The Awesome Power of Yeast Genetics: Spontaneous and Induced Mutagenesis and Complementation Analysis using *Saccharomyces cerevisiae*.

Mutations occur as a consequence of normal cellular physiology and because of the interaction of cells with environmental agents called mutagens. The Awesome Power of Yeast Genetics lab provides you with an opportunity to explore both spontaneous and induced mutagenesis using a simple, positive selection for yeast mutants. This powerful selection will allow you to:

1) Rapidly determine the relative spontaneous mutation rate of three yeast strains
2) Observe the effect of UV-irradiation on mutation rate and cell viability
3) Develop testable hypotheses to explain mutation rate differences among the yeast strains
4) Create new yeast strains that differ from wild type by a single gene mutation
5) Use complementation analysis to unambiguously determine the gene mutated in your strains

UV-induced Mutations

Exposure to ultraviolet radiation (UV) is a common topic of discussion in society today. UV wavelengths are below the visible energy spectrum, typically defined as between 400 and 200 nm. The UV spectrum is often divided into three different energy levels, UVA (400 to 315), UVB (315 – 280) and UVC (280 – 200). UVB and UVC radiation are higher energy than UVA and are responsible for DNA damage, which leads to cell death or the accumulation of mutations in surviving cells. The accumulation of mutations in surviving cells over the course of your life increases your risk of skin cancer. You will use a UV irradiation device in the lab to simulate levels of UV energy exposure that correspond to about 100 minutes in the sun on a cloudless day in south Florida and observe the effect of such a UV exposure on the mutation rate and viability of the yeast, *Saccharomyces cerevisiae*.

Selection for aminoadipate resistance as a tool to study mutagenesis (Zaret and Sherman, 1985. J. Bacteriology 162: pp579-583)

