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The Way of Life

The process of development as seen through the window of modern biology

by James Bonner

The time has come for direct attack upon the central problem of biology, the problem of how it is that a single cell, the fertilized egg, gives rise to an adult creature made of many different kinds of cells. This process, which is known as development, has been described and thought about by biologists for as long as there has been a science of biology. Its nature has remained a mystery because we have not heretofore understood enough about the nature of life itself. Today we do. We know in detail what makes a cell be alive. We know that all cells contain the directions for cell life written in the DNA of their chromosomes and that these directions include specification of how to make the many kinds of protein enzyme molecules by means of which the cell converts available building blocks into substances suitable for making more cells.

We know that to make enzyme molecules, the DNA prints off RNA copies of itself, messenger RNA molecules, and that these messenger RNA molecules are decoded by ribosomes, also made by the DNA, and that the ribosome as it decodes a messenger RNA molecule uses the information to assemble a specific kind of enzyme molecule.

This picture of life is that given to us by molecular biology and it is general: it applies to all cells of all creatures. It is a description of the manner in which all cells are similar. All cells possess DNA and this DNA makes messenger RNA, ribosomes, and hence enzymes. But higher creatures such as people or pea plants possess different kinds of cells. Some cells make hemoglobin, others do not. Some cells make pea seed globulin, others do not. The time has come for us to find out what molecular biology can tell us about why different cells in the same body are different from one another and how such differences arise.

The first thing we can say about the cells of the body of a higher organism is that they all have exactly the same amount and kind of DNA, the same genetic information. A single cell, the fertilized egg, divides into two cells and each of these receives a complete set of the genetic DNA. The daughter cells divide and divide, each cell continuing to receive a complete copy of the genetic book. But pretty soon in the course of embryonic development the cells of the embryo begin to become different from one another. Some produce hemoglobin, some produce muscle enzymes, some liver enzymes, and so on. The genetic information for making hemoglobin, for example, is in all cells but it is used only in a few cells, those which are to be red blood cells. In the other cells of the body the genetic information for making hemoglobin is turned off, repressed. To find out what causes development and differentiation, then, we must find out what it is in the cell that determines that particular units of the genetic information, particular genes, shall be active and make their characteristic messenger RNA, and what it is that determines that other genes shall be repressed, inactive in RNA-making.

Development is the orderly production by a single cell, the fertilized egg, of the several kinds of specialized cells which make up the adult creature. Specialized cells differ from one another in the kinds of enzymes which they contain. We are inexorably led by logic to the conclusion that the cause of development is a properly programmed expression and utilization of the genetic information. The genetic information is contained in the genes and these in turn are fastened together into the chromosomes which are housed in the cell nucleus. The new study of development must be oc-

cupied with the study of chromosomes since it is in the chromosome that the master plan for the architecture of the body resides.

