aspects of bio physics

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preface

This book is intended for those who have a basic knowledge of physics, chemistry, and biology and who wish to see how some of the more elementary parts of physics may be applied to the study of living matter. The treatment of the applications given here is, of course, not encyclopedic; it could not and was not intended to be. Instead, some representative topics from biophysics are presented, along with references that can guide the student to more detailed and thorough discussions of these topics.

Biophysics is a vigorous and still growing subject. In many cases, it would be easy to carry the development of certain topics into regions of far too uncertain knowledge. I have tried to avoid this by focusing on general results, methods, and analyses, so that the material is not quite so likely to be made useless by the natural progress of the subject.

I have profited from discussions with many, but especially with the late Dr. Peter Curran, who first introduced me to biophysics through his lectures on irreversible thermodynamics. Of course, those who have assisted me bear no responsibility for any errors in these pages.

William Hughes

to the student

In a recent National Academy of Sciences study, it was concluded that "the classical subdisciplines of biology are insufficiently instructive as approaches to current understanding and appreciation of life in its varigated manifestations." Thus classical zoology, botany, and microbiology, for example, are not used as subdivisions in the academy report. Instead the bases for living phenomena are examined at increasingly higher levels of organization, beginning with molecules and proceeding to organelles; cells; tissues and organs; organisms; and finally to species and ecosystems. To a considerable extent, the organization of this book, though not its scope, reflects the above view. Some comments on how the chapter topics fit into the above view of biology can now be made.

The first two chapters discuss the most basic ways of learning about the physical aspects of biological macromolecules, as opposed to their chemical properties, and the small-scale structure of biological material. The study of ultrastructure, that is, the description of how living matter is seen to be put together when studied at resolutions ~ 4 to $\sim 10^2$ nm, is a field all its own, marked by the use of many empirical techniques for preparing the material and extensive qualitative discussion of the observations. Discussions of much of this very detailed structure have been left out for two reasons: to keep the text from becoming too long and because the physical analysis does not yet carry into this realm, especially not at the mathematical level used here. Of course, the progress of the subject must eventually lead to a detailed analysis that will involve the use of such detailed structural knowledge.

The second chapter discusses several techniques for studying macromolecules and subcellular components. Again, the approach is general and does not aim to introduce any substantial amounts of detailed results. This is not because such results are not important but only because the level of the text does not require them. The methods discussed are in general use in both biophysics and biochemistry, but the precise details of any particular method will depend on its application. For example, just how one prepares a centrifuge tube depends on what one wants to separate, and how one adjusts a spectrophotometer depends on what one wants to study and what model of spectrophotometer is at hand. Clearly, these details are best left for discussion in the context of actual laboratory work.

Chapter 3 introduces some of the most elementary ideas for describing the behavior and properties of macromolecules. Two important classes of biological macromolecules are then discussed in Chapters 4 and 5. Again, much information that would be important in a purely biological context but that is not needed at this level of physics has been omitted; for example, there is no detailed discussion of a variety of specific enzyme reactions.

The discussion of the cell membrane in Chapter 6 follows my established trend in that it focuses on particular aspects of the membrane that are both important and can be treated at an appropriate level. In this case I am concentrating on transmembrane phenomena such as transport, and the many other critical biological phenomena in which the membrane plays a major role, such as the immune response, are not discussed. Of course, the study of membranes is now such an immense subject that this chapter can only serve to introduce the topic. In a similar way, all of the complications and detailed knowledge of neurons are not discussed, but instead a central problem, the conduction in the axon, receives the most attention in Chapter 7. Again, the biological details of neuron structure, function, and organization have outrun the ability of an analysis at this level to make much of a contribution. Finally, in Chapter 8, an introduction to the most fundamental aspects of artificial membranes is given, but the specific laboratory details of preparation and a discussion of the use of such membranes to investigate specific biological phenomena is best given in the context of actual laboratory problems.