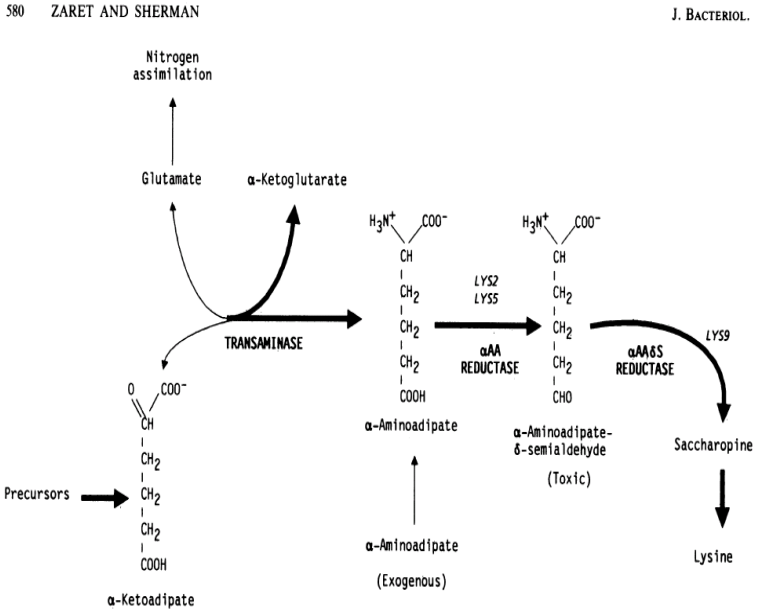
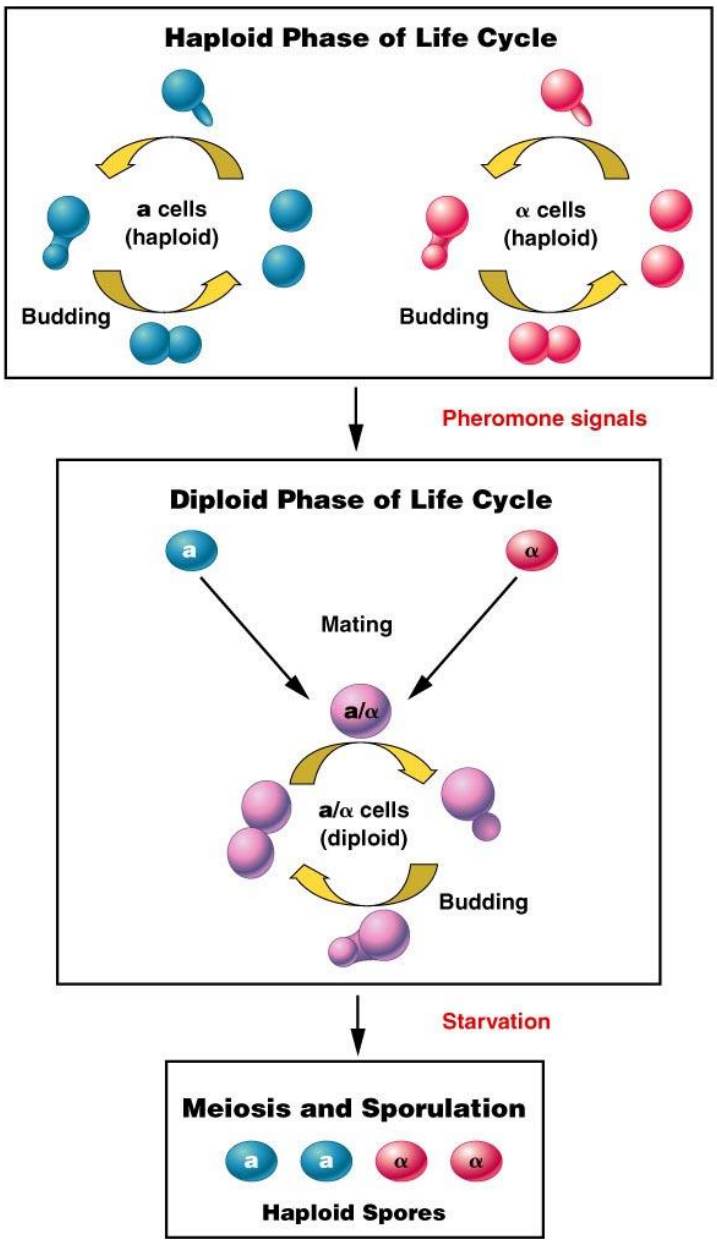


FIG. 1. Metabolic pathways of αAA utilization and lysine biosynthesis and the relationship to *LYS* genes. Some of the intermediates are not shown. (Adapted from Chatoo et al. [3].) The thick arrows indicate the predominant pathway in *LYS*$^+$ cells. The thin arrows indicate the predominant pathways in various *lys* mutants.

Aminoadipate (AA) is an intermediate in the lysine biosynthetic pathway in yeast. AA levels are normally low and harmless, however; high levels of AA added to culture medium leads to high intracellular levels of AA. In wild type yeast cells, high intracellular AA levels leads to accumulation of toxic levels of aminoadipate semialdehyde due to the action of the enzyme, AA-reductase. High aminoadipate semialdehyde kills yeast cells. The only way for yeast cells to survive on AA-containing medium is to be defective in AA-reductase. AA reductase is a heterodimer formed by the products of the LYS2 and LYS5 genes. Expression of LYS2 and LYS5 depends on the transcription factor, LYS14. Because mutations in LYS2, LYS5, or LYS14 prevent/diminish synthesis of AA-reductase, these mutants are resistant to AA in the culture medium. Thus, a yeast strain that is resistant to aminoadipate should carry a mutation in one of these three lysine biosynthetic genes. These mutants can also be identified by their phenotype on medium lacking lysine: lys2Δ mutants and lys5Δ mutants are unable to grow and lys14Δ mutants grow slowly.

