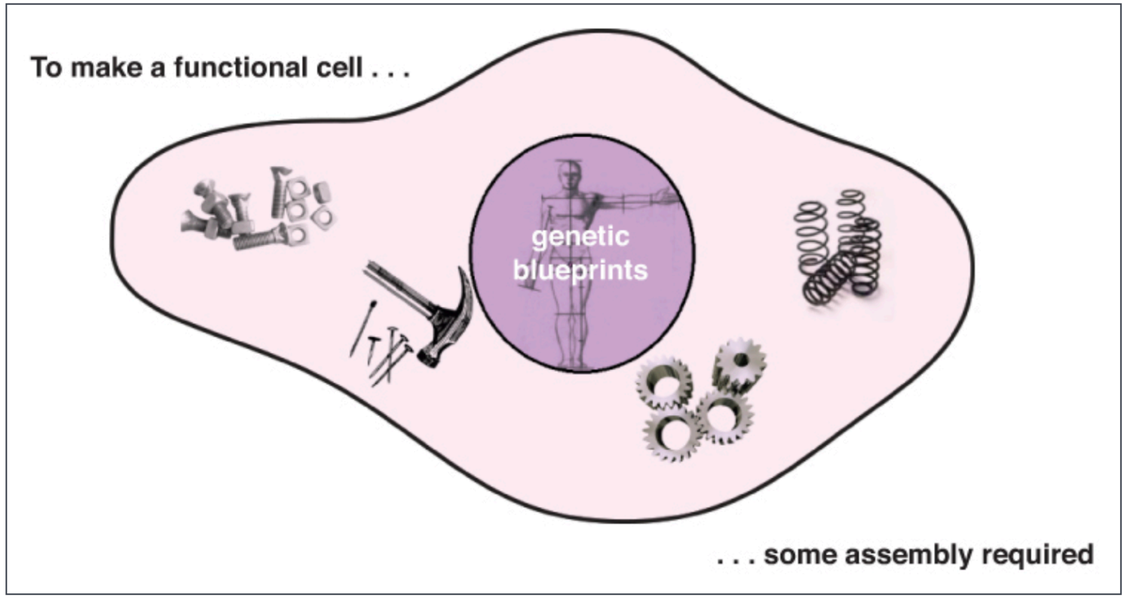
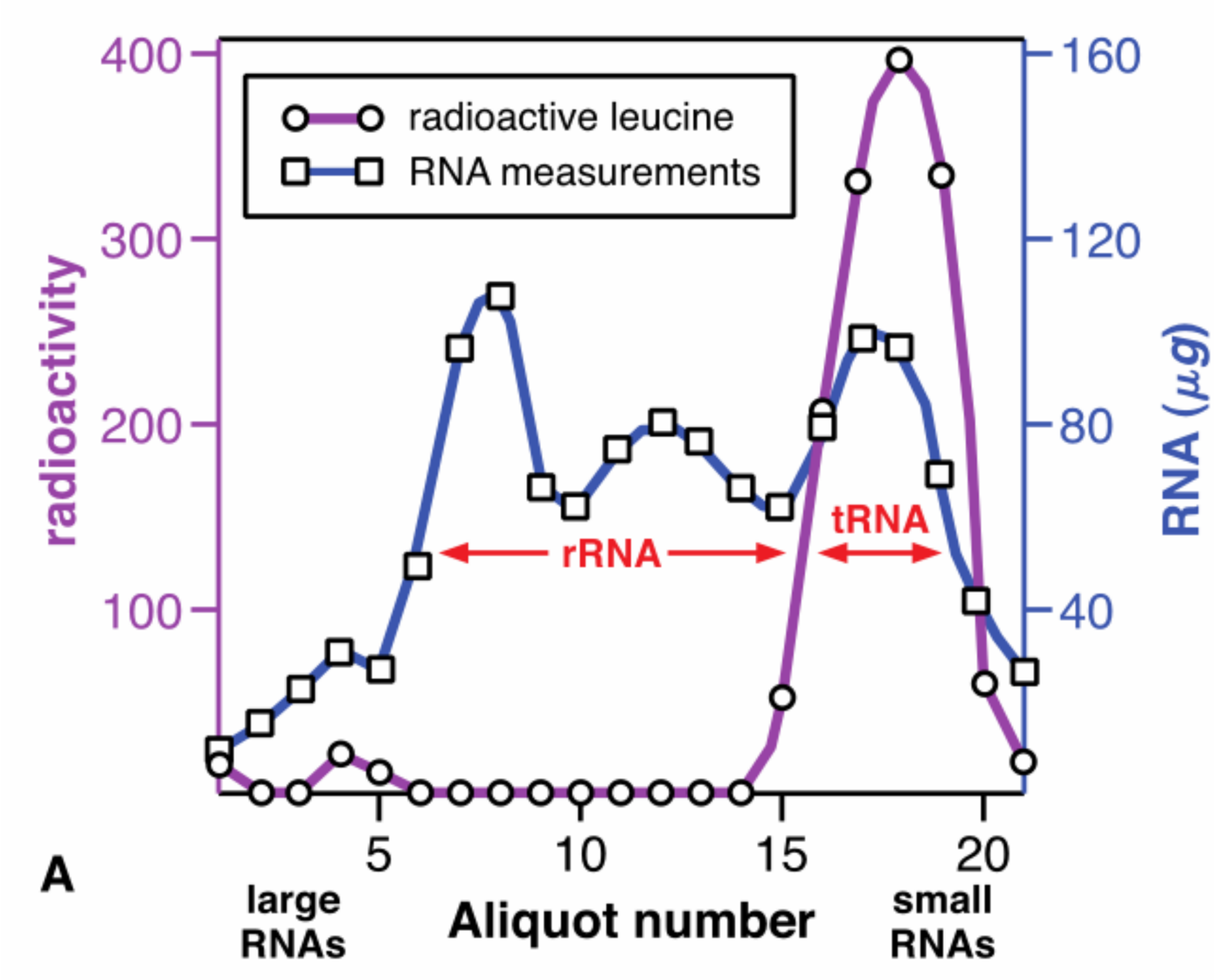
Chapter 2: Central Dogma

