made between piece-work constructions such as Mesoamerican earth or stone rubble pyramids, and moving of multi-ton stones. Huge earthworks can be built in a short time by many workers, or in a longer period by fewer workers. Unless we have a fairly clear idea of the construction time involved, it is only guesswork to suggest the number of man-days involved per month or per year. But with a 100-ton stone a minimum number of workers were necessarily involved, and it is for this reason that I believe transport of colossal stones offers a more definite avenue of inquiry into the energy organization of prehistoric societies. When numbers of megalithic stone monuments were moved by one people, it can be assumed that a large group effort was involved. Despite the persuasive arguments of Kaplan that the "chiefdom" type of society operating with an agricultural economy may have achieved such impressive construction projects as exist at Teotihuacán or at the major sites of the Olmec and Maya cultures in Mexico and lowland Guatemala, I consider the question of societal type still an open one until more precise information is available. It is difficult in any case to see Teotihuacán and Tikal as merely religious capitals of chiefdoms.

87. The La Venta pyramid contains about 140,000 cubic meters of earth, the stone-faced Pyramid of the Sun at Teotihuacán about 840,000 cubic meters, and the Pyramid of the Moon at Teotihuacán about 210,000 cubic meters of rubble.
88. A. Palerm, *Pan. Amer. Union Social Sci. Monograph* **1**, 28–42 (1955); G. Cowgill, *Southwestern J. Anthropol.* **20**, 145–159 (1964).
89. R. J. Forbes, *Man the Maker* (Schuman, New York, 1950), p. 44; W. A. Fairservis, *The Ancient Kingdoms of the Nile* (Crowell, New York, 1962), pp. 80–81.
90. M. Coe, *The Jaguar's Children* (New York Graphic Society, New York, 1965), p. 122, thinks that Olmec Society was not organized on a theocratic basis but rather, like that of the Maya, was controlled by "secular lords who drew their power from lineage and from conquest."
91. F. Lopez de Gomara, *Cortes*, L. B. Simpson, Ed. and Transl. (Univ. of California Press, Berkeley, 1964).
92. S. Linné, *Ethnogr. Mus. Sweden, Publ.* No. 7 (1942).
93. W. T. Sanders, *The Cultural Ecology of the Teotihuacán Valley* (Pennsylvania State Univ., University Park, 1965).
94. A point made earlier by J. W. Fewkes, *Smithsonian Inst. Misc. Coll.* **61** (No. 6), 4 (1913), who wrote, "Monoliths, as expressions of a desire to perpetuate the memory or to commemorate past events, are naturally found only where the race had arrived at a self-consciousness of its own power." G. Clark and S. Piggott, *Prehistoric Societies* (Knopf, New York, 1965), p. 158, discuss the theme of the "Neolithic enlargement of the conceptual horizon in terms of an increased [time] perspective."
95. A. L. Kroeber, *A Roster of Civilizations and Culture* (Univ. of Chicago Press, Chicago, 1962). See especially pp. 73–86, "Presences and absences: Old and New World civilizations."
96. A. Caso, *Cuadernos Amer.* **6**, 147–152 (1965).
97. J. H. Rowe, *Amer. Antiquity* **31**, 334–337 (1966).
98. Acknowledgement is made to the Guggenheim Foundation for a fellowship during 1964, to the Miller Institute for Basic Research in Science for a professorship in the fall of 1964, to the Wenner-Gren Foundation for Anthropological Research and to the National Geographic Society for travel grants in 1963 and 1964 in connection with field studies on the subject of this paper. The data for this article have been drawn from a much larger collection being prepared for a book to be published by A. Knopf, Inc.

Multiple Causes and Controls in Differentiation

The variety and interdependence of these causes may be essential to the stability of morphogenesis.

Barbara E. Wright

Entities are not to be multiplied without necessity.—OCKHAM

The correct hypothesis for the solution of a problem often turns out to be the least complicated one that can be thought of at the time. Experience has taught us that "nature operates in the shortest way possible," and that the least complex explanation usually corresponds to reality. This, of course, depends on how complicated the problem really is, and on how close we are to its solution. The investigator may be unaware that the shortest way possible is in fact long and tortuous, and may cling to the security of an oversimplified interpretation which interferes with a search for relevant new facts and avoids recognition of the complexity of the problem. In dealing with complicated phenomena which are brought about by varied and independent forces, searching for a single cause or trigger mechanism can only delay our eventual understanding of the problems involved. Differentiation appears to be such a phenomenon and, with Ockham's permission, we shall now proceed, out of necessity, to multiply entities; show that they are *all* required; and even suggest that their very number is an essential aspect of differentiation.

