MOLECULAR BIOLOGY AND BIOCHEMISTRY

Problems and Applications

DAVID FREIFELDER

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BRANDEIS UNIVERSITY



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Preface

In any experimental science it is important that students have practice in dealing with experimental observations. Carefully selected problems and questions can give students such practice; furthermore, these give a student an opportunity to test his or her understanding of textbook and lecture material and to put together various facts in order to draw conclusions not explicitly stated elsewhere. In the past, in my own course in molecular biology, I provided homework problems which I myself wrote, since the existing textbooks contained few problems. Writing problems for homework is a time-consuming operation compounded by the worry that a problem might be thought ambiguous or be misconstrued by the student who perceives details more sharply than concepts or, even worse, might not be answerable. All teachers face this problem but, after several years, usually accumulate a set of unambiguous, solvable problems.

In an effort to provide students and teachers of molecular biology, genetics, and biochemistry with useful problems and questions, I have collected homework sets and examinations compiled by several well-known molecular biologists who have been teaching—and assigning problems—for many years. These sets of problems form the core of this book. They range in difficulty from medium to challenging. As I arranged the material for this book, I felt it best to add about 300 relatively simple problems and questions for the beginning student.

It is intended that this book be used with the most widely used texts in molecular biology, genetics, and biochemistry. Since the chapter headings I have used in this book do not match those of every text, I have included lists at the front of the book of the problems appropriate for chapters in the major texts. Furthermore, an index is provided in which the problems are sorted by topic.

How to Use This Book

Molecular Biology and Biochemistry is meant to be a study guide, to teach as well as to test one's understanding. It is assumed that students using this book are taking a course in molecular biology or genetics and are reading an adequate textbook.

Each chapter deals with a separate subject and begins with several pages of explanatory material. These introductions are not meant to replace a textbook, and some cover only some of the topics within the chapters. Their purpose is to summarize basic material, define terms that must be known to solve the problems, provide some material that frequently is not contained in the usual textbooks, and to restate those points experience has shown students in molecular biology courses often miss or forget.

Students who supplement the introductions with the literature referenced at the end of each introduction should be able to solve all the problems.

The problems marked with an open circle (0) are elementary (though not necessarily simple) and are included so that students can be sure they understand various terms and know basic facts and concepts. The elementary problems should be answered first. If unable to answer any of these, a student should not proceed further until having done some review.

The problems marked with a solid circle (•) are difficult and often are beyond the range of a beginning course.

Answers to selected problems—those designated with boldface numbers—are given at the end of the book. Inasmuch as the problems have been grouped by subject, when several problems deal with the same subject, generally only one is answered. Similarly, if a problem has several parts which differ only slightly, only a few parts are answered. Answers are usually not as brief as a single word, except for very simple problems; in general, the answer is in the form of an explanation, which should itself be a teaching guide.

The section "Problems Useful for Particular Textbooks" at the beginning of the book and the "Index of Problems" at the end are intended to help students help themselves to a great extent: the former offers a convenient way to locate problems pertinent for use with various texts, and the latter is organized by concept so that (1) if a student wishes to test his or her ability in a given subject, appropriate problems can be located, and (2) if a difficult problem is encountered and one thinks it deals with a particular topic, a student can look up that topic to confirm that it is indeed applicable to the problem being solved.

I would like to express thanks to Bruce Alberts, Rich Calendar, Hatch Echols, Julie Marmur, Frank Stahl, and Bob Warner for supplying problems, to many of my students for reading the problems and reporting difficulties they experienced while solving them, to Ric Davern, who suggested that I gather the best problems of experienced molecular biologists, to Phil Hanawalt, who suggested that a problems book would be more useful if it were also an informative study guide, to Jay Magno, for the art work, and to Mildred Kravitz and Barbara Nagy, who typed the many drafts of the manuscript.

Waltham, Mássachusetts July 1978 David Freifelder

Problems Useful for Particular Textbooks

In the following lists, beneath each textbook citation, "chapter" refers to chapters within that textbook and "problems" refer to problems within this book.

Davis, B. D., R. Dulbecco, H. N. Eisen, H. S. Ginsberg, and W. B. Wood. 1973. *Microbiology*: New York. Harper & Row.