Complementation Analysis

In order to determine which LYS gene was mutated in your mutants, you will use known lys2, lys5 and lys14 mutants to perform a complementation test. In yeast, a complementation test is done by mating haploid yeast mutants of opposite mating types (MAT***a*** and MAT***α***) and asking whether the resulting diploid cell has the mutant or wild type phenotype. You will mate your aminoadipate-resistant mutants, which all have the "***a***" mating type, with a lys2, lys5, and lys14 mutants of the "**α**" mating type to form diploids, and then determine whether the diploids are able to grow on medium lacking lysine (a result called "complementation", which means that the mutations are in different genes) or are unable to grow on medium lacking lysine (a result called "noncomplementation", which means that the mutations are in the same gene).



The Awesome Power of Yeast Genetic to Study Mutagenesis Day1: Analysis of Yeast Strains and Selection for Mutants using Replica Plating Technique.

You have a plate containing patches of four yeast strains. This is your "Master Plate" for this lab problem. Three of the strains are LYS+, which means that they are lysine prototrophs, can grow on medium lacking lysine and are also AA sensitive. One strain is lys-, which means it is a lysine auxotroph, cannot grow on medium lacking lysine and is AA resisant. The LYS+ strains differ in the rate at which they accumulate mutations. You will observe this during your experiments and will use your observations to derive a genetic hypothesis to explain the differences. **Label the strains on your master plate A, B, C and D. You will use these names when referring to the strains.**

Replica plating yeast to make copies of your Master Plate on YPD medium, SC-lysine medium, and Aminoadipate (AA) medium.

YPD medium contains all nutrients required for yeast to grow. It contains lysine and does not contain aminoadipate. AA medium also contains all nutrients needed for your yeast strains to grow, but additionally contains aminoadipate at levels that are toxic to LYS+ yeast but not to lysine auxotrophs. SC-lysine medium contains all nutrients required for growth except for the amino acid lysine.

- Observe a demonstration of replica plating and then replica plate your Master Plate as follows.
- Stretch a piece of sterile velveteen on the replica plating block.
- Wet the velveteen with your YPD plate.
- Print your Master Plate onto the velveteen. Tap gently all over the plate to make sure the entire plate contacts the surface of the velveteen, BUT DON'T CRUSH THE PLATE ON THE VELVETEEN. Mark a location of the plate that corresponds to the mark on the collar with a sharpie. Take the Master Plate off the block and cover it and leave it on your bench.
- Press an AA plate on the velveteen, gently tapping all over the plate. Mark the plate with a sharpie. Take the plate off the velveteen and place the lid back on it.
- Repeat with the SC-lysine plate.
- Repeat with the YPD plate.
- Label the bottom of your plates with your group number and section number and initials.

Expose ½ of the AA and YPD plates to UV radiation

- Place your SC-lysine plate in the box.
- When your group is called, take your AA and YPD plates AND YOUR BOX to the TA at the UV cross-linker. Your TA will help you irradiate each plate at a level of 10mJoules/cm^2. This is a level equivalent to about 100 minutes in the sun at a south Florida beach. One half of each plate will be covered with foil, allowing you to observe the difference in frequency of mutations with and without exposure to UV light. NOTE: THE PETRI PLATE LID IS NOT UV-TRANSPARENT SO REMOVE IT WHEN IRRADIATING.
- Place the lid back on your plates and place your plates in your box and cover the box **immediately after irradiation**. Yeast have photoreactivation repair, therefore; if you do not keep your irradiated cells out of the light during the first few hours after irradiation, you will see less of an effect of UV irradiation on mutation rate.
- Take the plates back to your bench in the box and keep the box closed.

Practice streaking yeast for single colony purification

- Each student should draw four quadrants one YPD plate on the bottom of the plate using a sharpie.
- Observe demonstration for streaking yeast and then streak yeast cells to single colony – four streaks per plate. Label the plate with your section #, group #, and initials. The TA will collect your box and plates.

The Awesome Power of Yeast Genetic to Study Mutagenesis Day2: Observations and Interpretation of Results and Purification of AA-resistant Mutants by Streaking to Single Colony.

Today you are going to

1) Observe the results from your replica plating experiment from Day 1
2) purify four, aminoadipate resistant mutants (per student).

