BIO302：Modern Biotechnology

Lectures: coordinator: Prof. Huang Wei, 慧园4栋213

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Office hour: Thursday 10:20-11:50AM
or by appointment

lecturers, Profs. Huang Wei，Deng Yi,
Tang Bin, Tian Ruijun, Zhang Hongming

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Laboratory: Instructor: Dr. Yu Chunhong

Why study this course?

- Biology is an technology-driven science
  - New critical technology leads to major scientific advances
    - Number of technology-related Nobel Prizes
  - Benefits both research and development
- Clearly understanding it helps:
  - Design your study and individual experiment
  - Improve it by applying to new field or modify it for better use
  - Inspire new technology development

What do you need to do if you want to?

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    research)
  - Know other
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Pulse and chase labeling with [35S]-Methionine

Lucho Fuentealba 2008

Biotechnology is not protocol

This protocol is prepared for 12 well-plates. Adjust volume accordingly for different plates. It is recommended to plate cells in Fibronectin 20 µg/ml and use them at a confluence of 70-80%

Solutions

- Pulse-labeling medium: Met- Cys-free DMEM (Gibco Cat # 21013). It can also contain 10% dialyzed serum
- Chase medium: Met-Cys-free medium containing 7.5 g/L of Methionine. It is recommended to use Met at a concentration of 15 mg/L

Labeling of cells

- Thaw [35S]-Methionine (Perkin-Elmer, 5mCi. Cat # NEG009L005MC) at RT and prepare a 0.1-0.2 mCi/ml working solution in pre-warmed (37°C) pulse-labeling medium
- Wash cells twice with 1 ml of pulse-labeling medium
- Add 0.5 ml pulse-labeling medium and incubate 15 min at 37°C (to deplete intracellular pool of Meth)
- *Pulse*: Add 0.35 ml of [35S]-Meth working solution. Incubate 30 min at 37°C
- *Chase*: Add 0.75 ml of chase medium to the [35S]-Meth solution and remove
- Wash once with 1 ml of pre-warmed chase medium
- Add complete medium (DMEM + 10% FBS). Treatments, such as Wnt3a or Lactacystin, can also be added at this point
- Extract cells and determine [35S]-Meth incorporation by TCA precipitation

Syllabus

Modules	Professor	Department/ Research area	Date Lecture, JC
1. pulse-chase	黄巍	Biology Quantitative Biology	2-28, 3-7
2. super resolution microscopy	黄巍	Biology Quantitative Biology	3-14, 3-21
3. lineage tracing	黄巍	Biology Quantitative Biology	3-28,4-4
4. optogenetics	黄巍	Biology Quantitative Biology	4-11,4-18
5. mechanobiology	唐斌	Material sciences Biomechanics	4-25,5-2
6. proteomics	田瑞军	Chemistry Analytical Chemistry Proteomics	5-9,5-16
7. de novo protein design and engineering	张宏民	Biology Structural Biology	5-23,5-30
8. protein engineering by evolution	黄巍	Biology	6-6,6-13
Final proposal turn in			6-17

成绩评定:（Grading）

组成（Components）	所占比例 (%)
实验课	33
文献讨论（Pre），文章简评，及课堂讨论（含出勤）	36(12+12+12)
半期小论文	7
期末论文及答辩	24

1. 半期交（**4/25**）：Write an essay about a biological techniques developed in the past 10 years, which you think is the most important. In English, 1-3 pages, double space. You need to describe it clearly and convincing.

2. 期末交 (**6/17**）：Write a real research proposal that utilize at least one of the recent developed biotechnologies.

3. Journal Club (文献讨论), totally 8 times, 24 papers, each paper will be presented by a group of 4 students, each group will present twice.

3.1. The presenters will be scored by their presentatiosn and answers

3.2. Every students will need to write a referee report for one paper each times, and turned them in by the end of the week. Each student will write for 8 reports and be scored. The referee report is nomally 100-300 words/

4. The attendance and participation in class will be marked.

Modern Biotechnology

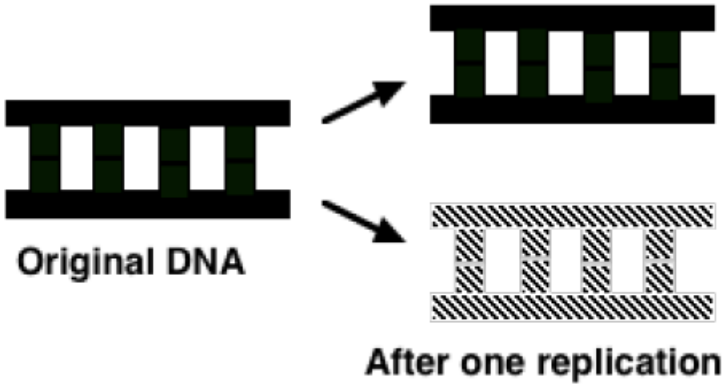
Module 1: Pulse-Chase and its reincarnations

Week 1, 2/28/2018

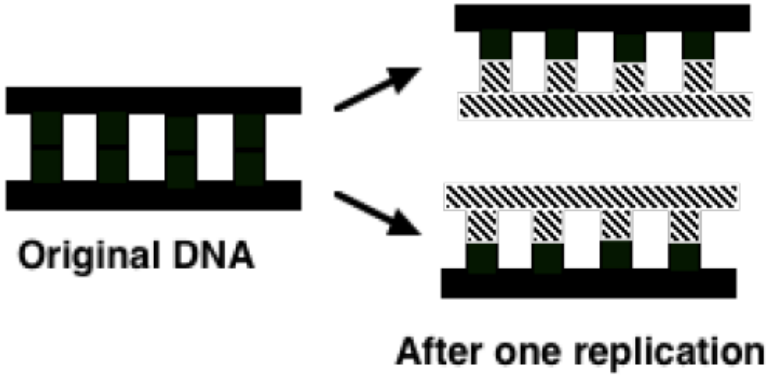
Pulse-chase assay: an “old” biotechniques

In the 1950s, there were three DNA replication models being proposed:

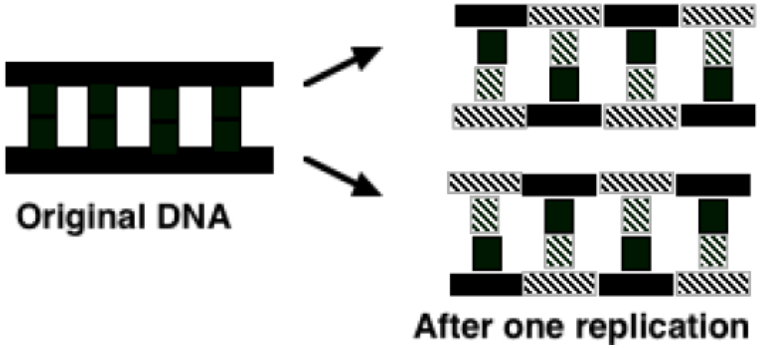
1. Conservative replication



2. Semiconservative replication (Watson and Crick)



3. Dispersive replication



In 1958, Meselson and Stahl conducted the classic **pulse-chase** experiment

•The key was the used of stable isotope nitrogen (^{14}N and ^{15}N) in the E. coli growth media.

•As E. coli reproduced, new DNA molecules were synthesized incorporating these isotopes.

1. Those grown with ^{15}N created "heavy" DNA.
2. Those grown with ^{14}N created "light" DNA.
3. They extracted equal amount of DNA from each culture, and centrifuged the mixture to separate the heavy from the light.

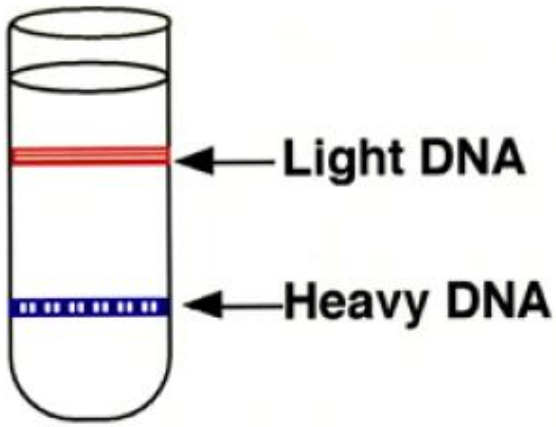
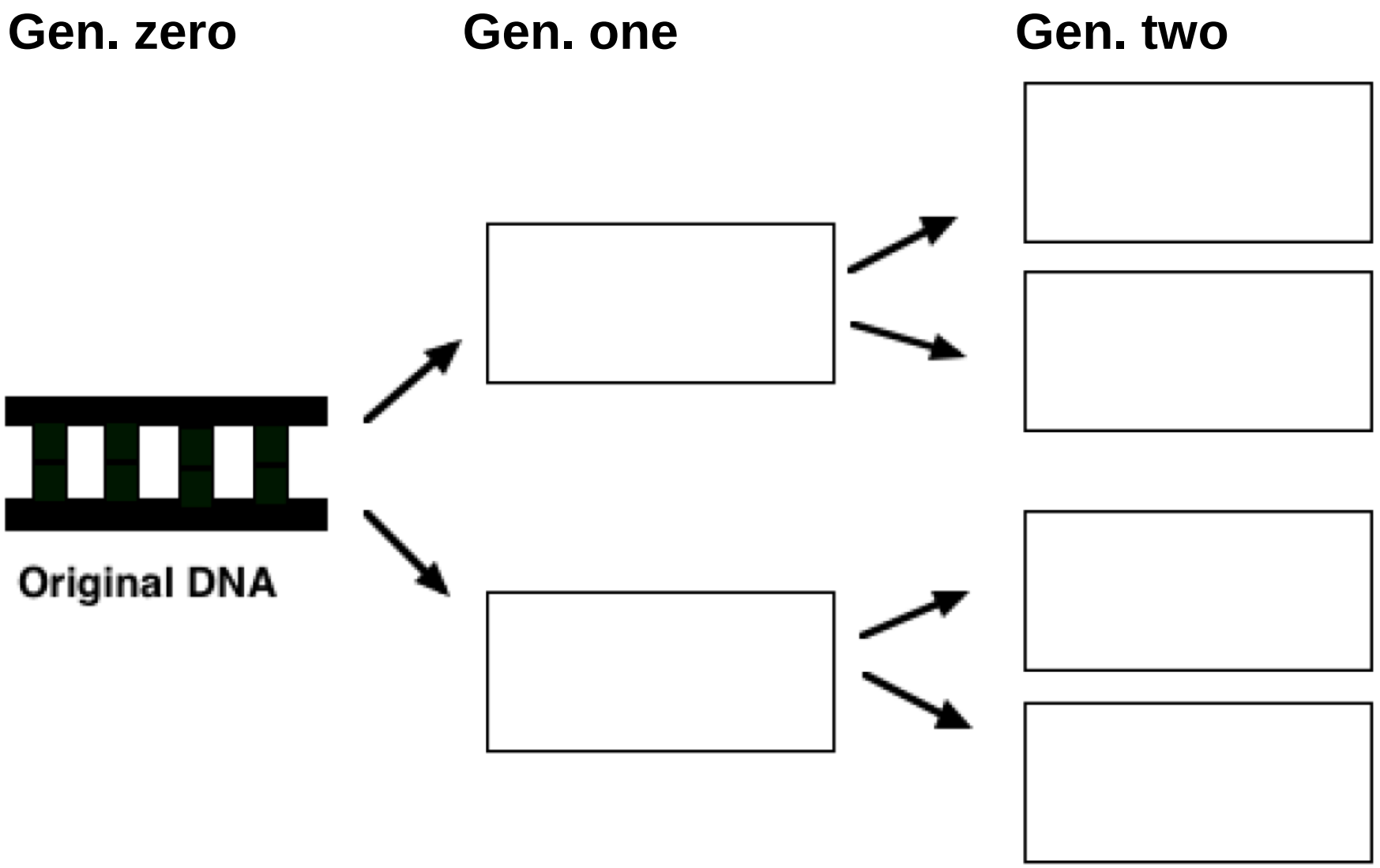