Chapter	Problems
9	2-2 to 2-6; 17-1 to 17-24, 17-26, 17-35, 17-40, 17-42
10.	4-1 to 4-12, 4-18 to 4-61; 5-1 to 5-33, 5-35, 5-39 to 5-47; 7-1
11	1-24 to 1-27; 5-34; 7-1 to 7-13, 7-16, 7-18, 7-20 to 7-34; 8-1 to 8-15, 8-51 to 8-54
12	7-13, 7-23; 9-1 to 9-10; 10-1 to 10-25; 11-1 to 11-17
13	8-16 to 8-36, 8-39 to 8-55; 12-1 to 12-17
44	14-27; 17-18 to 17-28
45	15-1 to 15-44, 15-79, 15-80; 16-3, 16-31 to 16-33, 16-44 to 16-50
46	15-45 to 15-72, 15-76, 15-77, 15-81 to 15-85; 16-4 to 16-6, 16-30, 16-36 to 16-38, 16-41
47	1-1 to 1-5, 1-17

Kornberg, A. 1974. DNA Synthesis. San Francisco. W. H. Freeman and Company.

Chapter	Problems
1	4-1 to 4-72
4	5-1, 5-2, 5-17, 5-24; 6-1, 6-2; 7-1, 7-3, 7-5, 7-23
5	5-1, 5-2, 5-4, 5-17
6	6-13; 16-15
7	5-1 to 5-47
8	5-3; 6-3; 15-27, 15-28, 15-29
9	6-5, 6-16, 13-1 to 13-13, 16-44, 16-45, 16-47, 16-48, 16-50
10	8-1 to 8-56
11	6-15

Lehninger, A. L. 1974. Biochemistry. New York. Worth Publishers, Inc.

Chapter	Problems*
5	3-1 to 3-10
6	3-11 to 3-24
9	12-1 to 12-17
31	2-1 to 2-16; 4-1 to 4-73; 7-1 to 7-33
32	5-1 to 5-47; 6-1 to 6-17; 13-1 to 13-13
33	8-1 to 8-16; 10-1 to 10-25
34	9-1 to 9-21; 11-1 to 11-17
35	8-17 to 8-56

^{*} Many of these problems are at a more advanced level of molecular biology than the sections in Lehninger. They are included because many could be worked if supplementary information were given by the instructor.

Lewin, B. 1974. Gene Expression, Volume I. New York. Wiley-Interscience.

Chapter	Problems
1	7-6, 7-8, 7-10, 7-18, 7-28; 9-1 to 9-19; 11-1 to 11-17
2	10-1 to 10-25
3	7-7, 7-17; 8-38; 10-1 to 10-25
5	9-1 to 9-19; 10-1 to 10-25; 11-1 to 11-17; 15-35, 15-37, 15-41 to 15-44, 15-48
6	8-1 to 8-56; 15-46
7	8-19, 8-20, 8-23, 8-26, 8-28 to 8-35, 8-38 to 8-40
8	8-37; 15-45 to 15-68, 15-70; 16-8, 16-9
10	5-1 to 5-47; 6-1 to 6-17; 7-1, 7-4, 7-23
11	13-1 to 13-12
12	16-1 to 16-50
13	5-19, 5-37 to 5-40

Stryer, L. 1975. Biochemistry. San Francisco. W. H. Freeman and Company.

Chapter	Problems
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29	6-5, 6-13; 15-1, 15-27 to 15-31, 15-43, 15-45, 15-46, 15-55; 17-1 to 17-28

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5	1-17 to 1-21; 7-19; 8-5
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8	4-1 to 4-61; 5-1 to 5-47; 6-1 to 6-17; 7-1, 7-13; 9-1 to 9-20
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16	7-13; 9-1 to 9-20
17	None
18	5-1 to 5-47
19	13-1 to 13-13
20	5-37, 5-38; 8-16 to 8-36, 8-39 to 8-53; 15-46 to 15-51
21	None

Suzuki, D. T., and A. J. F. Griffiths. 1976. An Introduction to Genetic Analysis. San Francisco. W. H. Freeman and Company.