DNA contains instructions but each cell must construct its own components to perform a function. Original art.



What makes cells function as productive members of your body? How do cells control which genes are activated at different times or in different organs of your body? As you learned in Chapter 1, DNA is the molecular information passed down from generation to generation. However, DNA does not perform cellular functions, it only provides a molecular blueprint for cells. Proteins perform most cellular functions. Although the structure and function of DNA were understood by the late 1950's, researchers still did not understand how the different forms of RNA functioned. In this chapter, you will follow the path of researchers who made many ground-breaking discoveries about how cells produce proteins. This path will lead you to the fundamental concept of "central dogma," which explains how cells process molecular information from DNA to RNA to protein. It may surprise you to know that the path of discovery has not ended. Biologists of your generation will continue to make many important discoveries, building on the foundation of ideas you will learn in Chapter 2. The four Sections of Chapter 2 focus on information at the cellular level.

you are here		Big Ideas of biology				
		Information	Evolution	Cells	Homeostasis	Emergent Properties
levels of the biological hierarchy	molecules	1	4	7	10	13
	cells	2	5	8	11	14
	organisms I	3	6	9	12	15
	organisms II	16	19	22	28	25
	populations	17	20	23	29	26
	ecological systems	18	21	24	30	27



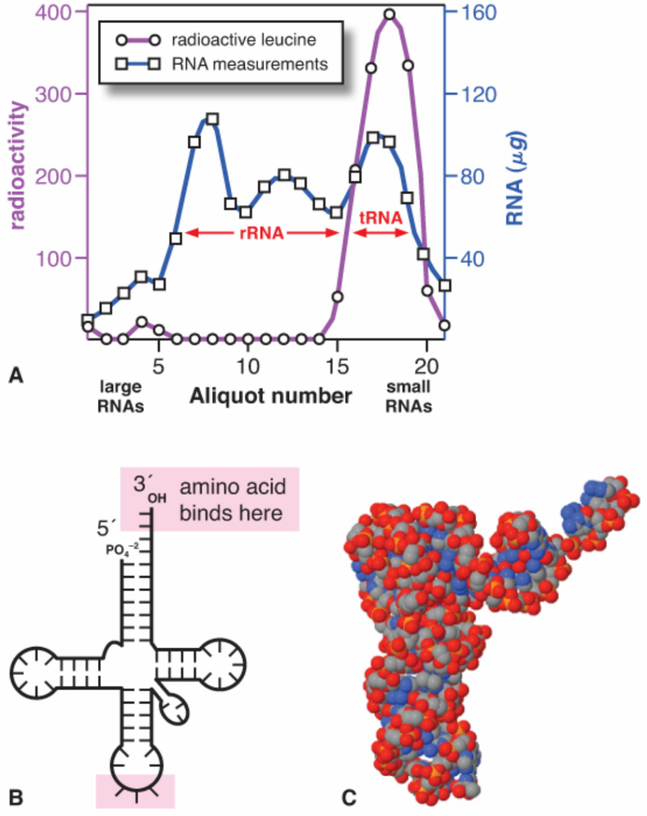


Figure 2.5 Structure and function of tRNA. **A,** RNA molecules interact with radioactive leucine. Total RNA from peas was extracted and incubated with radioactive leucine in the presence of cytoplasm from the peas. RNA molecules were separated by size. Radioactive leucine and non-radioactive RNA were detected separately as shown. **B,** Two-dimensional representation of tRNA as determined by base paring within the molecule. **C,** Space filling three-dimensional image of tRNA; red, oxygen; blue, nitrogen; orange, phosphorous; and gray, carbon. Interactive Jsmol views of tRNA are available online. (A) Modified from Chipchase and Birnstiel, 1963. Figure 2. Copyright Margaret I.H. Chipchase and Max L. Birnstiel, 1963. (B,C) original art based on

Trifecta?

