

CHAPTER 11

JACOB/MESELSON/BRENNER: DISCOVERY OF MESSENGER RNA (mRNA)

François Jacob and Matthew Meselson, working together in 1960, determined that proteins are assembled on ribosomes in the cytoplasm of the cell. This finding demanded that there be a link between chromosome and ribosome—some way to transfer the information. Thus was born the messenger RNA hypothesis. Sydney Brenner went on with them to confirm the mRNA hypothesis in 1964.

HOW IS INFORMATION IN DNA EXPRESSED?

While for 25 years it had been clear the DNA contained the basic genetic information, by 1960 it was still not at all clear how that information was expressed. How did a difference in the sequence of nucleotide bases translate into the differences between an elephant and a flea? The boundaries of the problem had been roughed out even *before* the era of Watson-Crick DNA. It was shown that all of the enzymatic proteins that determine a cell's physiology, morphology, and development are specifically encoded as individual genes in the chromosomes. Thus the genetic information in DNA gains its expression via the synthesis of specific enzymes that act to determine the appearance (phenotype) of the individual. One might imagine DNA as consisting of a linear series of such genes, each specifying a particular enzyme. So the problem of gene expression is to understand how a linear sequence of nuclear bases is used to produce a corresponding linear sequence of amino acids in a protein.

IS THE CHROMOSOME A “PROTEIN TEMPLATE”?

How does the cell manage to do it? The simplest hypothesis would be that in some manner the proteins are put together as amino acid strings directly upon the DNA of the chromosomes. From the beginning, however, this hypothesis could be rejected, as it was known that proteins are synthesized in the cytoplasm (injecting mice with radioactive amino acids and using a radioactive-sensitive photographic emulsion clearly demonstrates that proteins are synthesized in the cytoplasm) while the chromosomes remain at all times in the nucleus. Indeed, protein synthesis seemed almost always associated not with DNA but rather with RNA, which occurred in small, concentrated particles throughout the cytoplasm. These particles, usually associated with cellular membranes, were called *ribosomes*, referring to their ribonucleic acid content.

RIBOSOMES AND PROTEIN SYNTHESIS

Were these ribosomes then the site of protein synthesis? It was possible to show that this was true in a very straightforward way: working with bacteria (their ribosomes are easy to purify), protein synthesis was monitored by adding radioactive sulfur to a growing culture (^{35}S ends up in the amino acids cysteine and methionine, and not in DNA or carbohydrate). The ribosomes could then be harvested and purified from cells by gently breaking open the cells and centrifuging the contents (the ribosomes band out on a sucrose gradient at a specific place). The radioactivity quickly appeared in the RNA band; newly-made protein was therefore in (or on) the ribosomes: these purified ribosomes apparently had, attached to them, proteins still in the process of being made. If the ^{35}S was administered as a short pulse only, and followed by a “chase” of normal cold ^{34}S , then the radioactivity remained associated with the ribosomes for only a brief period. Ribosomes harvested later after the pulse had lost most of the radioactivity seen when harvested sooner, and the ^{35}S label now appeared among the soluble proteins. The proteins newly-made during the period of

the pulse had been completed and released from the ribosomes. This experiment established that proteins are synthesized from amino acids on the RNA-containing particles—ribosomes—and are released from them when completed.

THE MESSENGER RNA HYPOTHESIS

The fact that ribosomes contained significant amounts of RNA suggested an alternative hypothesis for the mechanism of gene expression: perhaps the ribosomal RNA carried the genetic information from the nucleus to the cytoplasm, and used it to construct proteins there. This also proved not to be the case. If it were so, there should be many different kinds of ribosomes with different amount of RNA, just as there are many different genes coding for proteins of widely differing sizes. When ribosome were investigated however, they were found not to be heterogeneous but rather all were identical to one another.

If the genetic information encoded in nuclear DNA was expressed on the cytoplasmic ribosomes, and if the rRNA was not the vehicle that transferred the information from nucleus to cytoplasm, then some other element must have been acting as the carrier, or information vector. While most cellular RNA was rRNA, not all of it was—perhaps some other class of RNA acted as the genetic “messenger.” Reasoning along these lines, François Jacob and Jacques Monod hypothesized in 1961 that there might exist a special species of RNA synthesized directly from the DNA template of genes, which is then transported to the ribosomes where the *messenger RNA* (mRNA) base sequence, complementary to the genetic DNA sequence, provides the information for protein synthesis. After the protein is made, the mRNA would leave the ribosome, making way for other, potentially different, mRNAs.

