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Cell Differentiation: Some Aspects of the Problem

The interaction of a temporal sequence of gene action and metabolic factors may direct differentiation.

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In a classical sense cytodifferentiation depends mainly upon the histological characteristics of cells. These morphological qualities, which provide a basis for classifying cells as differentiated, appear to be attributable primarily to structural properties of the proteins of the cells. For example, myosin of muscle and hemoglobin of erythrocytes are proteins which are end products of differentiation which typify these cells. The structural organization of proteins into cellular organelles is yet another facet of cytodifferentiation. Other cells may be characterized less easily by various cellular products which are synthesized under the control of specific enzymes or through certain physiological or biochemical activities having an enzymatic basis. Differentiation in an embryo involves the selective association of cells and tissues to form organs which attain a topographic pattern characterizing the organism, but this level of differentiation undoubtedly is also influenced by specific proteins of the cell membranes, which play a major role in the morphogenetic events. Since the properties of proteins are attributable to the activity of chromosomal genes, it would seem that the genes are the specific determinants of cytodifferentiation, although their activity may be modified by environmental factors.

According to the working definition of differentiation just given, the proteins that distinguish cells as differentiated are synthesized in significant amounts when the cells differentiate. For example, immunological studies have shown that myosin (*1*) and lens antigens (*2*) appear at the time of differentiation, and Boell and Shen (*3*) have demonstrated the onset of cholinesterase activity when the neural plate is formed. It is true that antigens with combining groups characteristic of myosin and actin (*4*), the globin portion of hemoglobin (*5*), and lens proteins (*6*) may be present in trace amounts before differentiation. This is of particular interest when the tissue-specific antigens are localized in cells that normally are directed into this type of differentiation. For instance, Ebert (*4*) has found that antigens which react with antisera against cardiac actin and myosin become localized in the prospective heart-forming areas of the early chick embryo.

Although specific proteins, when present, define or characterize cells as differentiated, it seems probable that the process of cell differentiation is allied with the mechanisms of directed or specific protein synthesis, rather than with the ultimate appearance of protein molecules. Schechtman (*7*) and Ebert (*8*) have emphasized that the appearance of tissue-specific antigens may reflect only the end point of differentiation. It appears profitable, therefore, to examine the role of DNA and of specific RNA's in directing protein synthesis, since their activities occur prior to protein synthesis and differentiation. This approach may be particularly fruitful in cases where the pathway of future differentiation of embryonic cells is set, or "determined," some time before the actual synthesis of the tissue-specific proteins occurring at the time of differentiation.

Recent studies in the field of molecular biology have revealed that a linear order of triplet combinations of nucleotides in RNA specifies the sequence of amino acids which are polymerized to form a polypeptide. The most convincing evidence for such a polynucleotide code was the demonstration by Nirenberg and Matthaei (*9*) that polyuridylic acid (polyU), a polynucleotide composed only of uridine, stimulated the formation of a polypeptide composed only of phenylalanine. In terms of a triplet coding unit, it appears that the triplet (UUU) codes for the utilization of phenylalanine in protein synthesis. This remarkable discovery tells us that a specific polyribonucleotide sequence can be transcribed into a specific sequence of amino acids. It does not, however, explain why certain gene-produced polyribonucleotide sequences operate in some cells whereas other sequences are transcribed in other cells, when the genetic constitution, and therefore the DNA makeup, of all cells in the organism is

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the same. That different genes act to form different proteins in various cells is shown by the fact that cells are of one type or another—for example, nerve or muscle. This does not mean, however, that similar genes are not active in nerve and muscle, since one finds many enzymes which are common to the two types of cell.

Metabolic Control of Cell Differentiation

One of the most important approaches to the problem of differential gene action and differentiation is that of examining instances in which the pathway of differentiation can be controlled experimentally and then attempting to understand the molecular basis of such experiments. Bits of undifferentiated embryonic tissue have been cultured in vitro, in order to remove them from the inductive influences of neighboring tissues, and then stimulated to differentiate by the addition of various chemicals or of cell fractions obtained by differential centrifugation. The isolation of ectoderm of amphibian gastrulae into a buffered saline medium results in the differentiation of the cells into epidermis. However, the undifferentiated gastrula ectoderm can be stimulated to form pigment cells, neural tissue, and muscle by short exposure to solutions of low or high *p*H (*10*, *11*) or by the introduction of embryo extract (*12*), serum globulin (*13*), a protein fraction of guinea pig bone marrow and microsomal fractions of neurula stages of the salamander embryo (*14*), or nucleic acids (*15*) into the culture medium. Introduction of the fraction of guinea pig bone marrow normally induces formation only of mesodermal structures. It is known that solutions of high and low *p*H cause some of the material of the yolk platelets to become soluble (*16*), and recent electron-microscope investigations of developing ampribian embryos have revealed that the surface layer of yolk platelets is solubilized from some of the yolk platelets of differentiating embryonic cells in vivo (*17*), and solubilized in cells differentiating in vitro under the influence of the protein fraction of guinea pig bone marrow (*18*). This evidence supports the view that soluble protein and other compounds from yolk are required for stimulating cellular differentiation in amphibian embroys (see *19*).