Figure 1: Result of the centrifugation of light and heavy DNA

1. Using this technique, Meselson and Stahl grew E coli on an medium containing ^{15}N for many generation to ensure all the DNA would be labeled with ^{15}N. This was the **pulse** phase. The DNA prepared from it is called **Generation Zero**.
2. Next the E coli with the heavy DNA were moved to a culture media containing ^{14}N. This step marks the beginning of the **Chase** phase.
3. After 20 minutes (the E coli doubling time), a DNA sample was prepared, called **Generation one**.
4. After another 20 minutes, another DNA sample was prepared, called **Generation two**. And so on.

Please make predictions for all three models, assuming each cell divided exactly once per 20 min



Using the tube on the left as the standard, sketch where the bands of DNA would collect in the tube of generations Zero, One and Two if DNA replication is semiconservative (if Watson and Crick were right)

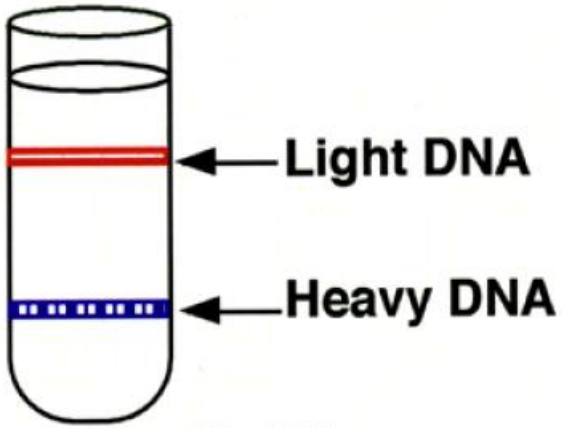
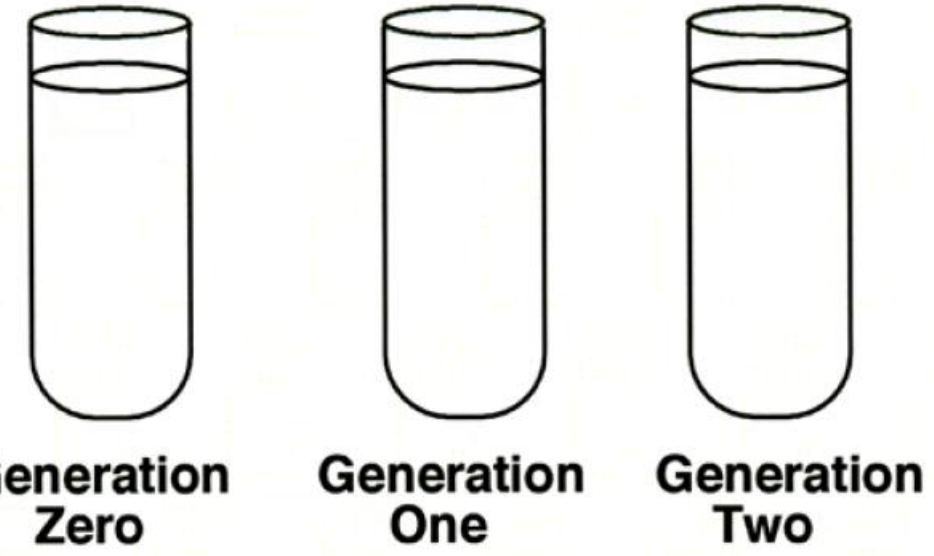


Figure 1: Result of the centrifugation of light and heavy DNA



The essence of a pulse-chase experiment

- Is a **two-phase** technique used to examine **dynamic** cellular processes
- During the **pulse** phase, cells are exposed to a labeled compound which is incorporated into the molecule or pathway being studied. Normally this step is **brief** comparing to the process studied.
- During the **chase** phase, an unlabeled form replaces the labeled compound. The reaction is monitored to see how long it takes the labeled form of the compound to be replaced by the unlabeled.
- The choice of label a compound: compromise between **detection** and **minimum interference** of cellular process. Typical: **radioisotope**, **staple isotope**, **chemical modified compound**, and **fluorescence proteins**.

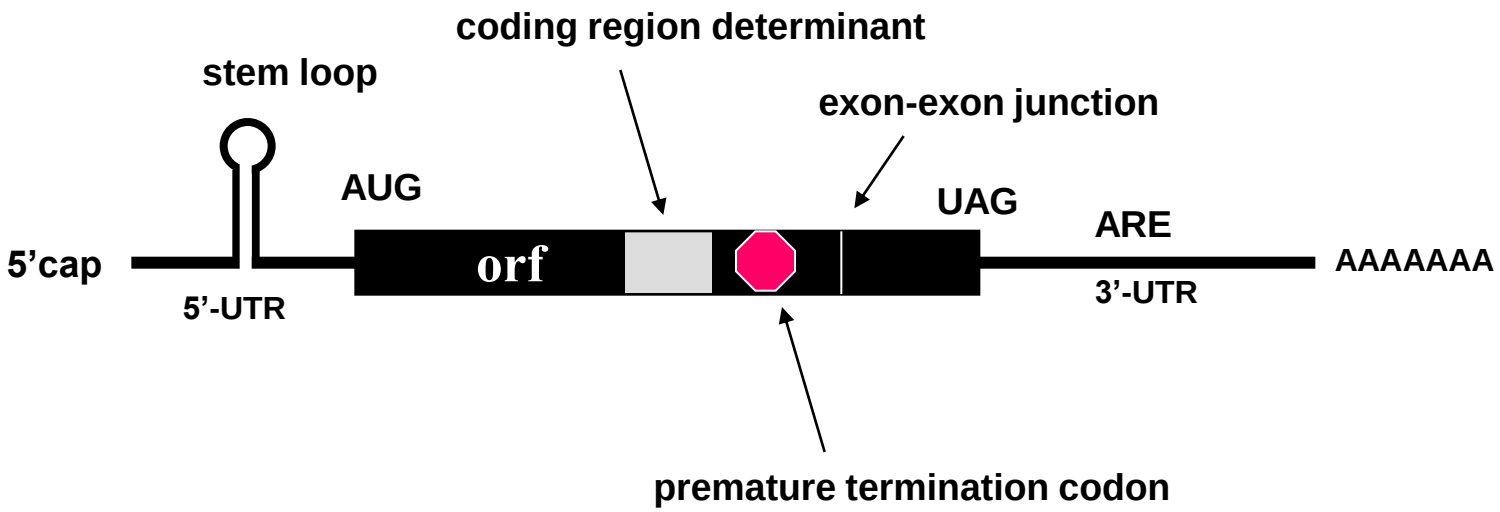
Pulse-chase to study mRNA decay process and dynamics in yeast

Using inducible GAL promoter: the transcription can be tuned with addition or removal of galactose

Transcriptional pulse-chase:

1. it was kept off using raffinose,
2. the mRNA is induced by adding galactose for a brief time
3. the transcription is shut off by adding glucose
4. This procedure generates a burst of newly synthesized transcripts whose decay can be followed by size using gel eletropheresis

Elements of an mRNA that affect its stability



5'-cap: protection against 5'-exonuclease

Stem loop: inhibition of translation can stabilize mRNA

Coding region determinant: can mask other mRNA elements to stabilize untranslated mRNA