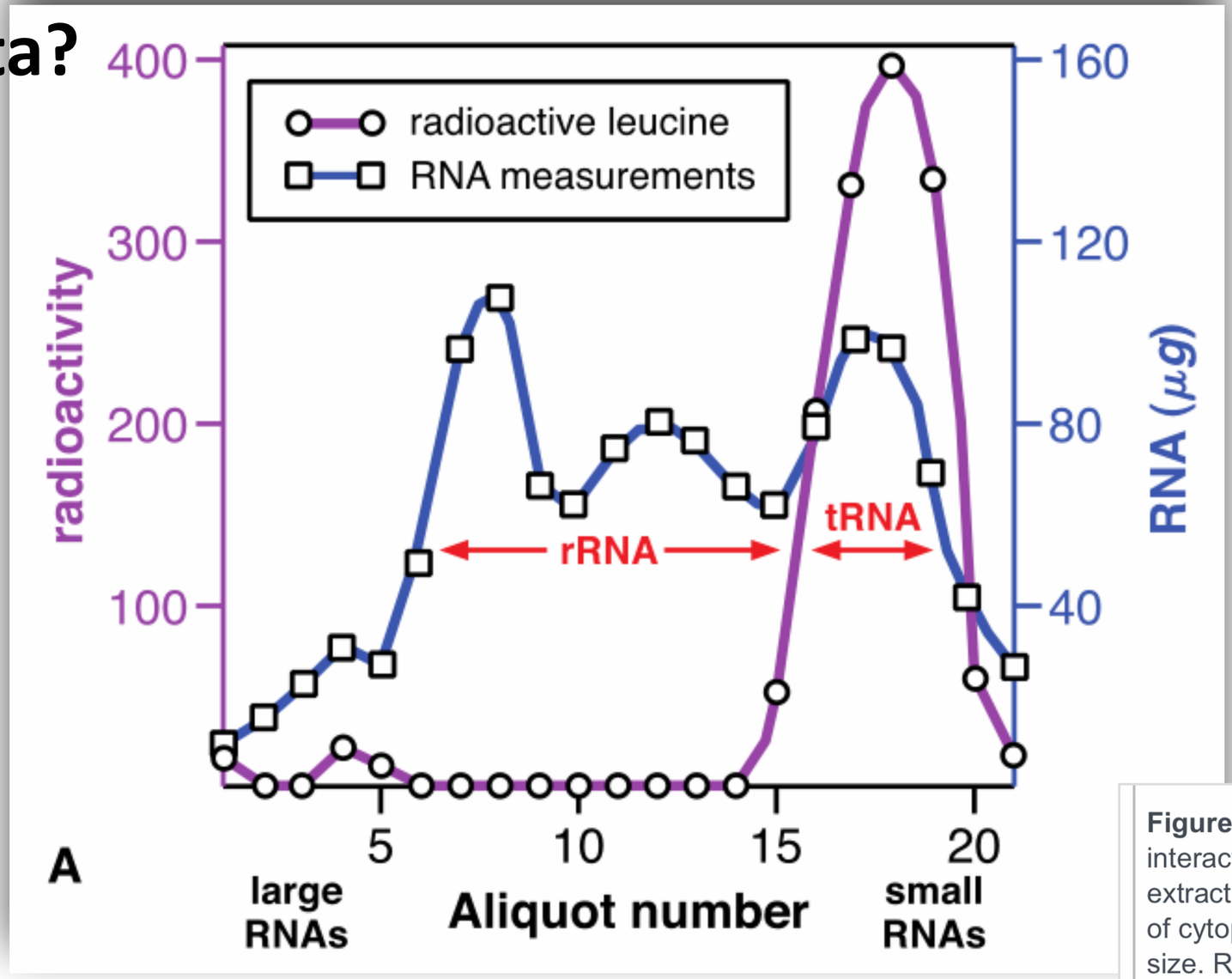
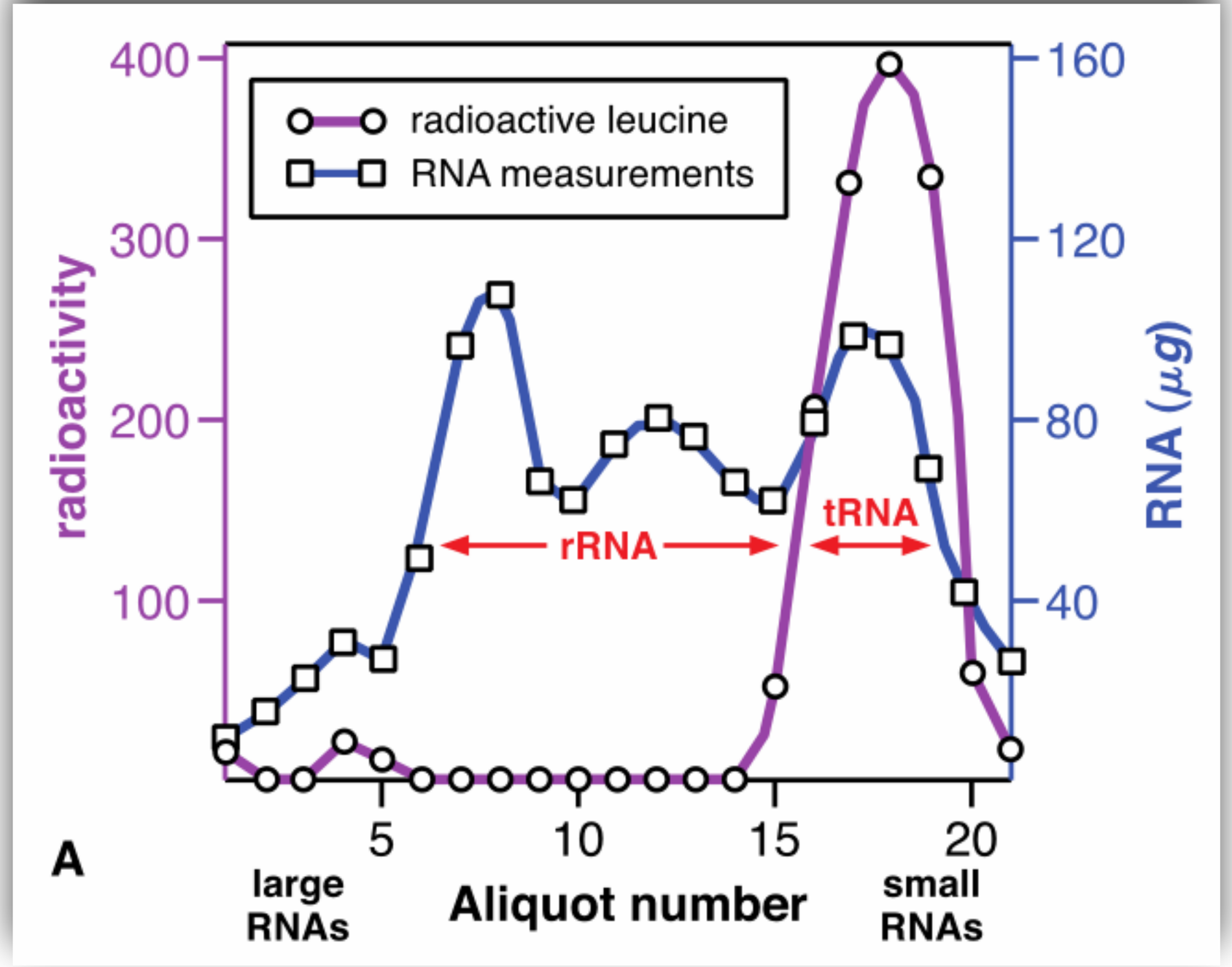