This view is also supported by recent unpublished work in our laboratory in which it was found that overnight exposure of gastrula ectoderm and ventral mesoderm of gastrulae to calcium chloride (concentration, 0.03 to 0.05*M*) and 0.5 percent guanidine hydrochloride can induce differentiation of nervous and muscular tissue. It is known that calcium chloride in these concentrations will solubilize some of the yolk platelets (*20*), and guanidine hydrochloride is also effective (*21*).

Niu (*22*) has claimed that ribonucleic acid from a given adult organ directs the differentiation of ectoderm from amphibian gastrula into an organ of the type from which the RNA was obtained, but his data, as well as those of others (*23*), indicate an absence of tissue-specific effects. It is more likely that the nucleic acids are hydrolyzed and that they then stimulate differentiation by supplying a pool of nucleotides to the reacting tissue. One would expect to find that the RNA that might be transferred between inducing and reacting tissues would induce the reacting tissue to differentiate in the same manner as the inductor, but this is not the rule; for example, differentiating notochord and somites induce the formation of neural tissue. Numerous experiments have demonstrated a lack of species specificity and tissue specificity when differentiation is induced in embryonic tissue, and this, together with the finding that differentiation is induced by low or high *p*H, offers a strong argument against the idea that the qualitative nature of protein synthesis and differentiation in embryonic cells is directed by the informational code of the RNA which may enter the cells of the reacting tissue from an inductor tissue.

From such studies is emerging the generalization that alterations in metabolism may switch the differentiative pathway of embryonic cells. As a result of experimental studies on yolk utilization in embryonic cells, I have suggested that the induction of cellular differentiation in embryonic tissue is a stimulus to protein synthesis in the cells of the reacting tissue, the genes then specifying what kinds of proteins are synthesized, and thus what kinds of cells differentiate (*24*). According to this view, the addition of factors needed for protein synthesis (for example, amino acids, or nucleotides for the synthesis of RNA, which plays a role in protein synthesis) might stimulate differentiation in the reacting tissue. It is apparent that this scheme refers primarily to the *differentiation* of cells and would not account directly for the morphogenesis of cells into certain patterns of tissues and organs. The action of chick embryo extract (*12*), serum globulin (*13*), and neurula microsomes (*15*) in stimulating differentiation possibly is due to the fact that, upon hydrolysis, such fractions provide amino acids and nucleotides which stimulate protein synthesis in the gastrula ectoderm.

It is possible that purified proteins that stimulate differentiation may not serve directly as a source of amino acids. Barth and Barth (*13*) suggest that serum globulin in the medium has a protective effect, allowing gastrula ectoderm to form nerve and muscle. The recent work of Yamada (*18*) indicates that the inducing activity of the protein fraction of guinea pig bone marrow may be attributable to its action in solubilizing the surface layers of yolk platelets. It is also possible that the inductive action of nucleic acid fractions (*15*, *22*, *23*) may be an indirect one. Organ culture of ectoderm and ventral mesoderm of the frog gastrula, in the presence of all the amino acids or of a mixture of the RNA mononucleotides, does not result in stimulation of differentiation into neural or muscle cells (*21*). It would be surprising if both amino acids and nucleotides were found to be rate-limiting factors for the synthesis of proteins at the time of differentiation, since the presence of either nucleic acid (*15*, *22*, *23*) or of protein alone (*18*) may stimulate differentiation. The system is complex, and there are probably a number of prerequisites for protein synthesis, such as the formation of an endoplasmic reticulum containing ribosomes.