THE EXPERIMENTS OF BRENNER, JACOB, AND MESELSON

The mRNA hypothesis was confirmed by Sydney Brenner, Jacob, and Matthew Meselson in a very simple way. They showed that when a virus infects a bacterial cell, a virus-specific RNA is made that is rapidly associated with *preexisting* bacterial ribosomes (figure 11.1). The bacterial ribosomes were normal and contained bacterial rRNA. The new viral RNA was something extra, not a permanent part of the ribosome, but only transiently associated with them. It was precisely the messenger RNA that their hypothesis had predicted should exist.

The essence of the mRNA hypothesis is that there exists a class of RNA molecule, the “messenger,” composed of many different individual mRNA molecules, each corresponding in base sequence to a particular segment of the DNA base sequence. Under this hypothesis, the ribosomal RNA is *not* gene-specific, and this is the key distinction of the messenger RNA hypothesis: the same ribosomes are seen as translating all the different mRNA molecules. The gene-specific information is in the postulated mRNA molecules, *not* in the ribosomes. The ribosomes under this hypothesis act as passive protein synthesis factories, fabricating protein from whatever blueprint they are provided by the mRNA.

What Brenner, Jacob, and Meselson did was to change the blueprint and watch to see if the old factory obeyed the new instructions. They first grew the bacterium *E. coli* for several generations on synthetic medium containing heavy isotopes ($^{15}\text{NH}_4\text{Cl}$ and ^{13}C glucose). As the bacteria used these heavy-isotope carbon and nitrogen sources as raw material to synthesize their carbohydrates, proteins, and nuclei acids, all of their components became heavy-labeled (because of being made up of carbon and nitrogen atoms containing additional neutrons), including the bacterial ribosomes.

They then changed the genetic instructions by infecting the bacterial cells with the T4 virus. It was known that such viruses destroy the bacterial DNA, substituting their own DNA as the genetic information that the bacteria would use to direct the synthesis of (viral) protein. If the ribosomes carried gene-specific information, then new virus-specified ribosome should be made as well. If, on the other hand, the ribosomes are passive sites of synthesis, the old bacterial ribosomes could be used by the virus-commandeered cell to make virus-directed protein. The distinction here concerned the source of the

information in protein synthesis, which occurred on the ribosomes. If the information could be shown not to reside in the ribosomes, then it had to have been transported there by some other element—the messenger.