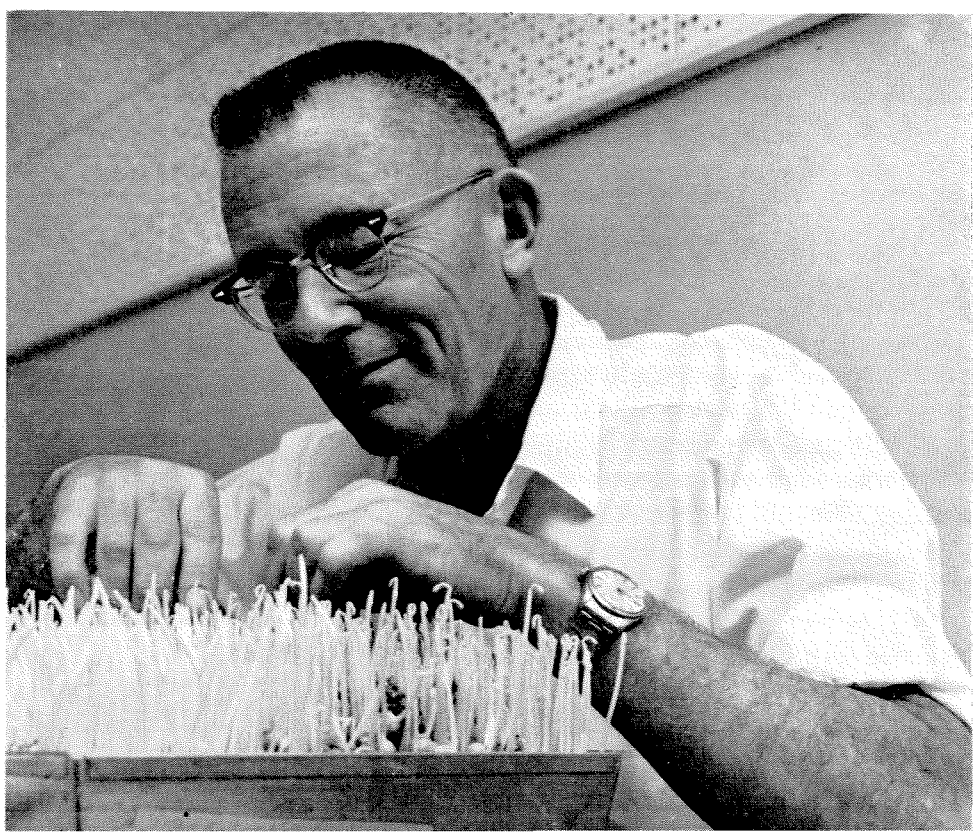
My colleague, Dr. Ru-chih C. Huang, and myself have started such a study. During the past four years we have found out how to isolate chromosomes and how to cause them to make their messenger RNA in the test tube. We have found out how to couple this messenger RNA production to ribosomes so that enzyme molecules are made in the test tube. We have found that chromosomes from different kinds of specialized cells make different kinds of messenger RNA's and hence different kinds of enzymes, kinds characteristic of the cell from which the chromosome was isolated. In this way we have shown that the control of genetic activity characteristic of the chromosome in life is preserved in our isolated chromosomes in the test tube.

We have studied the control and programming of genetic activity on three levels, namely:

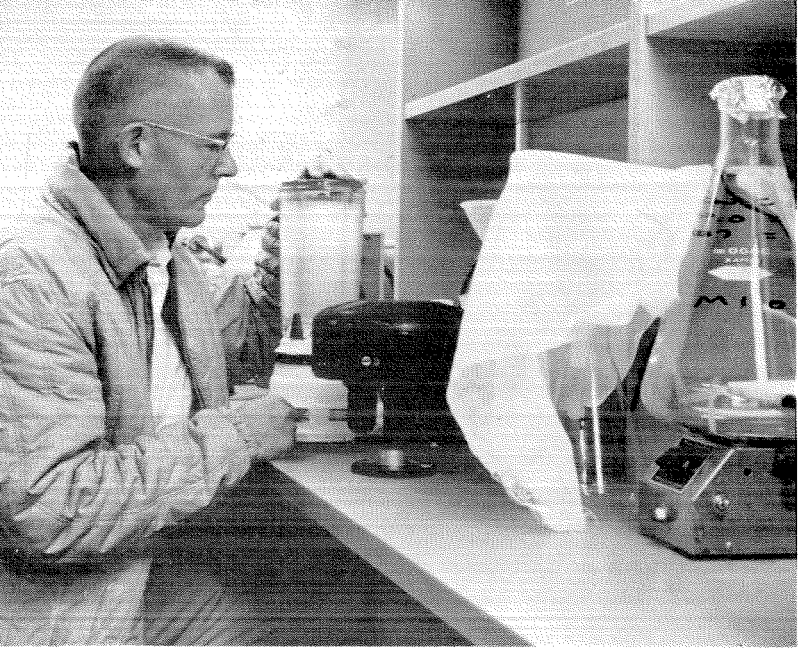
1. The hardware of genetic control – the nature of the material which represses gene activity.
2. The nature of the genetic switching unit – the nature of the act by which genetic activity is turned on and off, and
3. The nature of the switching network by means of which the individual genetic switching units are linked and integrated into a developmental system.