That the level of metabolism can influence the direction of differentiation is suggested by experiments in which the type of differentiation of an explant of embryonic tissue depends upon the mass of this group of cells. One somite plate of the salamander embryo, explanted in an ectodermal envelope in salt solution, shows poor differentiation of muscle, while nephric tubules and blood cells are often formed, but the isolation of two or more somite plates allows good differentiation of muscle, nephric tubules, notochord, neural tissue, and limb buds (*25*). Lopashov (*26*) demonstrated than an explant of a single piece of presumptive head mesoderm forms muscle, but that fusion of six or more such fragments

Table 1. Incorporation of C^{14}-labeled carbon dioxide by untreated worms and worms treated with chloramphenicol or with deacetylmethylcolchicine.

Radioactivity (count/min per mg of carbon)			
Region 1 (head)	Region 2	Region 3	Region 4 (tail)
Untreated			
8000	6800	3600	2200
*Treated with chloramphenicol**			
249	221	187	186
Treated with deacetylmethylcolchicine†			
554	561	455	571
796	631	531	714
594	364	408	407

*Dose, 1 mg/ml for 2 days. †Dose, 0.02 mg/ml for 1 day.

results in the differentiation of muscle, notochord, brain, sense organs, and epidermis. According to Trinkaus and Drake (*27*), the more complex differentiation in explants of larger mass may be due in part to increased carbon dioxide tension and to the accumulation of other metabolites. Since one is not introducing a heterologous tissue into these systems, the explanation that alteration in the level of metabolism is a factor seems appropriate.

There is a good deal of evidence that the quantity of the stimulating factor, or factors, dictates the nature of the differentiation. This conclusion is inferred from the work of Nieuwkoop *et al.* (*28*), in which a piece of gastrula ectoderm is folded together by the inner surfaces and grafted to a gastrula, coming to lie as a perpendicular flap of tissue upon the differentiating medullary plate. This results in differentiation into neural tissue of the cells of the flap that are proximal to the anterior part of the host medullary plate, in formation of neural-crest derivatives (ganglia and pigment cells) in a more distal part of the flap, and in formation of epidermis in the most distal region of the flap. A possible explanation of this result is that there is a gradient of inducing factor, or factors, in the flap, since the neural plate of the host embryo serves as the source of the factor.

The lability of the mesodermal mantle of the amphibian neurula seems to be another example of quantitative metabolic factors playing a role in specifying regional differentiation. Bits of the prospective blood-island tissue of the ventral region can form muscle if they are transplanted to the somite area, whereas bits of somite destined to form muscle may form blood cells in vitro or pronephros if they are grafted to the blood-island area (*29*). Yamada (*11*) has also stimulated notochord and muscle differentiation from the prospective ventral blood-cell area by shocking with media of high *p*H. This suggests that solubilized yolk materials may produce a shift in the direction of differentiation.

The principle that quantitative metabolic differences account for regional differentiation is illustrated also in recent experiments in our laboratory (*30*) in which the polarity of regenerating planarians has been reversed by exposure of the prospective anterior ends of the worms to chloramphenicol, an inhibitor of protein synthesis, or Colcemide (deacetylmethylcolchicine), which blocks mitosis. Worms with the head and tail cut off were embedded in thin sheets of agar, and the ends that normally form heads were placed in a solution of chloramphenicol (2 mg/ml) or Colcemide (0.02 percent) for 1 to 2 days. After removal of the worm from the agar, the progress of regeneration was followed to the time of head formation. At that time reversal of polarity was observed in approximately 22 percent of the treated worms. Pretreatment of intact planarians with a 0.02 percent solution of Colcemide for a day, followed by posterior sectioning, embedding of the worm in agar, and exposure of the head to Colcemide for a day, resulted in posterior differentiation of a head, despite the presence of the original head (Fig. 1). Control worms were cut, embedded in agar, and exposed to tap water that did not contain chloramphenicol or Colcemide, but none of these worms showed altered polarity. It has been demonstrated that there is an axial anterior-posterior gradient of incorporation of C^{14}-labeled carbon dioxide and glycine into the protein fraction of the planarian, and that this gradient can be greatly reduced, or abolished, by exposure of the intact worms to chloramphenicol or Colcemide (Table 1). These data support the view that chloramphenicol and Colcemide inhibit head formation at the prospective head end of the cut worm, reducing the level of protein synthesis and allowing head formation to occur at the cut end, which normally forms the tail. Here is an instance in which the biological alteration of polarity and the direction of differentiation of cells can be correlated with an alteration of the normal axial difference of activity of the protein-synthesizing mechanisms. It is known that an adequate supply of undifferentiated, or dedifferentiated, cells is needed for the formation of the regeneration blastemata. The action of chloramphenicol and Colcemide might be due to an inhibition of mitosis, but it must be remembered that heads often regenerate from the areas exposed to the inhibitor, so that there is a head at each end of the worm. Apparently the establishment of a gradient in the worms, rather than an absolute decrease in the number of cells or in metabolism, is the critical factor in altering biological polarity.

