From DNA To Development

By JAMES BONNER

In the higher organisms, cellular specialization of function is a key to efficiency. Specialization implies differential use of the inherited information available to every cell. How are the keys turned and the switches thrown?

Of all of the insights that the dramatic new developments in biology have given to us, none has more profound impact upon so many aspects of our thought and culture than our vision of the great sweep of evolution of living things. Today we know, as we did not even a few short years ago, that our planet and its elements were formed some 4.8 billion years ago and that the early earth possessed a reducing atmosphere quite different from today's—an atmosphere containing large quantities of methane, water vapor, and ammonia.

Our earth is nicely sized and appropriately situated with respect to the sun, so that it is neither too hot nor too cold, nor too big and therefore a sun, nor too small and therefore unable to hold its atmosphere. How fortunate! These facts made all else possible.

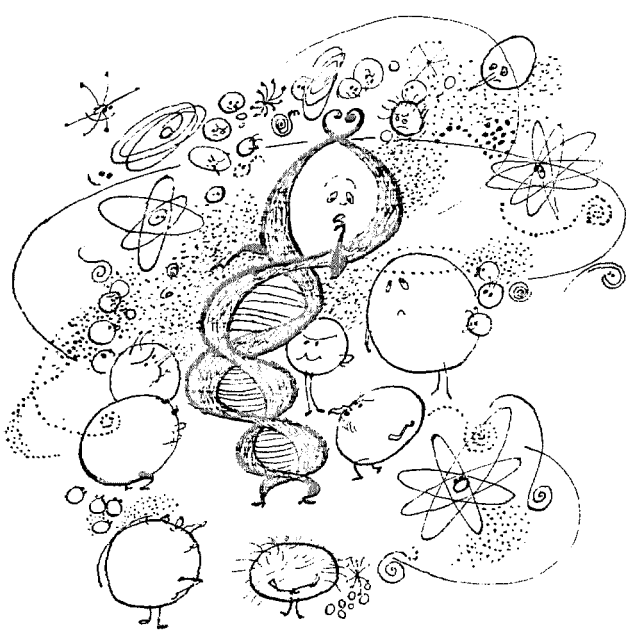
On the early earth, the rains rained, the lightning lightened, atoms were ionized, and chemistry took place—random chemistry, in which atoms were combined in all of the many permutations and combinations to form a vast variety of molecules, including, as we now know, those characteristic of today's living beings. And we know too that, as this random chemistry continued, organic molecules accumulated because there were no living creatures to eat them up as there are today. And then one day, as we see it now, a dramatic new event took place. The first DNA molecule—a long, linear molecule made of four kinds of building blocks stapled together, and thus capable of encoding information—was formed by just this kind of random chemistry. The appearance of the first lonely little DNA molecule introduced a whole new dimension into chemistry on earth; for the DNA molecule possessed what no molecule before it had possessed, the power to replicate itself, build copies identical to the original.

With the appearance of the first DNA molecule—a molecule because of its structure able to encode information, capable of self-replication, capable of mutation by occasional mistakes in replication—life as we see it now may be said to have begun. From that moment, one billion years after the creation of the elements, life on earth has been a continuum of mutation and selection, all based on survival of the fittest among the descendants of that first prototype DNA molecule.

By three billion years ago, living forms had evolved akin to those we know on earth today—creatures vastly more complex than the single DNA

molecule of early life. There were bacterial cells, and cells of blue-green algae, DNA molecules surrounded by semipermeable membranes, cells in which the information encoded in DNA served to generate—by transcription into messenger RNA—the many kinds of enzyme molecules which today's living creatures use to convert food to the building blocks for DNA replication and for the formation of other cellular structures. Indeed, the genetic code for translation from the 4-letter nucleic acid language to the 20-letter language of the enzymes was already established in its present form. Photosynthetic organisms, capable of transducing the energy of visible light into chemistry with the evolution of oxygen from water and the reduction of CO_2 to plant material, had appeared and were busily converting our atmosphere into an oxidizing one. Very possibly, by three billion years ago, the mechanisms responsible for interchange of DNA-encoded genetic information between bacterial cells had been developed, the kind of primitive sexuality which persists to this day in the bacteria.