Chapter 9 is concerned with energy transduction, but of necessity there can be no discussion of the very successful efforts in biochemistry that have produced detailed knowledge about the chemical reactions of metabolism, nor is there any more than the most basic remarks about the structures involved in these processes.

In Chapter 10 the basic ideas involved in understanding radiation effects are presented. Of course, there is a great deal of information on the radiation responses of particular organisms, as well as for particular tissues and organs, which does not appear here. In addition, information about the therapeutic uses of radiation has been omitted and should be sought in the appropriate medical volumes and journals.

Chapters 11 to 15 deal with physiological matters and it is fair to say that each gives some analysis of a topic that is of central importance to the subject. One should be aware, however, that a very large amount of information on the detailed structure and physiological responses of the relevant organs and tissues has been accumulated and can be found in the appropriate physiology books and journals. The structures shown here give only the basic information needed for the physical analysis.

The problem of the origin of life is one that is far less speculative and far more promising as a "proper" subject than was once the case. Nevertheless, the mathematical demands are impressive, especially in model building, and a substantial knowledge of biochemistry is probably going to be required. Recognizing these limits, in Chapter 16, I introduce the problem in a generally qualitative way and point the way to the quantitative treatments.

The last chapter shows some areas where technology based on biophysics is already important. Bioengineering is already an independent subject and this chapter can only serve to introduce it to the reader.

W.H.

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intro duc tion

"Life is a little current of electricity, driven by sunlight." No one would deny that the study of electricity and light are proper topics of physics, and thus Szent-Gyorgyi's remark is all the justification needed for the study of biological materials by physicists. Of course, there are more significant justifications. For one, an understanding of the intricate processes and complex structural arrangements of living matter seems unlikely to be achieved except through insights that only physics and chemistry can provide. The contributions to those insights that come from physics make up the subject that we call, for convenience, biophysics.

Although it is scarcely a new part of science, the precise origin of biophysics is a matter of debate. The importance of investigating biological phenomena was realized by physicists such as Helmholtz (1821–1894), who, because of his outstanding studies of the physics of hearing and vision, is often cited as the founder of biophysics. These early applications of physics to physiology are both well-known and important, but it should also be noted that cellular processes were not neglected; for example, the electrical properties of cells were the subject of fundamental studies in the late 1800s. However, these first investigations also represent relatively isolated efforts; biophysics during this time was waiting upon the growth of physics, the development of the capacity to investigate, both experimentally and theoretically, increasingly complex states of matter.

In a certain sense, physics has only recently reached a state at which matter as complexly organized as that in biological systems can be studied with understanding. The interest in applying modern physics to such systems certainly was strongly stimulated by Erwin Schrodinger's famous essay, 'What is Life?," in which he argued that the study of these complex systems, in addition to being intrinsically interesting, might also profit physics through the discovery of new physical laws. Although this prediction has not yet proved to be true, the recent successes of physicists and chemists in unraveling biological puzzles has provided ample evidence of the intriguing mechanisms and behavior exhibited by living matter. Of course, biophysics knows no shortage of real problems with as yet unknown solutions, nor is there a guarantee that the solutions of some outstanding problems do not depend explicitly on further developments. Schrodinger may yet be proved correct: the complexity of biological systems may provide the stimulus for the development of intriguing new pieces of physics whose applications will range over both biological and nonbiological problems.