In another type of experiment, worms were cut just back of the pharynx, and the anterior ends of the posterior parts of the worms were embedded in agar slabs and exposed to β-mercaptoethanol ($1 \times 10^{-9} M$) for a day. Subsequently, eye spots differentiated in the uncut tails of these worms (Fig. 2). This indicates that the biological polarity may be reversed without a wound stimulus at the site where the new head forms.

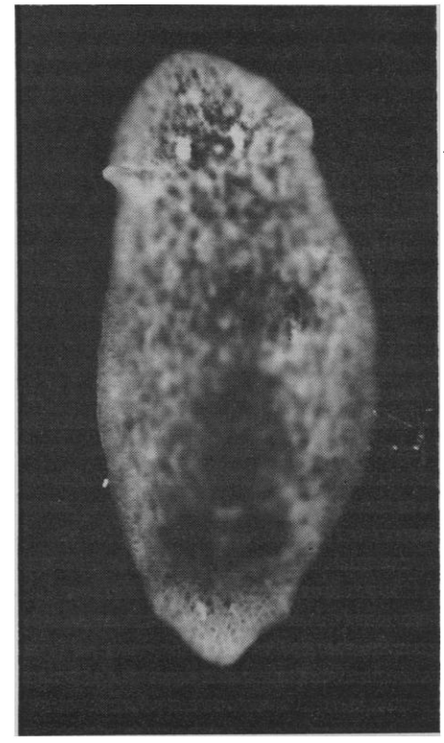

Fig. 1. Posterior head formation (at bottom) in a worm that had been treated with Colcemide (0.02 percent), then cut and embedded in agar so that the original head was further exposed to Colcemide for a day.

Temporal Pattern of Gene Action

In the field of experimental embryology there are numerous examples of compounds or factors that alter the rate of metabolism and the differentiation pathway of undifferentiated cells, and it has been suggested by Child (*31*) that there is a causal relationship between metabolism and differentiation. The critical problem becomes that of translating quantitative differences of metabolism into qualitative cell differentiation. I have suggested that the activation of different genes occurs in a temporal sequence, and that the type of cellular differentiation depends upon *when* the factors needed for protein synthesis (amino acids, nucleotides, and so on) reach a threshold level (*24*). The supply of factors needed for protein synthesis corresponds to what the embryologist terms induction, while the sequential activity of genes corresponds to embryonic competence. According to this view, the qualitative nature of cell differentiation depends upon what genes are active at the time a sufficient level of the materials necessary for protein synthesis are provided. A biological example of this principle is illustrated by the work of Lasarev (*32*). He removed prospective lens ectoderm from the embryo prior to lens induction by the optic cup and cultured the prospective lens ectoderm in vitro for several days. This ectoderm was then grafted back over an optic cup of an age at which the lens is normally induced. The ectoderm that had been cultured in vitro and grafted back then differentiated as corneal tissue. In this experiment, the inductive stimulus that normally promotes lens differentiation had been provided to cells that were older than the cells that normally receive this stimulus, and the differentiation response was specific for the age of the reacting tissue. Apparently, different genes had attained a state of potential activity, and a supply of materials allowed synthesis of proteins that characterize the cornea. Selection of the pathway of differentiation has been ascribed to an interaction of two variables: (i) a supply of factors needed for protein synthesis, and (ii) sequential gene action (*24*). However, these two activities are not independent, since they have a common metabolic dependence upon a number of biochemical substrates.