Your observations of the replica plating experiment should include:

1) Identifying aminoadipate resistant colonies
2) Judging the relative mutation frequency of the strains
3) Determining the effect of UV irradiation on mutation rate for each strain
4) Determining the effect of UV irradiation on viability for each strain

TIPS about making observations of your replica plates:

The pattern of growth on YPD with no-irradiation is the control showing growth of all cells transferred by replica plating. A typical result for all four strains on YPD with no irradiation is a patch of growth resembling the original master plate. If the half of the YPD plate that was not UV-irradiated shows individual colonies instead of a patch of growth, then there was a problem with replica plating. The interpretation of results on other plates will have to take that into account.

Individual colonies on a replica plate are assumed to be derived from a single yeast cell that was deposited on that site by replica plating. If replica plating was done properly, then the vast majority of yeast cells adjacent to that cell did not grow. On AA plates, this indicates a single AA-resistant cell among a sea of AA-sensitive cells. On the UV-irradiated portion of the YPD plate, this indicates a single survivor of UV-irradiation among a sea of cells killed by UV. On the non-UV-irradiated side of a YPD plate, this indicates poor transfer during replica plating.

Record your observations and your interpretation of those results on the following pages. Use these observations to fill out the report of yeast lab 2.

Purifying AA-resistant mutants by streaking to single colonies on AA medium.

Each student should select 4 AA-resistant colonies derived from one strain A from the AA plate that was not irradiated (spontaneous mutants). Sector one AA plate into four quadrants as illustrated by the instructor and streak yeast cells from each colony using the single-colony streaking technique you learned last week. These plates will be incubated and returned to you next week along with plates that the TAs will prepare for you to perform a complementation test that will determine which LYS gene was mutated in each of your mutant strains.

Name the strains: Section #, Group #, your initials, Strain Number. For example, four mutants isolated by Thomas Morgan of group 0 section 0 would be called 0-0-TM-1, 0-0-TM-2, 0-0-TM-3, and 0-0-TM-4.

Observations from Day 2 of the Yeast Mutagenesis Lab

Observe the YPD replica plate. For each strain, compare the side that was irradiated to the side that was not. Use your yeast strain designations (A, B, C and D) to refer to the strains.

1) What do you see?

2) What does this indicate about the relative effect of exposure to UV irradiation (100 minutes in the bright South Florida sun) for the different yeast strains?

3) Write a simple genetic hypothesis for any difference between strains that is observed.

Observe the “minus lysine” plate. Compare the strains to each other.

4) What do you see?

5) What does this indicate about the ability of each strain to synthesize lysine?

6) Write a simple genetic hypothesis to explain the results.

Observe the side of the AA replica plate that was NOT UV-irradiated. Compare the strains to each other.

7) What do you see?

8) What does this indicate about the relationship between the ability to synthesize lysine and the ability to grow on AA medium?

9) What does this indicate about the relative rate of mutation for these strains?

10) Write a simple genetic hypothesis for any difference between strains that is observed.

Observe AA replica plate. For each strain, compare the side that was irradiated to the side that was not.

11) What do you see?

12) What does this indicate about the effect of exposure to UV irradiation on the mutation frequency for each strain? NOTE: remember to take the effect of UV-irradiation on growth on YPD into account.

Final Question: Write a genotype for each strain taking all your genetic hypotheses into consideration.

The Awesome Power of Yeast Genetic to Study Mutagenesis Day3: Making Master Plates for Use in Complementation Analysis of AA-resistant Mutants.

You have isolated AA resistant mutants from a designated "wild-type" strain and purified them by streaking to single colony on AA-containing medium. Each colony is assumed to be derived from an AA-resistant mutant and each mutant is assumed to result from a different mutational event. Today you set up "master plates" that will be used to perform a complementation test during the next lab period.

On your bench you will find:

AA plates you streaked last time.
YPD plates containing control strains that you will use in the complementation test.

Control Strains are:
1. MATa met15
2. MATa met15 lys2
3. MATa met15 lys5
4. MATa met15 lys14
5. MAT***a*** trp1
6. MAT***a*** lys2
7. MAT***a*** lys2 lys5
8. MAT***a*** lys2 lys14

Two fresh YPD plates per student for preparing master plates.

