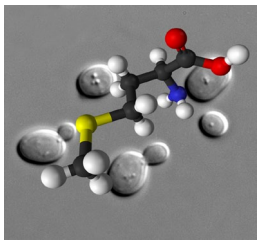
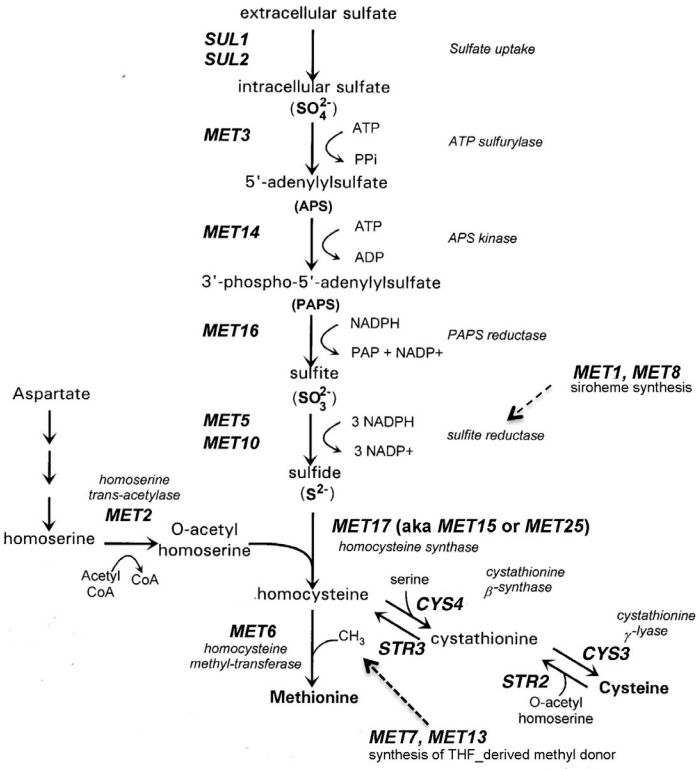
Follow the Sulfur: A Genetic Analysis of Methionine Synthesis



The budding yeast, *Saccharomyces cerevisiae*, has played an important role in human civilization. Before the advent of clean water sources and refrigeration, humans used yeast to produce bread and beer, foods that could be stored safely for periods of time. Both bread and beer production depend on fermentation, a complex set of metabolic reactions. Befitting its commercial importance, *S. cerevisiae* has been the object of scientific investigations for over a century. *S. cerevisiae* is a favorite organism for genetic studies, because it is small, simple to cultivate and reproduces every few hours. Geneticists have generated collections of mutant strains with many different phenotypes and used these strains to elucidate the biochemical pathways that comprise yeast metabolism.

In this experiment, you will work with yeast mutant strains to study the reactions involved in methionine (Met) biosynthesis. The *met* mutants are Met auxotrophs, meaning that they are unable to grow in media that does not contain a sulfur source that they can convert to Met. Each of the strains is missing a single gene involved in Met synthesis. All of the strains have additional auxotrophies, since they were derived from the BY4742 strain (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*), which carries mutations in genes involved in the synthesis of uracil, leucine and lysine.

The original *met* mutants were isolated in large genetic screens for yeast that were unable to live in the absence of Met. In studies spanning several decades, scientists were able to associate particular mutations with individual steps involved in Met synthesis. The figure on the right summarizes our current understanding of Met (and Cys) synthesis. Most of the *MET* genes encode an enzyme or enzyme subunit that catalyzes the conversion of one sulfur-containing metabolite to a second sulfur-containing metabolite. Note that a few *MET* genes encode proteins that do not interact directly with the sulfur-containing metabolites on the pathway. The *MET1* and *MET8* genes encode the siroheme cofactor for sulfite reductase, which is a heterotetramer composed of two Met5p and two Met10p subunits. The proteins encoded by the *MET7* and *MET13* genes are involved the synthesis of a methyl group that is added to homocysteine, the direct precursor to Met.



You will use selective and indicator media to identify the genes that are deleted in the mutant strains. Think of each mutation as erasing one of the arrows shown in the pathway. The selective media contain either Met, Cys or sulfite as the sole sulfur source. Depending on the position of the mutation in the pathway, the strains may or may not be able to survive. You will also culture the strains on BiGGY agar, an indicator medium that contains bismuth. Bismuth reacts with sulfide to form a brownish precipitate. The color of the colony reflects the quantity of sulfide produced by the cells. BiGGY contains yeast extract that supplies enough methionine for all strains to grow, as well as a small quantity of sulfate. The major sulfur source in BiGGY is sulfite, the immediate precursor to sulfide.

Exercise 1 - Predict the growth of mutant strains

Predict the ability of *met* mutants to grow on various media in the table below. YPD is a rich, but undefined, medium that contains all the nutrients required for yeast to grow. YC Complete is a synthetic medium that contains all the nutrients, including Met, required for BY4742 to grow. Place a plus (+) when you predict that the strain will grow and a minus (-) when you do not expect the strain to grow.

BiGGY agar plates are used to detect sulfide production. Use upward- and downward-facing arrows to predict whether strains will give rise to darker or lighter colonies than BY4742. Which strains have the lightest color on BiGGY? Why?

	YPD	**YC Complete**	**YC - Met**	**YC-Met +Cys**	**YC-Met +SO_3**	**BiGGY**
met3	+					
met14	+					
met16						
met5						
met10						
met1						
met8						
met2						
met17						
met6						
met7						
met13						

Exercise 2 - Identifying mutant strains using selective and indicator media

Your team will be given cultures of 3 different strains, each of which carries a different *MET* gene deletion, as well as a culture of BY742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*). The team should prepare spot plates on each of the six media listed above. Each row should have five spots from a single strain. ***Use the same row pattern on each of the six plates.*** (If you are unfamiliar with spot plates, see the separate document on preparing spot plates.)

1. Prepare a series of four 1:10 dilutions of each culture with STERILE deionized water. Vortex each culture before making a transfer.

2. Spot 5 µL aliquots of each dilution series in one row on a spot plate, beginning with the least concentrated culture dilution. Spot the complete dilution series of a strain on one plate before proceding to the second plate. ***Make sure that the plates are properly labeled!***

3. Allow at least 30 minutes for the cells to settle and attach to the media. Invert the plates and incubate the plates at 30°C until individual colonies become apparent. Note that strains may grow more slowly on some media than others!

4. Remove the plates from the incubator and store them inverted in the refrigerator or cold room.