

## Bio 230 General Genetics Lab Exercise 3

### SITES OF SYNTHESIS. EXAMINING CYTOPLASMIC AND ORGANELLE PROTEIN SYNTHESIS.

**Warning:** in this exercise you will be working with radioactive material. Be sure to read the attached safety sheet. There will be a safety quiz prior to starting the exercise. If you fail the quiz you will not be allowed to participate and will receive a grade of zero for the lab.



#### Introduction



All protein synthesis uses the same basic components: mRNA, amino acids, tRNAs and ribosomes. Prokaryotes only have what are termed 70S ribosomes, but in eukaryotes there are two different types of ribosomes: 80S, which are found in the cytoplasm and 70S, which are found in mitochondria and chloroplasts. The theory of endosymbiosis, first proposed by Lynn Margulis, suggests that these two organelles were once free-living prokaryotes (see pp604-606 of your text) and retain the prokaryotic type of ribosome. The two types of ribosome differ in size, and in the number of RNA and protein molecules present. They also respond differently to certain antibiotics that interfere with the ability of ribosomes to perform their role in protein synthesis. In this exercise you will use three protein synthesis inhibitors, cycloheximide, erythromycin and tetracycline. Cycloheximide is produced by the bacterium *Streptomyces griseus* and exerts its effect by interfering with the translocation step in protein synthesis thus blocking elongation, but only in 80S ribosomes. It has no effect on translocation in 70S ribosomes. By contrast, erythromycin (made by the actinomycete *Saccharopolyspora erythraea*) interferes with aminoacyl translocation, preventing the transfer of the tRNA bound at the A site of the rRNA complex to the P site of the rRNA complex, but only in 70S ribosomes. As a result, cells treated with cycloheximide (10µg/ml) will continue to make only organelle proteins and those treated with erythromycin (100µg/ml) will make only cytoplasmic proteins. The final antibiotic, tetracycline (made in *Streptomyces*) is capable of affecting both 70S and 80S ribosomes. It works by inhibiting the binding of aminoacyl-tRNA to the acceptor site on both types of ribosomes.

#### Radioactive pulse-labeling

To determine how much protein is made under each of these treatments, as well as in an untreated cell, it is necessary to measure only the protein made during the course of the treatment, as opposed to what was already present prior to the experiment. The simplest way to do this is to add radioactive <sup>35</sup>S during the treatment. *Chlamydomonas* will take up the sulfur, convert it to methionine and cysteine and incorporate the radioactive amino acids into proteins that are being made, but not into pre-existing

proteins. At the end of the treatment the amount of radioactivity in proteins will reflect how much protein was made.

### ***Chlamydomonas***

Analysis of cytoplasmic vs organelle protein synthesis will be easier if both constitute a significant portion of the total. The single celled green alga *Chlamydomonas* has a single chloroplast, that makes up about 40% of the total cell volume. In this case preventing either 70S or 80S ribosome activity should lead to a significant decrease in protein synthesis. This will help us understand where the proteins are being made, but not where they are going. Specifically, it would be useful to know if the proteins are remaining soluble, or are they ending up in membranes?

### **Freeze/thaw**

If cells are broken open by mechanical means then the membranes remain largely intact and can be pelleted by high speed centrifugation. This will leave the soluble proteins in solution in the supernatant and the membrane-bound proteins in the pellet. There are several ways to break open cells, the simplest is a series of freeze:thaw cycles. This method of lysis causes cells to swell and ultimately break as ice crystals form during the freezing process and then contract during thawing, rupturing the cell membrane. Multiple cycles are necessary for efficient lysis but, even after several rounds, not all cells will have lysed.

### **Identification of specific proteins**

The procedure described above tells us **how much** of each type of protein is being made, but not **which ones** are in each category. To identify where each type of protein is being made, and where it is going, samples from each of the above will be run on an SDS gel. After the gels are stained and dried they will be placed under X-ray film for a week. Radioactive proteins will expose the film and show up as a black band. By comparing the banding pattern from each of the treatments, you can determine where each of the proteins was made, and where in the cell it ended up.

### **Procedure**

Follow the flowchart on the next page. Remember to wear gloves at all times, dispose of all radioactive material in the appropriate containers. You will need four tubes:

