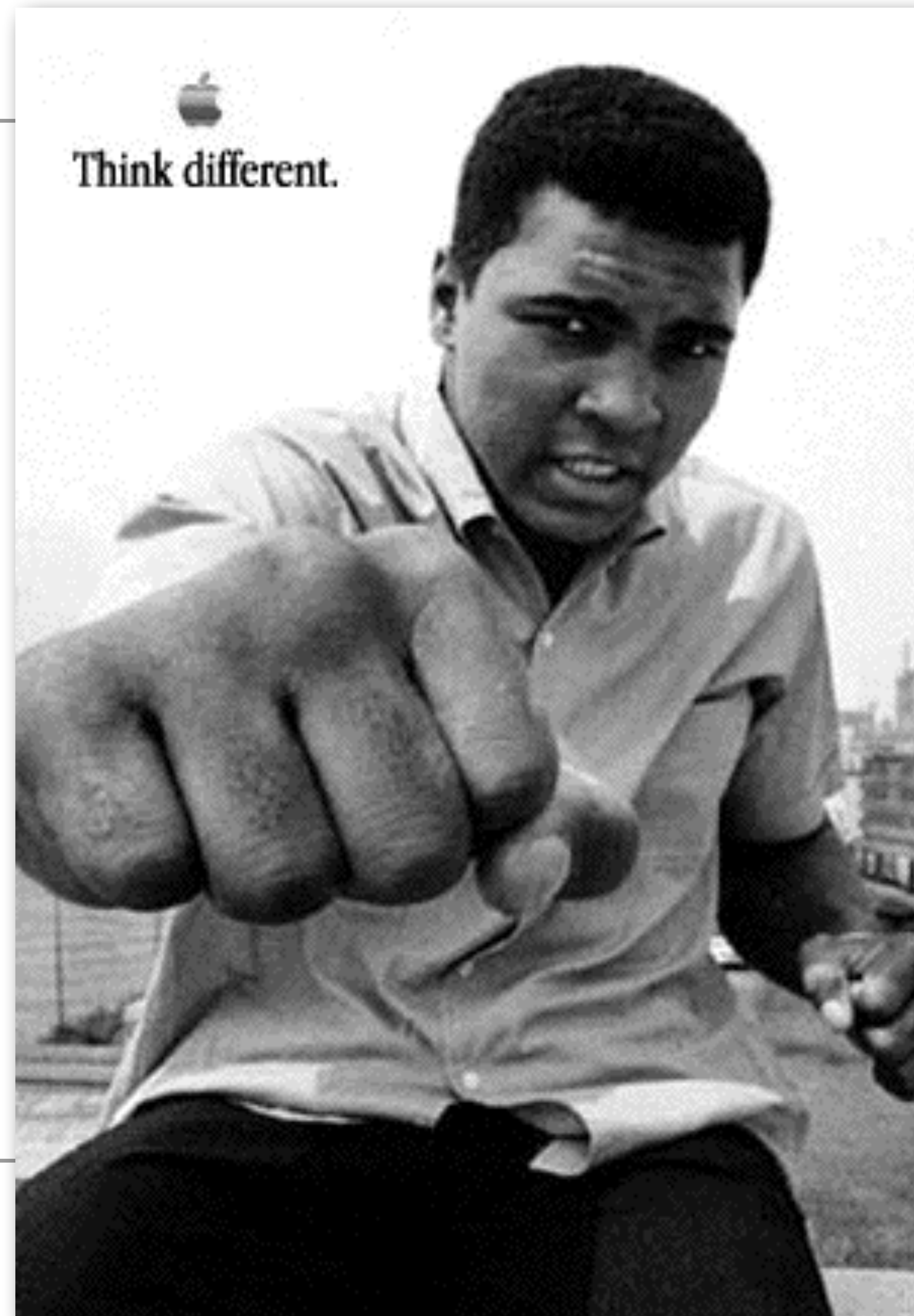
LB145-Spring *2025*



1. **Pick up** Name Folder
- Pick up name folder and set it up at seat.

2. **Sit** with your group.
- laptops on outer perimeter (avoid distracting)

3. **Clicker** Attendance
- Launch your Top Hat, and get ready to click.

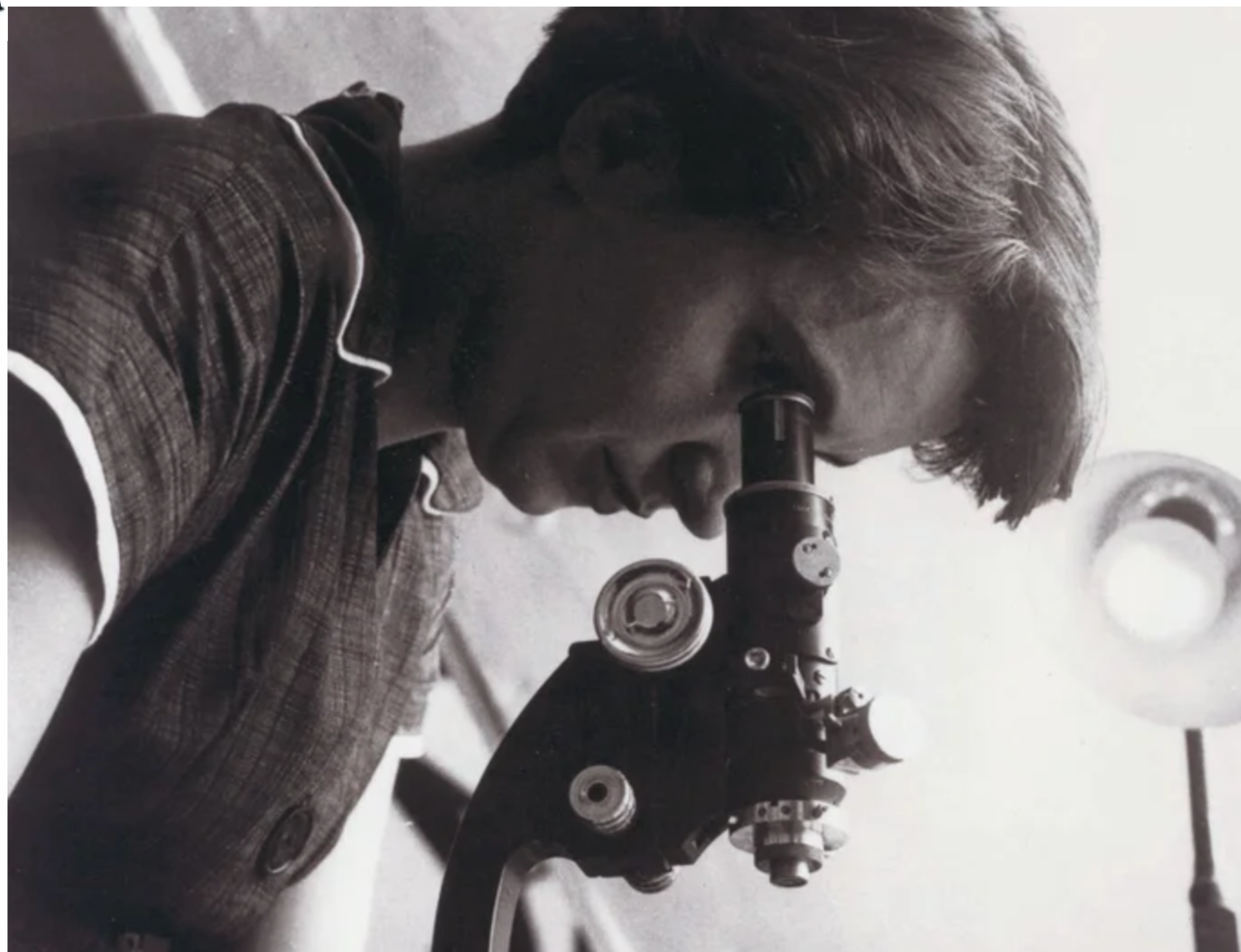
Announcements

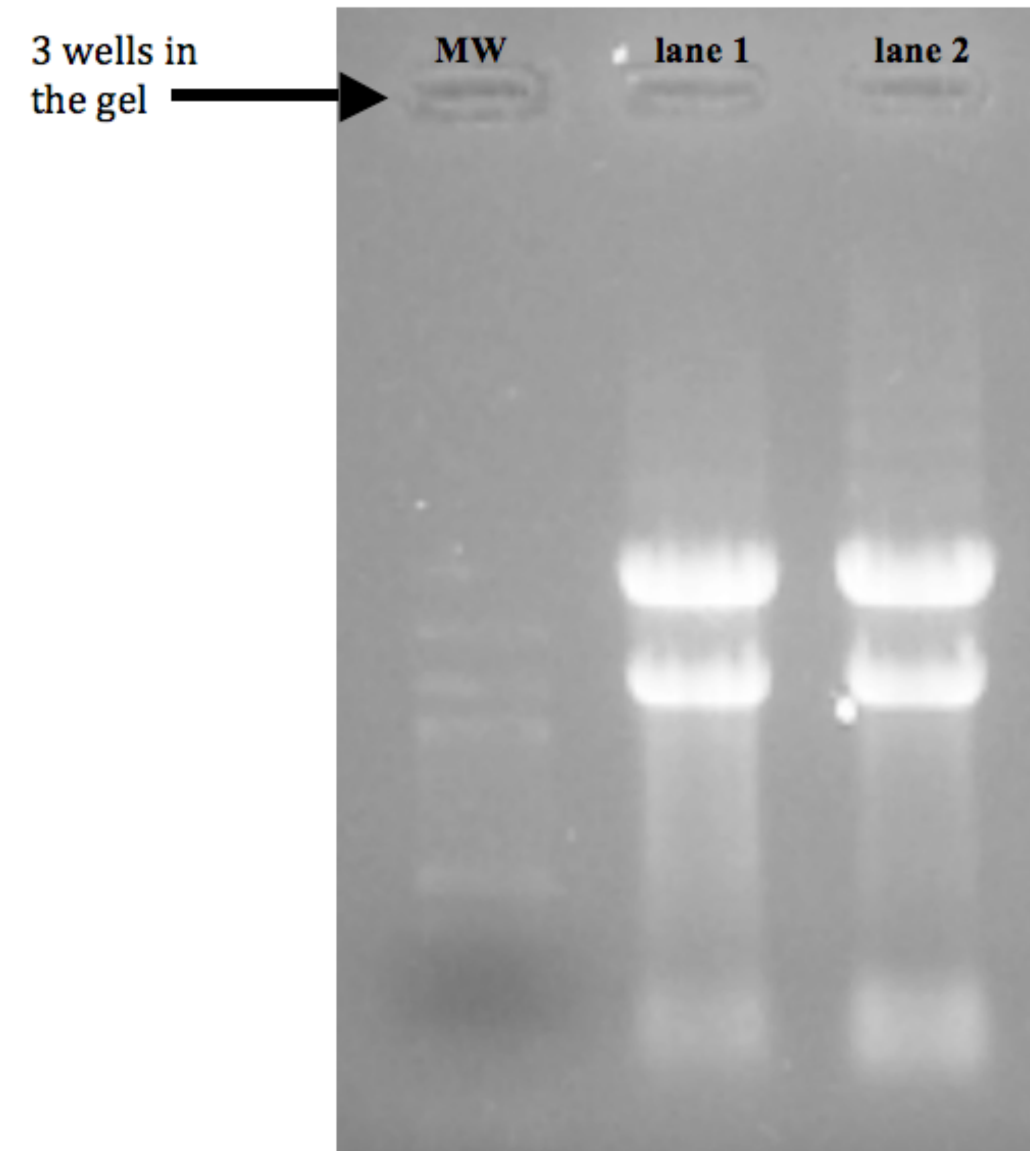
1. **On Monday 20min & two questions will be provided for feedback on course evaluation.**

We will provide this time to make it easier for all students to participate. More feedback is better!

As a result of reading midsemester feedback from students, we are curious about each student's experience in the course and want your ideas and answers to two questions.