FIG. 2.—Size distribution of nuclear RNA and localization of transfer RNA on a sucrose density gradient. Sucrose density gradient (20 ml) of 20–5% sucrose in .01 *M* tris (pH 7.1) and 1% phenol was set up according to Bolton *et al.*[17] and overlayered with an inverse gradient of approximately 1 mg 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.

Figure 2.5 Structure and function of tRNA. **A,** RNA molecules interact with radioactive leucine. Total RNA from peas was extracted and incubated with radioactive leucine in the presence of cytoplasm from the peas. RNA molecules were separated by size. Radioactive leucine and non-radioactive RNA were detected separately as shown. **B,** Two-dimensional representation of tRNA


radioactive leucine
RNA measurements
radioactivity
RNA (μg)
400
300
200
100
160
120
80
40
rRNA
tRNA
5
10
15
20
A
large RNAs
Aliquot number
small RNAs

Trifecta?

SYNTHESIS OF TRANSFER RNA BY ISOLATED NUCLEI*

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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

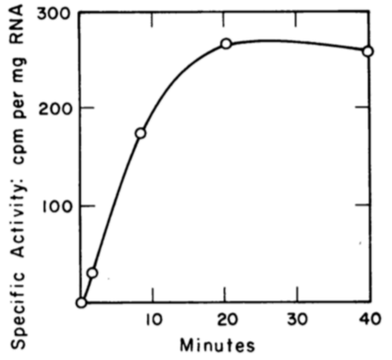


FIG. 1.—Formation of amino-acyl RNA in isolated nuclei. Incubation mixture: ATP, CTP, GTP, and UTP, .0001 *M* each; tris .02 *M*; phosphocreatine .02 *M*; creatine phosphokinase 100 μg/ml; $CaCl_2$ .003 *M*; $MgCl_2$ .0001 *M*; C^{14}-protein hydrolysate 2 μc/ml; final pH 7.0, incubation at 37°C. Aliquots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