The sequential differentiation of different organs of the embyro is relatively inviolate. Whereas one can experimentally prevent the differentiation of certain parts, cause them to duplicate, or cause them to change location, it is ordinarily not possible to cause differentiation of an organ to occur out of order—for example, to cause the liver to differentiate before the nervous system in the frog embryo. It is obvious that many differentiations are not widely separated in time, but along any given axis one usually sees a temporal pattern of differentiation. Regeneration would appear to be an exception to the concept of sequential gene action, but if one considers only the parts of the regenerating system, the plan of sequential generation appears to hold true, with the modification that only a small part of the sequence is activated. For example, regenerating planarians form the brain before they form the eye spots, and the eye spots differentiate in advance of the pharynx. Also, in regenerating limbs of salamanders there is a proximodistal sequence of differentiation. Gurdon (*33*) has shown that when nuclei from differentiated intestinal epithelial cells of larval *Xenopus* tadpoles are injected into enucleated eggs, the eggs develop to adult stages. It appears that the pattern of sequential gene action can be completely recycled in nuclei from the intestinal epithelial cells after they have undergone repeated mitotic divisions, since the daughter nuclei participate in the various cellular differentiations that occur in the embryo. This remarkable demonstration implies that the cell nuclei of regeneration blastemata may become totipotent —that is, dedifferentiated—after a number of cell divisions.

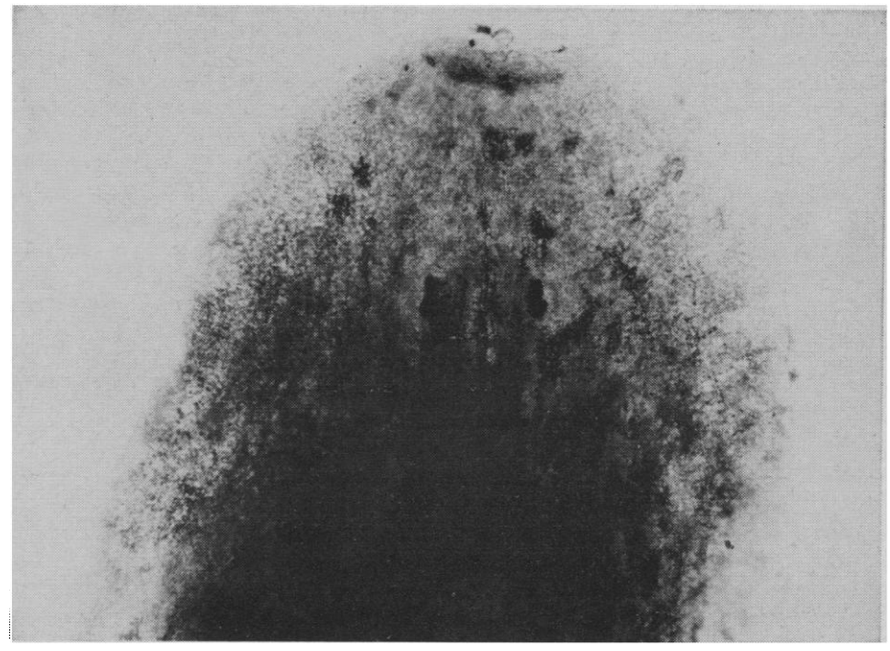

Fig. 2. Formation of eye spots in the uncut tail of a worm whose anterior cut surface had been exposed to β-mercaptoethanol ($1 \times 10^{-3} M$) for a day while the cut worm was embedded in agar.

Sequential Synthesis of RNA by DNA

Observation of developing embryos shows that tissues differentiate at various times. I have already pointed out that the synthesis of tissue-specific proteins usually begins at the time of differentiation, but it is necessary to learn whether earlier phases of gene action may have occurred some time prior to visible differentiation. It is possible that chromosomal DNA produces tissue-specific RNA molecules that are present in the cytoplasm for some time before differentiation, and that these molecules become active when they are supplied with materials for protein synthesis. If this thesis is valid, the cytoplasm has not been irreversibly "programmed" by RNA produced by chromosomal DNA; one has only to examine the various pathways of differentiation still available to the cells of the mesodermal mantle of the neurula

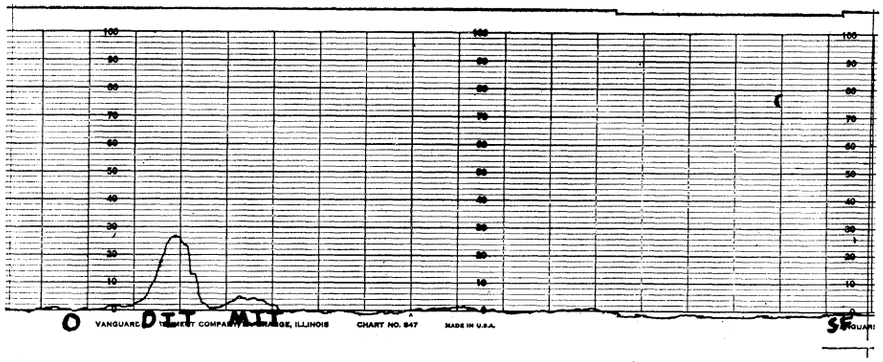

Fig. 3. Radiochromatographic tracing (collidine-H_2O-NH_3 system) of a butanol extract from the thyroid area of an 11-millimeter frog larva which had been exposed to NaI^{131} (5 μc/ml). Both monoiodotyrosine and diiodotyrosine may be observed.

to see that their fate is not yet irreversibly set.