Thus, in an exuberant burst of evolution, life on earth passed in a little over one billion years from simple, naked DNA molecules to complex single-celled organisms. At this stage, however, as we see it now, evolution took a long breathing spell—a breathing spell of almost two billion years. And then, quite suddenly, less than one billion years ago, a new kind of living organism appeared, an organism that we do not know today, but from which all of today's higher organisms are evolved. This new creature possessed cells much more complicated than those of bacteria. Its genetic material was composed of several DNA molecules, organized into chromosomes, which replicated and separated at each cell division by a complex molecular ballet, mitosis.



A dramatic event—the first lonely little DNA molecule.

The road to the complete understanding of the developmental process is at long last open before us.

These new organisms possessed regular programs for the periodic exchange of genetic information between organisms, the features of sexuality as we know them today. Their chromosomes were housed within an intracellular membrane, the whole structure forming the nucleus of each cell. Most surprisingly of all, these new creatures were not single creatures, but in fact societies of creatures. Each cell of the new organism contained within it, as symbionts, bacterial cells which happily evolved into today's mitochondria—bodies which the cells of all of today's higher creatures possess and which conduct the process of respiration. Others of the new kind of cells possessed not only mitochondria but also intracellular guests akin to the primitive blue-green algae. These have developed into the chloroplasts of today's green plants. Indeed, the cells of today's higher organisms are veritable barnyards of many kinds of self-duplicating entities housed within the cell of the organism. And it is from this new kind of cell that today's multicellular organisms have evolved. During the last billion years there has been a second explosion of evolutionary activity, and this burst has given rise to all of today's plants and animals.

How exciting it would be to find somewhere on earth a surviving prototype of that first aboriginal higher creature with its typical higher creature cellular structure.

Today's creatures, different as they are in form, habits, and life style, are basically very much alike, each composed of a great many cells of the new type. The human, for example, contains about a

thousand billion cells; yet each human being, and indeed each higher organism, starts his individual life as a single cell, the fertilized egg. The fertilized egg divides and divides, into two cells, four cells, eight cells—and in the course of time these cells start to become different from one another, to turn into the many kinds of specialized cells that characterize the adult organism. This is the process of development, and of today's problems of biology none is more exciting than that of exactly how development takes place—how it is that the individual cells of the body of the higher organism come to be different from one another, even though descended from a common ancestral cell, the fertilized egg. And it is to this problem that my colleagues and I have paid our full attention.

These last few years have been exciting ones in the study of development. Each day sees new excitement in the laboratory, new findings providing new insights into the logic of the organization and regulation of the genetic material of the higher organism. For development is a genetic problem; development too is controlled by the DNA of the chromosomes of the organism. Everybody knows about genes and how they control each and every one of our physical characteristics. Everybody knows that genes are contained in chromosomes, which are made of DNA. The form and features of each individual specialized cell of the higher organism are hereditary characteristics of that organism, and they are therefore encoded in that creature's DNA. How do genes work so as to control development? This is the question that our group asks.

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Much has been found out about how genes work by the study of the simple organisms, viruses and bacteria, for they too have genes arranged in (albeit simple) chromosomes. They have, however, fewer genes than we do. Each cell of the colon bacterium, *E. coli*, for example, contains only one one-thousandth of the amount of DNA contained in a human cell. It is a sobering thought. We are really only one thousand times more complicated than a miserable bacterium. By the study of bacteria we have come to understand how the information contained in DNA is transcribed by the enzyme RNA polymerase to form informational copies, RNA molecules, each containing the message of one or a few genes. We have come to understand how the messenger RNA is translated by the ribosomal protein-synthesizing system to make the enzyme molecules, each assembled from the 20 amino acids in accordance with the instructions contained in the message of a single gene. But simple organisms, bacteria and viruses, do not grow into complex, multicellular creatures, with different kinds of specialized cells. The problems of development must be studied with the higher organisms.

Each day sees new excitement in the laboratory.

The first great truism of the study of development is that each and every cell of the higher organism, no matter what its external appearance, contains the same amount and kind of DNA. That each cell contains the same amount of DNA is measurable by chemistry; that each specialized cell contains all of the DNA required to make the whole organism is shown by such simple facts as that an individual specialized cell may be caused, under appropriate experimental conditions, to behave like a fertilized egg and regenerate the entire organism. In any single, particular kind of specialized cell, however, only a portion of the genes contained in the chromosomes of the nucleus of that cell are actively engaged in producing their messenger

RNA, and thence the enzyme molecules for which they contain information. That this is so is evident from such elementary considerations as the fact that humans contain, for example, genes for making hemoglobin molecules. These genes are turned on in those cells which give rise to the red blood cells, and they are not active—are turned off—in all other cells of the human body. The genes for making muscle proteins are turned on in muscle cells but turned off in nerve cells. And so it goes.