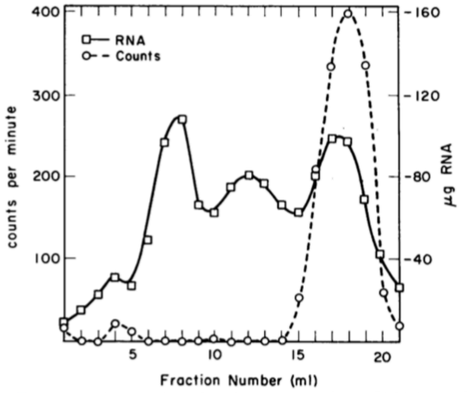
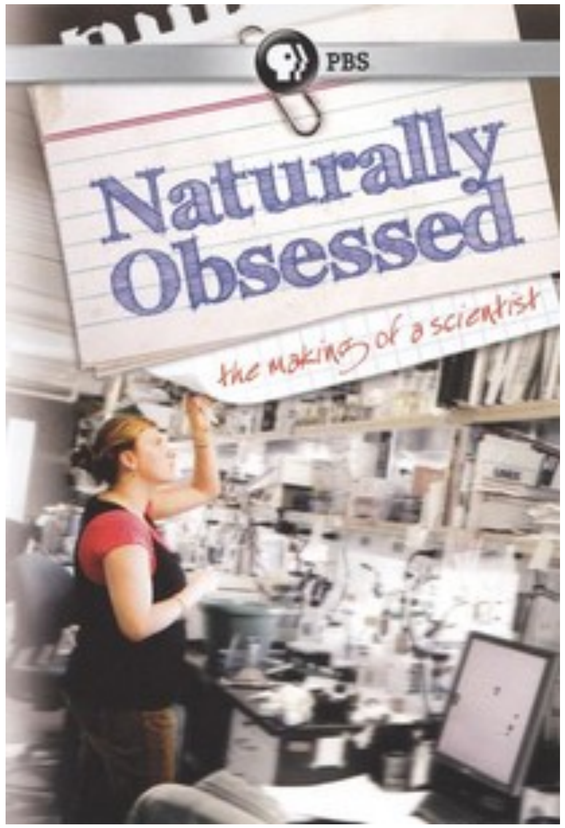


FIG. 2.—Size distribution of nuclear RNA and localization of transfer RNA on a sucrose density gradient. Sucrose density gradient (20 ml) of 20–5% sucrose in .01 *M* tris (pH 7.1) and 1% phenol was set up according to Bolton *et al.*[17] and overlayered with an inverse gradient of approximately 1 mg 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