Team#1- Trinity, Jay, Ethan, Kierin

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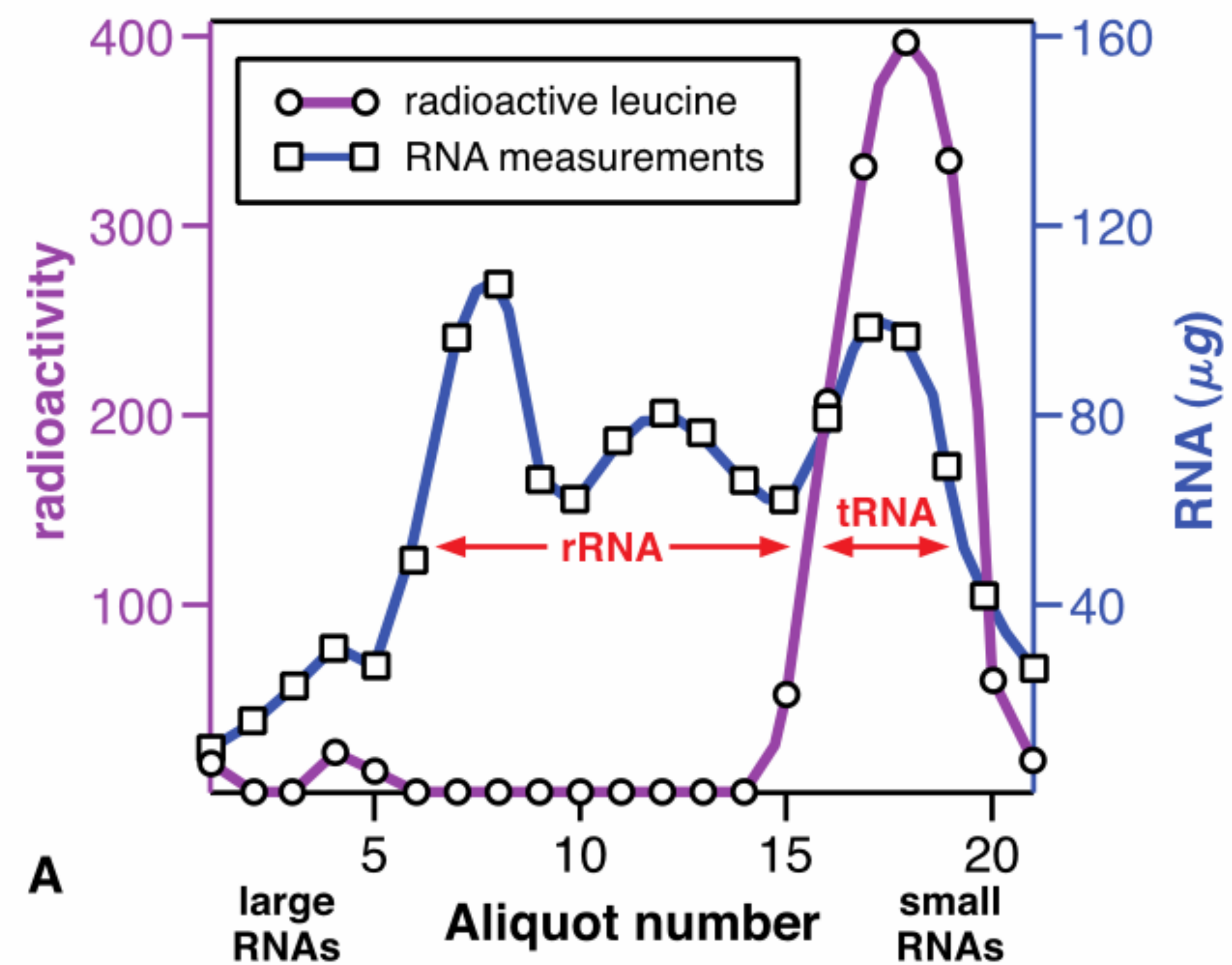
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RNA measurements
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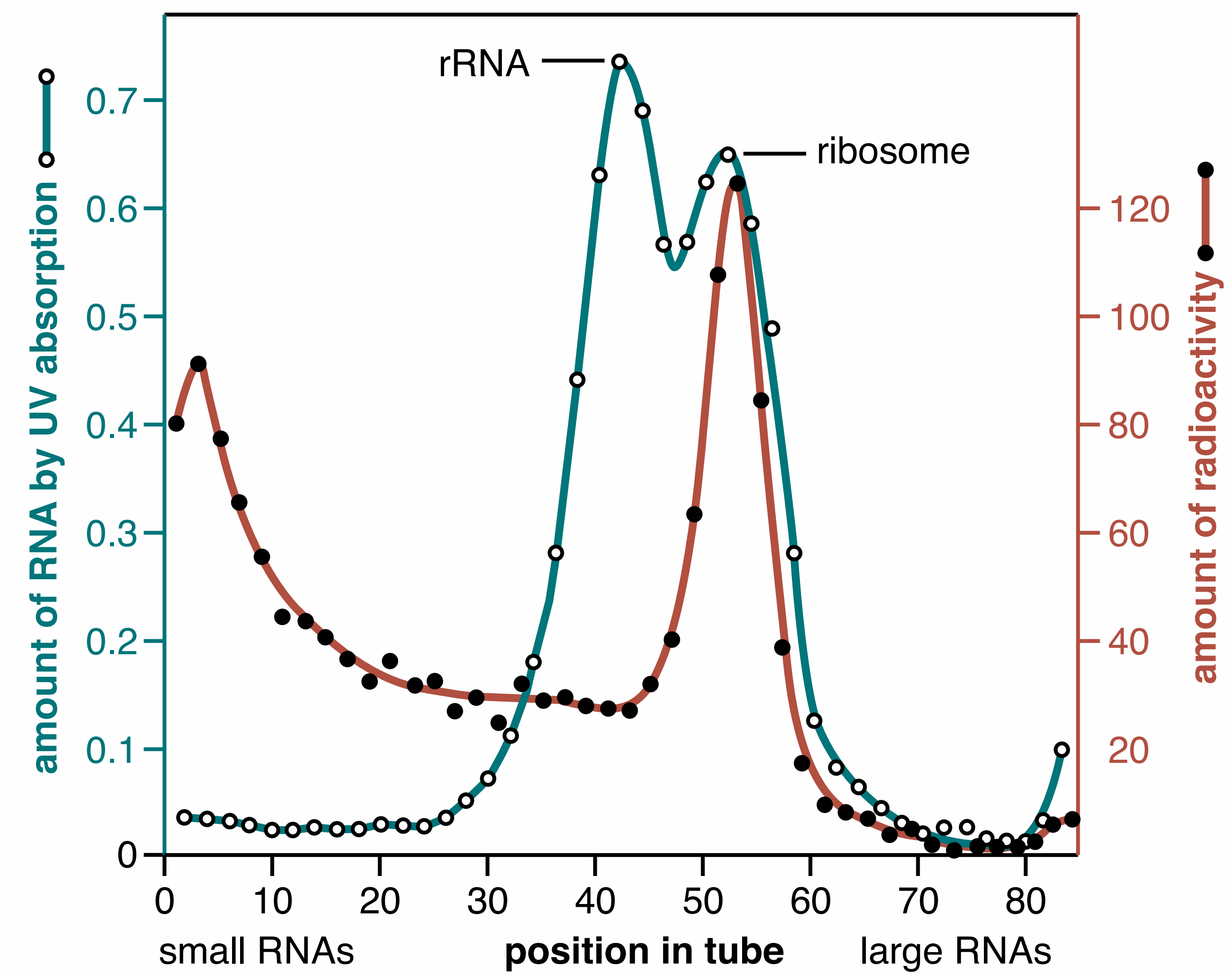
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amount of RNA by UV absorption
amount of radioactivity
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position in tube
large RNAs

3 wells in the gel
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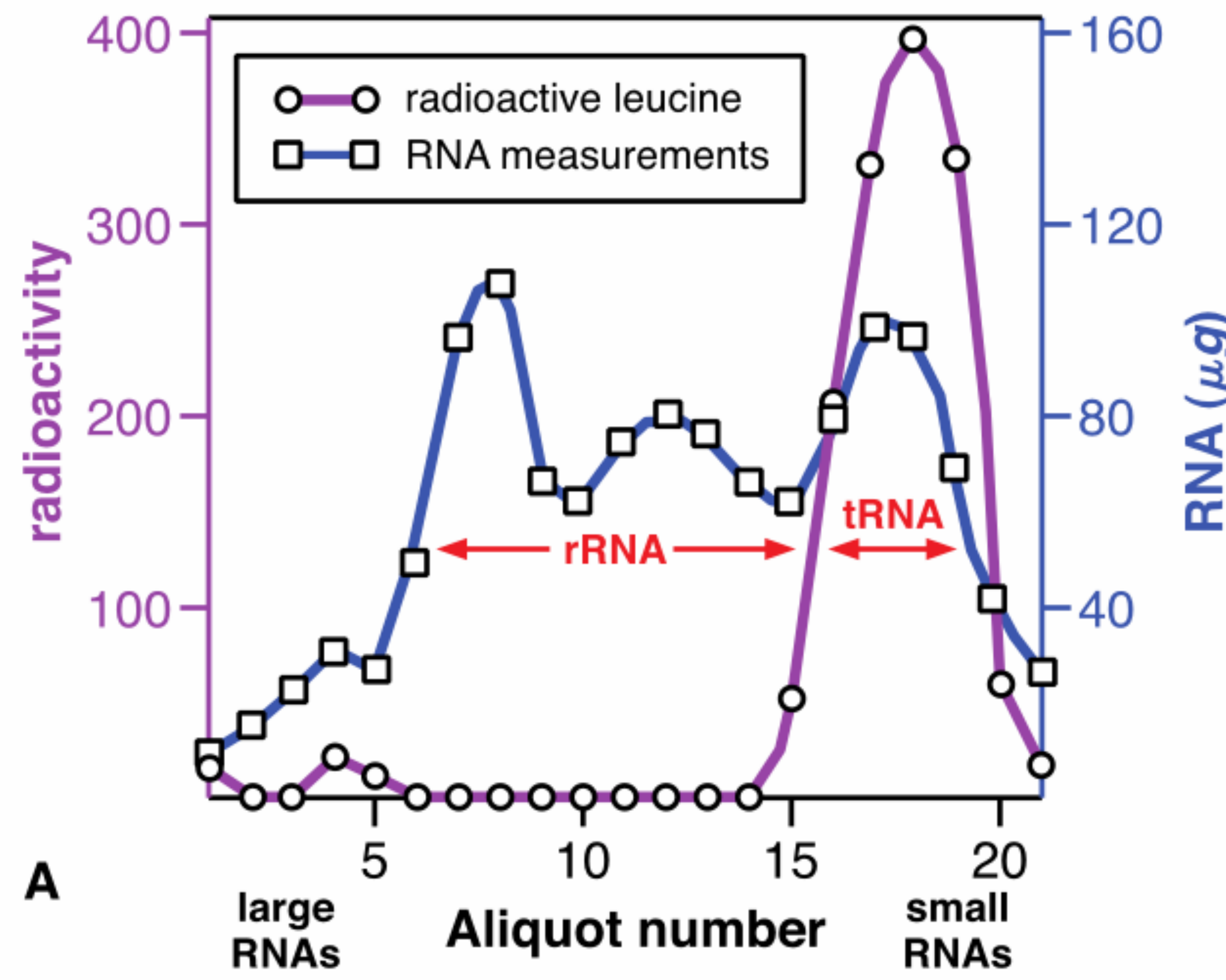
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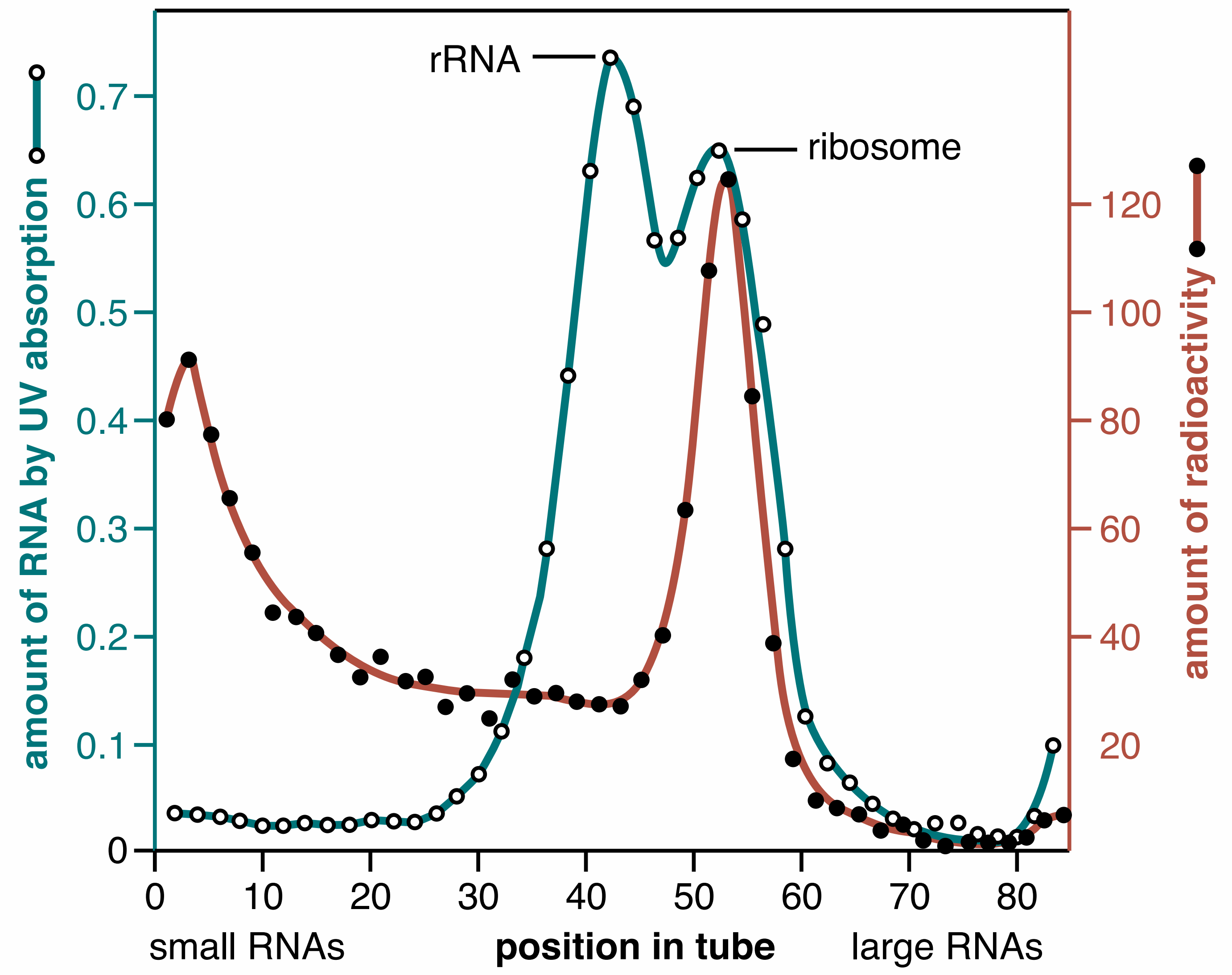
rRNA
ribosome
amount of RNA by UV absorption
amount of radioactivity
small RNAs
position in tube
large RNAs

*SYNTHESIS OF TRANSFER RNA BY ISOLATED NUCLEI**

By Margaret I. H. Chipchase and Max L. Birnstiel

Division of Biology, California Institute of Technology

Communicated by James Bonner, March 15, 1963

In the course of earlier experiments on the incorporation of labeled nucleosides into RNA by isolated nuclei[1] we observed that much of the newly synthesized RNA is soluble in 1 M NaCl, as is transfer RNA.[2–4] Sirlin has reported the incorporation of pseudo-uridine into nuclei, allegedly into transfer RNA,[5, 6] and the presence of amino-acyl RNA in thymus nuclei has been shown by Hopkins.[7] Since the pea nuclei with which we work are capable of protein synthesis,[8] they might therefore be suspected of containing transfer RNA. It will be shown below that isolated pea nuclei not only contain, but possess the ability to synthesize, transfer RNA.

