Chapter 2 Answers

1.   Griffith showed that nonvirulent bacteria could be transformed to a virulent state if they were mixed with heat-killed virulent bacteria. He demonstrated that this transformation was hereditary by showing that the virulence was not only present in the transformed bacteria, but it was present in their descendents as well. In other words, these transformed cells were capable of passing the genetic information for virulence to their progeny.

Without knowing whether or not subsequent generations were also virulent, it was also possible to conclude that the cells became virulent because they received a factor directly responsible for virulence, rather than the genetic information coding for the factor. In that case, however, the virulence factor would have been diluted upon division, and would not have been present in subsequent generations.

2.   Both Avery et al. and Hershey and Chase tried to pinpoint the biochemical source of the genetic material; the specific ways in which they did this, however, differed in several respects.

Avery et al. used an indirect, functional approach to identify the genetic material. For example, their assay was based entirely upon the ability to render rough bacteria virulent. Also, they demonstrated the essential role of nucleic acids in the process not by showing that nucleic acids were *sufficient* for the transforming activity, but by showing that they were simply *necessary*. The destruction of nucleic acids in their sample using nucleases was enough to eliminate the transforming ability.

In contrast, Hershey and Chase took a more direct, physical approach to finding the genetic material. At that time, scientists already had a relatively clear understanding as to what molecules were present in many viruses. This allowed Hershey and Chase to ask which molecules are physically transmitted by the virus to the bacterial cell upon infection. To do this, they used two different radioactive labels to specifically tag both the protein and nucleic acid components of the virus, and then asked which of these components ends up in the bacterial cell following infection and in the progeny phage. Their assay was thus based on the detection of a specific radioactive isotope in the infected cells (and their descendents), rather than on any functional property of the cells following infection.

3.   The absence of a role for nucleic acids was demonstrated by treating the material responsible for the disease with agents that destroy nucleic acids, such as nucleases or UV

irradiation. While these treatments should destroy whatever nucleic acids are present in the material, they were found to have no effect on infectivity. In contrast, methods designed to eliminate proteins in the material, such as treatment with phenol or proteases, were capable of reducing the infectivity.

4.   Chargaff examined the relative proportions of adenine, cytosine, guanine, and thymine in DNA, and made the key discoveries that the different nucleotides were not all present at the same concentrations in cells; that their relative levels differed in different organisms; and that adenosine and thymine, and cytosine and guanine, are present at similar levels. This work suggested that the genetic information may be somehow encoded by the precise arrangement of the different nucleotides within the DNA.

5.   The high-quality X-ray diffraction images obtained by Wilkins and Franklin suggested that DNA was helical and was composed of more than one polynucleotide chain. This knowledge helped Watson and Crick narrow down the possibilities in their work on modeling the structure of DNA.

6.   Meselson and Stahl wanted to address whether replication occurs in a conservative manner, in which the parental strands are copied but then remain together after replication, a semi-conservative manner, in which the duplicated DNA includes one parental strand and one newly synthesized strand, or a distributive manner, in which the new DNA is present in both strands of the new DNA.

To distinguish between these three possibilities, Meselson and Stahl developed a method that allowed them to separately label the parental and daughter strands of DNA during replication and then to ask where each of the strands were in the replicated DNA. In this way, they were able to address whether all of the "parental" label remained intact after replication, or whether the parental label and the daughter labels were present in a single double helix of DNA.

In their experiments, they grew bacteria in culture medium that contained heavy isotopes, such as N^{15}, so that all of the DNA in the cells contained the isotope. They then shifted the cells to ("light") medium that didn't contain the heavy isotope and let the cells undergo one round of DNA replication. They then isolated the DNA from the cells and separated it in a very precise way that allowed them to distinguish DNA that contained two heavy strands, two light strands, or one heavy and one light strand. In so doing, they observed

that the newly replicated DNA contained one heavy and one light strand, which is entirely consistent with semi-conservative replication. Conservative replication, in contrast, would have yielded DNA with either two heavy or two light strands and distributive replication would have included strands with an intermediate density between heavy and light.

7.   See Figure 2-7 for a depiction of the polymerase reaction. The sequence of the new strand of DNA is determined by the sequence of the template DNA. This was demonstrated by incubating DNA of varying nucleotide composition in the presence of polymerase and nucleoside triphosphates. The nucleotide composition of the DNA strand produced in this reaction precisely mirrors that of the DNA originally added to the reaction mixture.