We have studied the hardware of genetic control by focusing our attention on a particular gene of the pea plant. (We say, "If pea plants were good enough for Mendel to invent genetics with, they are good enough for us.") This gene is that which controls the manufacture of pea seed globulin, the reserve protein of the seed. It is made in growing pea seeds and is not made in any other part of the pea plant at any time during development. Its behavior is typical of development. Chromosomes isolated from growing pea seeds make messenger RNA in the test tube and this messenger RNA supports the synthesis of protein. This protein contains pea seed globulin, just as in life. Chromosomes from pea buds also make messenger RNA and this also supports protein synthesis. The protein includes no pea seed globulin. This also is as in life. But we know that the gene for pea seed globulin-making is in the pea bud chromosomes. It is merely repressed. How can we de-repress it? Simply. All that is required is to remove from the chromosome a characteristic chromosomal component, a particular kind of protein which is always associated with chromosomes, a class of protein called histone. A portion of the DNA in chromosomes is wrapped in histone. DNA that is thus wrapped cannot make messenger RNA. It is repressed. When we remove the histone from the chromosomes of pea buds, the gene for globulin-making is de-repressed, as are indeed all of the previously repressed genes of the pea bud chromosome.

We know, then, a little about the material nature of the repression of gene activity. The repressers include histones, although we do not know whether histones are the sole kind of repressers. The logic by which represser histones discover the proper



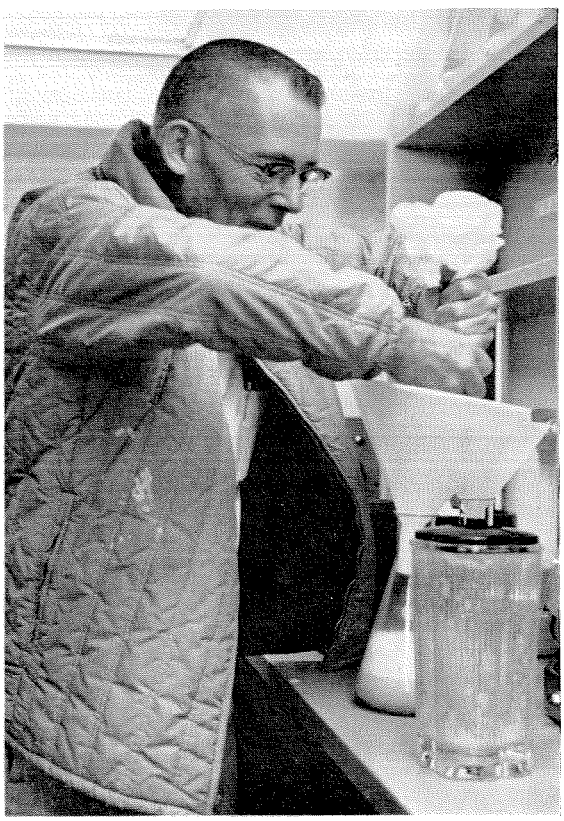
Dr. James Bonner, professor of biology, cuts buds from pea plants to obtain genetic material. The buds contain many small cells and are rich in chromosomes.