Control-

Cycloheximide

Erythromycin

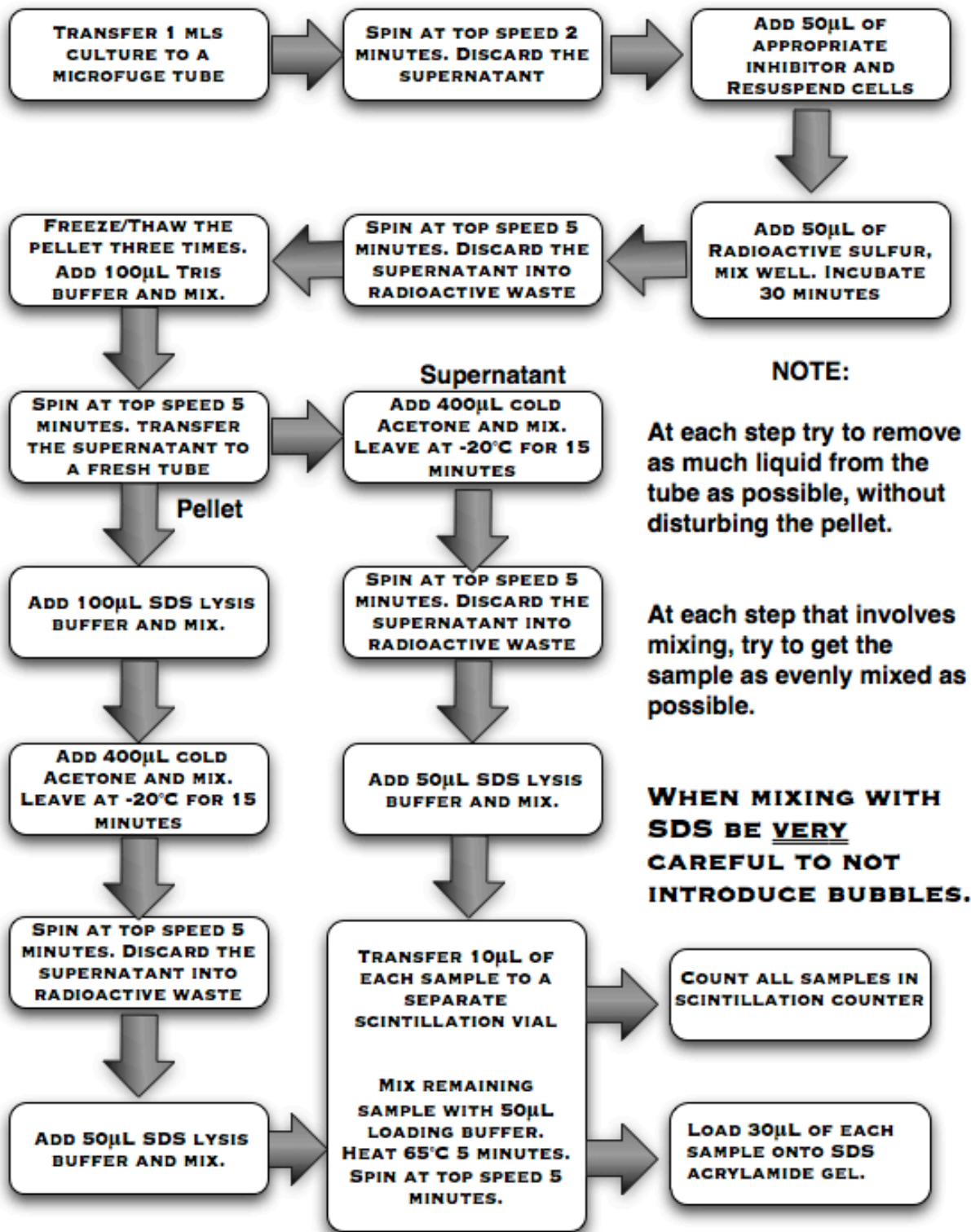
Tetracycline

After the freeze/thaw you will need another four tubes, to hold the soluble fraction for each of these. The membrane fraction will be the pellet in the original tube.

Prepare three additional scintillation vials for the group, as well as vial one for each person. Two will remain empty, and give an estimate of background radiation. The third vial should contain a 2cm x 2cm piece of KimWipe wiped on the benchtop around your work site. Each member of the group should also perform a swipe on themselves

LOAD 30 $\mu$ L ONTO GEL

FOR EACH OF THE SAMPLES:



### **Data analysis and write-up**

You should look at your own group's results first, and then look at the results from all four groups. Think about the following, and address them in your discussion: are the numbers similar for each treatment? Do the soluble+insoluble pairs add up to the same for each group? Did adding each of the antibiotics have an effect on protein production? Did keeping the cells in the light/dark make a difference: does it depend on which antibiotic is used? You may find it more convenient to express all numbers in terms of the counts in each treatment as a percentage of the total (soluble + pellet) seen in the light control tubes.

All citations must be available in the library or from a web site operated by a university or a refereed journal. In all cases, the first page of the article must be attached to the lab report.

### **Questions to be answered prior to lab.**

Every step is there for a reason. Think about the reasoning behind each of the following and write a short explanation.

- 1) Why are the cells incubated in sulfur-free medium for an hour before starting the experiment?
- 2) Why is the inhibitor added before the radioactive sulfur: why not together?
- 3) Why do an acetone precipitation? All of the protein is present in the solution anyway.



## Radioisotope Safety Instructions



**Read these instructions before the next laboratory session. You will be tested on this material at the start of the laboratory period.**

1) In the following exercise you will be using the radioisotope  $^{35}\text{S}$ . Any time you work with radioactive materials special safety precautions must be taken. Anyone disregarding the safety procedures will be asked to leave the laboratory and will receive a grade of zero for this exercise.

2)  $^{35}\text{S}$  is a beta emitter of moderate energy, with a half-life of approximately 3 months. Since sulfur is an important component of a variety of cell compounds it can become incorporated into the body if ingested. Surface contamination is less dangerous, but should still be avoided. In order to check that no bodily exposure has occurred, each student will be monitored for  $^{35}\text{S}$  contamination after the lab period by use of a 'swipe' test. We will also perform a swipe test on your area of the lab bench: a positive test means you have failed to follow safety procedures and you will be penalized.

Take a piece of Kimwipe (about 2.5 cm x 2.5 cm) and wipe your face, palms and fingers with it. Place the Kimwipe in a scintillation vial and put your initials on the lid. **NOTE:** No student will be allowed to leave the laboratory until the instructor has seen the swipe test results. This means that casual entering or leaving the lab is forbidden without expressed permission of the lab instructor.

### 3) Safety rules

- Wear plastic gloves and safety aprons at all times when working with the radioisotope.
- Avoid touching anything that will not be thrown out in the safety bags. This includes your face, clothing, partner, chairs, *etc.*
- Perform all work over the metal tray on the safety cloth, (which will absorb any spills). Place all used materials in the disposal bags provided. The only radioactive material that leaves the bench should be in sealed centrifuge tubes or in vials to be counted.
- **In the event of an accident or spill, do not move - inform the instructor at once!**