Chapter	Problems
1	1-17 to 1-22
4	1-23 to 1-27
6	16-1 to 16-9
8	2-2 to 2-4; 16-1, 16-2
10	2-5, 2-6; .4-1 to 4-4, 4-6 to 4-9, 4-17, 4-19 to 4-21, 4-34; 5-5 to 5-8, 5-31, 5-32, 5-37, 5-38, 5-42; 11-1 to 11-17
11	9-1 to 9-20; 10-1 to 10-25
12	7-1 to 7-33
13	8-17 to 8-23, 8-27 to 8-31, 8-33 to 8-35.

Watson, J. D. 1976. Molecular Biology of the Gene. Third Edition. Menlo Park, Calif., W. A. Benjamin.

Chapter	Problems
1	1-1 to 1-6; 17-2 to 17-7
2	1-10 to 1-16; 2-2 to 2-6; 4-3
3	None
4	3-1 to 3-24; 4-6 to 4-11, 1-18 to 4-20, 4-23 to 4-30
5	None
6	3-1 to 3-34
7	1-24 to 1-27; 15-1 to 15-11; 16-1 to 16-27, 16-31, 16-34, 16-35, 16-42
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11	7-16, 8-1 to 8-15, 8-51 to 8-54
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1

A Few Basics: Cells, Biochemistry, Classical Genetics

Introduction

The study of molecular biology and biochemistry combines information from biology, chemistry, physics, and physical chemistry. Usually, textbooks of molecular biology and biochemistry restate fundamental material that students are expected to know from prior courses; however, biology is often considered to be so elementary that a review of biology is omitted. In this chapter, problems are provided to enable you to determine whether essential biological concepts are part of your repertoire. Necessarily, many of the problems ask only for definitions, since it is important to know the basic vocabulary of biology.

Several of the problems (1-4 through 1-7) are designed to make you think about both the smallness of cells which are used in molecular biological studies and the number of molecules contained in these cells. Some types of cells are quite small and the number of molecules per cell is likewise very small. Per cell, the number of some molecules, for example DNA, is strictly regulated and is the same for all cells of the population. However, those molecules that can freely diffuse through a cell wall or those which are themselves regulators may be present within a cell in numbers determined by the Poisson distribution. This means that individual cells in a population may at any instant be quite different from one another with respect to the concentra-

Chapter 1

2

tion of a particular molecule. Averaged over a long period of time, fluctuations in the number of a particular molecule may be unimportant, but they are important if the particular molecule is an inhibitor and its concentration drops at any time below a critical value (or even to zero), for then an inhibited process may suddenly be turned on. Molecular numbers are also important when considering molecules that are synthesized on demand (for example, inducible enzymes), since a relatively small change in the number of a particular molecule per cell can result in a very large percentage change in concentration.

A large part of this chapter (problems 1-17 through 1-27) reviews simple Mendelian genetics and genetic recombination. As one studies molecular biology, it will rapidly become apparent that theories about the behavior of important biological molecules frequently grew out of simple genetic experiments. In the early period of molecular biology. these experiments merely involved either determining the relative order and positions of genes with respect to one another (that is, genetic mapping) or determining dominance relations. Problems 1-17 through 1-21 are concerned with the simplest aspect of the genetics of diploid organisms. A diploid organism containing a normal (N) and mutant (n) copy of a particular gene is said to have the genetic composition or genoture Nn. That is, each cell contains one copy of the N-type gene (called the N allele) and one of the n-type (n allele). When gametogenesis (the production of either sperm or egg) occurs and haploid germ cells are produced, the N and n alleles segregate at random; this means that in a large population of sperm cells, on the average, half of the sperm will contain the N allele and half the n allele. This will be true of the population of eggs also. If a single egg is produced. this means that there is a 50 percent chance of the egg having genotype N and a 50 percent chance of n. Thus, when a collection of sperm meets one or more eggs, since the probability of fertilization of an egg is independent of the genotype of either sperm or egg, on the average, the fertilized egg (the zugote) has the same probability (that is, 25 percent) of having each of the genotypes nn, Nn, nN, and NN. Nn and nN are the same genotypes, so this results in the familiar 1:2:1 Mendelian ratio of the progeny: 25 percent nn, 50 percent Nn, and 25 percent NN. An extension of this idea to a system having two or more genes is simple if one remembers that, in general, different genes assort independently. This means that if an organism has the genotype AaBb, the gametes AB, Ab, aB, and ab will each be produced with 25 percent probability.