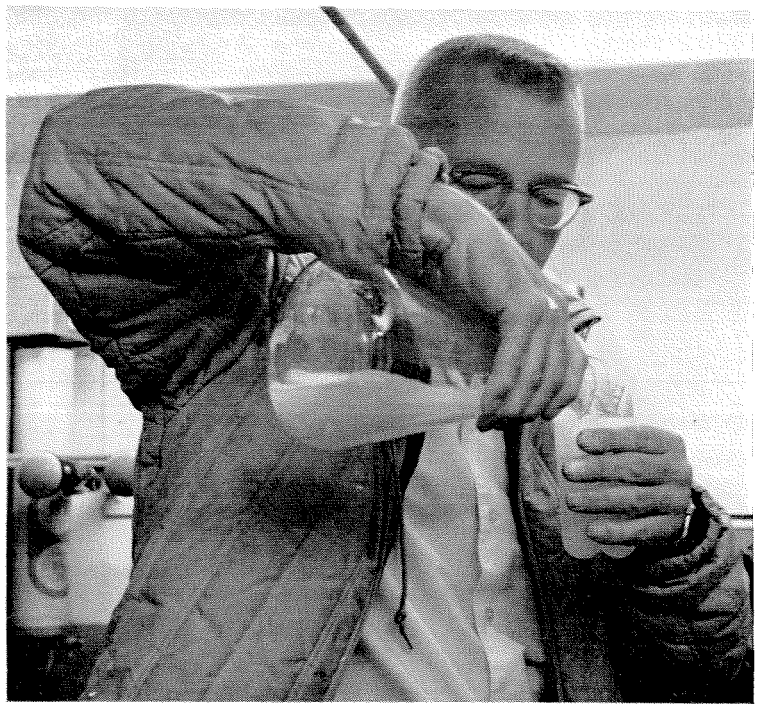


Left — Pea buds are ground in a blender, which ruptures the cells and releases the chromosomes. This and all subsequent operations are carried on in the cold room to minimize chemical changes which would otherwise occur after disruption of the cells.

Left, below — Ground tissue is filtered to remove membranes and other unwanted material.

Below — Filtered extract is dispensed into centrifuge tubes. These will be centrifuged at 4000 times gravity — a field which sediments the large and heavy chromosomes but does not sediment other cell constituents.

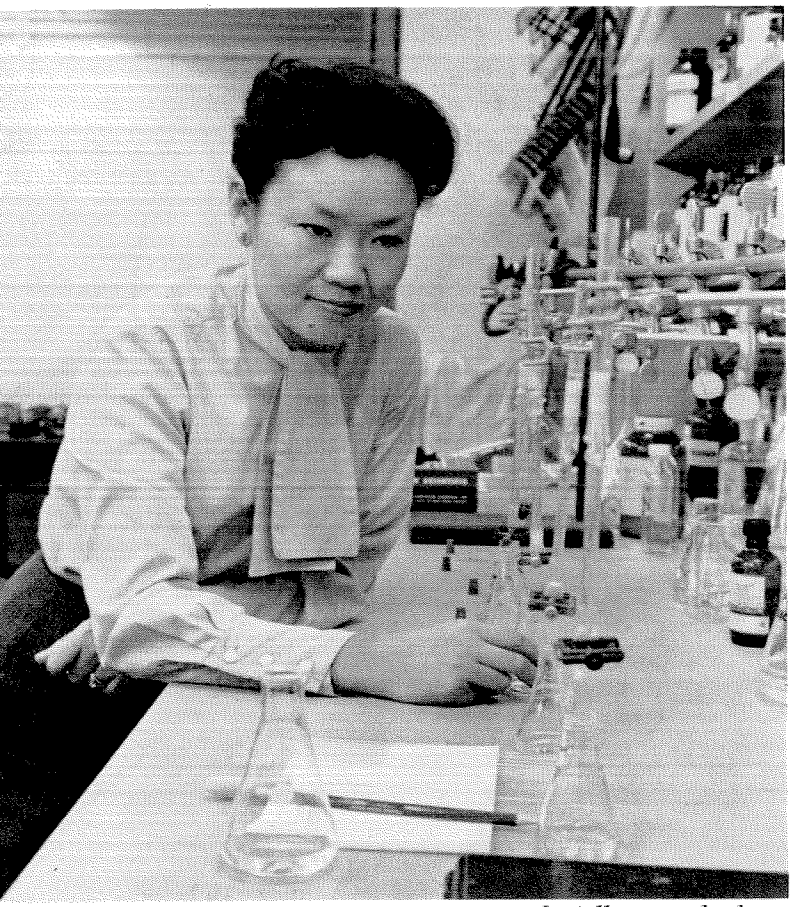




genes to repress also remains to be found out. At least, however, the matter is now accessible to experimental study.