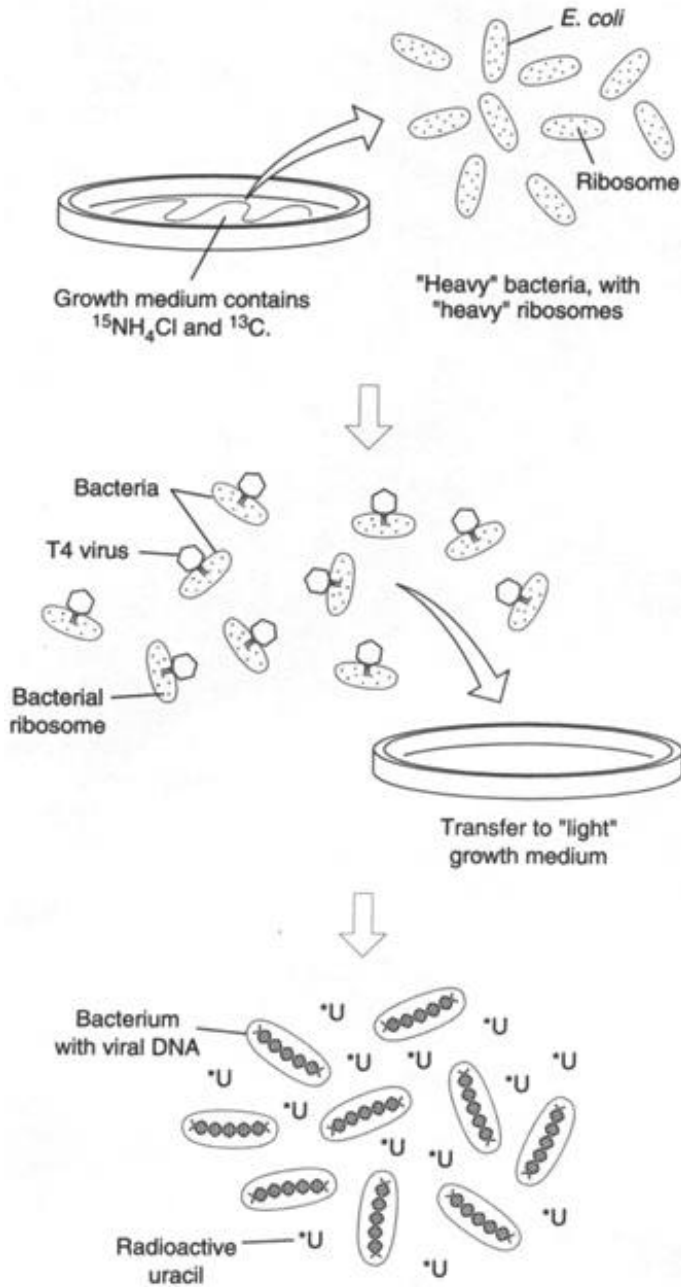
The experimental issue, then, was whether or not the ribosome used by infected cells to make virus proteins needed to be newly-made according to virus specifications, or whether old bacterial ribosomes would do the job. Brenner, Jacob, and Meselson, by starting with density-labeled cells, were able to choose between these two alternatives in a direct way: they transferred the bacterial cells to “light” normal medium at the time of bacterial infection by the T4 virus. Any newly-made ribosomes would be expected to be light in density, compared to the heavy bacterial ones. Radioactive RNA precursor (uracil) was added after infection to see if new RNA was made, and if so, where it went. After a short incubation time to permit the virus-directed synthesis, the bacteria were lysed and the ribosomes centrifuged in a CsCl density gradient.

CONFIRMATION OF THE mRNA HYPOTHESIS

The virus-infected cells did not make new “light” ribosomes. The only ribosomes seen in their results were the original “heavy” bacterial ones. Thus, the T4 virus indeed utilized the old bacterial ribosomes to synthesize new virus protein. This result established the messenger hypothesis. The researchers concluded that the nature of the messenger was RNA. New virus-directed RNA was made after infection. ¹⁴C-labeled RNA was detected, and the new radioactively-labeled RNA was associated with *old* ribosomes!

The newly-made radioactive RNA could be dissociated from the bacterial ribosomes, and tested for similarity to T4 virus DNA and to bacterial DNA. The base sequences were then compared to determine if there were enough similarities for complexing with single-stranded form of DNA. Such DNA/RNA hybrids readily formed between the newly-isolated radioactive RNA and T4 virus DNA, but did not form with bacterial DNA. Clearly, the new RNA was viral in nature.