These considerations enable one to determine the genotypes of the progeny. However, the *phenotype* (the observable character) depends upon whether the N or n allele is *dominant*. N is defined to be the dominant allele if the phenotype of Nn is the same as that of NN.

Problems 1-17 through 1-20 give the student practice in going through the mechanics of gametogenesis, zygote formation, and determination of the genotypes and phenotypes of the offspring.

In problem 1-21 the student is asked to perform a pedigree analysis—that is, to guess the genotypes of various members of a family tree if the phenotypes of some members are known. To perform such an analysis, one first writes down the genotypes of each family member whose genotype is unambiguously defined by the phenotype. For instance, if black (B) is dominant to blue (b) and a mating between a black and a blue gives one or more blue offspring, the black must have had the genotype Bb and not BB in order to contribute a blue (b) gamete to the blue (bb) offspring. Conversely, if a blue (bb) organism mates with an unknown parent and a black (BB or Bb) offspring results, the unknown parent must contain a B allele. One does not know however whether this parent was BB or Bb. Furthermore if two black (BB or Bb) parents give rise to a blue (bb) offspring, both parents must have had a b allele (each must have the genotype Bb).

Genetic mapping is based upon a simple principle: when two chromosomes (or DNA molecules) interact and exchange material by genetic recombination, the probability of exchange at any one point is the same as that at anu other point. This means that the probability of an exchange occurring between two genetic markers is proportional to the distance separating these markers. The recombination frequency, the ratio of recombinant types to parental types, is an expression of this probability, so we may say that the recombination frequency is proportional to the distance between the two markers. This can best be seen in the following elementary example. We cross two parents with genotypes Ab and aB and measure the recombination frequency, (AB + ab)/(Ab + aB), between A and B. Suppose this is 1 percent. This is often written: $A \times B$ is 1 percent. In another cross in which the parental genotypes were Ac and aC, we might find that $A \times C$ gives a recombination frequency of 2 percent. Thus, we may conclude that the distance between A and C is twice that of A and B (that is, 2 percent divided by 1 percent). We now know the distances but not the gene order. This can be determined by performing a third cross. Of the three possible orders ABC, ACB, and BAC, only one-ACB-is excluded by the data above since that order would require that the recombination frequency for $A \times B$ be greater than that of $A \times C$, which is not the case. The other possibilities can be distinguished by performing the cross $B \times C$, since for the two orders ABC and BAC, the recombination frequency for $B \times C$ would be 1 percent and 3 percent, respectively. You can gain practice in these manipulations by doing problems 1-22 through 1-27. Note that in some of the problems the gene order can be determined from a single cross; for these problems

one need only remember that the probability of the occurrence of two events is the product of the probabilities of occurrence of each event. Thus, if a cross is to be made with three markers, the production of some recombinant types requires one exchange and the production of others requires two exchanges; recombinants needing two exchanges will occur with lesser probability than those needing one. Problems 1-25 and 1-27 illustrate this method.

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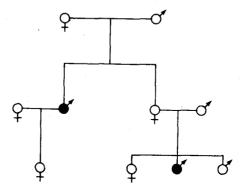
Problems

- ol-1. Define the following terms: Prokaryote; eukaryote; nucleolus; prophase; metaphase; anaphase; telophase; karyotype; haploid; diploid.
- o1-2. Which of the following cellular components contain DNA and which contain RNA? Mitochondrion; endoplasmic reticulum; nucleus; nucleolus; chloroplast; cytoplasm.
- o1-3. Describe some of the experimental evidence that chromosomes contain DNA.
- ol-4. Roughly how many DNA molecules are contained in a single eukaryote chromosome?
 - 1-5. Roughly how many DNA molecules are contained in the nucleus of a single human cell and in the cytoplasm of a single human cell? (The second number can be estimated.)
- •1-6. A cell of Escherichia coli is cylindrical, about 1μ in diameter and 3μ long. At pH 7, how many H⁺ ions are there in a volume the size of one cell? Note that, statistically, this number would vary greatly from cell to cell. Since many reactions are strongly pH-dependent, do you think that such fluctuations, if they in fact occur, would

introduce significant heterogeneity among the individual cells of a population? Explain. E. coli is capable of normal growth in nutrient media having a wide range of pH values. This is probably because the pH within the cell is not the same as that of the surrounding medium. How do you think a cell might regulate its internal pH?