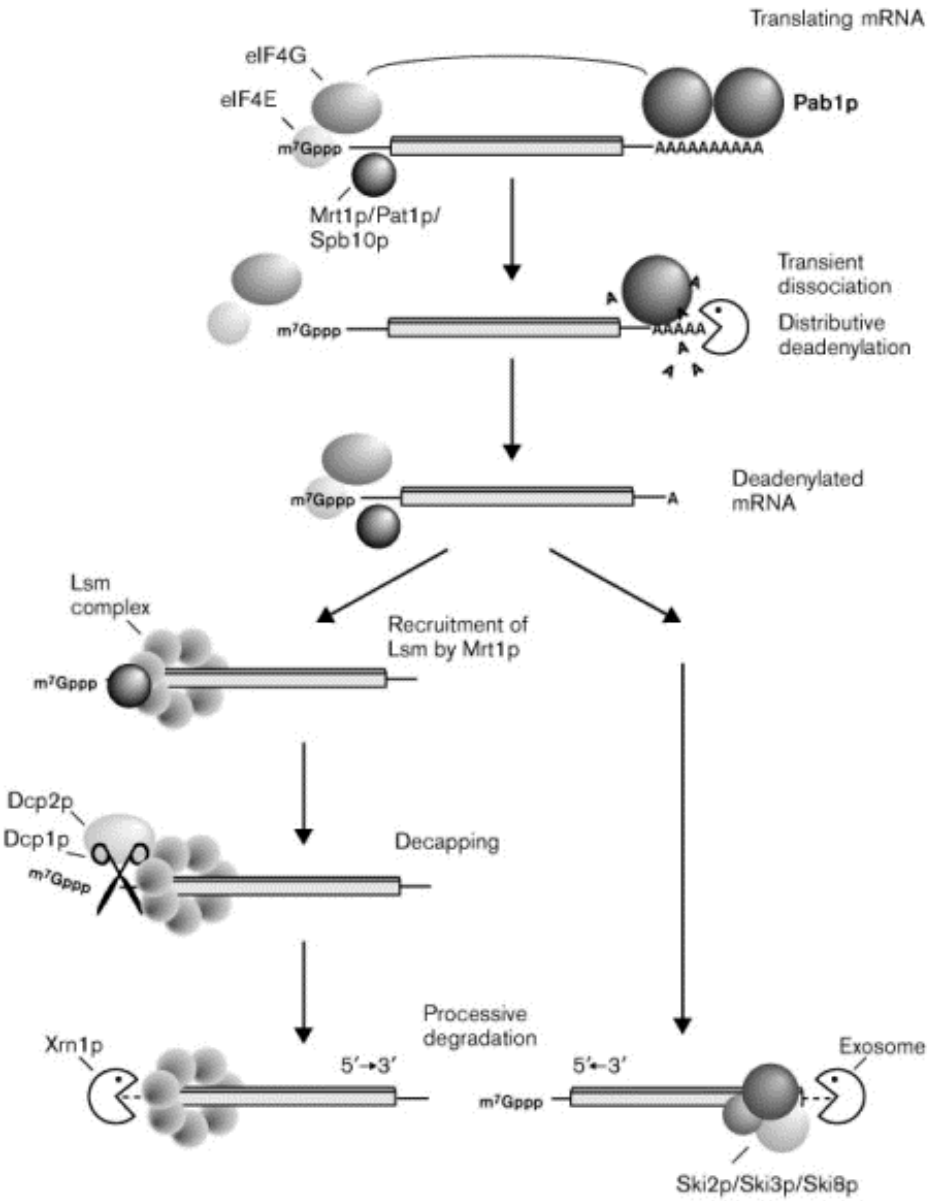
Premature termination codon: target transcript for nonsense-mediated decay (NMD)

Exon-exon junction: binding site for nuclear shuttling proteins - determinant for NMD

AU-rich element (ARE): binding site for destabilizing RNA

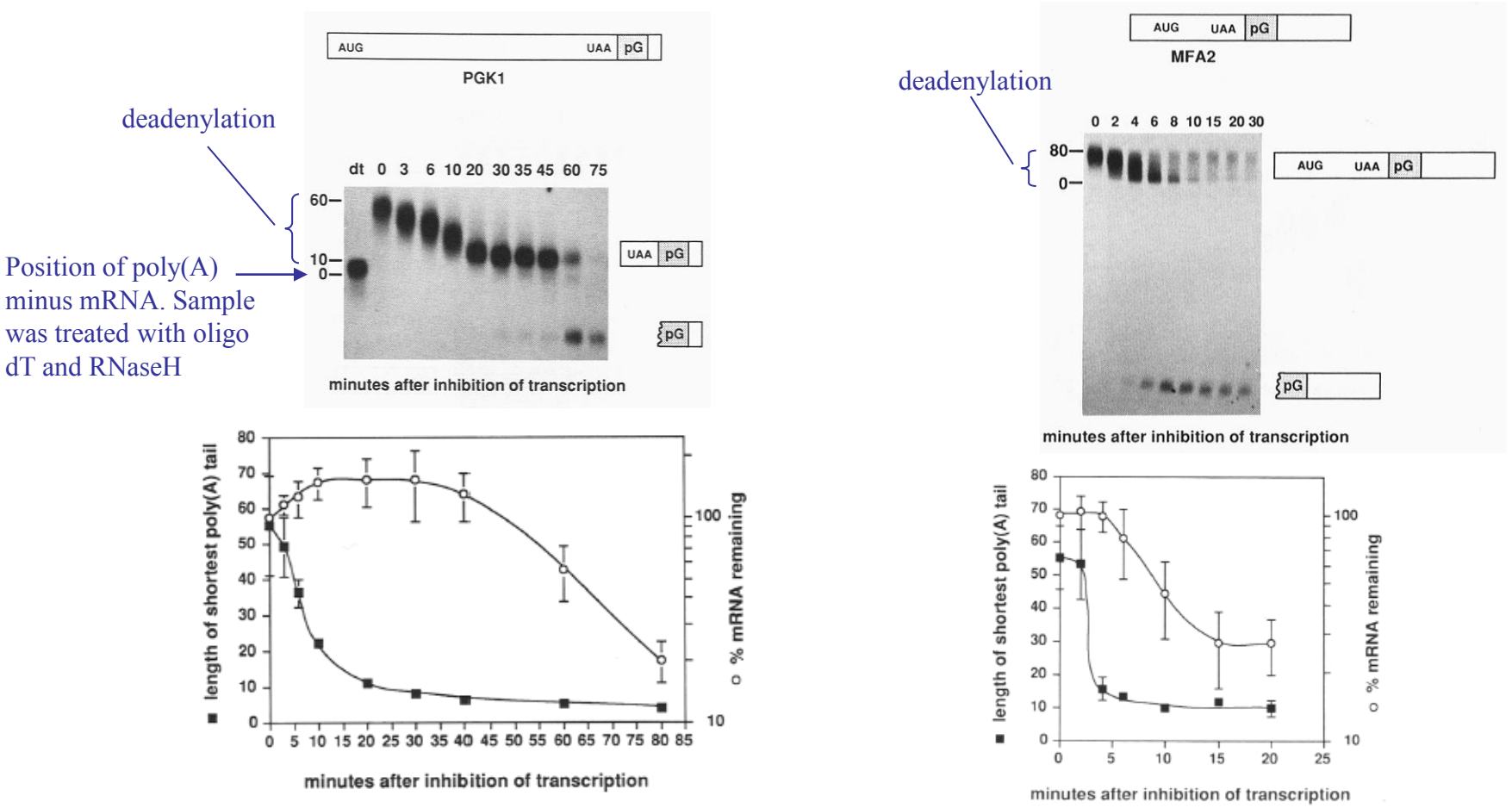
siRNA and miRNA-mediated degradation:..

Pathways of general mRNA decay in yeast



Current Opinion in Genetics & Development

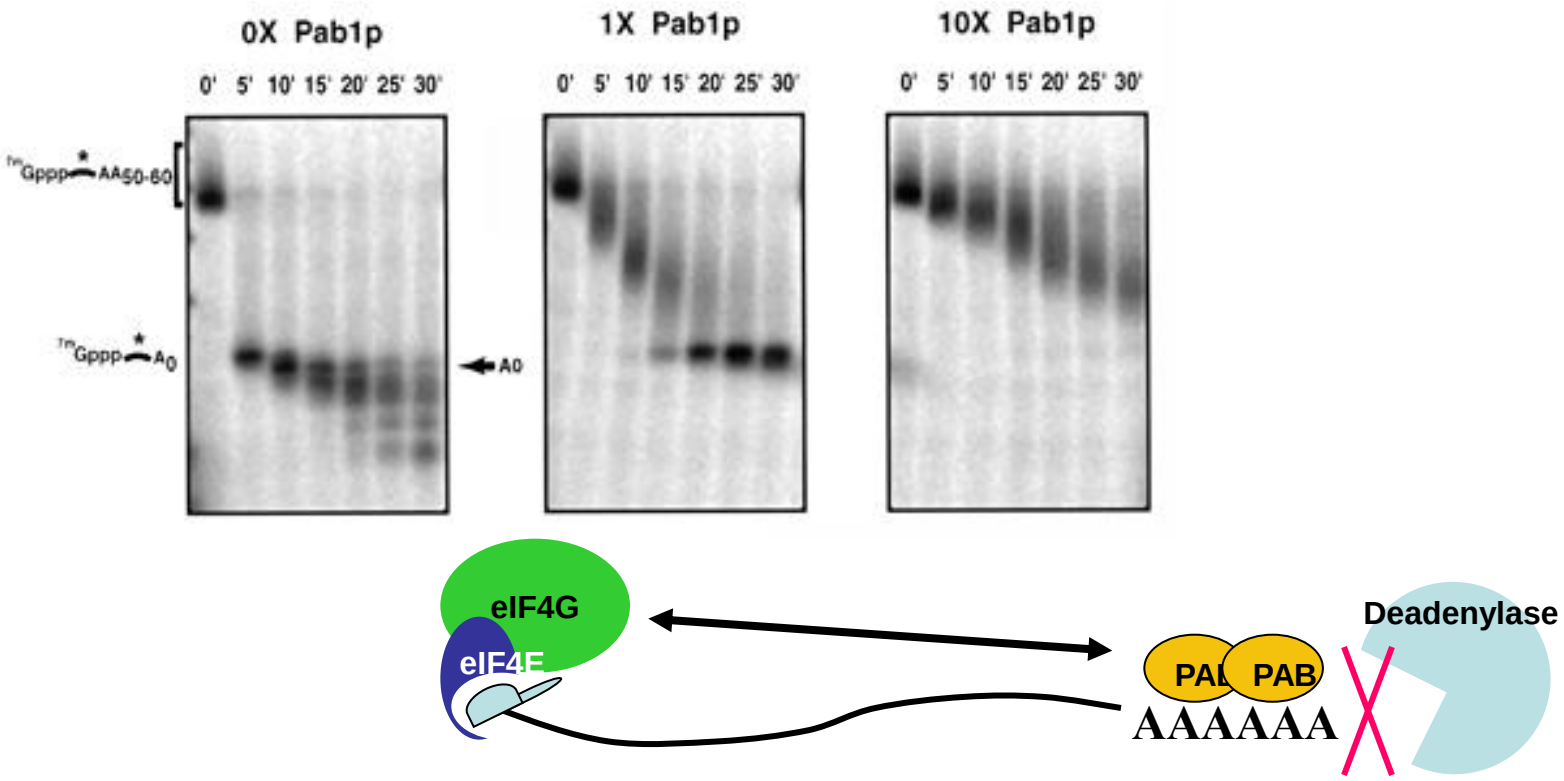
Decay of stable and unstable mRNAs in yeast initiates with deadenylation followed by degradation 5' to 3'



Transcriptional pulse-chase of PGK1 (stable, $t_{1/2}$=45min) and MFA2 (unstable,$t_{1/2}$=3.5min) in yeast. Tracts of 18 consecutive guanosines (pG) were engineered into the mRNAs for strong secondary structures to block exonuclease degradation.