Materials and Methods.—Analytical reagent grade chemicals were used throughout. ATP, CTP, GTP, UTP, UMP, uridine, phosphocreatine, and crystalline DNAse were obtained from Sigma. Creatine phosphokinase was obtained from the California Corp. for Biochemical Research. Sodium penicillin-G was a gift of Chas. Pfizer and Co., New York. 2-hydroxy-3-naphthoic

phosphate system.[13] Alternatively, an ammonium sulfate fractionation was used. Nine volumes of ice-cold 2.5 M ammonium sulfate, pH 5, were added to the aqueous solution to yield a final concentration of 2.25 M.[14] This solution was kept at 0°C for 10 min and the fine precipitate then centrifuged down at 35,000 × g for 15 min. This procedure precipitates approximately 90% of the dye-bound RNA[14] leaving the dye-non-bound amino-acyl RNA in solution. The precipitate of dye-bound RNA was washed once with 5% TCA, and then twice with 70% ethanol +0.5% sodium acetate pH 5 and once with absolute ethanol. The dye-non-bound RNA was precipitated from the dialyzed sulfate solution by the addition of TCA to give a final concentration of 5%, the mixture kept at 0°C for 10 min, and the precipitate sedimented at 35,000 × g in the Servall for 15 min. The precipitate was washed as above. Each precipitate was dissolved in distilled water, 560 mμ and 260 mμ absorptions determined, and an aliquot counted.

Experimental Results.—That nuclei contain an active transfer RNA is shown by the following experiment: isolated pea stem nuclei were incubated and extracted as described in the legend of Figure 1. The data of Figure 1 show that labeled

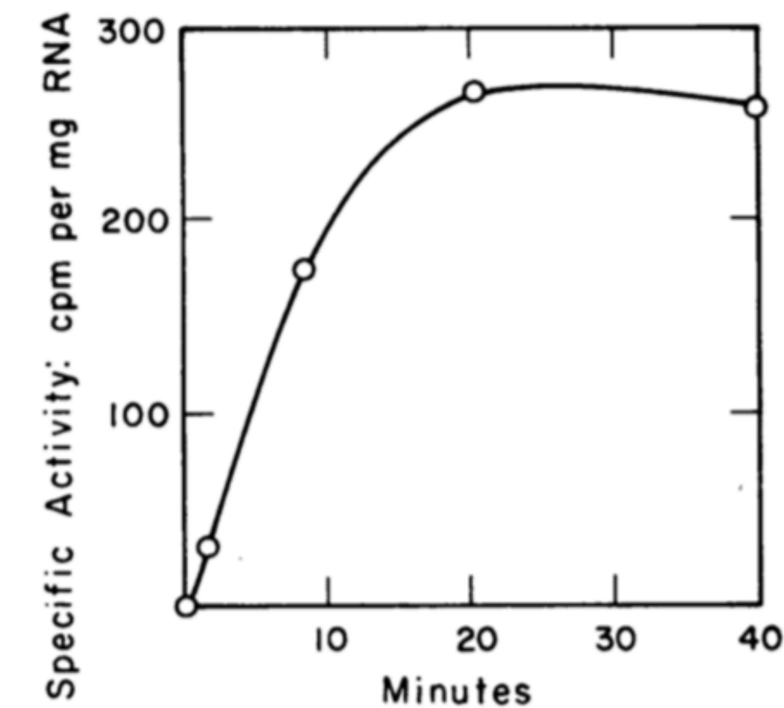

Fig. 1.—Formation of amino-acyl RNA in isolated nuclei. Incubation mixture: ATP, CTP, GTP, and UTP, .0001 M each; tris .02 M; phosphocreatine .02 M; creatine phosphokinase 100 μg/ml; $CaCl_2$ .003 M; $MgCl_2$ .0001 M; C^{14}-protein hydrolysate 2 μc/ml; final pH 7.0, incubation at 37°C. Aliquots were precipitated at intervals by addition of an equal volume of phenol. RNA was extracted[9] and washed as described in Table 1.

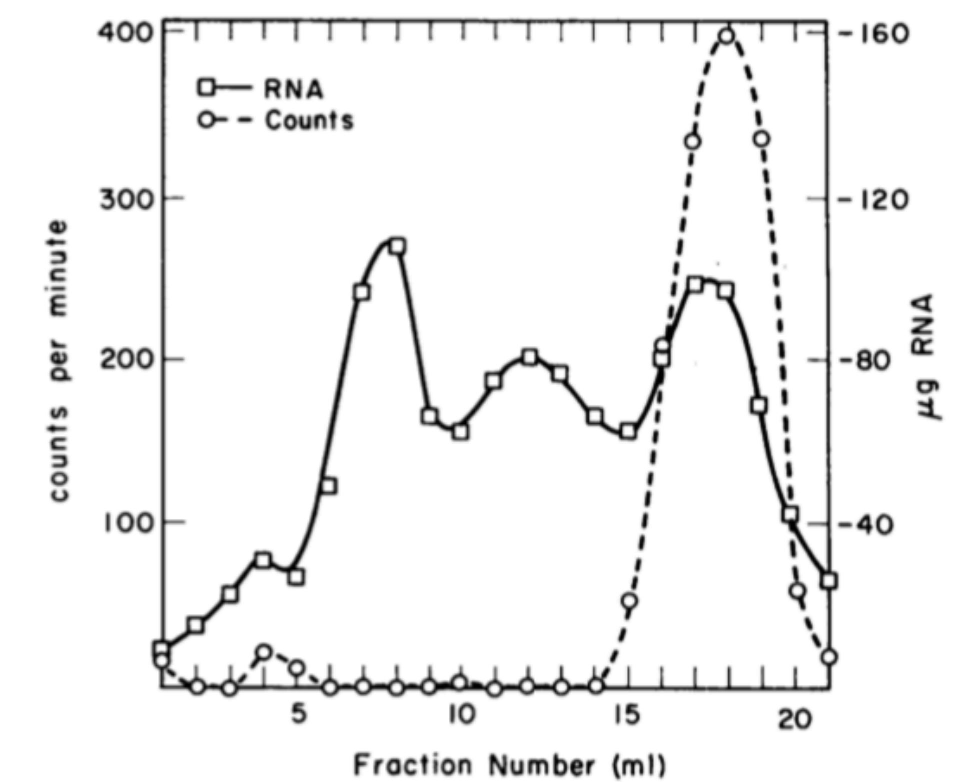

Fig. 2.—Size distribution of nuclear RNA and localization of transfer RNA on a sucrose density gradient. Sucrose density gradient (20 ml) of 20–5% sucrose in .01 M tris (pH 7.1) and 1% phenol was set up according to Bolton *et al.*[17] and overlayered with an inverse gradient of approximately 1 mg RNA in a sucrose gradient of 4–0%. A covering layer of paraffin oil prevented tubes from collapsing and rendered gradients more stable for handling. The tubes were centrifuged 12–14 hr in the Spinco rotor No. 25 at 24,000 rpm, then pierced and 1 ml fractions collected. Each fraction was precipitated with ethanol/acetate (66/2%), thoroughly washed to remove the phenol, and then tested for C^{14}-leucine incorporation as described in Table 1.

amino acids are incorporated into nuclear amino-acyl RNA.

That nuclear RNA can also bind amino acids *in vitro* is shown by the results of

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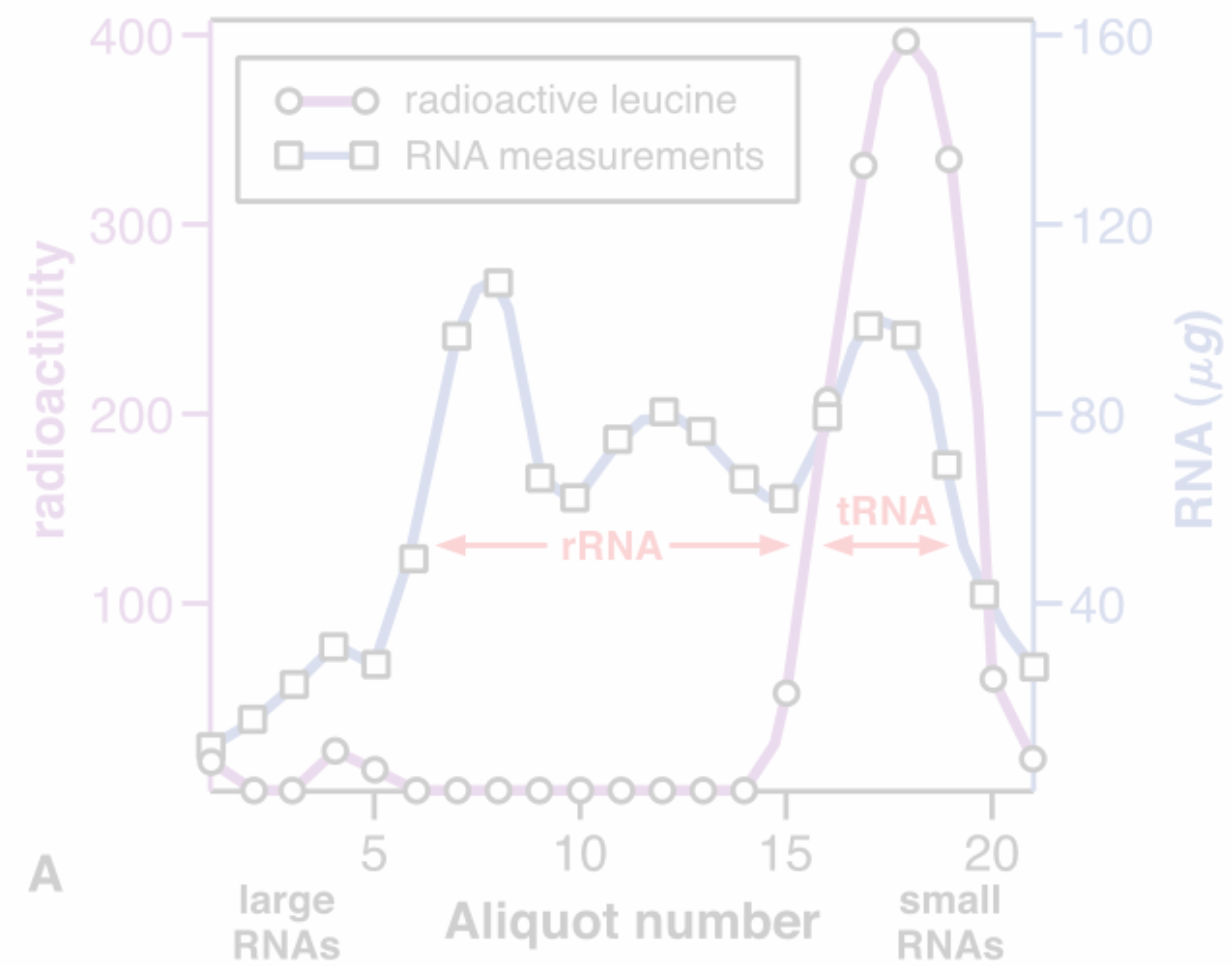
radioactive leucine
RNA measurements
radioactivity
RNA (µg)
rRNA
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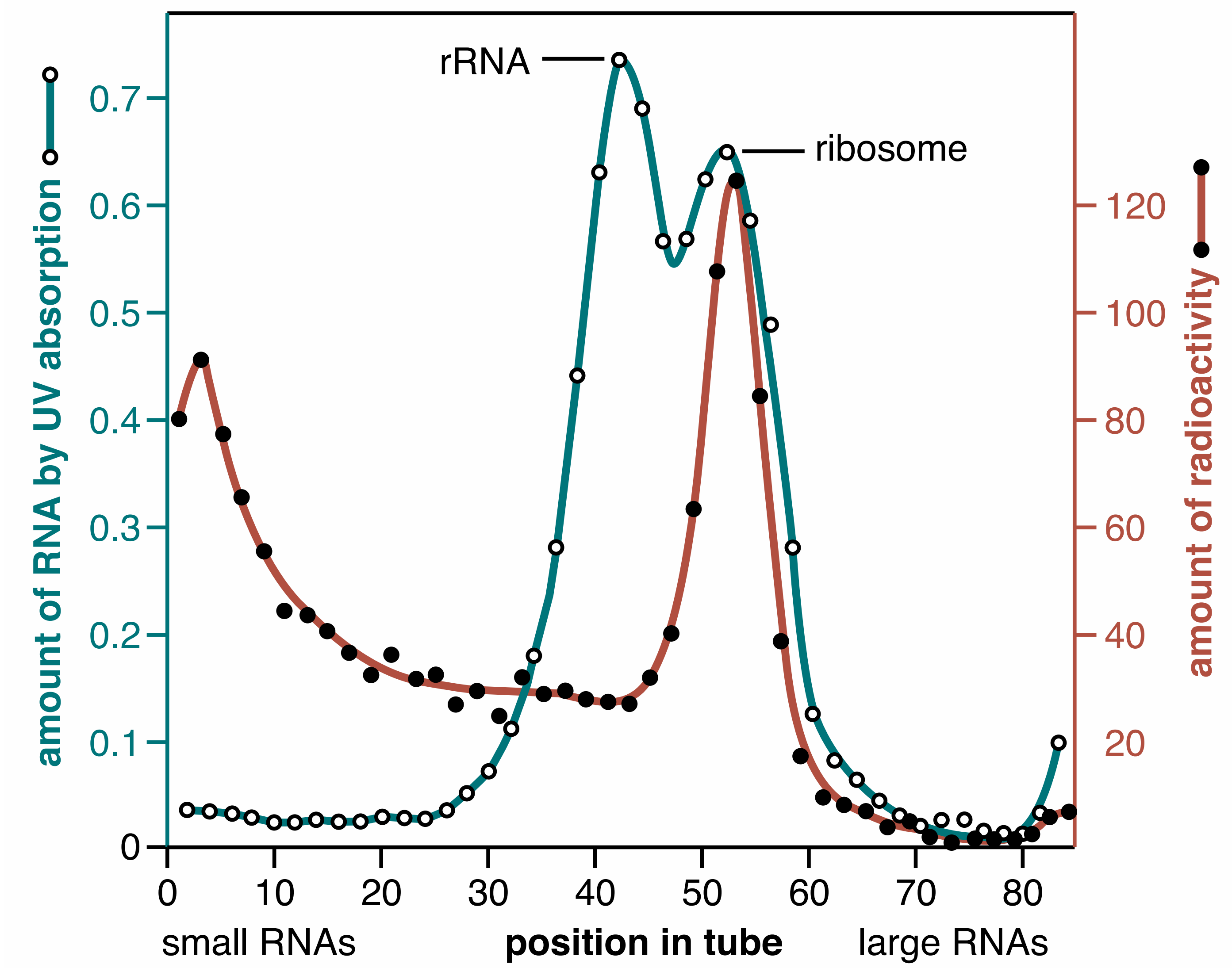
A
large RNAs
Aliquot number
small RNAs


