Recombinant DNA

SECOND EDITION

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CHAPTER 1

Establishing the Role of Genes Within Cells

There is no substance so important as DNA. Because it carries within its structure the hereditary information that determines the structures of proteins, it is the prime molecule of life. The instructions that direct cells to grow and divide are encoded by it; so are the messages that bring about the differentiation of fertilized eggs into the multitude of specialized cells that are necessary for the successful functioning of higher plants and animals. And because it has been present in virtually an infinite number of interchangeable chemical species, DNA has provided the basis for the evolutionary process that has generated the many millions of different life-forms that have occupied the earth since the first living organisms came into existence some 3 to 4 billion years ago.

This extraordinary capacity of altered DNA molecules to give rise to new life-forms that are better adapted for survival than were their immediate progenitors has made possible the emergence of our own species, with its ability to perceive the nature of its environment and to utilize this information to build the civilizations of modern man. As a result of our ability for rapid conceptual thought, we have for several centuries been asking ever deeper questions about the nature of inanimate objects like water, rocks, and air, as well as about the stars of surrounding space. And biology, the science of living objects, which only

40 years ago was generally perceived to be a much inferior science, has swiftly come of age. By now there exists an almost total consensus of informed minds that the essence of life can be explained by the same laws of physics and chemistry that have helped us understand, for example, why apples fall to the ground and why the moon does not, or why water is transformed into gaseous vapor when its boiling point is exceeded.

The key to our optimism that all secrets of life are within the grasp of future generations of perceptive biologists is the ever accelerating speed at which we have been able to probe the secrets of DNA. Now we know so much that it is difficult to remember the intellectual chaos that still existed in 1944—when DNA was first reported to carry genetic information—and that was to disappear effectively only in 1953, when the structure of DNA was revealed to be a complementary double helix. Since then it has been clear to all that the "brain," so to speak, of all cells is DNA: From DNA issue the commands that regulate the nature and number of virtually every type of cellular molecule. So, increasingly, our attention has been devoted to unlocking the information within DNA. We know that if we can reveal the exact form of our genetic blueprints, we shall have taken a giant step toward eventually understanding the many complex sets of interconnected chemical reactions that cause fertilized eggs to develop into highly complex multicellular organisms.

Before we examine DNA itself in more detail, we shall first look briefly at the cells in which it resides, to determine the nature of the commands that DNA must generate.

The Building Blocks of All Life Are Cells

The smallest irreducible units of life are cells. They were first seen over 300 years ago, soon after the construction of the first microscopes. By the middle of the nineteenth century, it had become clear that all living organisms are built from cells.

Generally, cells are very small, with diameters much less than 1 mm, so they are invisible to the naked eye. In the simplest cells, bacteria, a cell wall surrounds a very thin fatty acid–containing outer (*plasma*) membrane which in turn surrounds a superficially unstructured inner region. Within this inner region is located the bacterial DNA that carries the genetic information. The plasma membrane is effectively impermeable, except to selected food molecules and ions, so the inner cell contents are contained and are not lost to the outside. The integrity of this outer membrane is thus essential for the life of the cell, and it is constructed in such a way that minor accidental tears or openings are sealed automatically, like punctures in a self-sealing automobile tire.

In virtually all cells other than bacteria, the inner cellular mass is partitioned into a membrane-bounded, spherical body called the *nucleus* and an outer surrounding *cytoplasm.* In the nucleus is located the cellular DNA in the form of coiled rods known as *chromosomes.* Cells that contain a nucleus are referred to as *eukaryotic cells,* whereas the nuclei-free bacteria and their close relatives, the blue-green algae, are known as *prokaryotic cells.*

Cells Are Tiny Expandable Factories That Simultaneously Synthesize Several Thousand Different Molecules

The essence of a cell is its ability to grow and divide to produce progeny cells, which are likewise capable of generating new cellular molecules and replicating themselves. To perform these functions, cells must be chemically very sophisticated; indeed, even the very simplest cells contain more than 2500 different molecules. Thus, cells are, in effect, tiny factories that grow by taking in simple molecular building blocks, like glucose and carbon dioxide, and somehow converting them into the many diverse carbon-containing molecules that are required for cellular functioning. In growing and dividing, cells also require an external source of energy to ensure that the cellular chemical reactions proceed in the desired direction of biosynthesis. Cells are therefore governed by the same laws of thermodynamics that describe the energies of atoms to the physicist and the energies of molecules to the chemist. For most cells, the energy input necessary