From Decker and Parker Genes & Dev. 7:1632-1643 (1993).

Deadenylation in yeast requires Ccr4p in vivo and is blocked by Pab in vitro



Pab1p inhibits Ccr4p deadenylase activity in vitro. Analysis of deadenylation activity in Flag-Ccr4p purified fractions with addition of increasing amounts of purified Pab1p. Purified Pab1p was added to each time course in molar amounts relative to Flag-Ccr4p as indicated. Numbers above the lanes indicate time points taken after addition of substrate to the reaction. The asterisk indicates the position of the radiolabeled phosphate.

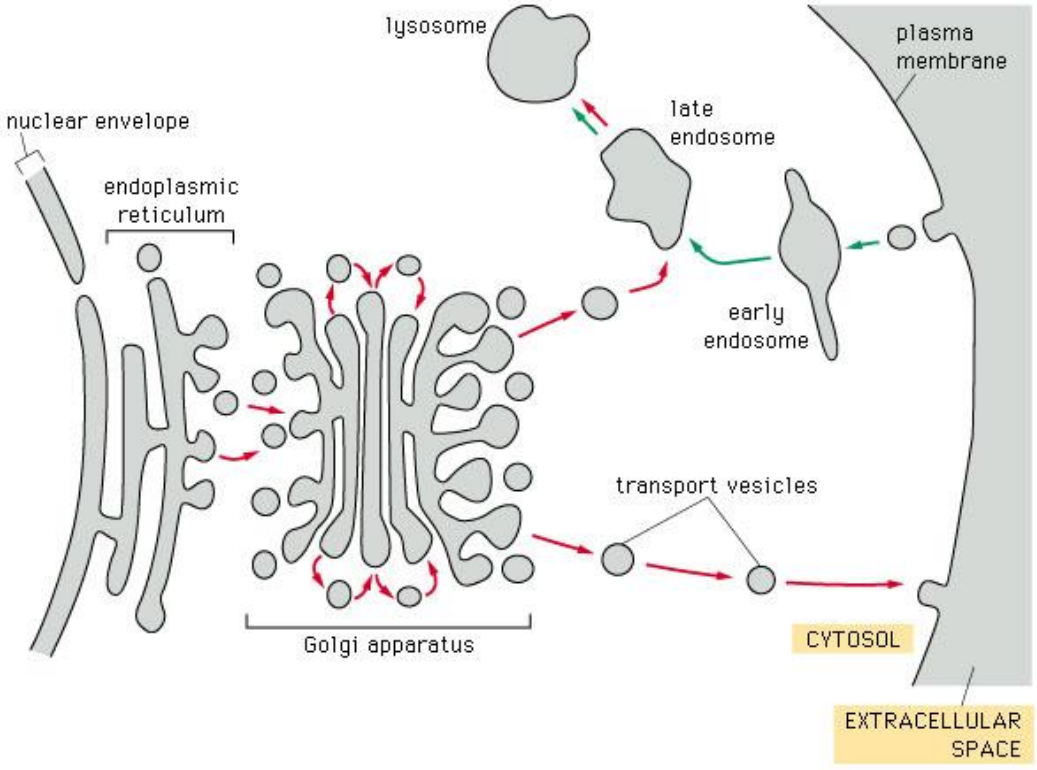
From Tucker et al EMBO,(2002) 21,1427-1436.

Pulse-Chase Autoradiography

An experimental approach to observing protein secretion dynamics

Secretory Pathway

- Proteins are synthesized on the Rough ER.
- Move via vesicles to Golgi
- Move via vesicles to Plasma membrane



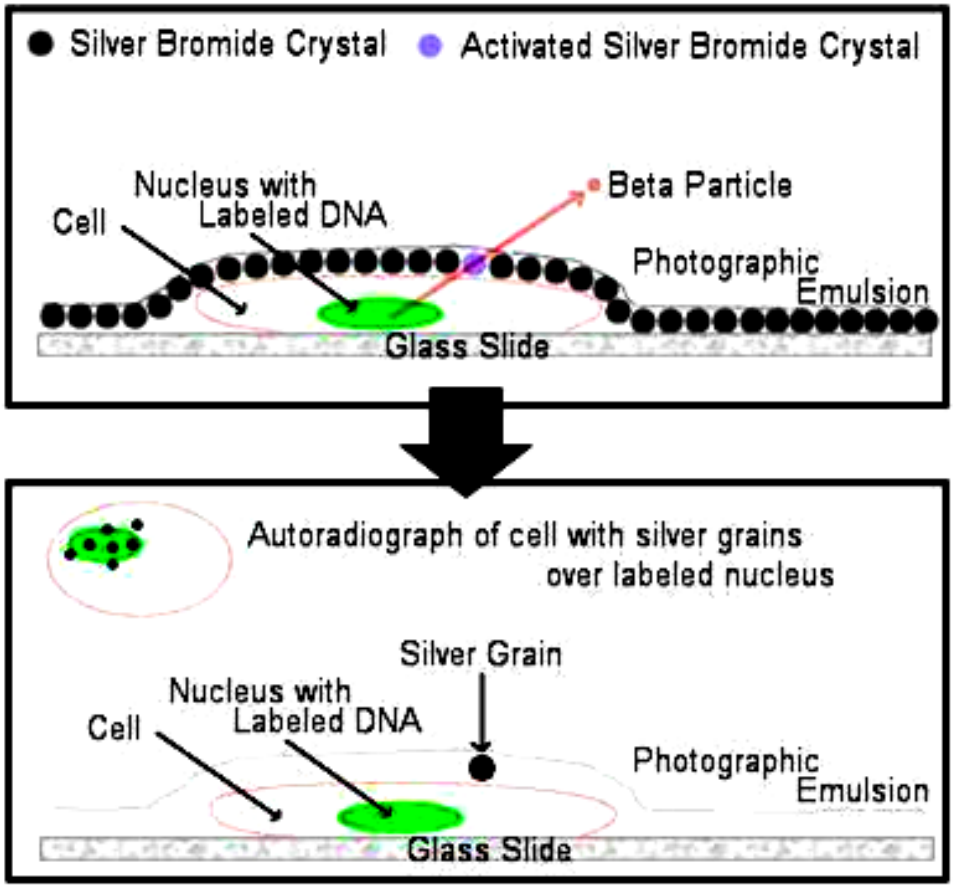
- **Budding** is through coated vesicles (ie. clathrin)

- **Docking** is through the SNAREs

What is Autoradiography?

- Autoradiography is the use of ***radioactively labeled*** molecules to look at cell processes.

- A labeled molecule can be located because its radioactivity develops the silver grains on a photographic emulsion.



“Tailing” a labeled molecule

- Allows us to observe the movement of molecules through the cell over time.
- Cellular pathways are revealed as the progress of molecules is monitored.

The “Pulse” and the “Chase”

- The "Pulse" consists of radioactive material added for a very brief period and then washed away.

- Then the “Chase” begins- non-radioactive molecules are added.

- This creates a group of labeled molecules, with unlabeled molecules in front and behind, that move through a particular metabolic pathway.

Now for a demonstration!

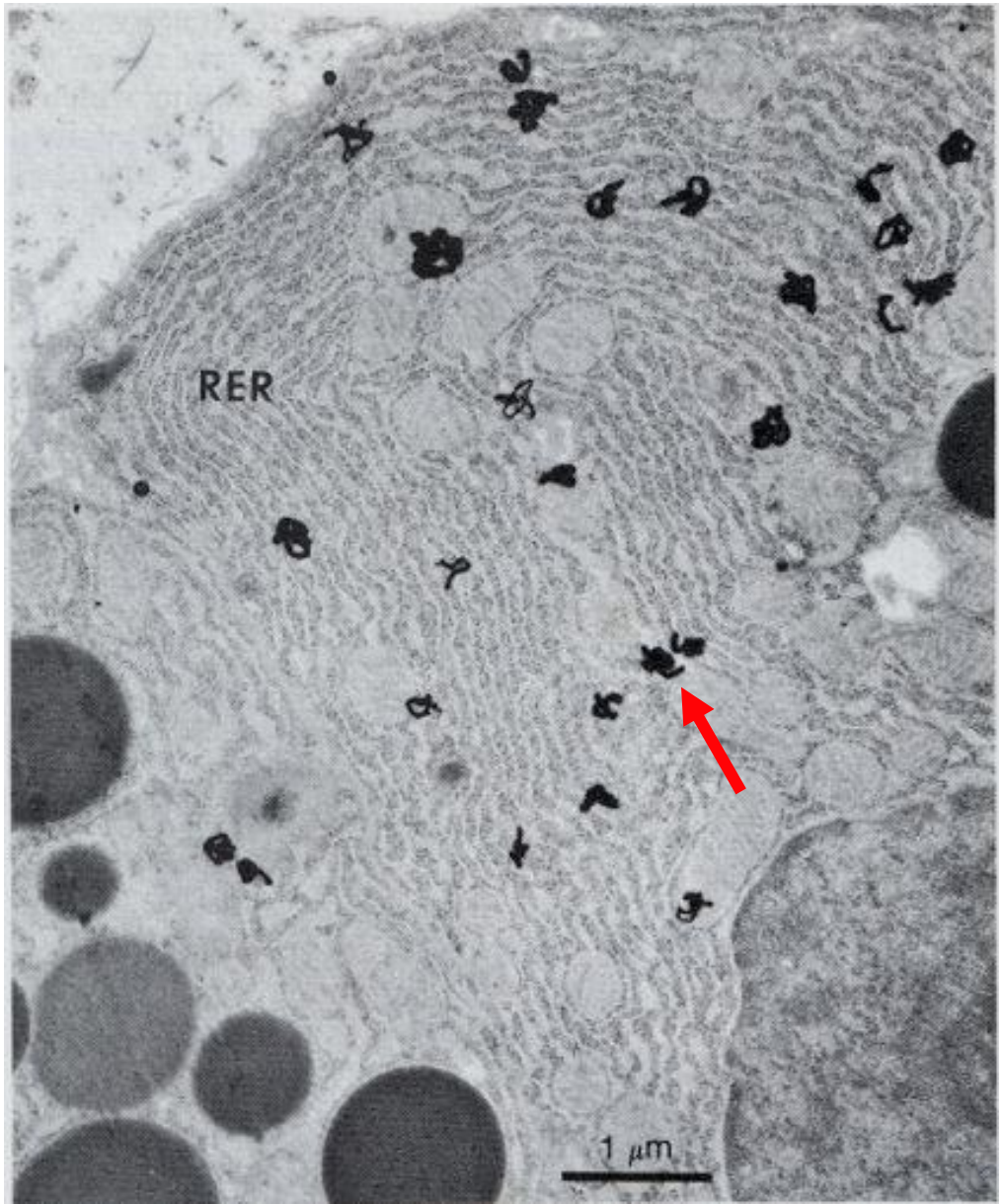
- Stomach cells which secrete digestive enzymes (zymogen) are supplied with radioactively labeled amino acids.