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The study of the developmental process is, then, the study of how it is that the activity of genes is controlled in the chromosomes of the higher organism. What determines whether genes are turned off or turned on? What is the material nature of the repressor molecules that cause genes to be turned off? How are genes transformed from the turned-off to turned-on state, and vice versa? How does the systematic, orderly programming of gene activity work so as to bring about orderly development?

The study of the developmental process is, then, the study of the biology of chromosomes. We study chromosomes by isolating them from the cell in pure form, causing them to generate their messenger RNA in the test tube, and finding out what it is that makes only particular kinds of messenger RNA be formed by the isolated chromosome. The method we use for the preparation of isolated chromosomes is simple. We take some cells or tissues, grind them in a blender so as to rupture the cell membrane as well as the nuclear membrane, and then filter the material through Miracloth, silicone-treated paper which magically removes membranes. We then subject the resulting suspension of cell particles to centrifugation in a centrifugal field too slight to pellet mitochondria or enzyme molecules, a centrifugal field in which only the biggest and heaviest things in the cell homogenate are pelleted. And luckily enough, the biggest and heaviest things in the cell homogenate are the chromosomes, which we then recover in 95 percent or higher yield and which may be purified by sucrose density gradient centrifugation, a procedure in which chromatin is layered over sucrose solution ranging in concentration from 0 percent at the top to 1.8 molar at the bottom. Chromosomes can pellet to the bottom through 1.8 M sucrose because they are large and dense; they are made of DNA, which is heavy. Membranous and proteinaceous materials are lighter and float at that point in the gradient in which they find their position of neutral buoyancy. Our methods appear to be applicable to a vast variety of living creatures, plant and animal alike, and serve to provide chromosomes not only for the study of their biology, but also for the study of their physical biochemistry—studies of their shape, size, and configuration.

Isolated chromosomes possess a non-trivial property, the property of producing RNA if supplied with the four RNA building blocks, the four riboside triphosphates. Such messenger RNA is formed because chromosomes contain bound RNA polymerase, the enzyme that catalyzes the transcription of DNA to form messenger RNA. Since isolated chromosomes of higher organisms contain in general only a small amount of RNA polymerase, we can add more of the separately prepared enzyme. RNA polymerase may be purified for this purpose from microorganisms which live at a rapid pace and possess a higher ratio of RNA polymerase to DNA than do the cells of the more placid higher organisms. In this way, then, isolated chromosomes can be transcribed by RNA polymerase to produce RNA in vast amounts, amounts large enough to make it possible to study the kinds of molecules present and the messages they contain.

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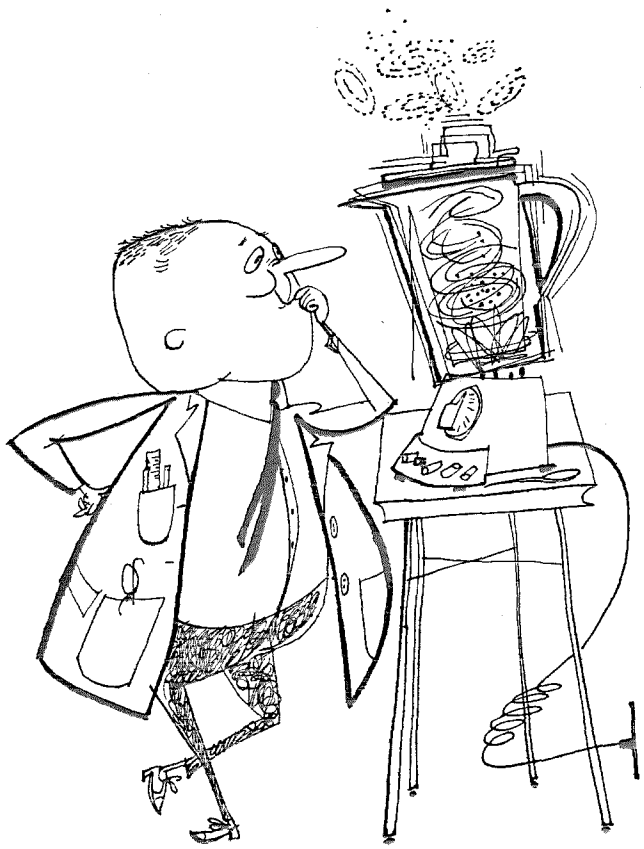
By this strategy it has been possible to show that the RNA molecules transcribed from chromosomes in the test tube are identical to the RNA molecules that are transcribed from the same chromosomes in life. No genes are repressed by the act of isolation; no genes are derepressed by the act of isolation.