rRNA
ribosome
amount of RNA by UV absorption
amount of radioactivity
small RNAs
position in tube
large RNAs

AN UNSTABLE INTERMEDIATE CARRYING INFORMATION FROM GENES TO RIBOSOMES FOR PROTEIN SYNTHESIS

By Dr. S. BRENNER

Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, University of Cambridge

Dr. F. JACOB

Institut Pasteur, Paris

AND

Dr. M. MESELSON

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California

A LARGE amount of evidence suggests that genetic information for protein structure is encoded in deoxyribonucleic acid (DNA) while the actual assembling of amino-acids into proteins occurs in cytoplasmic ribonucleoprotein particles called ribosomes. The fact that proteins are not synthesized directly on genes demands the existence of an intermediate information carrier. This intermediate template is generally assumed to be a stable ribonucleic acid (RNA) and more specifically the RNA of the ribosomes. According to the present view, each gene controls the synthesis of one kind of specialized ribosome, which in turn directs the synthesis of the corresponding protein—a scheme which could be epitomized as the one gene–one ribosome–one protein hypothesis. In the past few years, however, this model has encountered some difficulties: (1) The remarkable homogeneity in

RNA is not the intermediate carrier of information from gene to protein, but rather that ribosomes are non-specialized structures which receive genetic information from the gene in the form of an unstable intermediate or 'messenger'. We present here the results of experiments on phage-infected bacteria which give direct support to this hypothesis.

When growing bacteria are infected with a virulent bacteriophage such as $T2$, synthesis of DNA stops immediately, to resume 7 min. later[6], while protein synthesis continues at a constant rate[7]. After infection many bacterial enzymes are no longer produced[8]; in all likelihood, the new protein is genetically determined by the phage. A large number of new enzymatic activities appears in the infected cell during the first few minutes following infection[9], and from the tenth minute onwards

The third type of RNA is the most difficult to see for two reasons. First, this RNA is the least abundant—only about 2% of the total RNA. Second, this category of RNA is produced in many different sizes. You are familiar with the phenomenon of not seeing dispersed objects in your daily life. If 100 hairs from your head fall to the ground and are scattered about, they are difficult to see. But if you piled all the hairs together, they'd be more obvious because of their collective mass. The third type of RNA causes a faint white smear that extends from above the top rRNA band down to the smallest RNA molecules. The smear indicates this type of RNA comes in a wide range of sizes, and none are noticeably more abundant than any others in the smear.

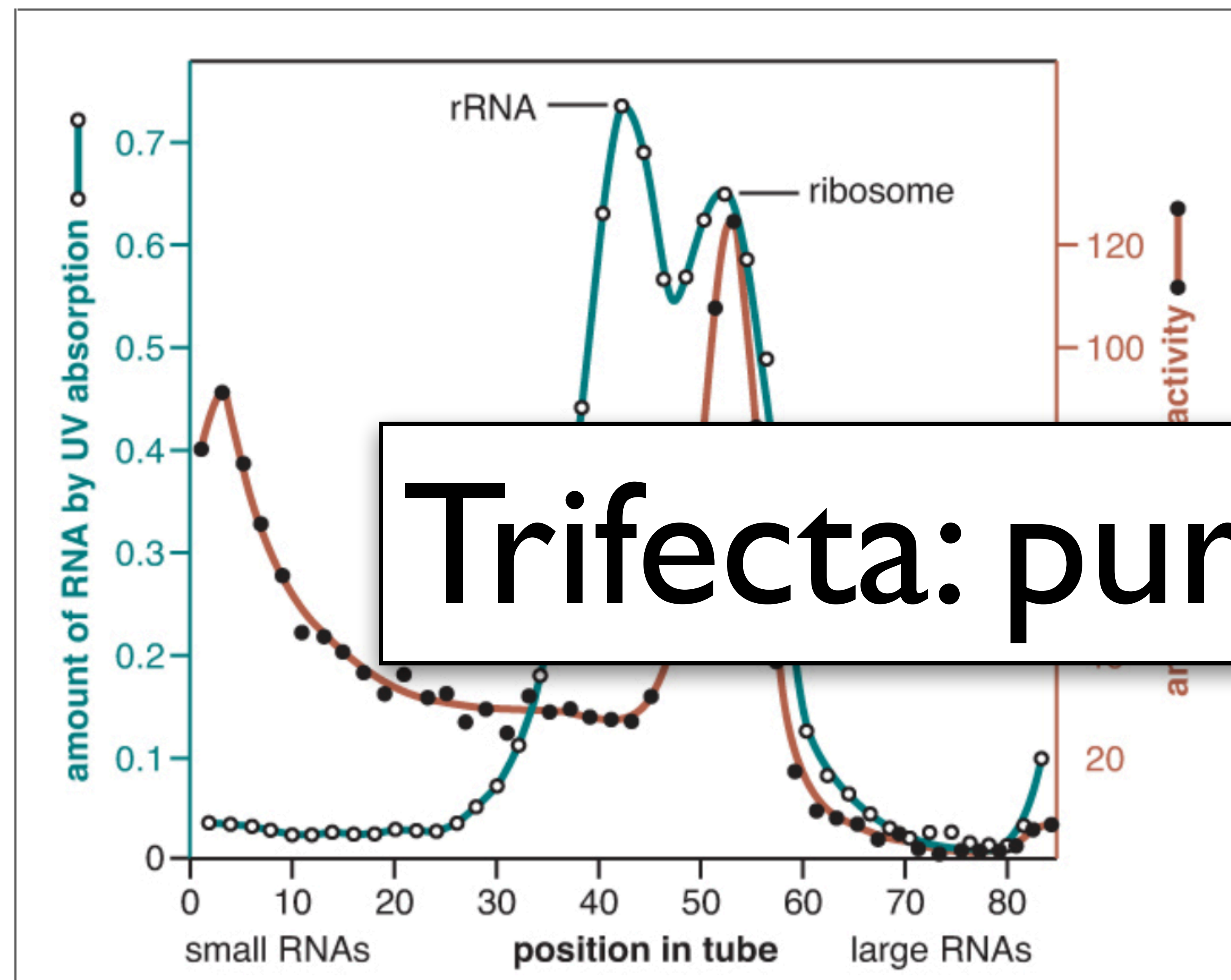


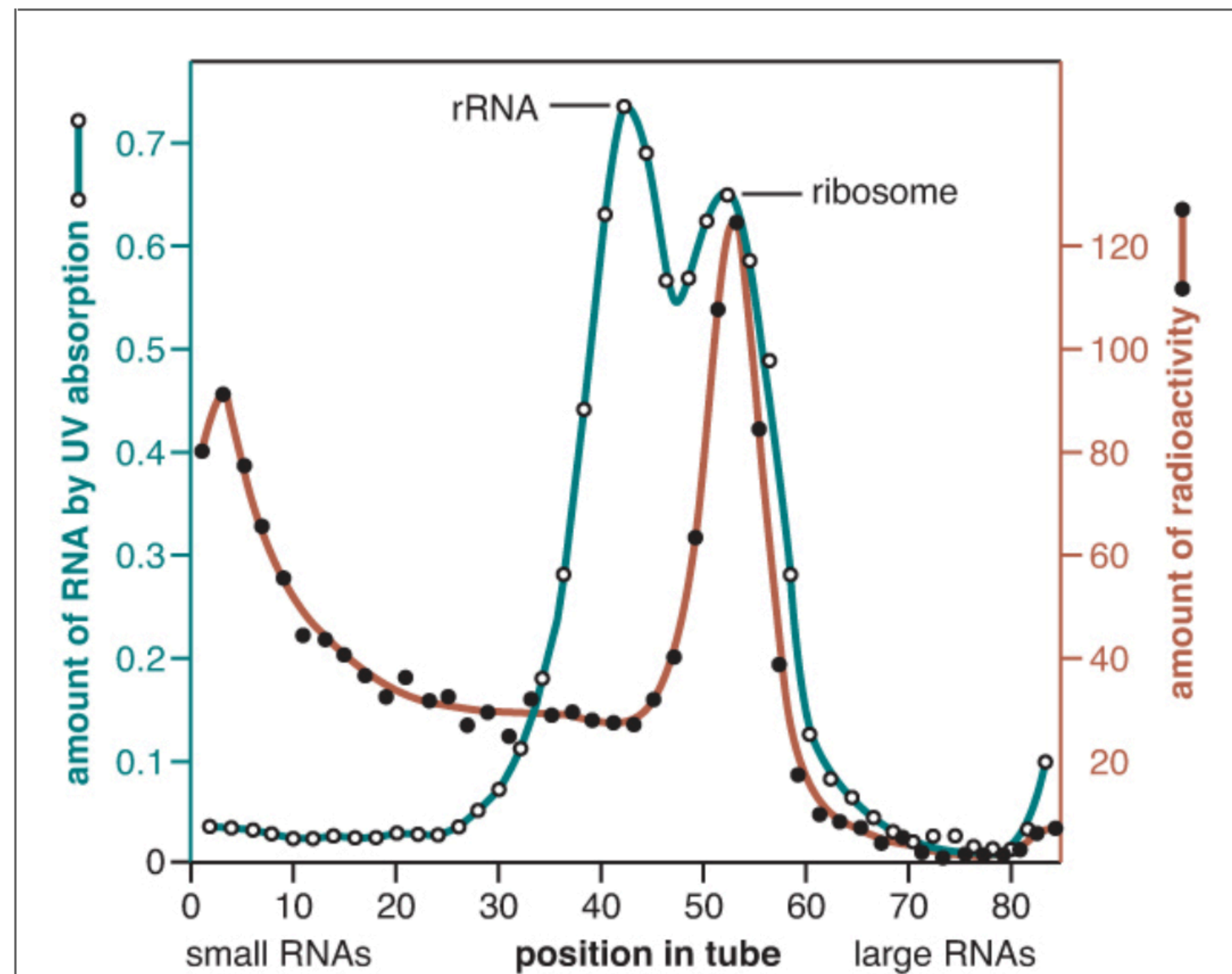
Figure 2.6 Centrifugation of RNA isolated from virally infected cells. The RNA was isolated in a way that protects ribosome stability. The RNA was separated by density, measured by UV absorption (teal line) and by radioactivity (brown line). After centrifugation, aliquots of liquid were removed from the indicated vertical positions in the tube (X-axis) and measured for RNA. Modified from Brenner *et al.*, 1961. Figure 4. Reprinted by permission from Macmillan Publishers Ltd: Nature, Brenner *et al.*, copyright 1961.