- After a brief period -the “Pulse” - the excess labeled amino acids are washed away.

- The cells are then supplied with unlabeled amino acids -the “Chase”

And in the TEM we see…

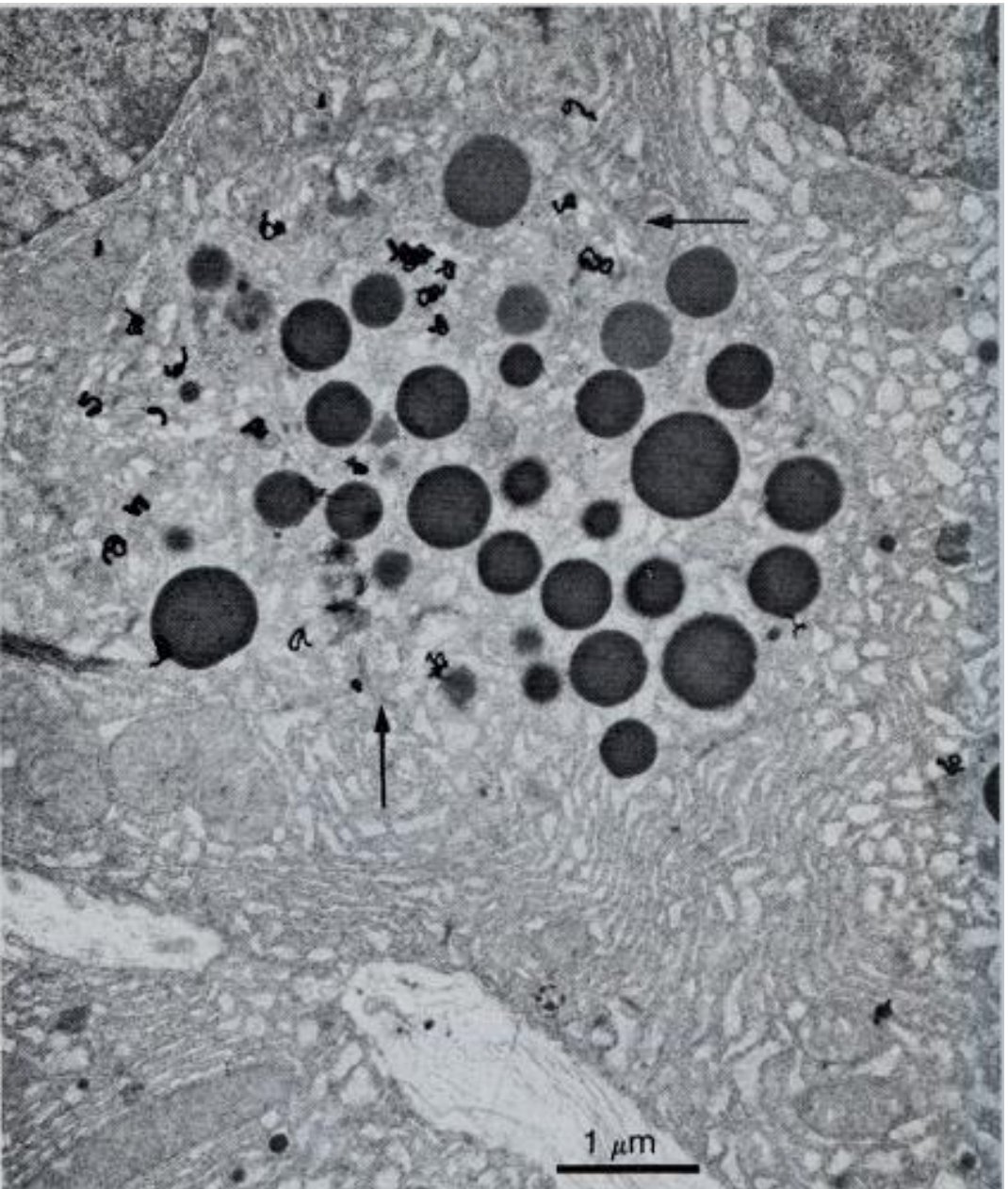
Three minutes after pulse…



Labeled amino acids (incorporated into newly synthesized proteins) are localized around **RER**.

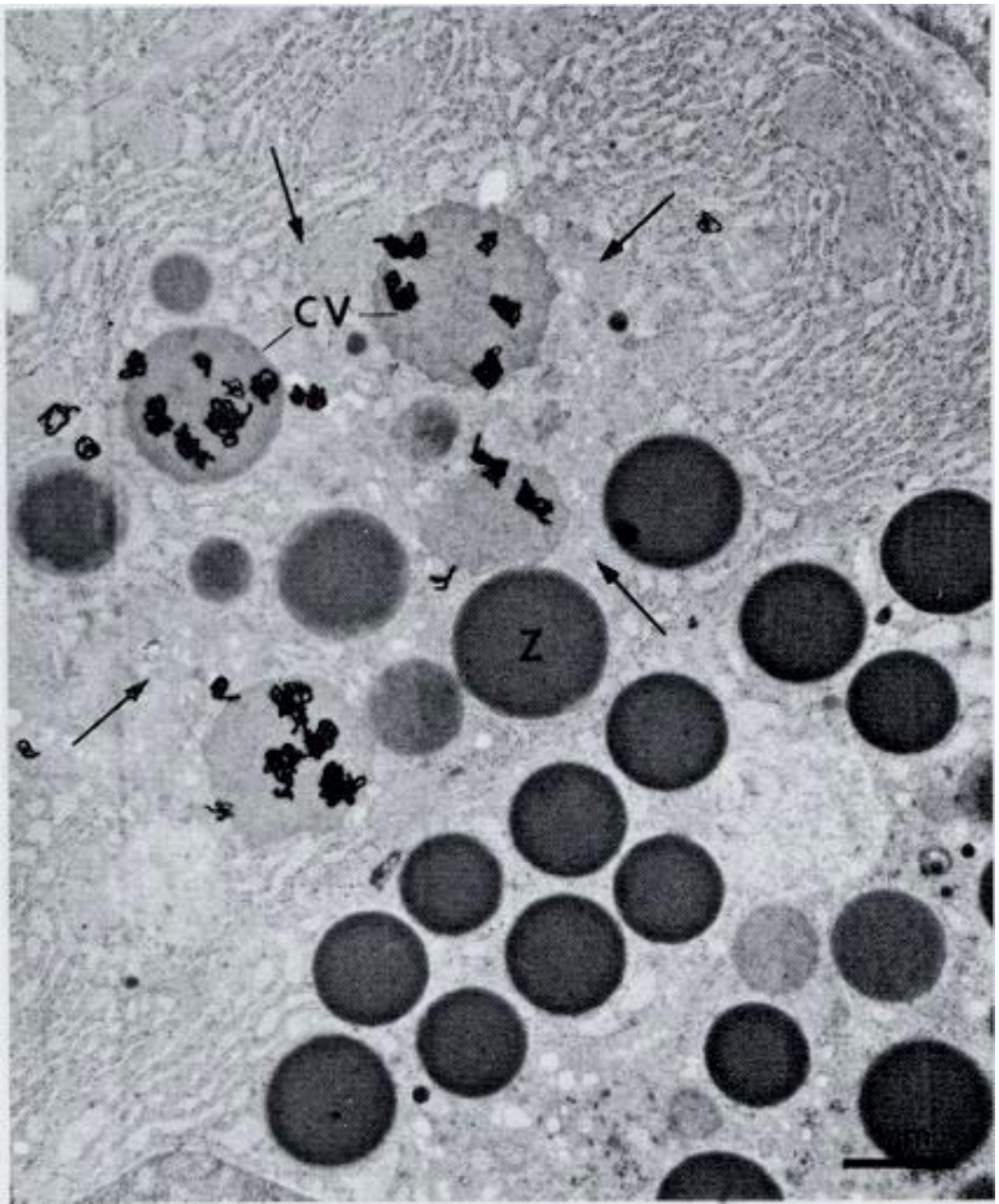
The "squiggles" show radioactivity.

