

Modeling the Organism: The Cell in Development

Laboratory 2: Cell-cell interactions and mating in *Saccharomyces*

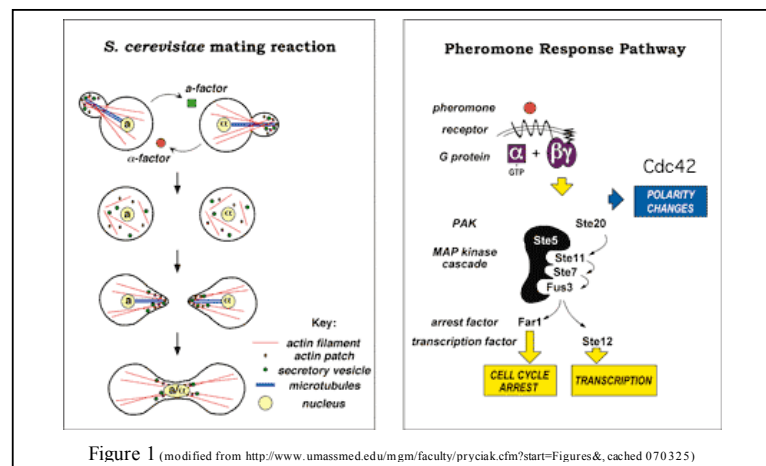
Introduction

In today's lab, we will observe the different life cycle stages of the budding or bakers' yeast, *Saccharomyces cerevisiae*. Wild-type strains typically consist of haploid cells that divide mitotically; following each division, mother cells undergo mating type switching upon reentry into G1 phase of the next cell cycle. Haploid cells express the single allele at the active MAT locus that determines their mating type identity: a or alpha (which I will designate @). Laboratory strains of yeast typically contain a dysfunctional mutant form of the HO gene (whose product is the endonuclease responsible for switching); hence they are unable to switch and are mitotically stable as either a or @ haploids. This gets around the issue of uncontrolled mating in the lab.

During vegetative growth, haploid cells secrete pheromones into their environment, advertising their presence to cells of the opposite mating type. Cells of mating type a secrete a factor and bear receptors for @ factor (encoded by Ste2) from the opposite mating type; cells of mating type @ secrete @ factor and bear a receptors (encoded by Ste3). The mating pheromones are short glycopeptides that diffuse through the surrounding medium.

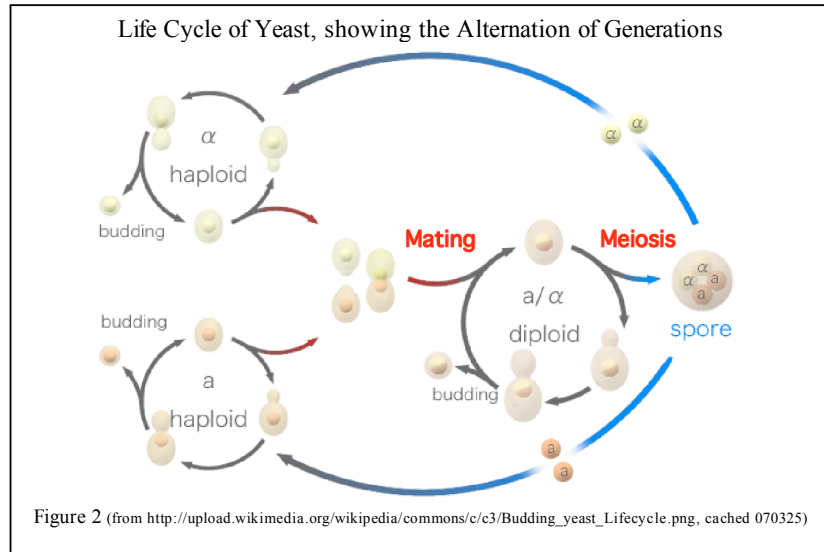
Upon sensing mating pheromones of the opposite mating type (remember opposites attract!), haploid cells stop dividing (i.e. cell cycle arrest) and form a schmoo (i.e. polarized growth) in the direction of the pheromone source (Figure 1). This response is integrated by pheromone binding to the extracellular domain of the pheromone cell membrane receptors, which in turn activates a heterotrimeric G-protein on the cytoplasmic side

of the cell membrane. This turns on a small (monomeric) G-protein Cdc42 to coordinate polarized cell outgrowth (schmoo formation), while also triggering a Mitogen Activated Protein Kinase (MAPK) cascade that triggers cell cycle arrest (via Far1) and initiates mating program transcription (via Ste12).



As the schmoos schmooze, they eventually contact schmoos of opposite mating types and lock in a passionate embrace (well, in molecular terms) and trigger a program of cell fusion. The two cells form a diploid cell that now contains copies of both MATa and MAT@. The new diploid organism reenters the mitotic cell cycle, dividing if nutrients are available. However, when nutrients become scarce the diploid cell enters a meiotic cycle to generate a spore. The spore can become dormant until conditions improve, at which point meiosis completes to release four haploid cells (two a and two @) and the cycle begins anew. Note that these offspring represent new genetic combinations, having passed through recombination and random assortment.

In today's lab we will examine the morphology of vegetative haploid strains, and then we observe a mating of a with @, observing the induction of polarized growth and the fusion of a/@ pairs to form diploid cells.



Protocol:

The center bench will have fresh plates of a and @ gametes and a mating plate upon which we plated a mixture of a and @ cells roughly 6 hours and 18 hours before the lab. Yeast cells take a while to grow (90 minutes per generation) and secrete the mating pheromones and they need to be undisturbed as they mate, so the process of schmooing and forming diploids takes several hours before changes are seen.

1. Select one of the haploid cultures and prepare a slide for examination under the microscope. Pipette 10 ul of sterile water onto the left half of a cleaned microscope slide (use a little 70% ethanol and a Kimwipe to clean the surface) and then use the pipette tip to transfer some cells from the plate to your slide; gently cover with a coverslip. Locate the cells using the 10x (Ph1 condenser setting) or 20x (Ph2) objective and then increase to 40x (Ph3). Record your observations of your living cells in your notebook. Are the cells moving? Why? Do you see buds? Sketch several cells in your notebook.
2. Repeat this process, using the right half of your slide to mount a sample of the other haploid culture. Do these cells look the same as the other gametes? Why or why not?
3. Taking a fresh slide, prepare samples of the two mating plates, noting which is which. Do you see any unusual cell forms? Schmoos? Zygotes? How can you tell?
4. Finally, prepare a slide of our other model organism, *E. coli*, from a culture on the main bench. Record in your notebook the appearance of *E. coli*.

Questions (answer in your lab notebook in place of a lab report):

1. Can you tell gametes of the two mating types apart? Design an experiment that would allow you to distinguish the two.
2. Compare and contrast the prokaryotic *E. coli* cells with your eukaryotic yeast. How are they the same? How are they different?

Finally, if the yeasts are mating well, we will have a special microscopy demonstration to give you a first hand experience with a technique described in lecture.