When we de-repress genes in the laboratory by removal of histone we do so by disassociating DNA from histone by the use of high concentrations of salt. It works nicely but not selectively; it de-represses all repressed genes. In the living cell de-repression is selective; one or a few genes may be turned on or off without influencing others. We are, however, beginning to know something about the nature of this genetic switching unit. Just as in bacteria, so also in high organisms it has been found that particular kinds of small molecules are able to turn off or on the activity of particular genes. In the bacteria these are small molecules and genes concerned with making everyday metabolites and the control serves the end of seeing to it that the bacterium does not make some particular kind of substance if that substance is available in the nutrient medium. In higher creatures this kind of regulation by small molecules serves the process of differentiation, and one important class of such molecules consists of the hormones.

A hormone on arrival at its target organ turns on individual or whole sets of genes, causing the pro-



Dr. Ru-chih C. Huang, research fellow in biology, studies isolated chromosomes in the laboratory. Such studies make use of the techniques of enzymology and biochemistry.

duction of characteristic enzyme molecules and setting a cell or cells on a new pathway of development. This is dramatically exemplified by the case of arousal from dormancy which has been studied by our colleague, Dorothy Tuan. The buds of freshly harvested potatoes do not grow. They are said to be dormant. The chromosomes of the cells of the dormant bud are almost completely repressed and cannot, therefore, make any messenger RNA. Dormancy can be ended at any time by supplying the bud with a particular hormone, gibberellic acid, or a synthetic substitute, ethylene chlorohydrin. Treatment with a minute amount of one of these materials causes the buds to grow – the chemical causing a substantial proportion of the genetic complement to be de-repressed and become active in RNA-making. Many hormones work in this way. Cortisone, when it arrives at the liver, causes the genes for making particular liver enzymes to be de-repressed. The flowering hormone, when it arrives at the bud, turns on the previously repressed genes for making flowers and fruit – turns on the flowering pathway.

There is much to find out about the unit genetic switch. We do not, for example, know in detail how a small molecule can bind to a larger represser molecule and change it so that it no longer represses. But again the matter is accessible to experimentation.

This leads us to the final aspect of the new biology of differentiation, the aspect which concerns how the individual genetic switches are linked together to bring about a sequential and orderly development. That the logic of development is based on such a developmental switching network there can be no doubt. The fact, for example, that the several hormones, each itself acting on a unit switch or switches, interact in their effects to bring about sequences of developmental processes shows at once that much interacting switching of genetic activity takes place in life.

How can a genetic switching network be mapped out in molecular detail? The task will certainly be a vast one. For the present, however, we can at least think about it. Let us think of the vegetative bud of a plant at the moment of arrival of the flowering hormone. The hormone turns on some previously repressed genes. The control settings responsible for direction along the vegetative, leaf-producing pathway are reset to bring the developmental pathway on to a fresh course. Once set upon this course the bud inexorably develops step by step into a flower. Certainly, during the course of flower development further switching must take place; fresh genes must be turned on at appropriate places and times, and others appropriately turned off. But this whole latter sequence of genetic switching is called into play only as a result of the initial induction caused by the flowering hormone. Once induction has occurred, the further programming of flower development flows on automatically. It is as though induction by the flowering hormones calls into action a pre-programmed subroutine which contains all of the further directions about how to make a flower.

Let us then try for fit and attractiveness the concept of the life cycle of development as consisting of a master program constituted in turn of a set of subprograms or subroutines. What might be required by way of genetic subroutines to program a creature through its life cycle? A plant, for example, would certainly need a subroutine on cell life, directions for making the subcellular organelles and enzymes needed for cell growth, multiplication, and maintenance. It would also need a subroutine on embryonic development, together with one – a short one, perhaps – about a life as a seed. Then, too, our plant would require subroutines containing information about the making of each of the kinds of plant organs, stems, leaves, roots, and flowers.