Seven minutes after pulse…



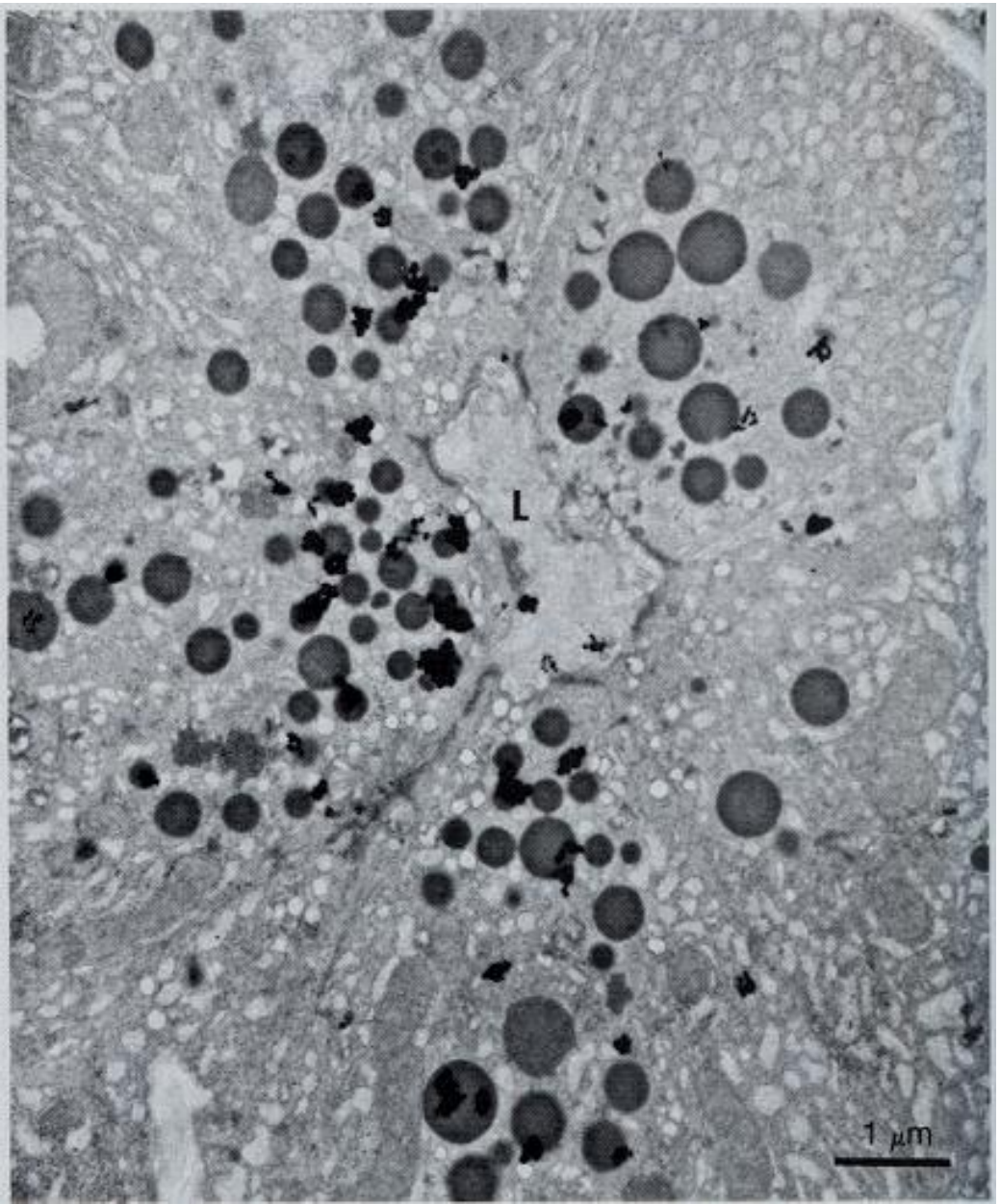
- The majority of the newly synthesized proteins have moved to the periphery of the **Golgi complex**.
- Arrows indicate periphery of Golgi complex.

37 minutes after pulse…



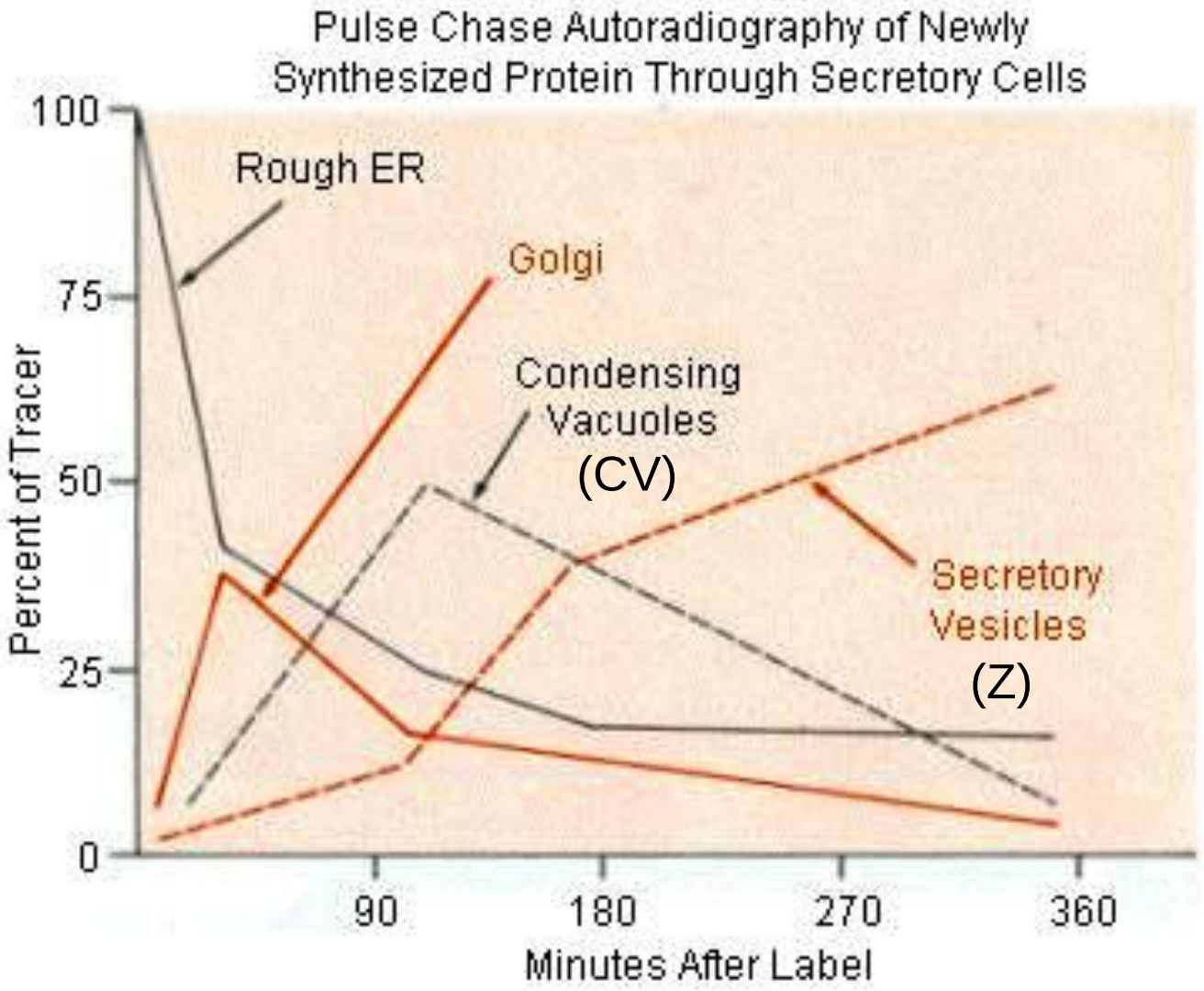
- Labeled proteins are concentrated over **secretory vesicles** called condensing vacuoles (CV).
- Arrows indicate periphery of Golgi complexes.
- Secretory vesicles containing zymogen are marked Z.

117 minutes after pulse…



- Radioactivity is mainly localized over **secretory vesicles** containing zymogen.
- Some labeled protein has already been secreted into the **lumen** (L) .

Where are they now?

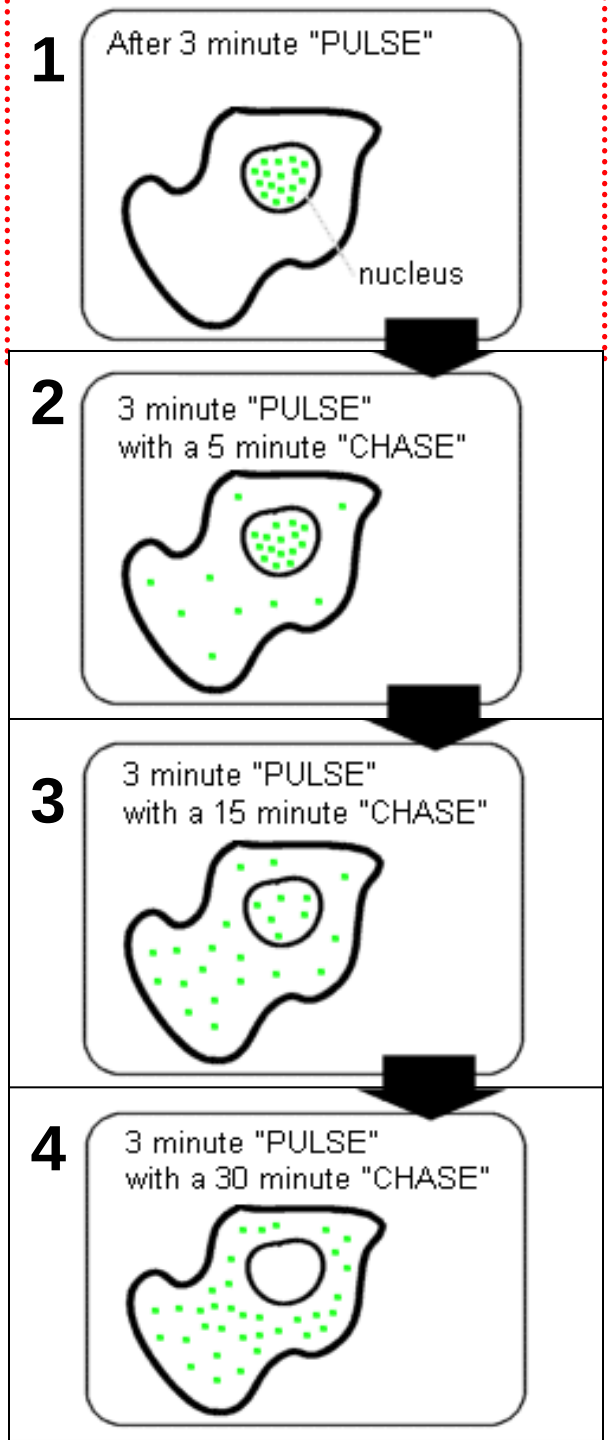


- Samples are taken after various **time** periods and the **location** of the labeled molecules is identified.