If a virus is able to dominate protein production within ten minutes of infecting a cell, then the RNA molecules that inform the cell to make its own proteins must not persist very long. For example, if the RNA that encodes proteins persisted for one hour, then you would expect protein production in an infected cell to be unchanged for at least one hour. With this in mind, the ... that ... chose ... because ... Meselson had already successfully used the same method to demonstrate the semiconservative replication of DNA. *{Connections: Section 1.4 revealed how DNA replicates in a semiconservative process.}*

Trifecta: purpose, methods, findings

Brenner and his colleagues grew bacteria in the presence of radioactive uracil, a nucleotide base found only in RNA molecules. They infected these bacteria with viruses at the same time they added the radioactive uracil. The team isolated all the RNA from the cells shortly after infection. They placed the RNA into the centrifuge tube but took care not to disrupt ribosome structure (Figure 2.6). Once the RNA reached its density-dependent location in the centrifuge tube, they quantified the RNA using UV light absorption. Large RNA-containing molecules are on the right side of the graph, and small RNA molecules are on the left. They also measured the radioactivity along the length of the centrifuge tube. About half of the ribosomes remained intact in this experiment; the two largest peaks are labeled as fully assembled ribosomes as well as disassociated rRNA. You can see the radioactive RNA distributed over a range of different sizes.

The third type of RNA is the most difficult to see for two reasons. First, this RNA is the least abundant—only about 2% of the total RNA. Second, this category of RNA is produced in many different sizes. You are familiar with the phenomenon of not seeing dispersed objects in your daily life. If 100 hairs from your head fall to the ground and are scattered about, they are difficult to see. But if you piled all the hairs together, they'd be more obvious because of their collective mass. The third type of RNA causes a faint white smear that extends from above the top rRNA band down to the smallest RNA molecules. The smear indicates this type of RNA comes in a wide range of sizes, and none are noticeably more abundant than any others in the smear.



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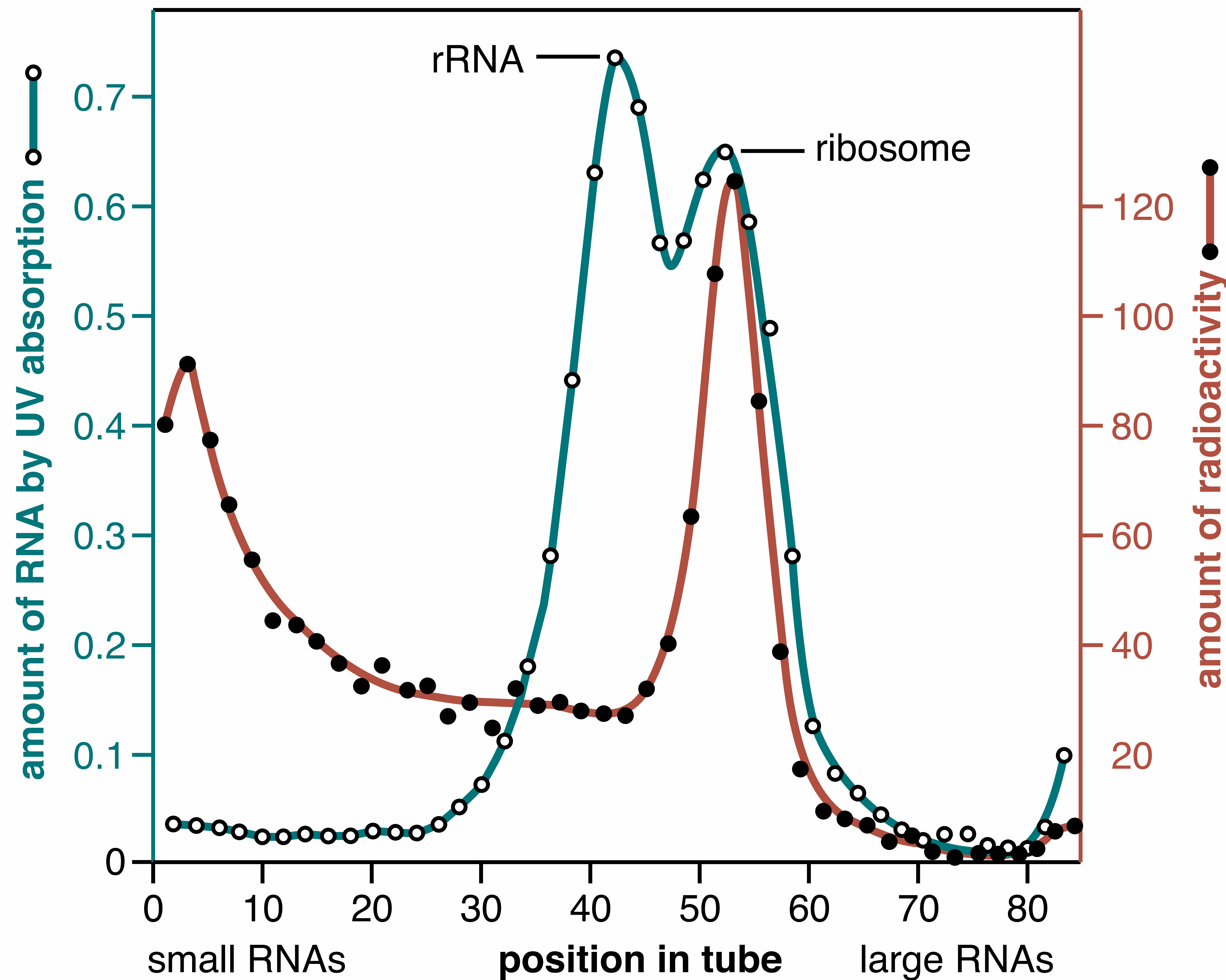
Brenner and his colleagues grew bacteria in the presence of radioactive uracil, a nucleotide base found only in RNA molecules. They infected these bacteria with viruses at the same time they added the radioactive uracil. The team isolated all the RNA from the cells shortly after infection. They placed the RNA into the centrifuge tube but took care not to disrupt ribosome structure (Figure 2.6). Once the RNA reached its density-dependent location in the centrifuge tube, they quantified the RNA using UV light absorption. Large RNA-containing molecules are on the right side of the graph, and small RNA molecules are on the left. They also measured the radioactivity along the length of the centrifuge tube. About half of the ribosomes remained intact in this experiment; the two largest peaks are labeled as fully assembled ribosomes as well as disassociated rRNA. You can see the radioactive RNA distributed over a range of different sizes.

05:00

Prepare to **Explain**: Purpose, Methods, Findings (trifecta)

from Brenner et al. 1961

Figs. 4 and 5. Distribution and turnover of RNA formed after phage infection. A 600 ml. culture of *E. coli B6* (mutant requiring arginine and uracil) was infected with *T4D* (multiplicity 30) and fed ^{14}C-uracil (10 mc./m*M*) from third to fifth min. after infection. One half of the culture was removed and ribosomes prepared (Fig. 4). The other half received a two hundred-fold excess of ^{12}C-uridine for a further 16 min. and ribosomes prepared (Fig. 5). In both experiments approximately 3 mgm. of purified ribosomes were centrifuged for 42 hr. at 37,000 r.p.m. in cæsium chloride containing 0·05 *M* magnesium acetate. Alternate drops were collected in *tris*-magnesium buffer for ultra-violet absorption (○) and on to 0·5 ml. of frozen 5 per cent trichloroacetic acid. These tubes were thawed, 1 mgm. of serum albumin added, and the precipitates separated and washed by filtration on membrane filters for assay of radioactivity (●)



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Trifecta!

from Brenner et al. 1961

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1. How do they visualize the molecules of interest?
2. How do they separate them in an orderly fashion?

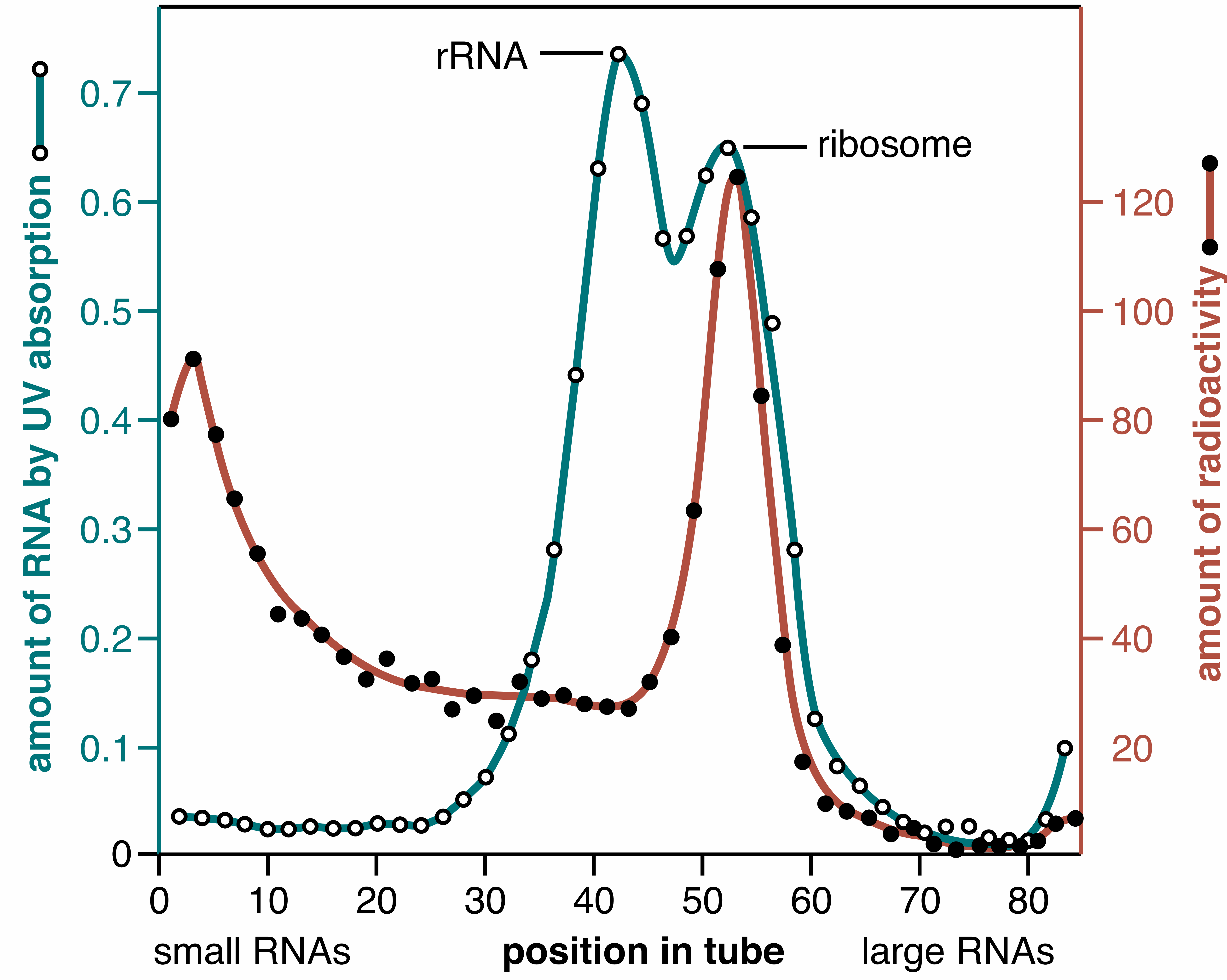


Fig. 2.6

modified from Brenner et al. 1961

Which RNA tells ribosomes what to make?