What are the fundamental properties exhibited by living matter? We can either take a very simple view, and thus be able to write down some general answers, or we can consider the many complexities and finally end bogged down in an attempt to "define life." Seymor Benzer once observed that scientists could be divided into "clarifiers" (obviously complimentary) and "turbidifiers" (obviously not) depending, respectively, on their willingness to simplify complex problems. Indeed, the whole strategy of physics when faced with a difficult problem often leads us to consider only the most essential aspects of the problem, even if the items eliminated from consideration are not necessarily trivial, but only of lesser importance. Thus we come down squarely on the side of the clarifiers and say, with some internal reservations, that living matter, as exemplified by a single cell, shows three important characteristics:

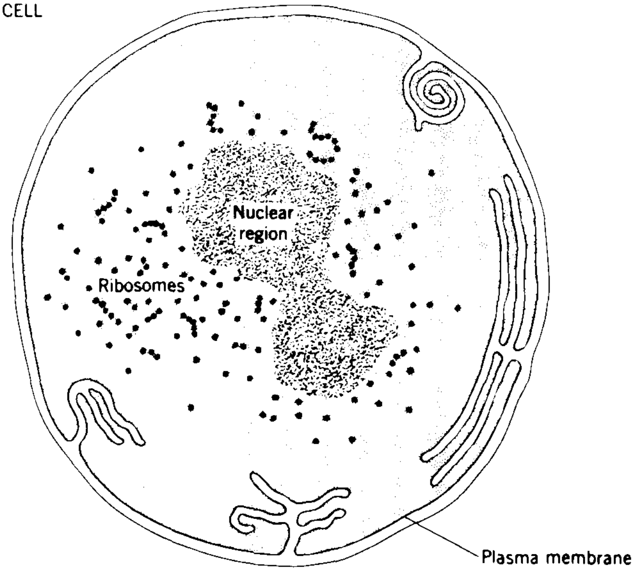
1. It is enveloped by a membrane structure and this structure is not a passive sack but a dynamic component of the system.
2. Chemical reactions occur from the membrane inward. These reactions provide energy, both for the various types of work done by the cell and for the production of some of the particular molecules required for the functioning of the system.
3. Information sufficient to permit the synthesis of required substances is stored as a molecular template in such a way that the living matter may reproduce itself.

Furthermore, we recognize that groups of cells that exhibit specialized features and properties exist in the form of tissues and organs; we assume that these features and properties are understandable in terms of the properties and features of single cells. Of course, it should be clear that these simple statements conceal very complex problems whose detailed solutions are great and as yet unrealized goals.

The identification and the determination of the precise composition of those molecules that are assembled to form living matter is one of the goals of biochemistry. From such studies, we have come to realize that the variety of molecules present in even a single cell is quite amazing. For example, a single cell of the bacterium *E. coli* is a cylinder about 3 μm long, with a radius of about 1 μm and a mass of 10^{-12} to 10^{-13} g. As Lehninger has pointed out this cell contains about 5000 different organic compounds; about 1000 of these are different nucleic acids and about 3000 are different kinds of proteins. In comparison, a human being has about 5×10^6 different kinds of proteins and, as far as we know, none of the protein molecules in *E. coli* is exactly the same as any one of the protein molecules found in human beings.

From this, it appears clear that the properties of the living state as a whole do not depend on specific individual molecules but rather on the organization of certain classes of molecules. How many different classes of molecules are needed? At first glance, this might appear to be a hopeless question. After all, as Figure 0.1 shows, the complicated structure of even a single cell is relatively obvious. There is certainly no reason to guess that this structure has its base in the properties of a relatively small class of organic compounds that are themselves composed of the members of a relatively small group of atoms. However, this turns out to be the case. Indeed, the existence of these "biochemical universals" greatly simplifies the physicist's task, because an enumeration of the immense number of different specific molecules becomes unnecessary; all that is required is a knowledge of the common features of the different categories. Let us now summarize the most elementary aspects of living matter, taking as an example a single unspecialized cell.