Kano-Sueoka and Spiegelman (*34*) used isotopic procedures and column chromatography to characterize RNA in *Escherichia coli* infected with T2 phage in which specific phage proteins appear sequentially. They found that RNA's synthesized at different times during infection differed either in base sequence or in composition—a finding that indicates a sequential synthesis of specific RNA molecules just prior to synthesis of the different proteins.

The use of the antibiotic actinomycin D, which inhibits DNA-dependent RNA synthesis, seems to offer another means of determining when this phase of gene action takes place. If specific RNA is produced by DNA and localized in the cytoplasm in a stable form, then protein synthesis should continue in the presence of actinomycin D. Higher concentrations of actinomycin D (20 to 100 μg/ml) block cleavage of the sea urchin egg (*35*) and gastrulation of the frog embryo (*36*)—findings that indicate the importance of DNA-dependent RNA synthesis for early development. When frog gastrulae are exposed to lower concentrations (8.0 μg/ml) of this inhibitor for 2 days, they frequently develop to the early tail-bud stage and appear normal, except that they do not show any of the flexure movements that are characteristic of normal larvae of this stage. If these treated embryos are then subsequently raised in saline medium, some of them renew their development and subsequently reach the feeding stage. They may be fairly normal in morphology, but they are immotile (*36*). These results suggest that this concentration of actinomycin D has blocked the synthesis of RNA which directs formation of proteins of the nervous or muscular system, or both, which normally account for motility. Histological examination of some of the more severely affected larvae reveals failure of the brain, spinal cord, and myotomes to differentiate in the anterior regions but normal differentiation of spinal cord and muscle in the tail, which develops later. The fact that eyes, lens, heart, and other late-differentiating structures develop normally in the embryos subsequently raised in saline medium indicates that the RNA molecules responsible for the synthesis of these tissue-specific proteins were produced at a later time. That actinomycin D in higher concentrations blocks gastrulation of frog embryos or cleavage of the sea urchin egg may be due to inhibition of DNA-dependent RNA synthesis, which is necessary for the formation of proteins, such as cytochrome oxidase, that have a critical metabolic role. In this regard, Davidson, Allfrey, and Mirsky (*37*) have found that exposing cultured cells of connective tissue to actinomycin D stops the synthesis of a characteristic polysaccharide protein within 17 hours, although it does not affect succinic dehydrogenase activity in that same period.

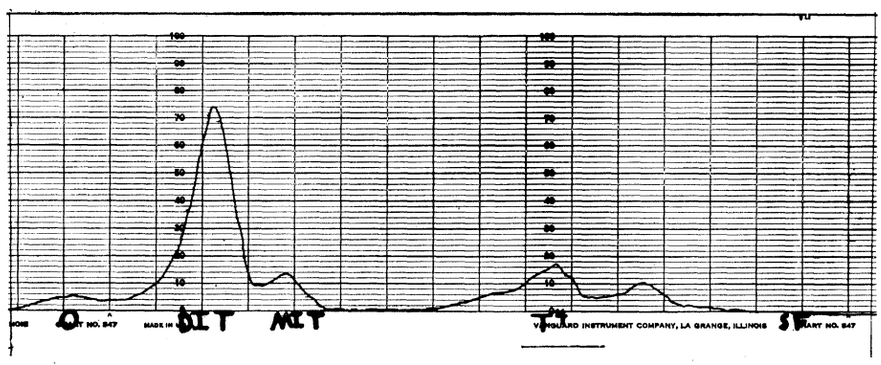

Fig. 4. A recording, similar to that of Fig. 3, from the thyroid area of a 16-millimeter frog larva, showing the presence of thyroxine (T^4).

O'Brien (*38*) has shown that 8-azaguanine, a base analog, will inhibit hemoglobin synthesis in the chick embryo if it is present just before the time of hemoglobin formation. Earlier or later exposure to 8-azaguanine has no effect. This is another demonstration that nucleic-acid synthesis specific for the formation of a given protein occurs just prior to the time of synthesis of that protein.