We take some cells and grind them in a blender.

Isolated chromosomes are not artifactual; they represent the state of repression characteristic of life itself.

The DNA of isolated chromosomes is a poor template for the support of RNA synthesis as compared to deproteinized DNA made from the same chromosomes. Studies of the kinds of messenger RNA produced from chromosomes or from deproteinized DNA show that in isolated chromosomes only a small proportion, between 1 and 10 percent in general, of the DNA is available for transcription by RNA polymerase; the rest is repressed, turned off, and unavailable. This is, then, as it is in life. The great majority of the DNA of the chromosomes of any particular kind of specialized cell is repressed and does not produce RNA containing the messages encoded in the repressed DNA. What is the agent of repression?

To answer this question we have made a detour into the study of chromosome chemistry. Chromosomes of higher organisms contain, in addition to DNA, proteins of a particular class, the histones, which are found only in chromosomes and in association with DNA. Histones are basic proteins in which one amino acid in four is a cation, and histones are bound to DNA by interaction of these groups with the anionic phosphate groups of the DNA.

Chromosomes also contain a small amount of RNA, a portion of it messenger RNA which was in the act of being born when the biologist came along and isolated the chromosomes, and, in part, RNA molecules of a special class, chromosomal RNA. And chromosomes contain also a small proportion of nonhistone protein, ordinary proteins, of which RNA polymerase itself constitutes one portion. Removal of histones from DNA causes all of the DNA of that chromosome to become available for transcription by RNA polymerase. It is the histones which are the agents of repression of gene activity in the chromosomes of higher organisms. To put it simply, it is that portion of the DNA that is complexed with histone which is repressed—not available for transcription—while that portion of the DNA which is not so complexed is the portion that is turned on and is available for transcription.

To digress still further into chemistry, we have made a detailed study of the chemistry of the histones. It has been shown, in particular by biology graduate student Douglas Fambrough, that there are eight kinds of histones in the chromosomes of higher organisms, and that these same eight are present in organisms as different from one another as peas, cows, humans, rats, frogs, and protozoa.

Fambrough, in association with our colleagues at UCLA, Emil Smith and Robert DeLange, has studied the amino acid sequences which characterize the structure of one particular histone, comparing the structures of that histone in peas and in cows. They have found that the amino acid sequences of the homologous histone of these two organisms are essentially identical, differing only in two amino acid residues. The histones would appear to be the most conserved, as the biologist says, the most resistant to evolution of all of the protein molecules which have yet been studied—much more constant during the course of evolution than say, hemoglobin, or even the respiratory cytochrome enzyme molecule.

It would appear that the genes for making the histones, the regulators of gene activity, were established long ago in evolution—were established in that early creature which constitutes the ancestor of all of today's higher organisms—and that the genes for making histone molecules have been pre-

served essentially unaltered since that time, perhaps one billion years ago.

It is interesting to note too that the primitive and early organisms, the bacteria and blue-green algae, do not possess histones. What regulation of gene activity they possess appears to be conducted in an entirely different way, with a great variety of different kinds of proteins. Perhaps it is due to their cumbersome mode of genetic control that they are still lowly bacteria. The higher organisms, those

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that have succeeded in becoming multicellular, and in exhibiting differentiation and production of specialized cells, all conduct their genetic repression operations with the same basic kit of eight kinds of histone molecules.

It is clear, therefore, that the repression of genes is accomplished by protein molecules of a special class, the histones. There are many genes to be repressed in any given kind of specialized cell. In the human, for example, whose genetic material contains about 2,500,000 genes, in any particular kind of specialized cell perhaps 99 percent of the genes are turned off, repressed. This leaves 25,000 turned on, to be sure, but there are still 2,475,000 different genes to be turned off. How is this accomplished with only eight kinds of histone molecules? This is the function of the chromosomal RNA. Chromosomal RNA molecules are short ones, 40 to 60 nucleotides in length in different organisms. In the chromosome, chromosomal RNA is bound to the DNA, apparently through the same complementarity rules that regulate DNA replication and transcription of RNA. Chromosomal RNA molecules are also bound at one end to a particular kind of chromosomal protein, which is in turn bound to the several different kinds of histone molecules.