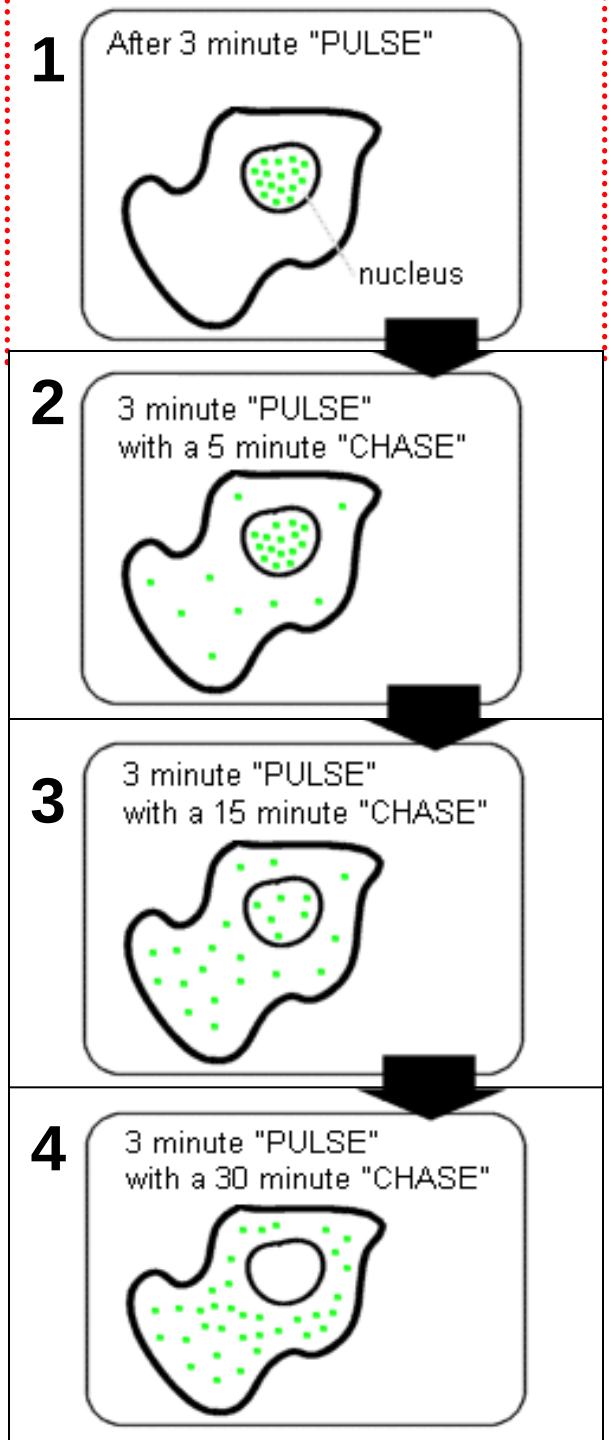
Got it?

Feed the cell a pulse of *radioactively labeled molecules* and see what it does with them.

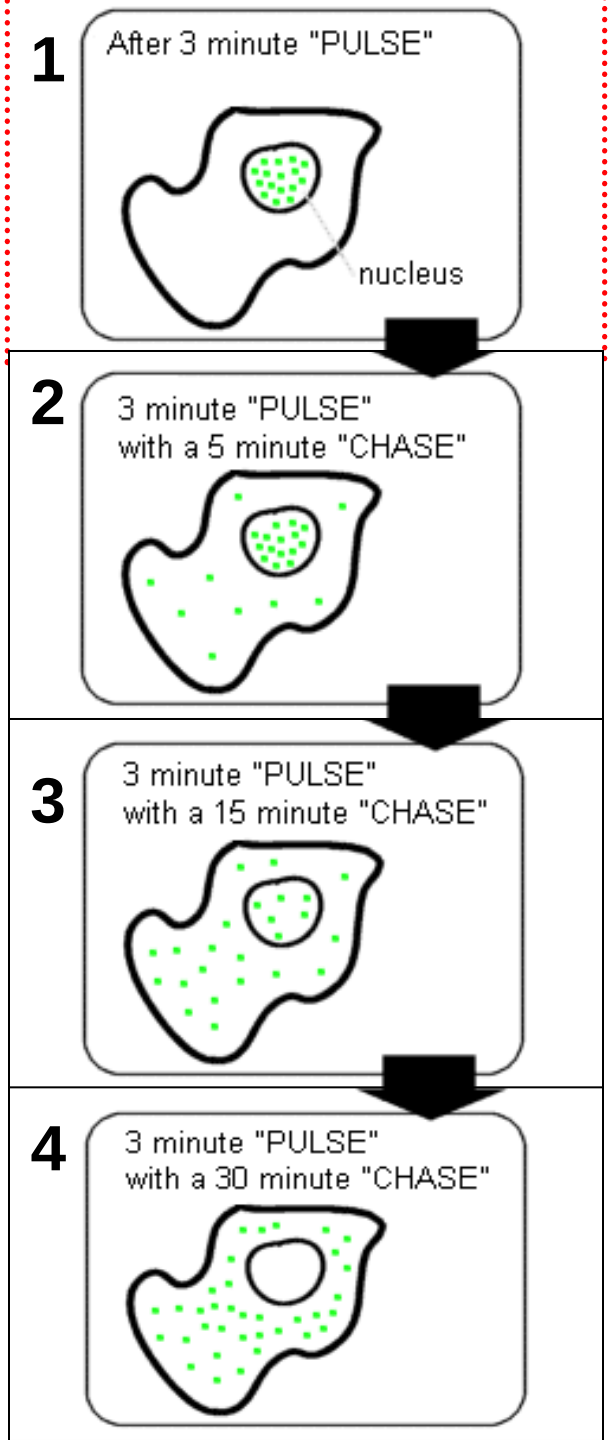
So here are a few questions…



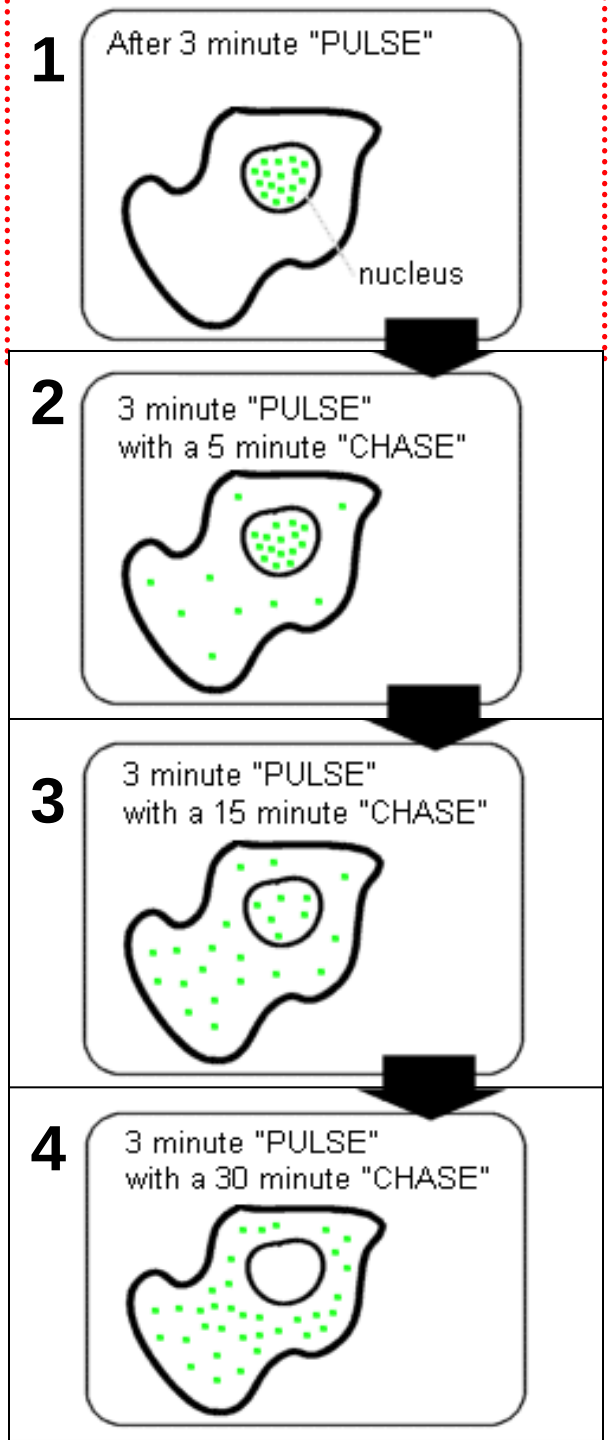
1. **PULSE:** Cell is exposed to radioactively-labelled nucleotides (**green**) for 3 minutes. The nucleotides are taken up by the nucleus.



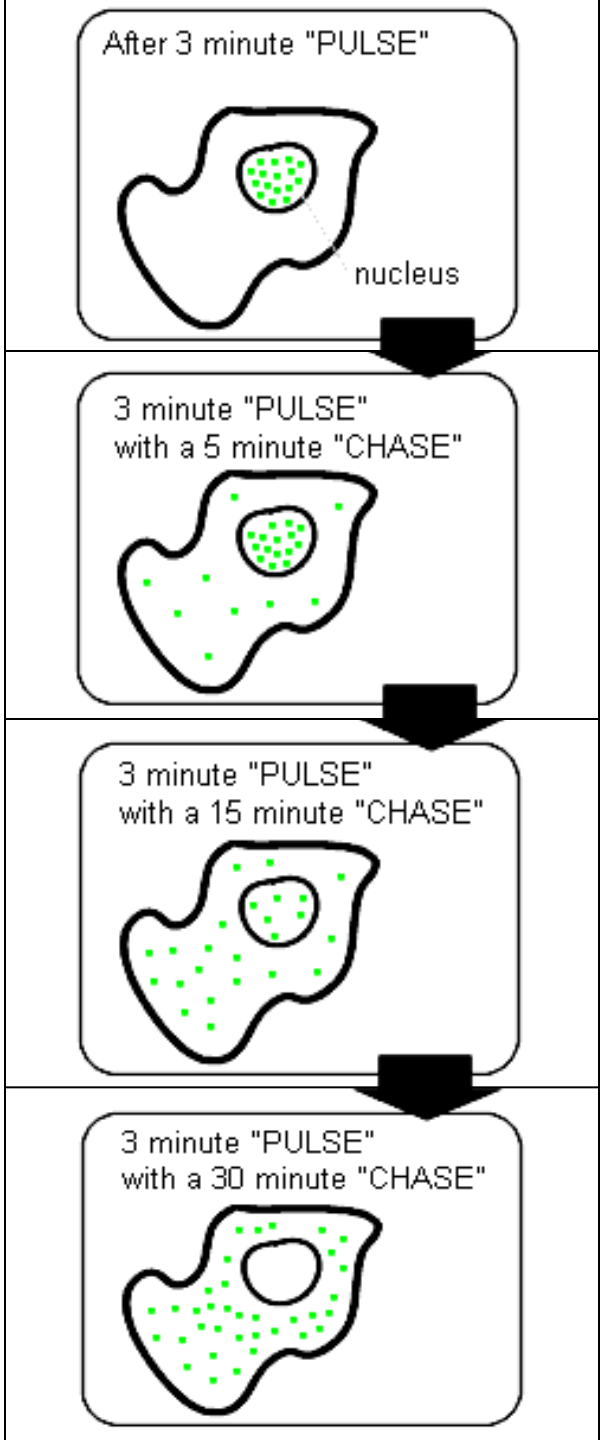
2. <u>CHASE:</u> The cell is exposed to an excess of non-radioactive nucleotides. After five minutes of chase, some radioactive molecules are found in the cytoplasm.