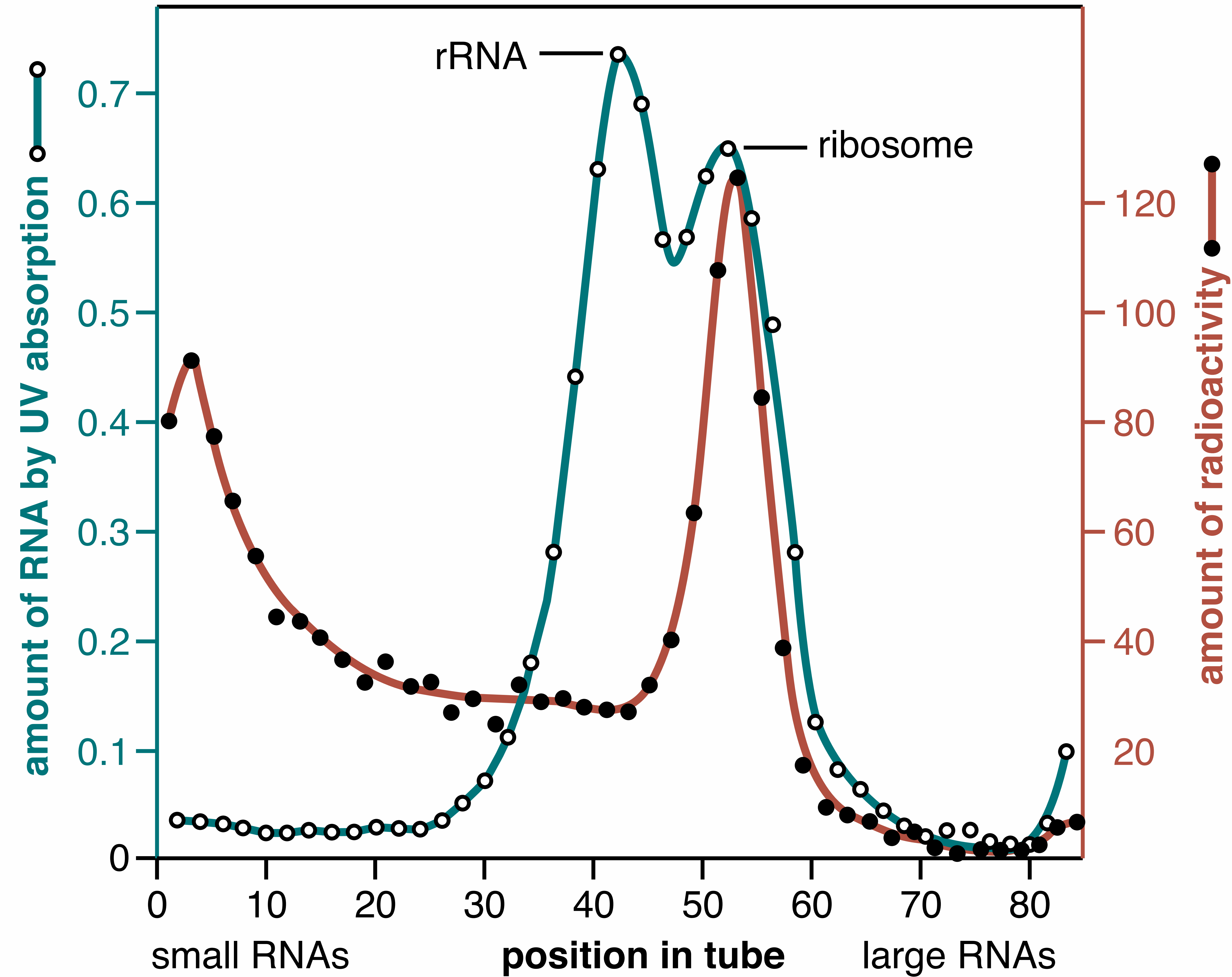


Fig. 2.6

modified from Brenner et al. 1961

Size Fractionation of RNA

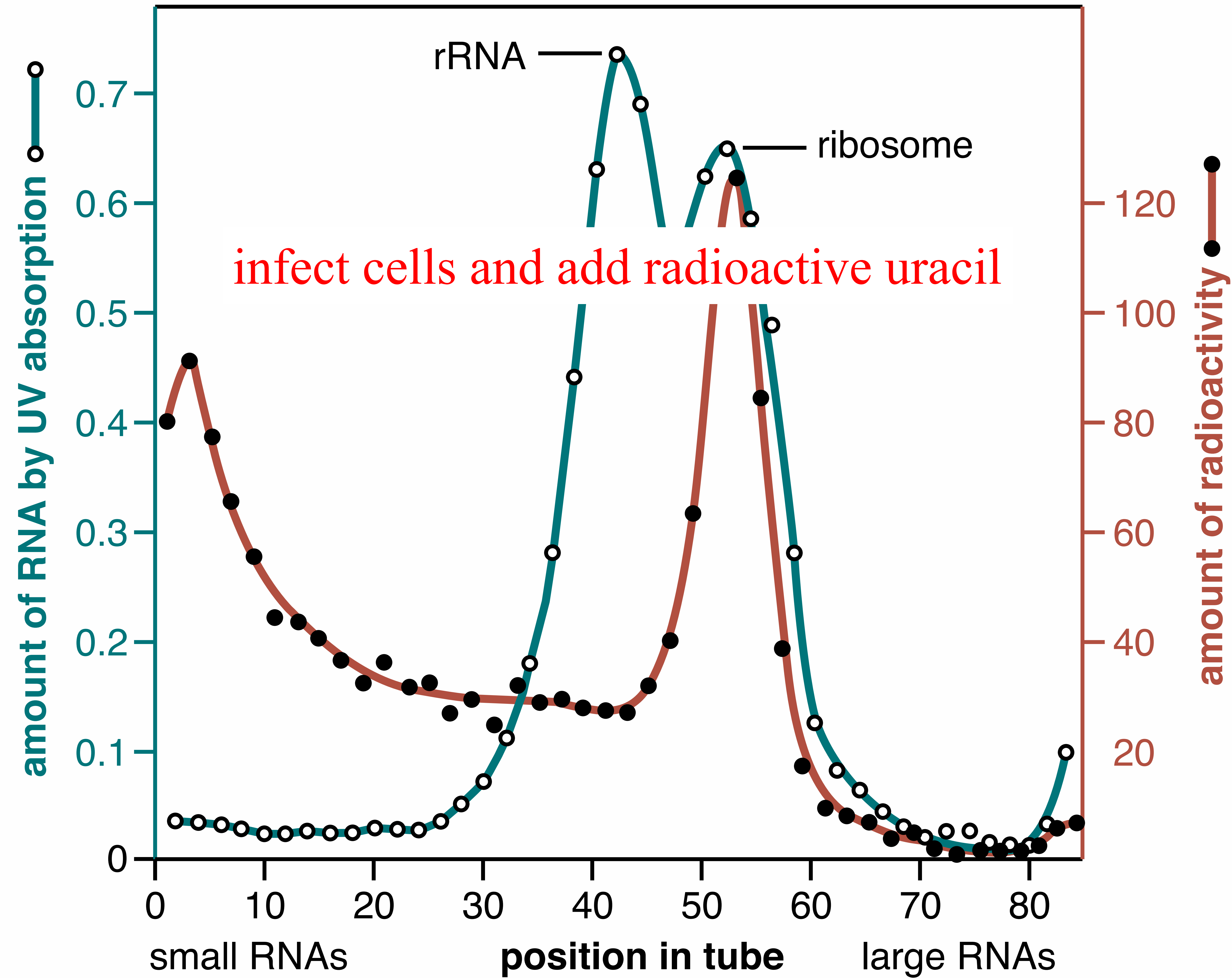


Fig. 2.6

modified from Brenner et al. 1961

Size Fractionation of RNA

Two graphs in the same space.