Thus we might view the information needed for development as subdivided into categories, each

category appropriate to a particular sub-task. How might these various subroutines be related to one another? How are they wired together so as to constitute the whole program?

Let us consider the problem by considering a simpler example which poses, however, the same questions. The individual subroutines provide the required example. Let us consider the subroutine for bud development. We cannot, of course, specify in detail all of the items which are contained in that part of the genetic book which tells how to make buds. We can, however, specify at least some of the kinds of things which must from the nature of bud development be contained in it.

The bud in some cases arises from a single apical cell which by continual division gives rise to the bud and thence the leaves and stem, always surmounted by the apical cell. Our apical cell must then have instructions about how to divide in appropriate planes and each daughter cell must have instructions about how to find out if it is now the apical one. Clearly, information must be provided about when and where to stop cell division. Such information might in some cases consist merely of a directive to divide so many times and then stop. This cannot be the case with the bud, however, since little pieces cut from the bud continue cell division until a complete bud is again produced.

The same is true of the development of embryos from isolated cells of the early animal embryo. We need a new principle and to this principle we assign the designation of test. In the case of the bud we imagine that the dividing and growing embryonic tissue continuously tests itself for size or for number of cells, each time comparing the value found to a value stated in the program as the desired one. When the correct value is ultimately reached, the cells of the bud can proceed to the next developmental step.

The concept of the test carries us, I believe, to the control core of the logic of differentiation. The growing bud tests itself against the required ultimate size. The same bud tests itself for the presence or absence of flowering hormone. A cell tests its neighbors for strangeness or similarity. In these and a myriad of further ways must we imagine that each cell in the developing organism keeps itself informed of where it is in the developmental path and what therefore is the appropriate next step. The test as one unit in the logic of differentiation is of course already known as an experimental fact, as in the case of the presence or absence of a hormone in its target organ. All that we do here is to extend the concept of the test to include other kinds of tests which, although not yet experimentally known, would seem to be essential to the machinery of development.

One of the clearest examples of the developmental test at work is provided by the example of the plant embryo. This normally develops as do all embryos from a single cell, the fertilized egg, and carries on its development inside an ovary full of the chemicals needed in embryonic growth. And these two conditions are all that are required to cause a cell to develop into an embryo: the cell must be single and be surrounded by the ovarian nutrients.

F. C. Steward at Cornell University has shown that fully differentiated cells of many different types start life anew and develop into embryos if these two conditions are fulfilled. We may imagine that the plant cell is continuously testing itself for the presence of neighbors. If it finds it has none, it then tests for the presence of the embryo nutrients. If the outcome of this test is positive, the cell must say to itself, "Well, it's strange, but I am all alone and here are the embryo nutrients, so I must be a fertilized egg and I will therefore develop into an embryo."

The developmental test as it is here conceived consists in the sensing by a particular gene of the concentration of a particular chemical substance. The concentration of this substance in the region of that gene determines whether the gene shall be active or repressed. In this way, through the use of a multitude of different sensor substances the cell keeps track of where it is in the developmental pathway.

We have found that, by using the concept of the developmental test, it is possible to write down model programs which, if carried out by a cell, would cause it to develop into an organ or organism. In such a model program, a successful outcome of one test leads to a directive to carry out a further and different test and so on. In format the developmental programs resemble those for computation by an electronic computor. For this reason our most lifelike program, one which causes a single apical cell to develop into a plant stem, is called "digital organ generator, model A," or, for short, DOGMA.

Such modeling of the developmental switching network is not done just for fun. Its usefulness lies in the fact that it not only helps to give us insight into the principles which underlie development but in that it also suggests which specific key control points in the developmental process will be most fruitful to investigate.

These are stirring times for the study of growth. The tools are at hand, the way is clear, and progress is being made at all levels in the deep probing of the processes of development and differentiation.