraction on the Y axis (log axis) and the UV dose on the X axis (linear).

Surviving fraction = Number of colonies on irradiated plate/ Number of colonies on unirradiated plates (UV time = 0) at the same culture dilution factor.

The 0 time point value will be 1. We should expect an inverse linear relationship of log survival to dose.

2. Using linear graph paper, plot the number of mutations (white colonies) per 100 survivors (total number of colonies on the plate) for each UV treatment on the Y axis against the UV dose on the X axis. Again make two curves on one plot to illustrate the effects of UV light and UV light followed by photoreactivation on mutation rate.

For the Discussion:

Discuss the effects of UV light on viability and on mutation frequency.

What kind of damage does UV light do to cause killing and mutagenesis?

How has exposure to sunlight affected both killing and mutation rate?

Explain the kind of mutation that is susceptible to photoreactivation and describe the mechanism of photoreactivation.

What kind of mutations will cause the colonies to be white?

GBS2730 has a mutation at the *rad2* locus. What effect does the *rad2* mutation have on UV survival, mutability and photoreactivation.

What is the function of the protein encoded by *rad2* ? You may use the web to find the answer.