It is evident that there are data supporting the idea that production of specific RNA molecules by chromosomal DNA (genes) occurs in a temporal sequence and is followed by synthesis of specific proteins. It has been suggested that there is a temporal sequence of activity of a series of genes, and that the time at which factors necessary for protein synthesis are supplied determines which genes will operate to direct the synthesis of their proteins, and so determines the *kind* of cellular differentiation that will take place (*24*). An obvious way to evaluate this suggestion experimentally is to ascertain whether the sequence of potential gene action is characterized by the production of specific RNA molecules. This problem is currently being investigated in our laboratory by isotopic methods in cell-free systems, in which the factors for protein synthesis can be manipulated in any desired manner. Another possibility is that the postulated sequence of gene activity may be represented, not by the production of specific RNA molecules by DNA, but instead by a series of activations, or derepressions, of various genes, which would account for a serial sequence of potential gene activity. It is obvious that any of these suggestions must be verified experimentally.

Basis of Sequential Gene Action

The critical and most difficult problem relative to the question of protein synthesis and differentiation is that of discovering the mechanism which insures the actual or potential sequential activity of the genes. Since this facet of the developmental mechanisms is the most inflexible, one is tempted to attribute this feature to a stable structural component of the cell. The chromosome or its polynucleotide molecules are the most obvious possibilities. The problem of whether a chromosomal deoxyribonucleotide sequence will merely replicate or will make a specific RNA molecule which will code for the synthesis of a specific protein is also seen in lysogenic bacteria. Some viruses can exist as prophages in lysogenic bacteria, where they are integrated into the bacterial chromosome, and these prophages replicate their DNA but do not form phage protein. When these prophages are "induced" to leave the host chromosome, they make phage protein, and the virulent phages subsequently lyse the bacteria. In this case the suppression of activity of the prophage DNA is dependent upon its presence in the host chromosome.

This analogy immediately suggests that chromosomal DNA is subjected to repression. It has been shown that removal of histone from chromosomal nucleohistones facilitates the production of RNA (*39*). The elegant work of Jacob and Monod (*40*) provides a scheme by which regulatory genes act to produce repressors which maintain the structural genes in an inactive state. At present there seems to be insufficient experimental evidence to relate this evidence for repressors to the problem of differentiation, but this situation may change with the acquisition of further experimental data.

In searching for the mechanism that accounts for the role sequential activity of gene families plays in cellular differentiation, one is faced with the problem of not knowing the identity of all the genes that have a role in the synthesis of those proteins that characterize a cell as differentiated. A simpler system is found in the bacterium *Salmonella,* in which there is a linear transcription of the genome in the cases of the genes that control the synthesis of the nine enzymes that promote histidine synthesis (*41*). The suggestion that the actual or potential sequential gene action in multicellular organisms has, as its basis, a serial order of genes whose time of action is a function of their linear position on the chromosome is not supported by genetic mapping experiments in higher organisms. These experiments show that the genes which control enzymes that act sequentially in a biochemical pathway are not always in a serial order on a chromosome, and are not necessarily even on the same chromosome.

However, there is evidence for the sequential appearance of compounds in certain biosynthetic pathways in the developing embryo. In the thyroid gland of the developing chick embryo there is a temporally sequential appearance of monoiodotyrosine, diiodotyrosine, and thyroxine (*42*). This sequence is also observed when developing frog larvae are exposed to NaI^{131} (*36*). With radiochromatographic techniques it is possible to detect labeled monoiodotryosine in the 7-millimeter larva, diiodotyrosine in the 11-millimeter larva (Fig. 3), and thyroxine in the 16-millimeter larva (Fig. 4). Caston (*43*) has detected dopamine, noradrenaline, and adrenaline in the developing frog embryo; they appeared in that sequence, and this is the order of their appearance in the biosynthetic pathway. These data imply that the enzymes that promote these reactions are synthesized in a temporal sequence. This might be due to sequential induction of enzymes, the product of one reaction inducing the synthesis of the next enzyme, whose product then induces synthesis of the next enzyme. Another possible explanation is that the genes that account for the synthesis of these enzymes become active in this sequence, due to their order of linkage in the chromosome. The evidence of Jacob and Monod (*40*) indicates that in *Escherichia coli* a group of closely linked structural genes (operons) is transcribed in a polarized manner. Structural genes of a single operon are controlled by one operator locus, which is the site of action of a repressor which is produced by a regulator gene. This system can be complex; in the case of arginine synthesis in *E. coli* there is one repressor gene for seven structural genes that control the synthesis of seven enzymes, but these structural genes are found in five different parts of the bacterial chromosome. Jacob and Monod (*40*) suggest that the operator portions of these operons have a common structure and that this accounts for their control by a single repressor.