PROCARYOTIC CELL



EUCARYOTIC CELL

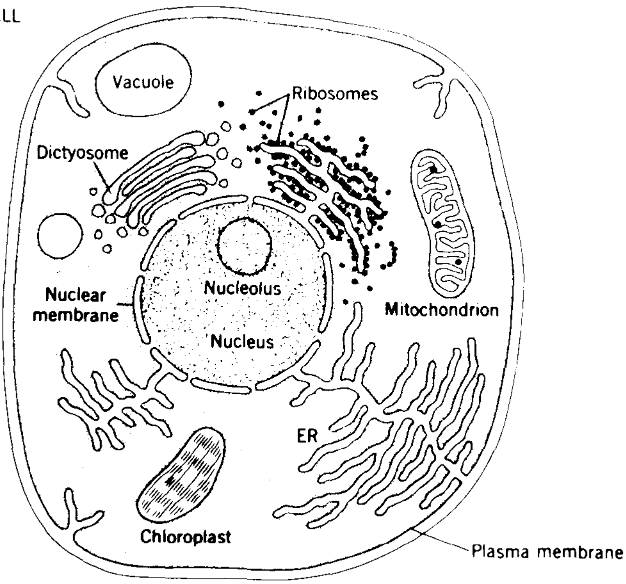


Figure 0.1 The major components of the two general types of cells are shown in the above drawings. The essential difference between the procaryotic and eucaryotic types is the presence of membrane-bound organelles, particularly the nucleus, in the eucaryotic cell. Bacteria and blue-green algae are procaryotic; the cells of all animals and higher plants are eucaryotic. Eucaryotic plant cells are distinguished from animal cells by a cellulose cell wall, chloroplasts for photosynthesis, and a central vacuole (see Figure 9.3).

Table 0.1 Critical Molecules

Compound	Comment	Example
Hexose sugars	Monosaccharides are compounds with the equation $(CH_2O)_n$, $n \geqslant 3$. Hexose sugars, $(CH_2O)_6$, are the most abundant monosaccharides.	Glucose, fructose
Trioses	The simplest monosaccharide, with the formula $(CH_2O)_3$.	Glyceraldehyde
Fatty acids	Long hydrocarbon chains terminated with COOH, the carboxylic group.	Palmitic acid, stearic acid
Purines	Complicated structures, formally known as nitrogenous bases.	Adenine, guanine
Pyrimidines		Thymine, uracil, cytosine
Steroids	Derivatives of compounds with three fused cyclohexane rings.	Cholesterol
Hydrocarbons	HC chains.	Squalene
Amino acids	Linkage of COOH, NH_2, and side chains.	Leucine, glycine

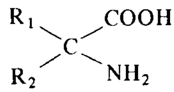
The key structural atoms from which living matter is built up are C, H, O, N, S, and P. These atoms occur more frequently in living matter than one might guess from their terrestrial abundances. The essential ancillary atoms are Na, K, Mg, Ca, Fe, Co, Mn, Cl, Cu, and Zn; these are less abundant in living matter than one might guess. Green has summarized the above situation very well: "There is no known case in which any of these atoms is replaced by an atom which is not part of the list. There may be additions, but never subtractions . . . (these) particular atoms are invariant for all forms of life."

These atoms occur in living matter as ions (e.g., K^+, Na^+), as components of simple molecules (e.g., H_2O), but most especially as the components of certain critical types of molecules, listed in Table 0.1. These molecules are themselves the principal units from which the important macromolecules – the proteins, the nucleic acids, the polysaccharides, the lipids and phospholipids – are built up.

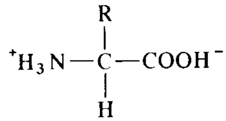
The construction of a protein molecule is based on linking amino acids, compounds formed by linking a carboxylic group, COOH, an amino group, NH_2, and certain specific radicals called side chains, as shown in Figure 0.2. The particular radicals that are linked determine the specific amino acid formed. All proteins are combinations of the 20 common amino acids. Although animals cannot synthesize all twenty from the simple molecules listed in Table 0.1, higher plants can, and hence animals can obtain these necessary amino acids.