Prepare one master plate containing your four mutants and the four MATa control strains (1-4 above) by using toothpicks to make streaks across the plate in the pattern shown below.

Prepare a second master plate containing the MAT***a*** control strains (5-8 above) by using toothpicks to make streaks across the plate in the pattern shown below.

The TA will incubate your plates and return them to you during the next lab period.

The Awesome Power of Yeast Genetic to Study Mutagenesis Day4: Mating Yeast Strains to Perform a Complementation Analysis of AA-resistant Mutants.

You will use the replica plating technique to transfer yeast from each strain onto several types of media. Be sure that you understand the overall goal and procedure before beginning.

You will have the following on your bench:

Your master plates from the previous lab
A complete" plates that contains everything all the strains require for growth (SC).
A "minus lysine" plate that contains everything except lysine (-Lys).
A "minus lysine, minus methionine, minus tryptophan" plate that contains everything except met, trp, and lys. (-Met, Trp, Lys)

Your goals is to replica plate the strains from each master plate in a criss-cross pattern onto the velveteen and then print that pattern onto each of the plates in the following order:

- Lys, Met, Trp first
- Lys second
- SC third

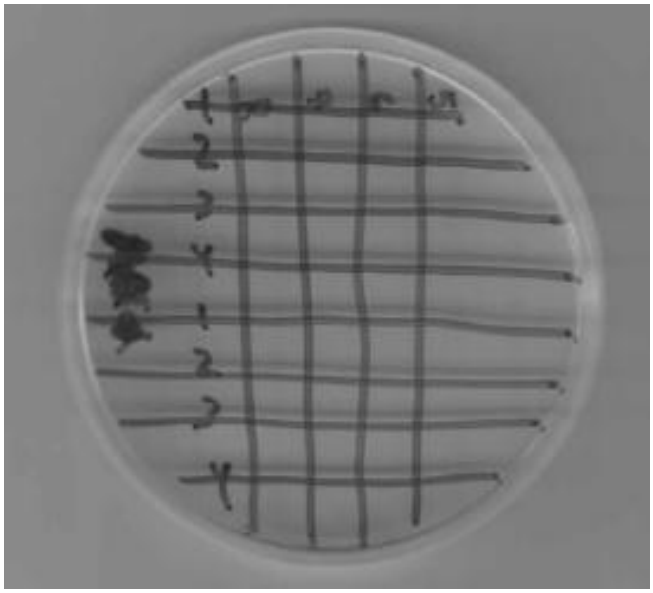
To accomplish the goals, follow the instructions below (your TA will walk you through this, step by step):

Observe a demonstration by the TA before proceeding.

1. Place a sterile velveteen square on your replica block and wet the velveteen using your minus lysine plates.

2. Print the MAT***α*** control strains on the velveteen first. Mark the control plate with a sharpie to indicate the orientation of the plate on the replica block. Remove the plate, replace the lid, and leave the plate on your bench. You should be able to see yeast on the velveteen in the same pattern as on the plate.

3. Print your Mat***a*** Master Plate on the velveteen at a 90o angle to the MAT***α*** strains so that the two patterns criss-cross. Mark the master plate with a sharpie to indicate the orientation of the plate on the replica block.



6. Print the yeast onto the – Lys, Met, Trp plate by pressing that plate down on the velveteen. Mark the plate to orient it.

7. Print the yeast onto the – Lys plate by pressing that plate down on the velveteen. Mark the plate to orient it.

8. Print the yeast onto the – SC plate by pressing that plate down on the velveteen. Mark the plate to orient it.

Leave the plates on your bench for the TAs to collect. They will be returned to you in the next lab period.

Yeast Mutagenesis Problem: Day 5.

On your bench you will find the plates you printed during the previous lab period. Generate a table displaying the results of the complementation analysis. Determine which LYS gene was mutated for each of your mutants.