Naturally Obsessed



tRNA and Amino Acids Interact

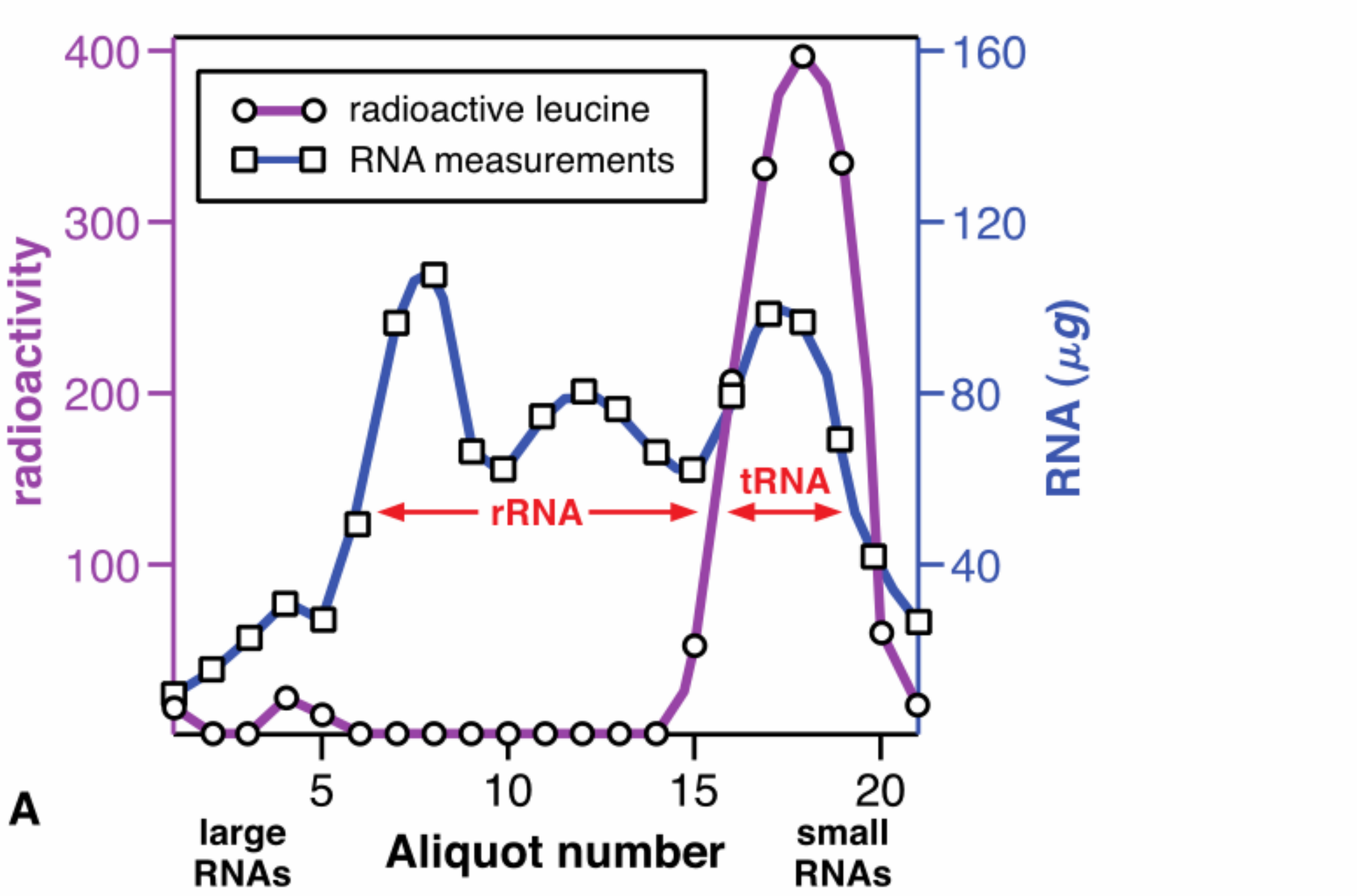


Figure 2.5A

modified from Chipchase and Birnstiel, 1963

RNA and Amino Acids Interact