The amino acids of a protein are linked to form a chain by means of a peptide bond, $\overbrace{-COCO-NH-}$, as shown in Figure 0.2. If the chain contains less than about a hundred links, it is usually called a peptide. If all the amino acids are the same, it is a

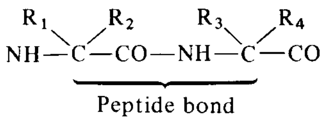
1. Amino acid structure:



which often appears as a polar combination of two ions



2. Amino acids are linked to form chains by a peptide bond:



3. Nucleic acids are formed by linking mononucleotides. Mononucleotides are formed from a combination of phosphoric acid, a nucleotide, and a pentose sugar. The combination of a pentose sugar and a purine or a pyrimidine is called a nucleoside. A nucleotide is formed by a PO_4 attached to the sugar of the nucleoside. The compound is then an acid, named by the nucleoside.

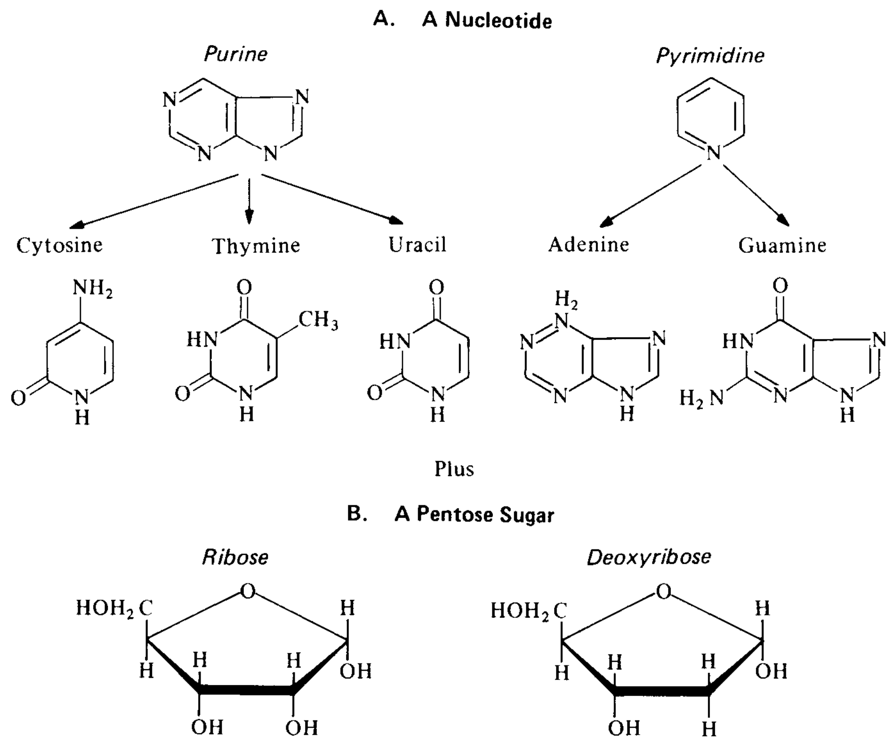


Figure 0.2 This drawing summarizes the general structural basis of proteins and nucleic acids.

polypeptide. Proteins are usually considered to be one or more chains of from 100 to 300 various amino acids.

A second important class of macromolecules is the nucleic acids. These are formed by linking mononucleotides, which are formed as shown in Figure 0.2.

A third group, the polysaccharides are built up in a straight forward way from identical repeating units of glucose or some more complex sugar. The complex sugar that forms the repeating unit may itself be formed by the linkage of simpler sugars.

Finally, the last category, the lipids, are formed from the combination of fatty acids, and usually take the form of long chain molecules of fatty acid with glycerol. An important variation replaces one of the fatty acid chains by phosphoric acid, producing a phospholipid.

It is principally the above macromolecules that are organized to form the cellular components. What are the general roles of each category of macromolecule in the cell? The proteins play a variety of roles. They are enzymes, catalysts for reactions in the cell; they may serve as structural elements; and they are the important component in the mechanism by which transport of certain substances across the cell membrane occurs. The nucleic acids are essential for the storage of the information that permits the cell to reproduce itself, and they also play an important role in the energy processes of the cell. The lipids and phospholipids are the critical components from which the cell membranes are formed.