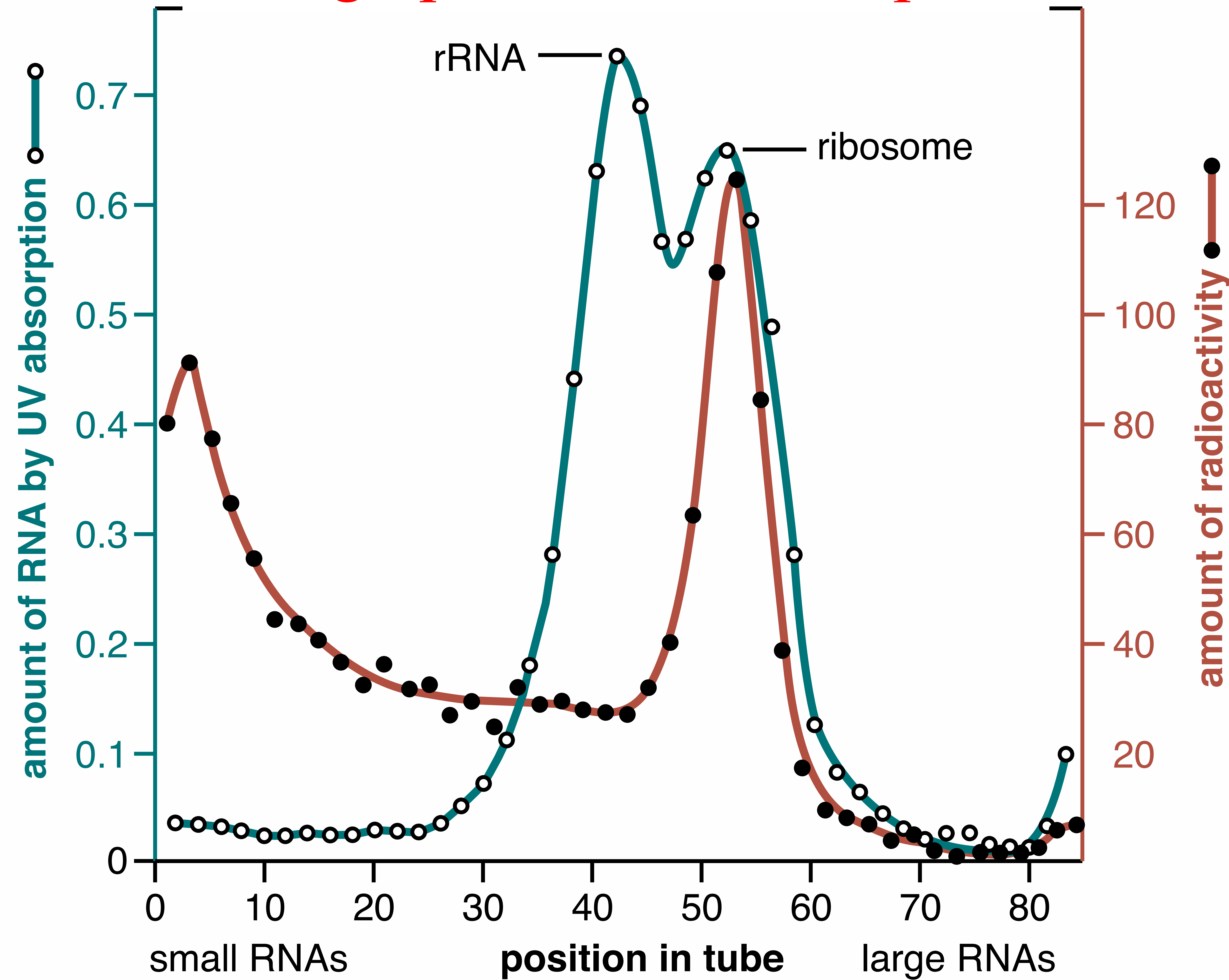


Fig. 2.6

modified from Brenner et al. 1961

Size Fractionation of RNA

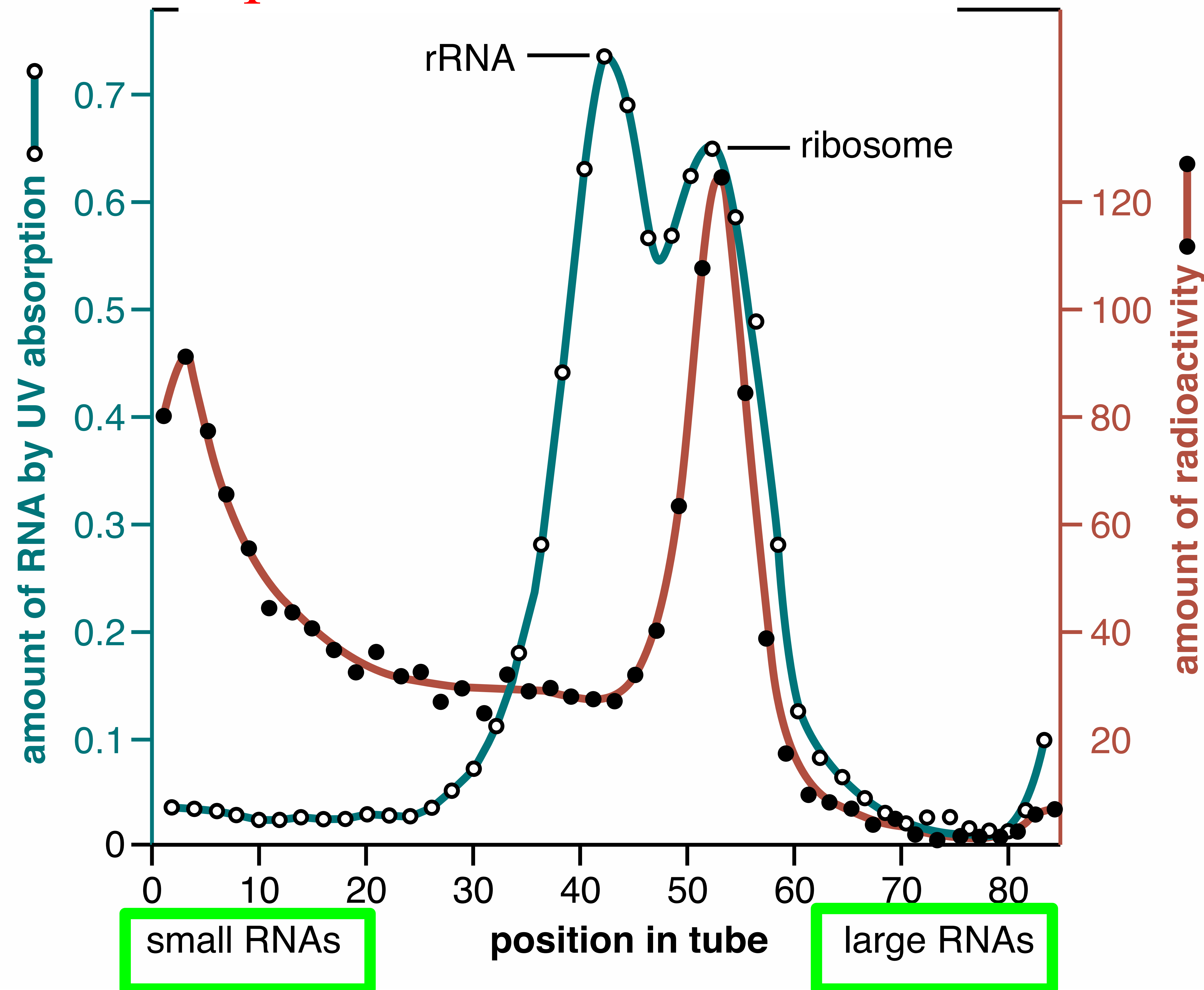


Fig. 2.6

modified from Brenner et al. 1961

Separation of Three RNAs

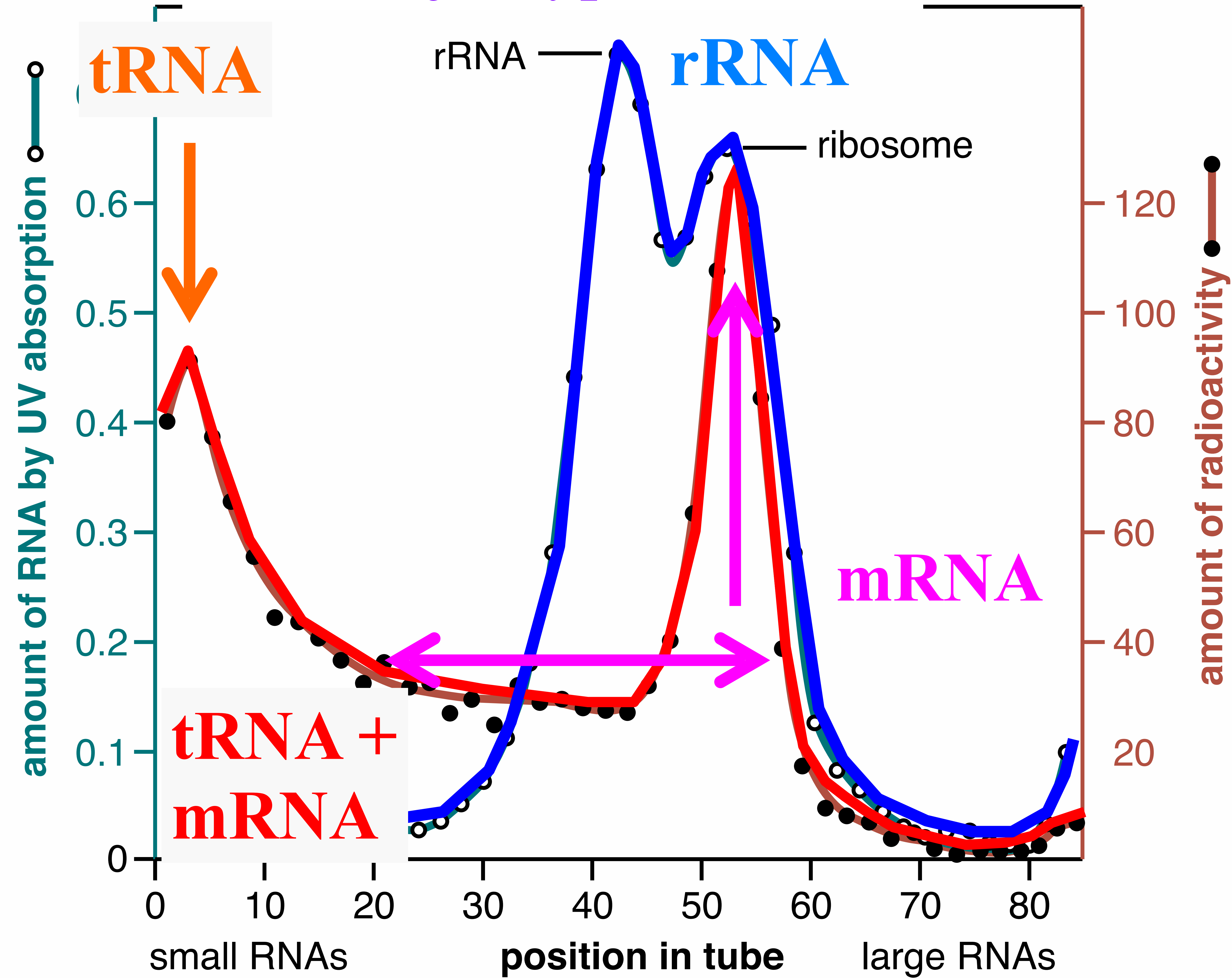
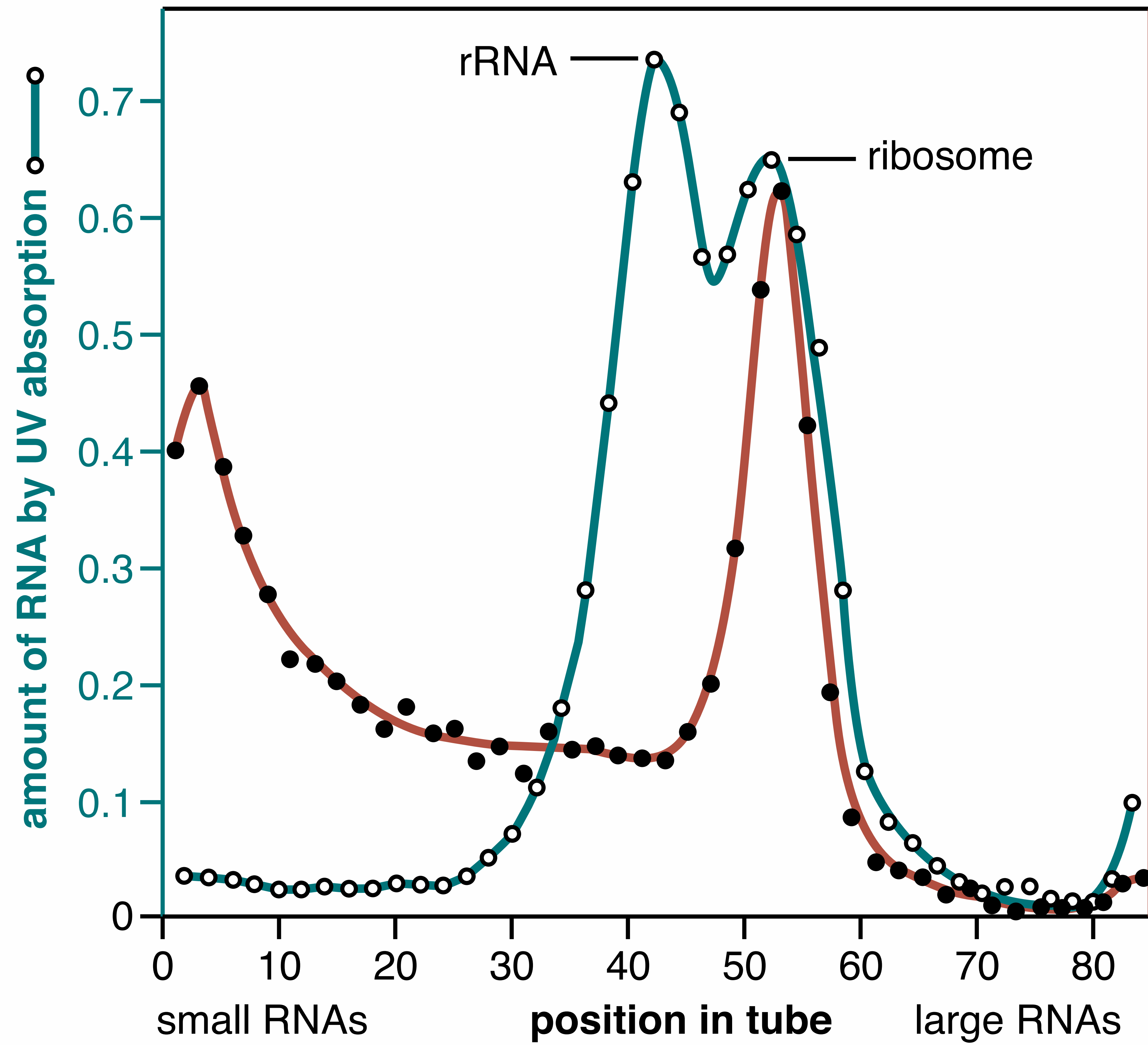
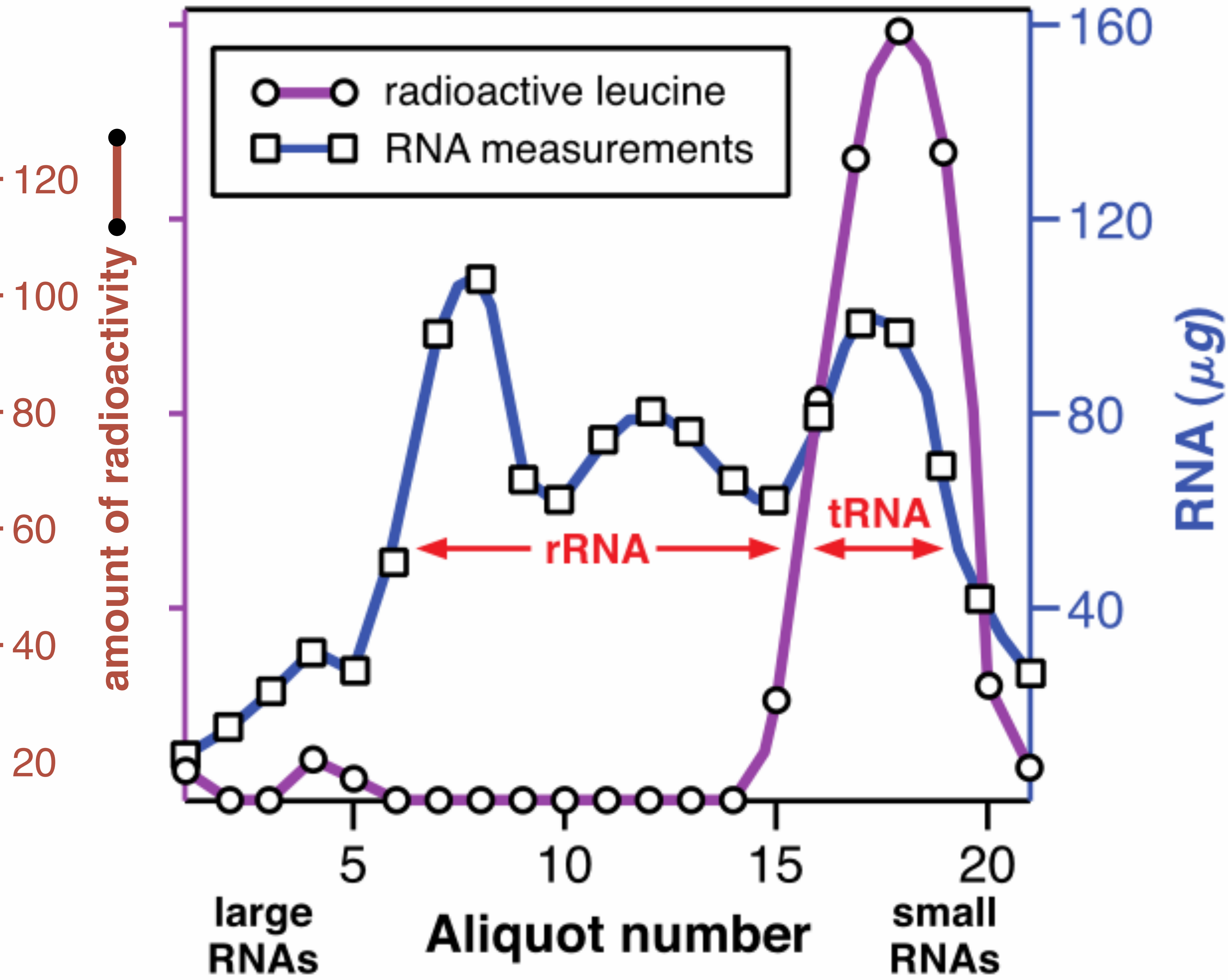


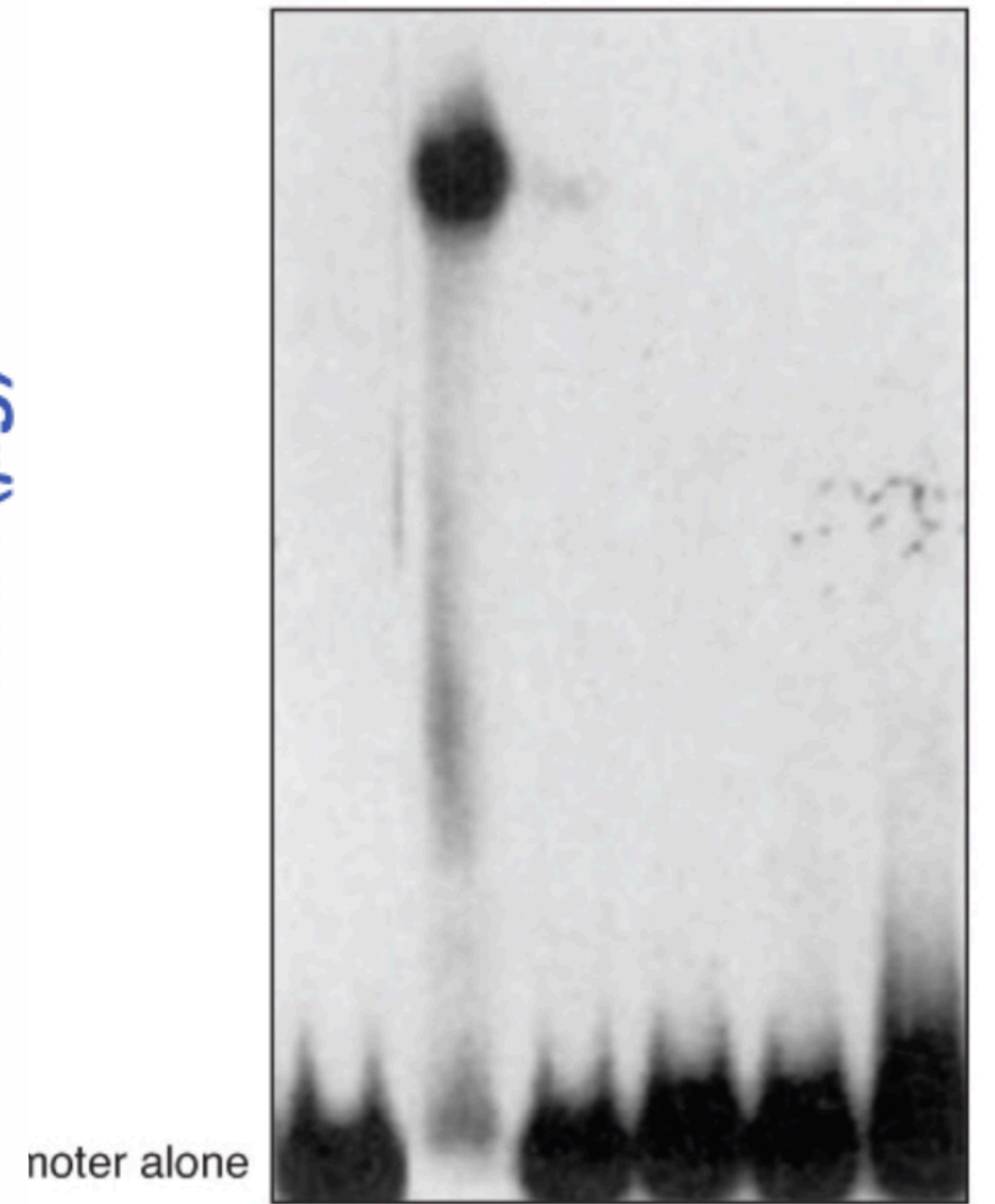
Fig. 2.6

modified from Brenner et al. 1961

Which similar NEW experimental strategy was used in these?