We have shown, during the past two years, that it is the chromosomal RNA molecules which guide the histone molecules to the correct genes to be repressed. Histone molecules of themselves cannot read the information content of DNA. This is the function of the chromosomal RNA molecules, which do it by the regular procedures by which nucleic acid molecules recognize one another. In the world of nucleic acid, "It takes one to know one," biologists say. We have found that we can, for example, prepare chromosomal RNA and chromosomal proteins from the cells of one organ, bind these components to the purified DNA of a second and different organ, and reconstitute chromosomes identical to those of the organ which served as the donor of the chromosomal RNA. Our understanding and control over the process of repression is therefore considerable and growing fast.

How do genes that are repressed become derepressed, and vice versa? Our insight into this matter is growing too, and is based again upon information gained originally by those who work on simple creatures, the bacteria. In bacteria we know that small molecules enter the cell and turn on genes that were previously turned off. This mechanism serves primarily to cause the cell to not make enzyme molecules to utilize metabolites that are not present in the cell. The biologist says that the genes for making the particular enzyme molecules are inducible, and they are induced to form the enzyme only when the inducer substrate molecule is present. Similarly, certain classes of small molecules regulate gene activity in higher organisms. Classic examples are the hormones.

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Hormones are small molecules, a few dozen atoms at most, small enough for chemists to be interested in them; and they are produced in one organ and travel to other so-called target organs upon which they exert their effects. The effect of hormones in general, and perhaps in most, if not all, cases of hormone action, is to cause derepression of genes previously repressed and hence the production in the target organ cells of new kinds of enzyme molecules which those cells did not previously produce. Examples are the activity of cortisone on liver cells, which causes liver cells to produce enzymes needed

for glucose and amino acid metabolism; or the sex hormones which go to their target organ and cause the cellular activities which result in the secondary sex characteristics.

The way in which hormones work can also be studied with isolated chromosomes. We have found that when hormones enter the cell they bind first to specific hormone-binding proteins, a different species of protein for each different hormone. The complex thus formed is then capable of binding to the chromosome, finding the right gene in a way perhaps analogous to the way in which histone molecules find the right gene to repress. The new complex of chromatin, hormone, and hormone-binding protein is derepressed with respect to the particular genes which the hormone controls. Although the way in which this happens is not yet completely elucidated, it is nonetheless clear that the detailed study of the molecular basis of the interaction between hormone-binding protein and chromatin provides a key to the understanding of the molecular basis of derepression.

How then are we to understand the developmental process as a whole—the detailed programming of gene repression and derepression? We may imagine that each gene in the cell is reposing in a repressed state, waiting for the proper specific small molecule substance to come along and turn it on. Some such small molecule effector substances, as they are known, are certainly not only hormones but also everyday metabolites, dissolved gases, perhaps water, specific substances produced by neighboring cells, and so on. We can begin to visualize too how the derepression of one gene may lead to the production of material which causes derepression of further genes, and how this may in turn bring about long chains of genetic switching which can result in the developmental process.

Each gene waits to be turned on by a molecular substance.

The developmental process behaves as though it were a preprogrammed routine written down in the genetic DNA.

Consider, for example, the developmental process in the case of flowering. The vegetative bud of a plant goes along producing leaves and stems. Genes for making flowers and fruits are all turned off. Their activity is not required for vegetative growth. Suddenly, in the case of a short-day plant, for example, the leaf sees a short day (and a long night) and produces flowering hormone. The flowering hormone goes to the bud and to the meristematic (actively dividing embryonic) cells of the bud. There the flowering hormone says to the repressed genes for making flowers, "Genes for making flowers, I have seen a short day. It is time to become derepressed and start upon the pathway to flower development." Once started upon the floral pathway, development flows on, as it were, automatically. The developmental process behaves as though it were a preprogrammed routine written down in the genetic DNA.

How can we hope to study in detail the sequential genetic switching which results in a programmed developmental process? Certainly the task will be an enormous one. Today, however, we know how to approach the matter; we see that the problems of development must be resolved one by one to the level of the repression and derepression of individual genes by the interaction with the gene of its appropriate effector substance. The road to the complete understanding of the developmental process is at long last open before us. □