The general molecular components of a cell are summarized in Table 0.2. We can now show the structural organization of an "elementary" cell. Two general forms occur. In the first, the nucleic acid is not confined to a clearly distinguishable region of the cell, nor does it occur in combination with protein. Such cells, known as procaryotes, are typical of bacteria and blue-green algae. In all other cases, the nucleic acid is complexed with protein and confined to a well-defined region of the cell known as the nucleus. In addition, such cells, known as eucaryotes, contain a variety of specialized structures, also well defined, known as organelles, which apparently carry out specific functions. These two general cell categories are illustrated in Figure 0.1.

Of course, these figures do not exactly represent any particular type of cell, but only a useful generalization. Real cells are specialized in varying degrees. In some cases,

Table 0.2 Typical Bacterial Cell Composition

Component	Percent Total Weight	Number of Each Kind
H_2O	70	1
Protein	15	~3000
DNA	1	1
RNA	6	~1000
Carbohydrates	3	~50
Lipids	2	~40
Various molecules	2	~500
Inorganic ions	1	~12

such as the exocrine cells in the pancreas, the cells in the proximal tubule of the kidney, or the pallisade cells in a leaf, the specialization is not particularly extreme. In other cases, such as nerve cells or muscle fiber cells, considerable specialization has obviously occurred, to the point that many features of such cells would be unintelligable without more information than appears in the figures. Of course, there are still common features; for example, many of the organelles such as the mitochondria are similar over a variety of specific types of cell. It is clear that the cell occupies a central position both as a general structural unit, especially for simple life forms, and as the basis for specialization in both structure and function. It should be noted that cellular specialization is a stable phenomenon. Using techniques of tissue culture, pioneered by R. G. Harrison, cardiac cells, retinal pigment cells, and cartilage cells have been cloned for some 50 cell divisions without loss of specialization.

Within cells occur the wide variety of chemical reactions required for the production of energy, the synthesis of required compounds, and reproduction of the cell. It is probably true that nearly every organic compound can be used by some organism as an energy source. Clearly, there is no hope of simply summarizing such possibilities, and no attempt to do so will be made here. The immensely detailed processes of metabolism and biosynthesis have been revealed by decades of biochemical investigation and those interested in these processes should consult the standard texts.

Nevertheless, it is possible to summarize the elementary features of protein synthesis. The determination of the protein synthesis mechanism was a step of the greatest importance because proteins have fundamental roles in all of the essential cellular processes. The general problem is clear: the base sequence of the DNA specifies a set of amino acids. Therefore, there must be a process that translates this code in the form of base sequences into the set of amino acids that are linked to form the protein. In eucaryotic cells, tracer studies show that the proteins are produced in the cytoplasm. Since the DNA is in the nucleus, protein synthesis obviously does not occur on the DNA molecules. It seems clear that this is a general result for both types of cells. The process by which protein synthesis is accomplished is illustrated in Figure 0.3. This process is conveniently expressed in the so-called "central dogma": DNA → RNA → Protein. Although Temin and Baltimore have shown that certain viruses can interact with cells and carry out processes that do not follow this rule, the principle applies to all normally functioning cells.

The results shown in the figures serve to emphasize that DNA, RNA, and protein are key substances and cooperate in the production of other cell protein. The capacity for cellular protein production can be estimated by considering a bacterial cell. The DNA in such a cell consists of about 10^6 nucleotides. An average protein consists of approximately 100 amino acids. Since three nucleotides are required to specify an amino acid, 10^6 nucleotides code for some 330×10^3 amino acid molecules, which is about 3300 different protein chains.