Team#2- Rachel, Grace, Daniel, Nathan

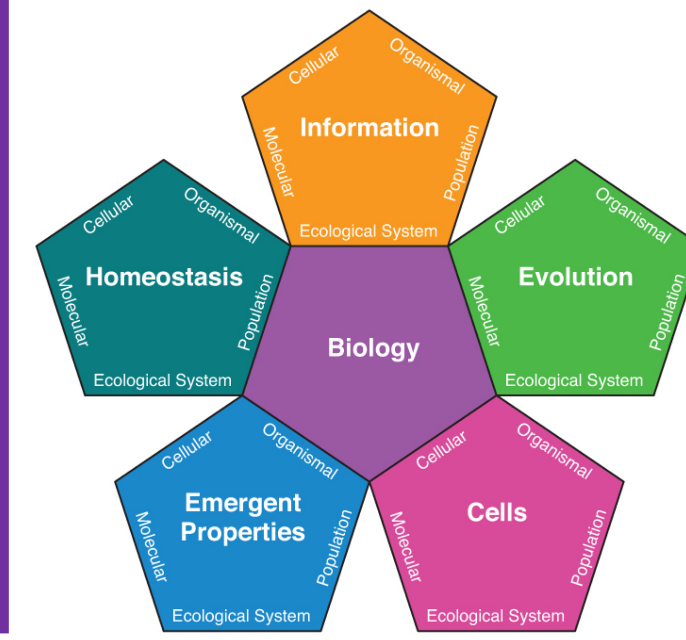
Team#1- Trinity, Jay, Ethan, Kierin

Team#2- Rachel, Grace, Daniel, Nathan

VOTE

Team#1- Mit, Ryan, Nicole, Costa

Integrating Concepts in Biology



PowerPoint Slides for Chapter 2: **Central Dogma**

2.4 Can cells pick and choose information?

by A. Malcolm Campbell, Laurie J. Heyer, & Christopher Paradise

Title Page

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NCBI Biological Information Database

NCBI Resources How To Sign in to NCBI

NCBI National Center for Biotechnology Information

All Databases Search

NCBI Home
Resource List (A-Z)
All Resources
Chemicals & Bioassays
Data & Software
DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
Genomes & Maps
Homology
Literature

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- Downloads: Get NCBI data or software
- How-To's: Learn how to accomplish specific tasks at NCBI
- Submissions: Submit data to GenBank or other NCBI databases

Popular Resources
PubMed
Bookshelf
PubMed Central
PubMed Health
BLAST
Nucleotide
Genome
SNP
Gene
Protein
PubChem

A

Fig. 2.24A

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Integrating Questions

35. Search NCBI using these directions: Search NCBI for accession number NM_000207. You will see the name of the mRNA that we are investigating—human insulin. The gene that encodes the insulin mRNA has the accession number NG_007114. Now you will use NCBI to align the gene and mRNA sequences encoding insulin. Use a program called BLAST2, paste the insulin mRNA accession number NM_000207 into both of the big boxes, choose the "megablast" option under Program Selection and then click on the "BLAST" button. When you get results, click on “Dot Plot” tab to see a graphical display showing how two sequences align when they are identical (see Figure 2.24B). *{Connections: You will explore dot plots in Bio-Math Exploration 5.1.}* These plots display the maximum alignment of two sequences by placing a dot where each amino acid occurs in both sequences. Identical sequences produce a diagonal line. Go back to the BLAST2 page and reload the page. Paste the insulin gene accession number, NG_007114, into the bottom box in place of the mRNA accession number, confirm the Program Selection is "megablast" and align these two sequences. You will see a very different picture in the dot plot where the insulin mRNA is on the X-axis and the insulin gene is on the Y-axis. Instead of one diagonal line, you will see three segments separated by small gaps. Use these data to answer Integrating Question 36, and keep this browser window open.
36. Which sequence is longer, the mRNA or the gene? How can you tell? By how many bases do these two coding segments differ? Click on the "Alignments" tab and examine the aligned sequences for Range 2 and Range 1 to determine if any mRNA bases (query sequence) are not present in the gene (subject sequence). Predict how many amino acids would be in the human insulin protein after translation, given the length of the mRNA (query length at the top of the alignments page).

Integrating Question 36 allowed you to discover an important fact: Usually, genes are longer than their encoded mRNAs. The insulin gene is longer because its mRNA has been processed inside the nucleus. When the gene was first transcribed, the RNA was longer that the final mRNA. However, the initial RNA contains some information that is not needed for translation, and these nucleotides are splic[...] and the intervening segments that are cut out of the RNA are calle[...]RNA is rare in prokaryotes but very common in eukaryotes. Splicin[...]slation. Exons and introns are one example of how DNA informati[...]

IQs 35-36 and 37-38

When you aligned the gene wit[...]nd two introns between the exons. The mRNA is 469 nucleotides long, so you could divide this number by 3 (the size of a codon) to estimate how many amino acids are in the insulin protein: 156.3. However, you should realize something is incorrect because proteins cannot contain 0.3 amino acids. The data are telling you something is wrong with your current interpretation, and more data mining at NCBI is needed. As with Integrating Questions 35 and 36, the following set of questions combines data mining with questions for you to answer.

Integrating Questions

37. Use the NCBI tool called **open reading frame (ORF)** finder. An ORF is defined as a continuous piece of DNA that begins with a start codon that encodes a methionine and ends with one of the three stop codons. Refer to the genetic code in Figure 2.23 to see which codons are start and stop. Have the ORF finder locate these for you by entering the mRNA accession number NM_000207, and click on the “submit” button.
The next page displays the results of searching the DNA forward on the top strand and backwards on the bottom strand. Each strand much be searched three times because codons are groups of three bases. Any given base could be the first, second or third one in a codon. If you see the nucleotide abbreviated A, it could be the A from the start codon of AUG in mRNA, or ATG in DNA. In this example, only three large ORFs were found in the DNA (two forwards and one backwards). Click on ORFs 1, 2 and 3 in the table below the colored graph to see the amino acid sequences appear to the left. Which ORF encodes the largest potential protein? You can choose to "Display the ORF as..." CDS translation to see each codon and its corresponding amino acid in the single letter code.
Notice in the upper graph with the number line that before the start codon and after the stop codon in the largest ORF, some portions of the mRNA are not translated. These nucleotides are part of the exons but are not translated into protein and are called **untranslated regions.** Again, it seems wasteful to produce parts of mRNA that are not translated, but evolution does not necessarily produce the most efficient mechanisms possible.
Now that you have found the correct reading frame (ORF2 in reading frame +3), click on the “Mark” button above the amino acid sequence in the left window. With only the protein sequence displayed, copy the sequence in the left window that starts with the letters MALW... and ends with ...NYCN. Integrating Question 38 asks you to compare the ORF amino acid sequence you just produced to the amino acid sequence of insulin purified from human blood:
FVNQHLCGSHLVEALYLVCGERGFFYTPKTGIVEQCCTSICSLYQLENYCN. Does the purified blood protein begin with methionine?
38. Align the two insulin protein sequences (translated ORF and purified from blood) with BLAST2. *Directions:* Paste the protein sequence you obtained from the ORF finder

Yeast RNA Separated by Size

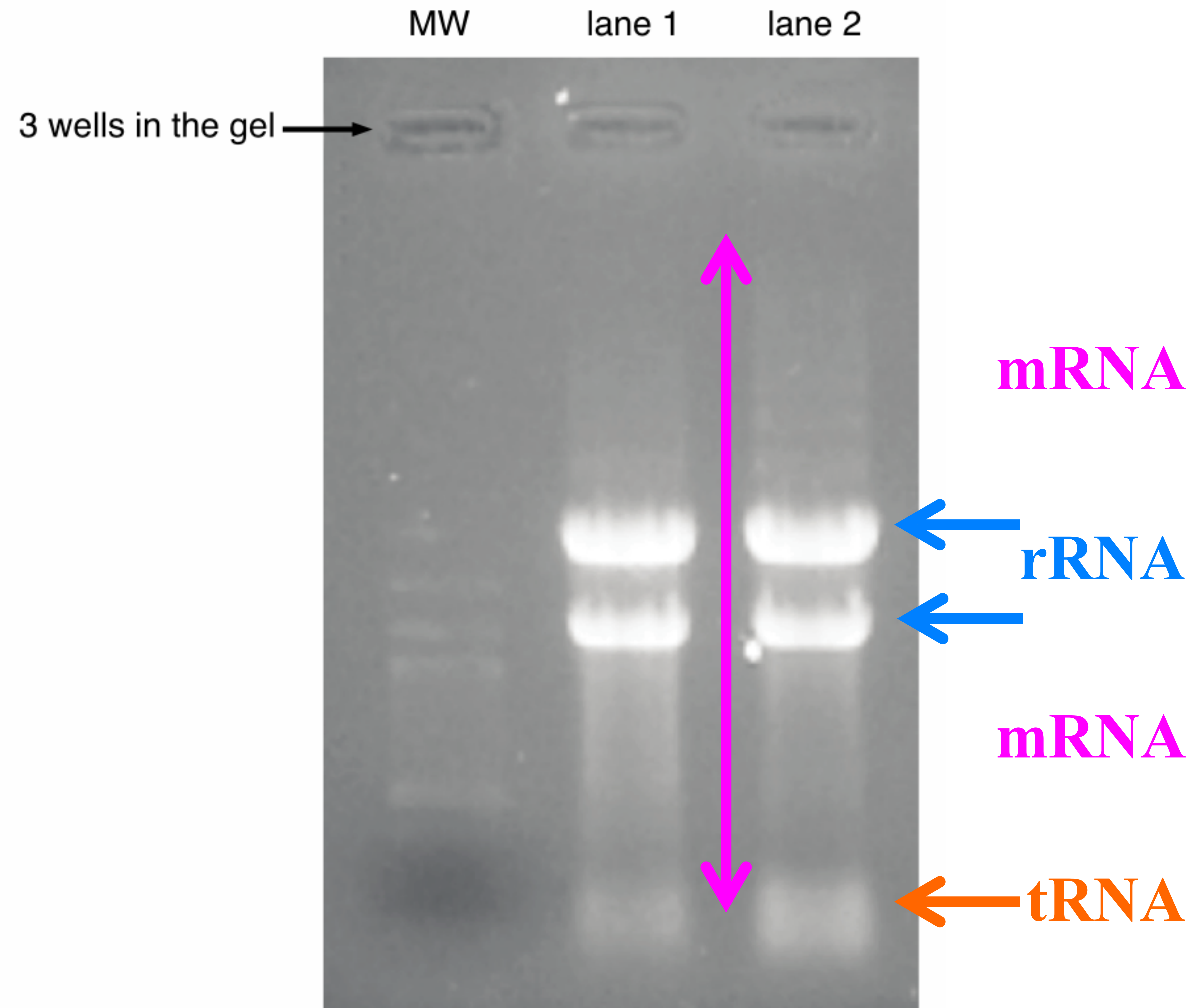

Fig. 2.3

courtesy Todd Eckdahl

(notebook)

Translation: 3 parts

- **Language** (AUG!)
- **Translators** (tRNA & synthetase)
- **Factory** (Ribosome subunits)

Language (AUG!)

What words are hidden in this mRNA sentence?

5’ 3’

UUAAGUUAAGAUGGGUUAUAAGUCAACUUGA UUG A

What additional *information* do you need?

?????????? I THE I DOG I AND I MAN I EAT I HAM I STOP

Frameshift mutation (delete O in DOG)

????????? I THE I DGA I NDM I ANE I ATH I AMS I TOP

Language (AUG!)

What words are hidden in this mRNA sentence?

5’ 3’

UUAAG | AUG | GGG | UAU | AAG | UCA | ACC | UUG A

What additional *information* do you need?

?????????? | THE | DOG | AND | MAN | EAT | HAM | STOP

In-frame mutation (delete AND)

?????????? | THE | DOG | MAN | EAT | HAM | STOP

Translators (tRNA & synth)

- **tRNA** is a translator/transfers amino acids.
- **Aminoacyl-tRNA Synthetase** (Charging enzyme loads aas on tRNAs)

- # Aminoacyl-tRNA Synthetase

In order for translation to occur, the tRNA molecules must be "charged" with the amino acid appropriate for its anti-codon. The enzyme that covalently links the amino acid with its cognate tRNA is called an amino-acyl tRNA synthetase, or charging enzyme.

Exit | Previous | Slower | Section 3 | Faster | Next | Play