3. <u>15 MINUTES:</u> Most of the radioactivity has moved from the nucleus to the cytoplasm after 15 minutes of the chase.

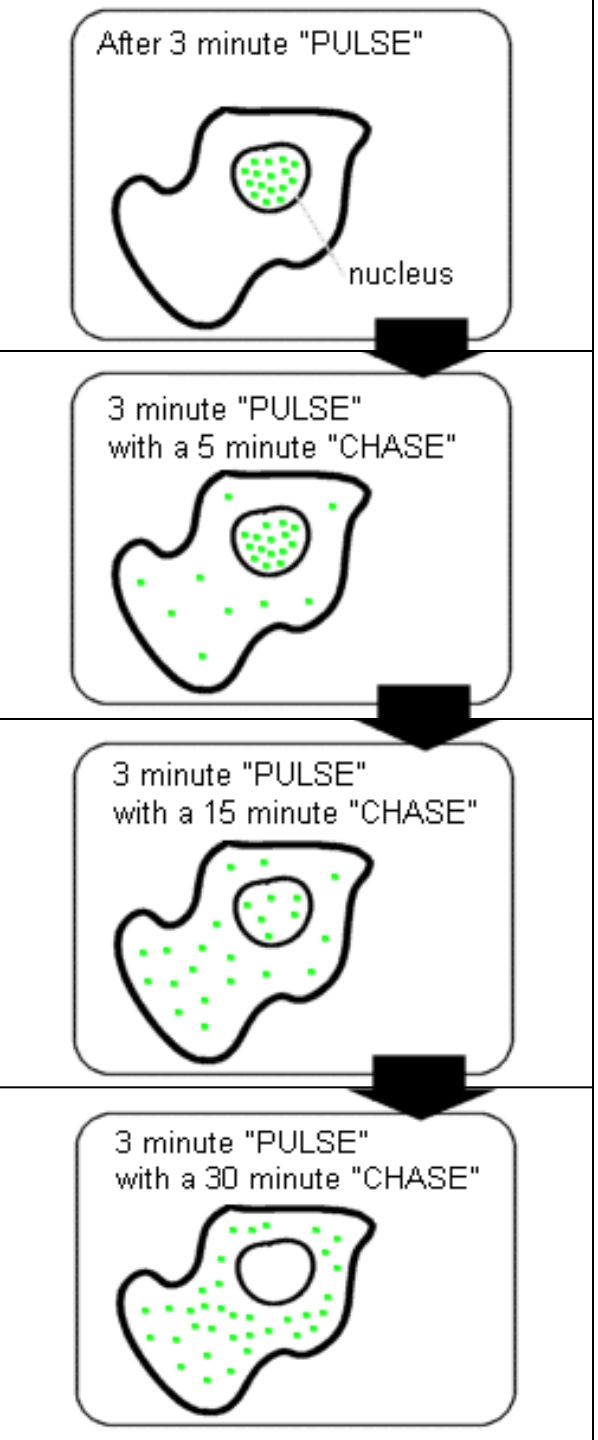


4. <u>30 MINUTES:</u> After 30 minutes, all of the radioactive label is found outside the nucleus.

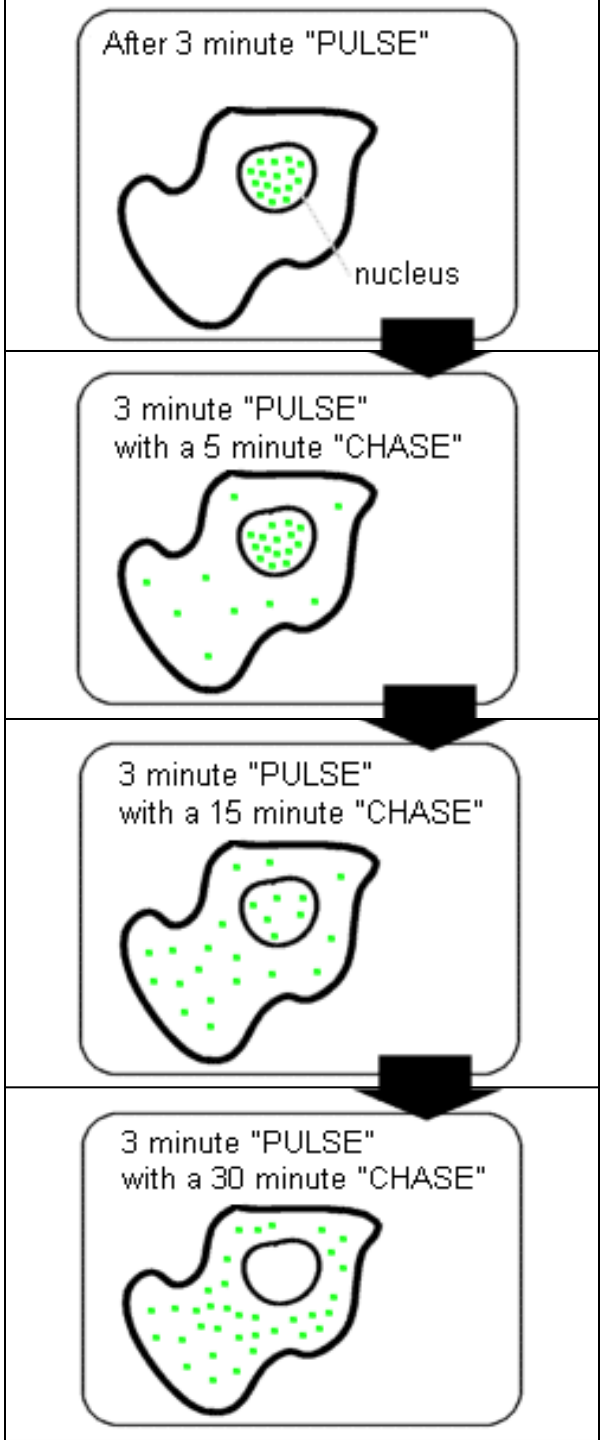


1) What macromolecule is being studied in this experiment? Why?

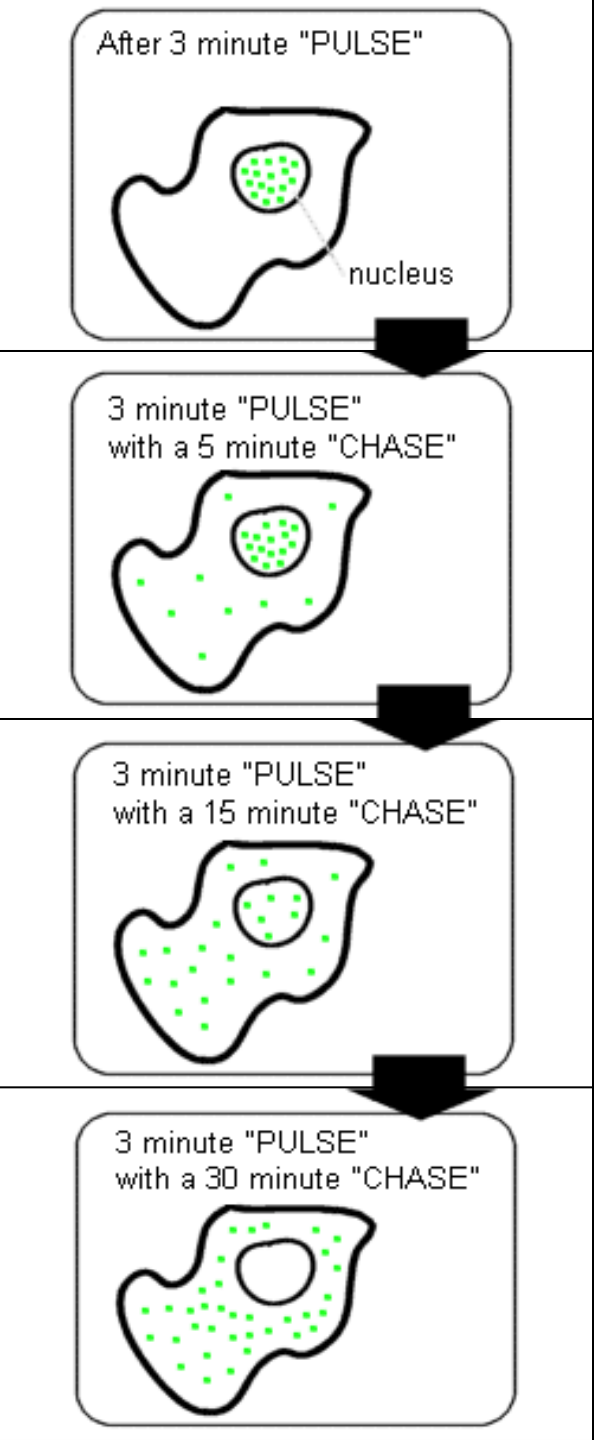
A. Polypeptides
B. Lipids
C. DNA
D. RNA
E. Carbohydrates



A. Nucleus
B. Golgi
C. Rough ER
D. Cytosol
E. Smooth ER



3. After thirty minutes, is the macromolecule still being synthesized?



4) Which is the best explanation for the observations?

A. Synthesis doesn't continue as there is no more labeled nucleotide.

B. Synthesis doesn't continue as the cell is not making any more RNA.

C. You cannot 'see' that synthesis is continuing as the nucleotides being incorporated are not labeled and hence can not be visualized by autoradiography.

Other examples of Pulse-chase studies you can share with us?

Student research proposal outlines (1/2)

1. **Title of the proposal**
    1. informative and precise
2. **Name/School year/Faculty Advisor etc**
3. **Abstract**
    1. 200-400 words, or less
    2. capture the attention of professors, colleagues, reviewers, using layman's language
4. **Purpose/Rationale**
    1. Brief introduction of background to bring up the questions you'd like to study, why it is important,
    2. State your hypothesis, the specific aims you will work on in order to proof your hypothesis.
    3. The impact of your research

Student research proposal outlines (2/2)

5. **Background, literature review**
    1. Overview of the literature closely related to the subject, shown that you are an expert and have done your homework
6. **Research plan**
    1. The material and methods session for each aim, what cell, gene, drug, techniques, how will you do it etc
    2. The data analysis session for your each aim, including the expected results, the method to analysis the data, and possible interpretations.
7. **References**

Using pictures or cartoons to illustrate your point. A picture is better than a thousand words.