One is drawn to the view that there is some type of limited polarized transcription of the genetic material, even if the transcription occurs in patches, since this would insure the sequential nature of actual or potential gene action. Edgar (*44*), in recent work with a bacteriophage, has demonstrated a linear order of genes which accounts for the sequential appearance of structural proteins of the phage. Although metazoan chromosomes are much more complex than the phage chromosome, Edgar's "embryological" study of a developing bacteriophage points to the importance of genetic mapping experiments with embryos and of studies of chromosome structure. With the availability of such information it may be possible to elucidate the mechanism of sequential gene action and determine why certain types of cell differentiate before others.

Summary

The pathway of differentiation of a group of cells can be directed experimentally, in some instances, by regulating the availability of the substrates and cofactors necessary for protein synthesis. Several lines of evidence indicate that, during development, there is a temporal sequence of activity of groups of genes that play a role in the differentiation of the various kinds of cells. It is possible that this activity may be expressed by the production of specific ribonucleic acid molecules by DNA, or perhaps only by derepression or activation, so that some of the genes of the chromosomal DNA are capable of forming specific RNA. The decision as to whether these derepressed or activated genes will go ahead and synthesize proteins that will specify what kind of cell will result from differentiation may depend upon the availability of substrates and energy for protein synthesis, and upon the level of activity of various components of the protein-synthesizing machinery. If the levels of substrates and cofactors needed for protein synthesis are too low at a time when the groups of genes that code for the synthesis of proteins of one cell type are capable of acting, then the synthesis of these specific proteins will not take place and the cells

will not differentiate into cells of that type. If the metabolic level becomes optimal in these cells later, when other group of genes are active, then these other genes will direct the synthesis of proteins that will specify differentiation into cells of another kind.

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Space Science and the Universities

Educational and research programs contribute to manpower and advances in the space sciences.

Frederick Seitz

There is a curious dualism in the mobility of man and his culture throughout history in that the species is distributed in essentially all places that are even remotely habitable, in spite of the fact that the average individual basically prefers to remain fixed. Most human beings spend their lives within a few miles of their birthplace, usually marrying someone from just around the corner. Yet in spite of this, man has managed to get to every spot or crevice of the earth's surface that he can reach by muscle or machine. Moreover, individuals have wandered far afield in spite of hardships. Man's motives in moving from one region to another, either in small missions or in large groups, are varied. Let me list a few of the principal ones.

Motives in Exploration

First, and perhaps most important, is the drive for self-preservation. It is this, for example, which brought the Eskimos to the Arctic wastes, Patagonians to the bleak tip of South America, and Bushmen to South Africa.

Second is the desire for trade. This led to the establishment of the Amber Route between the Mediterranean and the Baltic many thousands of years ago, and led the Chinese to develop the caravan routes across the Gobi Desert to Persia and Rome. This trade enriched Rome with silks but depleted it of gold. The same desire for trade brought the Phoenicians to England and around Africa, and ultimately brought Columbus to the New World.

Third is the desire for adventure or booty, which drove Alexander across Persia to India, Marco Polo to far-off Cathay, and Pizarro to Peru.

Then there are motives of military strategy, such as those which caused Hannibal to cross the Alps with his elephants to attack Rome from the north and caused Henry the Navigator to seek a route around Africa, thereby outflanking the Arabian citadels in the Near East.

Desire for prestige may play a very important role; it is this which led national teams to penetrate the polar regions and led Hillary to the top of Mount Everest.

Then there may be religious motives, such as those which led Livingstone to the heart of Africa, the Puritans and the Quakers to the New World, and the Italian Jesuit Matteo Ricci to Peking, where he lived the life of an expatriate 400 years ago.

Finally, there is the desire for unusual knowledge, one of the strongest of driving forces in one group of human beings. This quest for knowledge drove many men to the heart of Africa to search for the source of the Nile, drove others across the Arctic wastes to the Poles, and drives still others in bathyscaphes to the ocean depths.

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