

CHAPTER 12

SZYBALSKI: ONLY ONE STRAND OF DNA IS TRANSLATED

In 1967, Waclaw Szybalski and his collaborators used a simple virus to demonstrate that after the DNA separated into two strands during the transcription stage of protein synthesis, only one strand was copied by mRNA to make a protein.

WHY WOULD ONLY ONE STRAND OF DNA BE TRANSLATED?

Waclaw Szybalski and his colleagues investigated the phenomenon in a simple virus, T7 (figure 12.1). They were able to isolate the two DNA strands of T7 separately by making use of the fact that one strand had regions rich in cytosine. They heated the DNA to break it into separate strands and then added a synthetic RNA compound called poly-UG (composed of uridelic acid and guanylic acid), which had a high affinity for the cytosine regions on the DNA strand. By allowing the mixture to cool, the DNA strand rewound (reannealed) and incorporated the poly-UG RNA into the reassembly. The material was then centrifuged on a CsCl gradient. DNA-RNA hybrids are denser than DNA-DNA hybrids (RNA nucleotides have an extra oxygen atom in their ribose sugars and are heavier), so that the cytosine-rich T7 DNA strand with bound poly-UG was denser than the other T7 DNA strand, which binds far less RNA. In this fashion the two T7 DNA strands were separated as two distinct bands on the CsCl gradient. Szybalski could then test the two strands to see which one was able to bind T7 messenger RNA. Only one strand proved complementary to the virus mRNA: the “light” strand. Thus, only this strand of DNA was translated into mRNA. Similar experiments have been carried out in which ^{14}C -labeled natural mRNA is substituted for poly-UG. Again, it binds preferentially to one strand.

“EARLY” AND “LATE” GENES

Is it the same strand always translated? In more complicated organisms, could some genes be read from one strand while other genes are read from the other? Or must all genes be read from the same strand, as in the virus T7? Many investigators carried out experiments addressing this issue. Among the most elegant are those of Jayaraman and Goldberg. They worked with another virus, T4. They chose T4 because some of its genes are transcribed into mRNA early in infection, while others are transcribed later. Are “early” and “late” genes read from the same strand? Jayaraman and Goldberg separated the T4 DNA into heavy and light strands, and challenged each separately with “early” mRNA and “late” mRNA. They added a DNA endonuclease that degraded single-stranded DNA, so that any DNA not bound by the mRNA was degraded. They could then ask which DNA strand bound which mRNA by looking to see which gene survive the degradation step (e.g., if “early” mRNA bound light DNA, then in this combination viable early-gene DNA would survive).

Each of the four combinations was tested for ability to convert mutant virus to wild-type by *transformation* (the same technique used by Hershey and Chase). Jayaraman and Goldberg found that a T4 viral mutant in an early gene (*rIIB*⁻) could not be transformed to wild-type by either of the preparations made against the heavy strand; no early-gene, heavy-strand DNA survived the degradation. Thus, the early gene *rIIB* was not read from the heavy DNA strand. Looking at the two light DNA strand preparations, they found that when early mRNA was added, the corresponding early-gene DNA *did* survive, and was able to transform the early-gene mutant *rIIB*⁻ to *rIIB*⁺. As expected, late mRNA did not afford the early-gene DNA this protection. From these results one can conclude that the early gene *rIIB* is read from the light DNA strand. The late genes gave the reverse pattern, with transforming ability only being obtained from the heavy DNA

strand-late mRNA combination. These experiments established that DNA is indeed read into mRNA from only one strand, but that different genes are transcribed from different strands.

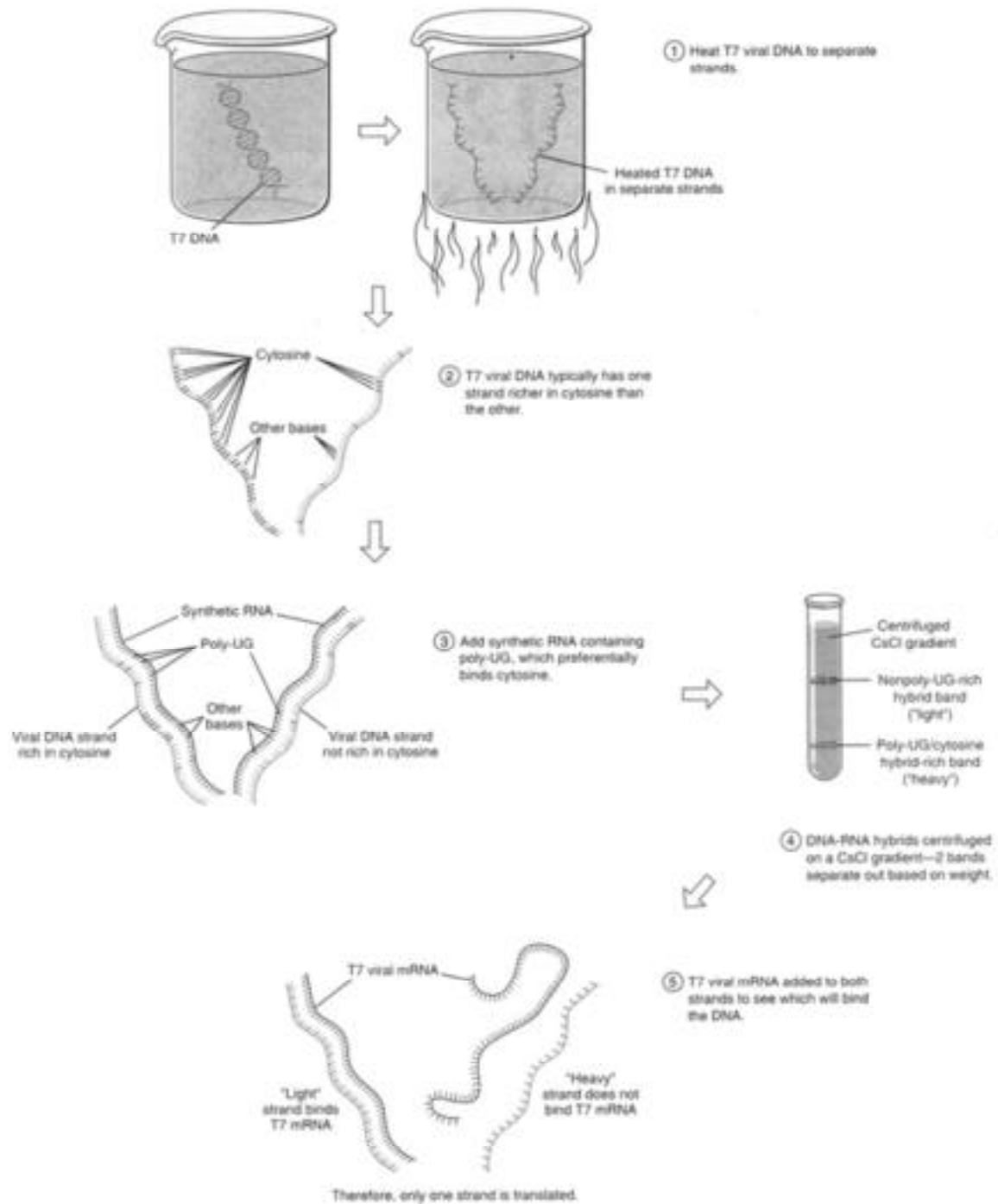


Figure 12.1
Szybalski's experiment.