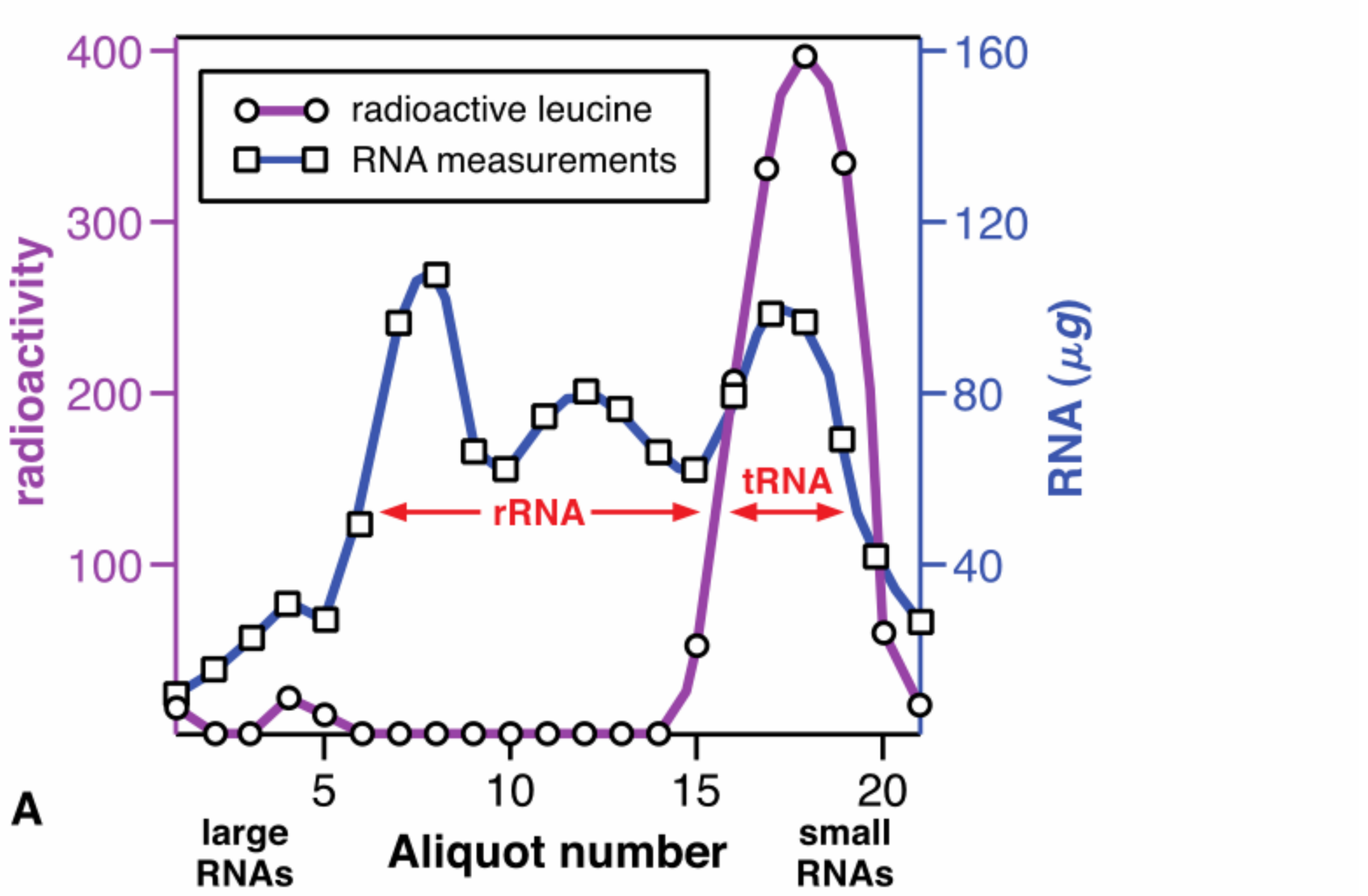


Figure 2.5A

modified from Chipchase and Birnstiel, 1963

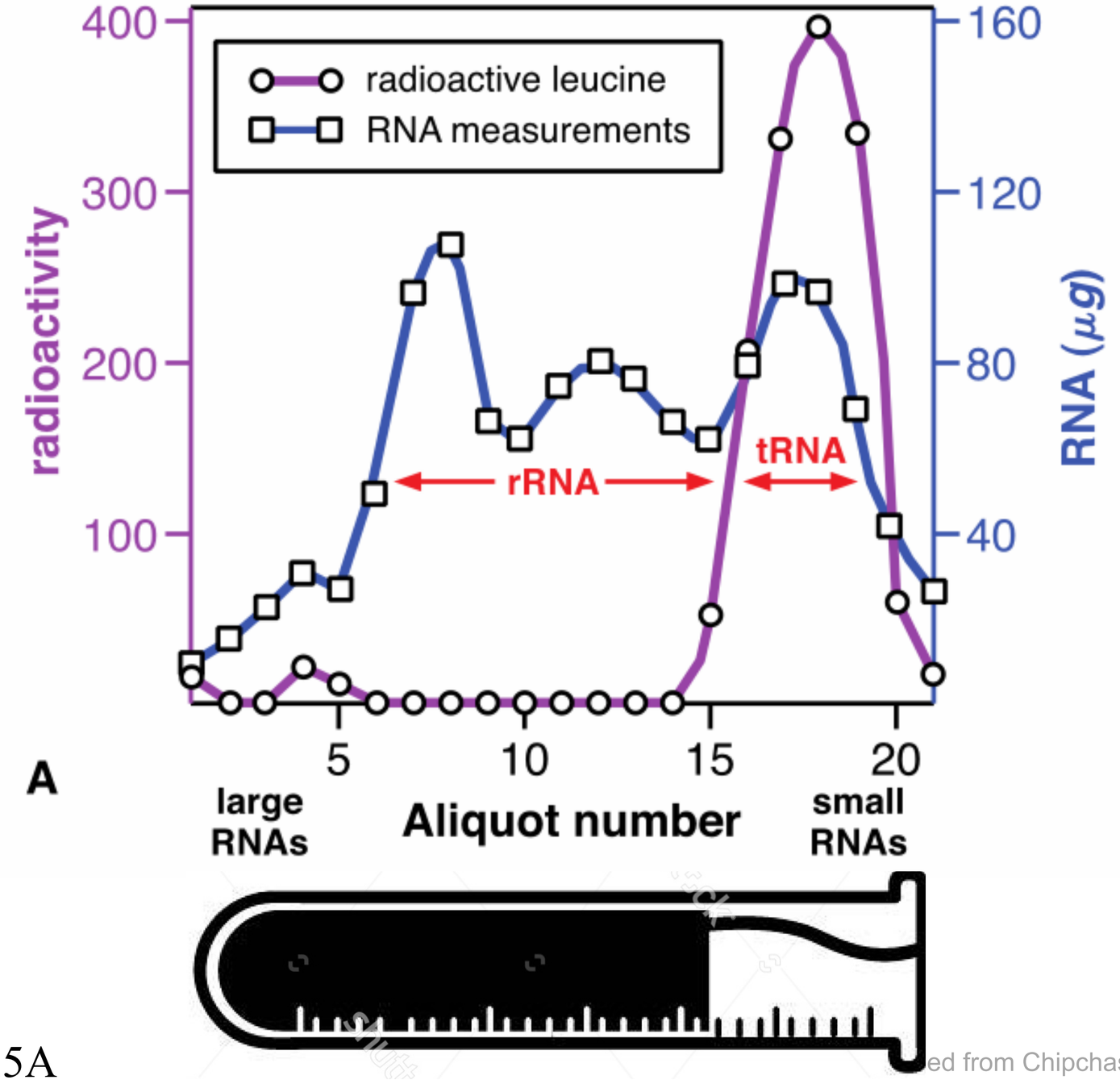


Figure 2.5A