- 1-7. A cell of *E. coli* contains about 10^{-14} g of DNA. A DNA strand is 20 Å wide and has a mass of about 2×10^6 daltons for each μ of length. What fraction of the volume of *E. coli* is DNA?
- 1-8. E. coli has a cylindrical shape about 1μ in diameter and 3μ long. The doubling time of E. coli when growing on nutrient agains about 25 minutes. After 12 hours of growth, a colony is roughly 2 mm in diameter and $\frac{1}{2}$ mm high. Have all of the cells been growing for 12 hours?
- •1-9. Some bacteria have the property that the colonies formed on an agar surface are very large—for example, 5–10 mm in diameter. It is known that, if methyl cellulose is incorporated into the agar (this has the effect of greatly increasing the viscosity of the agar), the colonies will be much smaller. Furthermore, these colonies will frequently be surrounded by a slightly translucent film that extends over a large area. What might be the cause of this colony morphology?
- 1-10. What is the essential difference between an enzyme and a coenzyme? What kinds of reactions are typically carried out by coenzymes?
- 01-11. Distinguish between anabolic and catabolic reactions.
- 01-12. What is the principal biological role of the glycolysis reaction and the Krebs cycle?
- 01-13. Which of the following are true statements?
 - (a) Enzymes affect the direction of a chemical reaction.
 - (b) Enzymes alter the speed of a chemical reaction.
 - (c) Enzymes are rarely, if ever, consumed in chemical reaction.
 - (d) Enzymes are always proteins.
 - (e) A particular enzyme can catalyze reactions involving many different substances.
 - (f) Enzymes frequently carry out different types of reactions involving a single chemical group.
- ol-14. How is the energy generated during metabolic processes usually stored for later use?
 - 1-15. Explain why, when glucose is the sole carbon source, bacteria grow much more slowly in the absence of oxygen than in the presence of oxygen.
 - 1-16. Bacteria can use a very large number of compounds, such as sugars, alcohols, and amino acids as a carbon source. Animal cells usually require a single sugar—that is, they are basically glucose burners. Why might this be expected?

- 1-17. Hair color in some animals can be black (BB), gray (Bb), or white (bb). If a black and gray mate and produce one offspring, what is the probability that it is gray? If there are two offspring, what is the probability that both are gray? If there are three offspring, what is the probability that only one is gray?
- 1-18. Two parents each with genotype AaBb mate. They have 16 offspring. How many would you expect to be homozygous recessive for both genes?
- 1-19. An animal can have red (RR or Rr) or blue (rr) eyes. If they are also tt, their eyes are colorless (TT and Tt give color). If RrTt mates with RRtt, what is the probability of getting a blue-eyed individual?
- 1-20. An animal has a single gene for tail shape. If a fat-tailed animal is mated to another fat-tailed one, only fat-tailed animals result. If fat and thin are mated, half are thin. If thin and thin are mated, there are always, on the average, twice as many thins as fats. Identify the genotypes of fat and of thin. *Hint:* Determine the allele that is dominant.
- 1-21. Color blindness in humans is inherited as a sex-linked recessive trait. Write as far as possible the genotype of each person represented in the pedigree shown in Figure 1-1. Black = colorblind.



- FIGURE 1-1
- 1-22. Following is a list of mutational changes. Which would be recessive in a heterozygote and which would be dominant?
 - (a) The mutant protein has no activity but the total number of proteins made by the gene is in excess of that needed for normal biological function.
 - (b) A protein contains 4 subunits. Both mutant and good protein units can interact. One defective subunit eliminates activity.
 - (c) A mutant enzyme fails to carry out a particular chemical reaction.