LAB MODULE 5B

The Yeast Cell Cycle

**Objective:** the goal of this exercise is to study the yeast cell cycle using quantitative light microscopy of synchronized cells.

**Introduction:**
The Nobel Prize in Physiology or Medicine was awarded in 2001 to three cell biologists, Drs. Tim Hunt, Leland Hartwell and Sir Paul Nurse for their pioneering work on the cell cycle. This work, which was carried out in the 1970’s and 80’s, was performed in embryonic cells of the sea urchin, which are amenable for isolation of proteins, and in two different species of yeast, *Saccharomyces cerevisiae*, budding yeast, and *Schizosaccharomyces pombe*, or fission yeast. Budding yeast is well known as the organism important in brewing beer and baking bread; *S. pombe* is named for a particular type of beer that it is used to brew. Although both are classified as fungi, they are distantly related. These organisms share several key features that make them ideal for study of the cell cycle. First, they grow rapidly and have a small genome. Second, molecular biological manipulations – including gene deletions and gene replacements– are easy to perform in these cells. Importantly, yeast can exist as haploids, where only a single copy of each gene is present, and are thus particularly useful for isolating mutants. A schematic diagram of the cell cycle in these yeast is shown in the Figure.

![Cell Cycle Diagram](image)

**Figure 17-5: Molecular Biology of the Cell 5/e (© Garland Science 2008)**
To examine the cell cycle in yeast, the Nurse and Hartwell groups designed genetic screens to isolate cells with mutations in cell cycle regulation. To do this, cells were mutagenized, and conditional mutants examined. These mutants can grow at the permissive temperature, but when the temperature is raised a few degrees, to the so-called restrictive temperature, the mutant protein becomes nonfunctional. The mutant cells were imaged using light microscopy at the restrictive temperature. Cells with mutations in key cell cycle regulators could not progress through the cycle, and they arrested at a particular stage of the cycle (see Figure). Thus by using the relatively straight-forward approach of morphological examination of cells, scientists could identify key regulators of the cell cycle. Genes that were important in regulating the cell cycle were called CDC-genes, for cell division cycle genes. Hartwell’s group identified more than 100 CDC-genes in *S. cerevisiae*.

![Figure 17-6: Molecular Biology of the Cell Cycle](© Garland Science 2008)

From these experiments, it became clear that the events of the cell cycle are precisely coordinated, and one event normally does not occur until prior events are completed. Cells monitor the major transitions in the cell cycle, often called checkpoints. The first checkpoint is referred to as ‘Start’ or the restriction point. Once a cell has passed through Start, it is committed to DNA synthesis. The next checkpoint is the G2-M checkpoint. Here, cells must determine if all the DNA has been replicated, prior to progressing to M phase. Finally, cells monitor the alignment of chromosomes in mitosis and do not progress into anaphase if the chromosomes are not aligned. For details on the molecular machinery of the cell cycle, many modern cell biology textbooks have detailed explanations.

In the experiment you will perform today, we will use budding yeast that have been arrested in the G1 phase of the cell cycle. For example, if yeast (and other cells) are removed from a nutrient rich medium, they will stop growing and arrest the cell cycle. Alternatively, if cells are exposed to mating pheromone, or cells of the opposite mating type, they arrest in G1 and form a ‘shmoo’ in preparation for forming diploid cells. *S. cerevisiae* exist in one of two different mating types.
called ‘A’ and ‘alpha’. In this experiment, yeast of mating type ‘A’ have been exposed to alpha factor (the pheromone secreted by yeast of the opposite mating type, ‘alpha’) to arrest them in G1. Thus, nutrients are a positive growth factor, and mating factor is a negative factor, which can override the presence of nutrients. After a 2 hour arrest, the cells were pelleted in a centrifuge, the medium with alpha factor was removed, and fresh medium added. This induces the cells to re-enter the cell cycle synchronously. Once the cells are released from alpha factor, they will progress through all the stages of the cell cycle in approximately 2 hours.

**Materials:**
Wild type, asynchronously growing yeast.
Cells arrested in the cell cycle with alpha factor.
This strain expresses CFP-tagged tubulin, so the spindle is fluorescent. It may be possible to view the fluorescence using your blue filter cube.

**Methods:**

1. **Examine the morphology of asynchronously growing yeast.**
The single celled organism, *Saccharomyces cerevisiae*, grows by forming a bud, which appears in G1. Obtain a sample of the asynchronous yeast culture.
Make a wet mount by placing a few microliters of yeast cells on the slide and covering it with a coverslip. You don’t need spacers because the yeast cell wall prevents the cells from getting squashed. Image the yeast at 10, 40 and 100X in phase contrast. Save several images at each magnification. Yeast are very small cells, so you will need to make the measurements from images collected at 100X. **Be sure that your microscope is properly aligned for phase contrast.**

Identify unbudded cells and cells with various sized buds. Collect images of each of the different stages in the cell cycle by using phase contrast; you can also see if the fluorescence is visible in your microscope using the blue filter set (it might not be a strong signal because the cells express a variant of GFP, called CFP, with different excitation and emission properties. Save these images.

2. **Analysis of the Cell Cycle using synchronized cells.** Yeast can be arrested in the cell cycle by adding alpha factor to yeast that express the a-mating type. This has been done for you. After arrest, the alpha factor is removed and the cells progress synchronously through the cell cycle.
You will have a total of 6 samples to analyze:

Time zero – this sample will be taken prior to release from alpha factor. No cells should be budded.
15 min
30 min
60 min
90 min
120 min
These time points are a recommended guideline for you to follow. When you take each sample of yeast, note the EXACT time that you make the sample. You can enter that in the spreadsheet. For each sample, remove 270 ul from the yeast culture and add to a tube that contains **30 ul of 37% formaldehyde**. The formaldehyde will fix the yeast (ie they will be dead) so that you can image them at the specified time. The yeast will be released from alpha factor at 2:15, so the last time point will be 4:15.

3. For each sample you need to collect at least 10 fields of view at 100X. Take phase and images (and fluorescence if possible). Do this in the lab period.

4. Data analysis. (can be done later, using your saved images).

For each field of view you will make several measurements and put the values in the spread sheet. Go to the Bioimaging web site and find the data acquisition page for yeast, open it, and familiarize yourself with the values that are needed. You will use **one line on the sheet for each field of view**. For each field of view, you will record: the number of cells and the number that have a bud. You will also measure the length of the bud and of the mother cell for 5 representative cells in the field of view. The class will pool the data, so there will be plenty of data. You will have 10 lines of data (one for each field of view) and each line will have measurements for 5 cells, for a total of 50 cells.

5. If the fluorescence images are sufficiently bright, you can determine how the mitotic spindle position changes as the cells progress through the cell cycle. The spindle is a bright bar of fluorescence. It is intranuclear, because in this organism the nuclear envelope is intact throughout mitosis. To accomplish chromosome segregation into the two daughter cells, the spindle is positioned at the neck and then the spindle elongates, resulting in each daughter cell receiving one set of chromosomes (see the diagram).

![Figure 2. Schematic diagram, and corresponding fluorescence images of microtubules, in budding yeast. Left side shows a cell in metaphase; right side shows a cell that has completed cell division. SPB is the spindle pole body (the yeast equivalent of the centrosome). Green lines are microtubules. Blue sphere is the nucleus. Note the increase in the length of the mitotic spindle.](image)
If your microscope filters are adequate for imaging the fluorescence, take images of yeast at 100X. Include images of cells without a bud, and with buds of different sizes.