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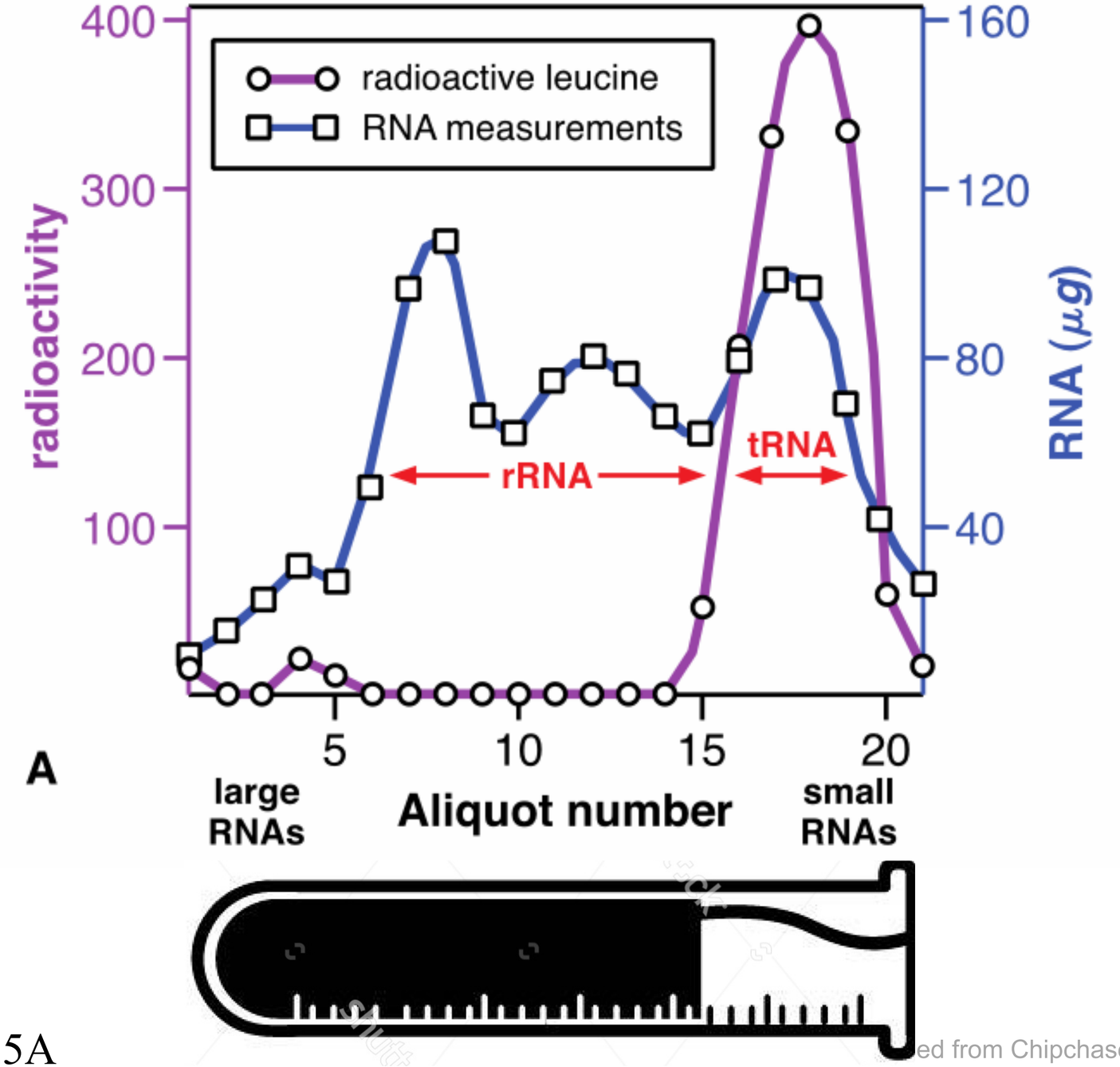

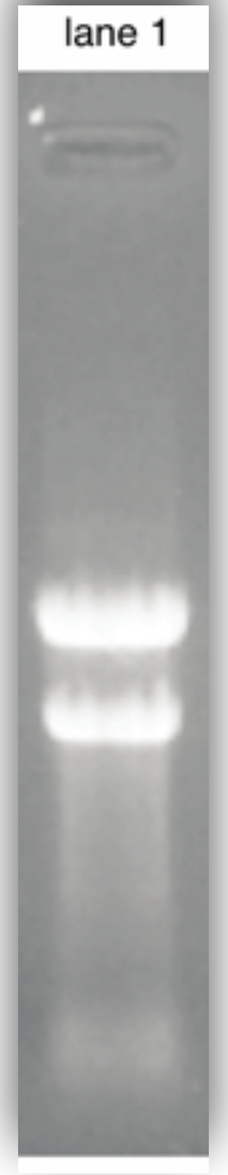

Figure 2.5A

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