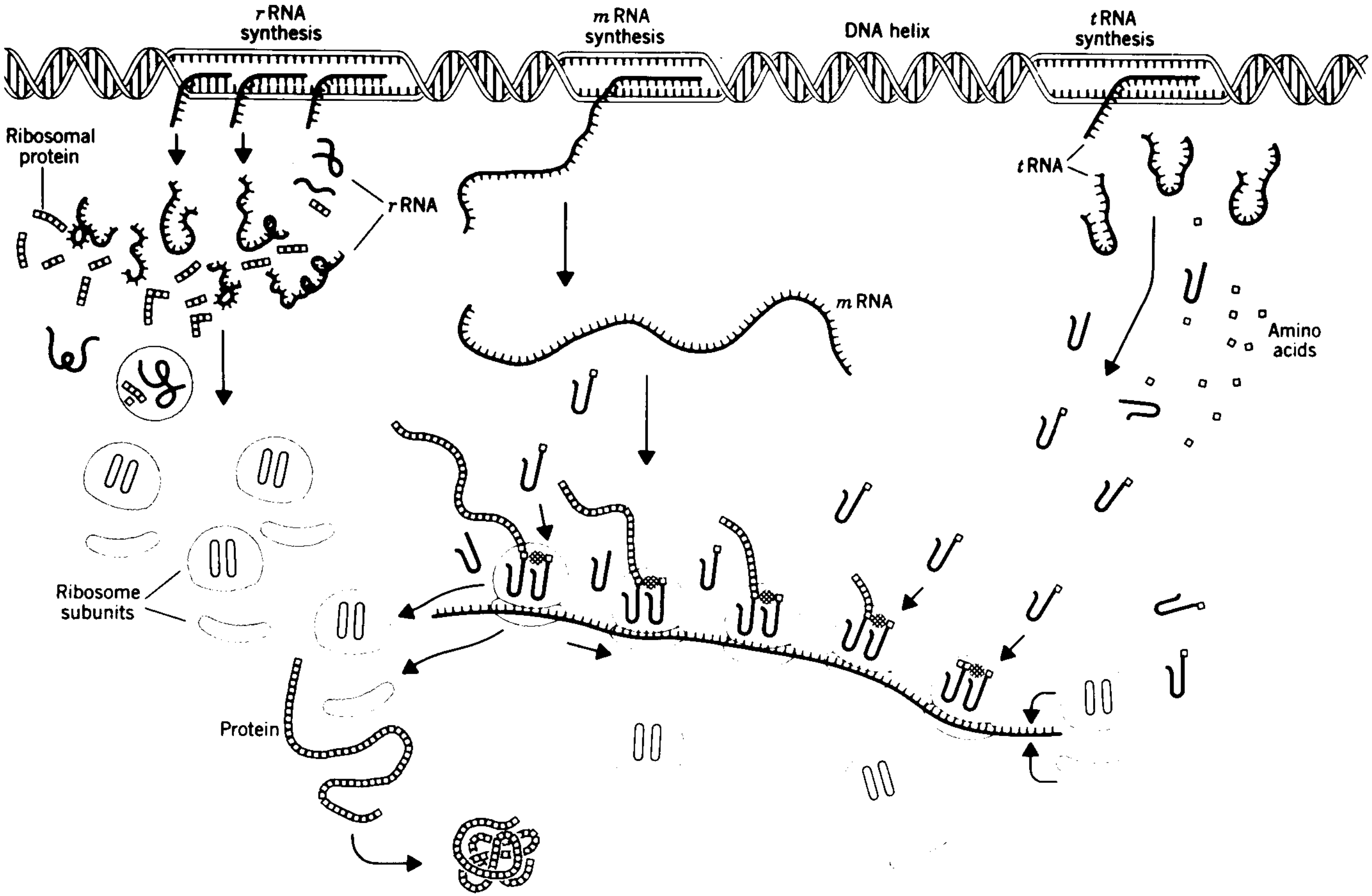


Figure 0.3 The general mechanism of protein synthesis appears to be essentially the same in all cells. The above figure shows, in a schematic way, the major steps in the process.