8.   The conclusion that DNA must not be the template for protein synthesis was made based on the observation in eukaryotic cells. In these cells, DNA is exclusively present in the nucleus, yet protein synthesis occurs in the cytoplasm. It was postulated that some sort of intermediate molecule must carry the information from the nucleus to the site of protein synthesis.

      RNA was identified as the likely template because of its presence in the cytoplasm, and because, given the structural similarity of RNA to DNA, it was easy to imagine how the DNA sequence could be copied as RNA, which could then move to the cytoplasm. Also, the development of cell-free extracts allowed researchers to isolate the various cellular components that are required for protein synthesis, and this indicated the importance of RNA in the process. Definitive evidence for the activity of mRNA in protein synthesis was provided by observations in T4 infected cells, where it was found that, upon infection, RNA is produced that has an identical base composition to the T4 DNA, and that this RNA binds to ribosomes, leading to protein synthesis.

9.   One major difference lies in the structure of the sugar moiety of the nucleic acid: ribonucleic acids include a hydroxyl group at the 2' carbon of the sugar moiety, whereas the 2' carbon of deoxyribose has only hydrogen. A second difference is that RNA contains the base uracil, whereas DNA contains thymine. Finally, DNA is almost exclusively present in its double-stranded form in vivo, whereas RNA is largely single-stranded.

10.   The central dogma states that genetic information flows from DNA to RNA to protein. In other words, DNA stores the genetic information within the chromosomes in the nucleus.

This information is transcribed onto RNA, which then exits the nucleus, and the RNA is then translated into protein. Even though the dogma is almost always valid, exceptions have been found such as the copying of DNA from RNA templates during the life cycle of retroviruses.

11.   Before Crick's proposal that an adaptor molecule might intervene between mRNA and amino acids, scientists had speculated that RNA may fold into precise three-dimensional forms that specifically recognize particular amino acids. Crick rejected this suggestion as unlikely, because RNA bases are mostly hydrophilic, yet the side chains of numerous amino acids are hydrophobic. In addition, the strong structural similarity between certain amino acid side chains made it difficult to imagine how different RNA structures could accurately distinguish between them.

        The adaptor was ultimately found to be tRNA.

12a.   These three types of RNA differ in multiple respects. For example, both rRNA and tRNA are quite different from mRNA in terms of size and complexity. tRNA molecules are quite small (less than 100 nucleotides), and are relatively uniform in sequence. rRNA molecules are rather large and fall into several discrete sizes, and are also relatively uniform in sequence. mRNA, in contrast, is extremely diverse, both in terms of size—ranging from very small messages of less than 100 nucleotides to very large messages of many thousands—and in terms of complexity, as the collection of mRNA sequences in the cell must be sufficiently diverse to code for all of the different proteins made in the cell. Also, the GC content of rRNA is relatively high. Finally, the abundance of the three types differs within the cell, with rRNA making up some 85 percent of the total RNA, tRNA about 10 percent, and the rest being mRNA.

b.   Whereas rRNA binds directly and stably to the ribosomal proteins, mRNA only interacts transiently with already assembled ribosomes. Also, while a given rRNA molecule forms part of a single ribosome, a single mRNA molecule can associate with multiple ribosomes (forming a polyribosome).

13.   The codons must have sufficient complexity to be able to code for all 20 amino acids used during protein synthesis. Codons of only two nucleotides could only specify (4^2) 16 different combinations and would therefore not be sufficient to specify all of the amino acids. While four-nucleotide codons would be possible, there would be a great excess of potential codons compared to the number of different amino acids. This would both needlessly

increase the size of the genome and also require the cell to wastefully produce a much larger, but unnecessary, number of different tRNA species.

14.   Mutant classes C and F should show very little phenotypic difference from the wild-type gene, whereas classes A, B, D, and E should show a mutant phenotype. In these classes, the addition of one, two, four, or five nucleotides would change the reading frame of the coding sequence, altering the identity of all subsequent amino acids and almost certainly destroying the function of the encoded protein. In contrast, the addition of three or six nucleotides (in classes C and F) would not change the reading frame but would simply add one or two amino acids to the protein sequence. While this may have some effect on the protein's activity, it is likely that the protein would still function even with these extra amino acids.

        The fact that insertions of any number of nucleotides that is not a multiple of 3 causes a mutant phenotype indicates that the codons must consist of three nucleotides.

15.   They created synthetic RNA molecules of a known sequence, added them to a cell-free translation system, and examined the amino acid sequence of the proteins that were produced. The first such molecule they made was poly-U, or UUUUU…, which gave rise to a polypeptide composed exclusively of the amino acid phenylalanine.