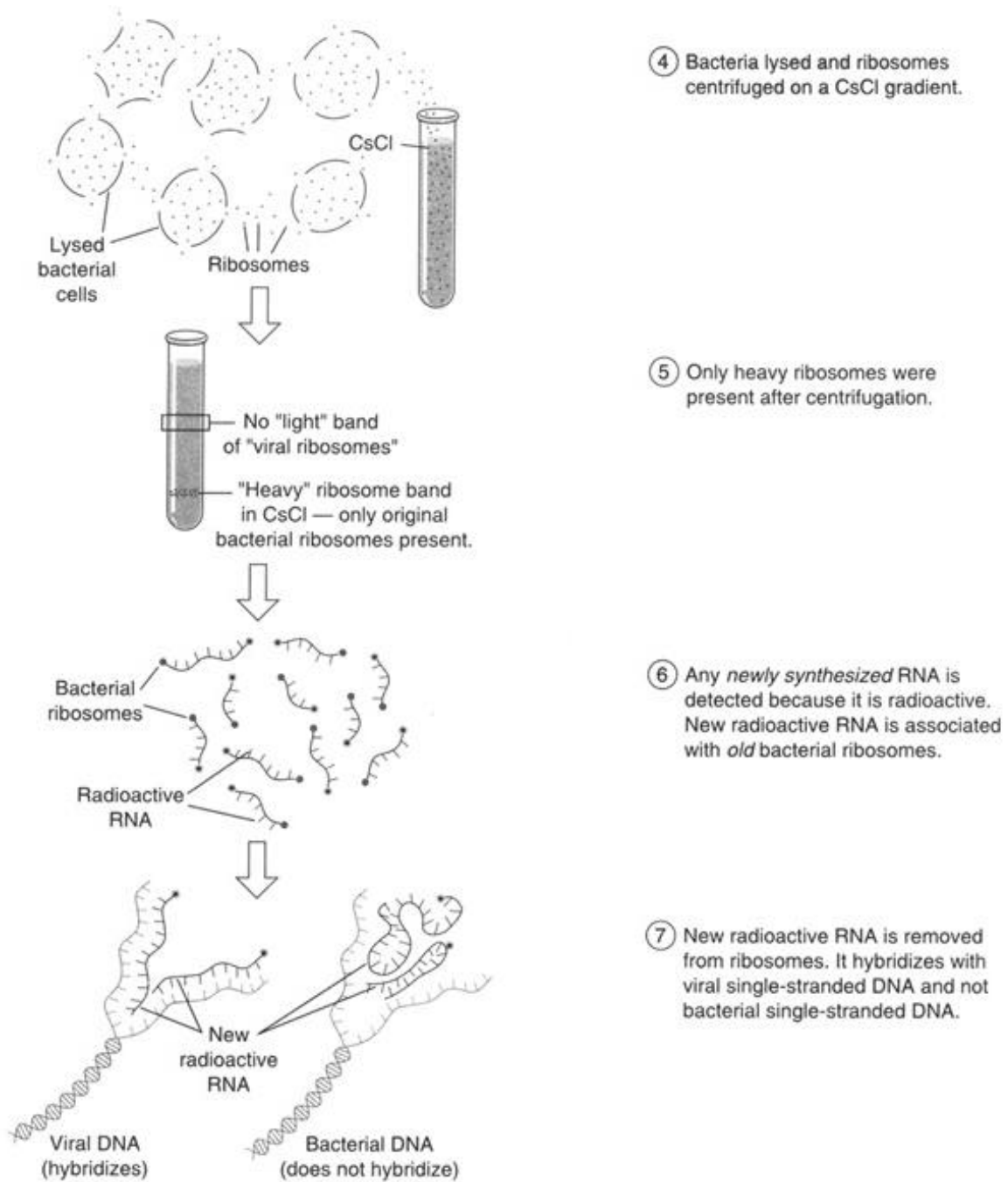
These experiments firmly established that: 1. The expression of the viral genes is associated with the formation of new virus-specific RNA molecules (mRNA). 2. Ribosomes are not involved in viral gene expression except as passive sites of synthesis. 3. The new messenger RNA has a base sequence complementary to DNA, and presumably originated there. 4. The new mRNA can be isolated complexed to ribosomes. It follows that these new RNA molecules are indeed the genetic messengers visualized by Crick, carrying information from DNA to the ribosome.



① Bacteria grow for several generations on heavy-isotope-laden media, yielding "heavy"-labeled bacteria.

② Bacteria infected with T4 virus, which destroys bacterial DNA, and substitutes viral DNA. Bacterial cells are simultaneously transferred to a "light" (non-heavy-isotope-labeled) medium.

③ If ribosomes have their own DNA, they'll now make viral ribosomes (which will not be "heavy" like the *E. coli* ribosomes). Radioactive RNA precursor (* uracil) was added and time was allowed for virus-directed synthesis to proceed.



④ Bacteria lysed and ribosomes centrifuged on a CsCl gradient.

⑤ Only heavy ribosomes were present after centrifugation.

⑥ Any newly synthesized RNA is detected because it is radioactive. New radioactive RNA is associated with old bacterial ribosomes.

⑦ New radioactive RNA is removed from ribosomes. It hybridizes with viral single-stranded DNA and not bacterial single-stranded DNA.

Figure 11.1
Brenner, Jacob, Meselson experiment.