Relatively few processes of morphogenesis are both simple enough and at present, well enough studied to allow an analysis of more than one of the responsible entities (or causes) involved. Many investigators have stressed the importance to differentiation of changes in (i) enzyme activities (*1*); (ii) RNA metabolism (*2*); (iii) gene activation (*3*); (iv) levels of specific substrates (*4, 5*); or (v) inhibitors (*6*). At any of these levels of control the rate of a reaction critical to morphogenesis may be influenced. Since disagreement and confusion frequently arise from unexpressed (and usually unknown) discrepancies in the definitions of the words *differentiation, morphogenesis*, and *development*, they are used interchangeably in this discussion for the sake of variety; their meaning in the particular context should be clear. If differentiation were always so complex as to simultaneously involve each of the types of control summarized above, it would indeed be difficult to analyze them all at this stage of our knowledge. For example, in view of the role of the gene in controlling the rate of an enzymic reaction necessary to differentiation, it is clear that its action is distant and indirect, being mediated through RNA templates, through enzymes, and through substrates. Since partial control of morphogenesis could (and does) occur independently at these "lower" levels, the extent of their contribution must be understood before we can clarify the role of selective gene activation.

As an example of the dependence of one level of control upon another, let me summarize two cases in which interpretation of data at the enzyme level was completely dependent on knowledge of alterations at the substrate and inhibitor level.

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1) Glutamate oxidation plays an important role during differentiation of the cellular slime mold *Dictyostelium discoideum*, since morphogenesis depends in part on the utilization of endogenous protein, the oxidation of amino acids, their entry into the Krebs cycle, and their eventual utilization for carbohydrate synthesis. In fact, when glutamate oxidation was measured in vivo (by means of radioactive glutamate-1-^{14}C, and following $^{14}CO_2$ evolution), the rate of the reaction was found to increase sevenfold during development. The concentration of the enzyme (glutamic acid dehydrogenase) does not change; however, glutamate concentrations in vivo increase during development, probably as a result of protein degradation. Knowing the K_m (that concentration of substrate at which the reaction velocity attains half its limiting value) of glutamic acid dehydrogenase, as well as the concentration of glutamate in the cells during development, we showed that the change in substrate-K_m ratio could fully account for the increase, in vivo, in the rate of glutamate oxidation (*5*). Thus the lack of change in enzyme concentration did not tell the whole story.

2) The slime mold also offers an example of a case in which the concentration of an enzyme *does* change, yet this change is not reflected in its activity in vivo, due to the presence of an inhibitor. This enzyme, an alkaline phosphatase specific for adenosine-5′-monophosphate, increases sixfold in concentration during development. However, at the same time a competitive inhibitor, inorganic phosphate, is accumulating in the cells. The increases in concentration of this inhibitor result in maximum activity of the enzyme in vivo, not at the end but in the middle of the differentiation process (*6*). Thus, knowledge of variables at the substrate level is essential in interpreting the significance of changes at other levels of control (*7*).

Fortunately, evidence is accumulating now in a number of systems which indicates that the levels of control—(i) through (v), above—do not always occur together; thus we can analyze some of them independently. Example 1, above, concerns a change in substrate concentration not accompanied by a change in enzyme concentration. At yet another level of control, it appears that changes in messenger RNA (mRNA) activity can occur in the absence of synthesis of mRNA. Thus, the formation of mRNA from DNA templates can occur *before* morphogenesis [as, for example, in the early stages of differentiation in the amphibian (*8*) or sea urchin (*9*), and in the formation of bacterial flagella (*10*)]. As D. D. Brown so clearly states (*8*): "The time of gene expression in a developing organism need not synchronize with the needs of the organism for the gene product." In other words, although the expression of genetic information at some time is essential in order for differentiation to occur, it does not follow that genetic activity necessarily accompanies each step of morphogenesis. This circumstance should, for example, allow an analysis of control at the mRNA or enzyme level in an environment of constant gene activity. Even more favorable to investigation are cases in which enzyme levels do not change. These should be amenable to relatively simple definition and interpretation because changes at the substrate level—including changes in concentrations of effectors and inhibitors—can be analyzed independently of simultaneous control by genes or mRNA.

A Model System for Studying Differentiation

The cellular slime mold *Dictyostelium discoideum* is an organism exhibiting one of the simplest kinds of morphogenesis, in which only two major cell types are involved (*11*). When a few of the complexities at the substrate and enzyme level of control in this organism have been described, it will become clear that a comparable analysis of systems involving other levels of control as well would be exceedingly difficult at present.

We can begin with a brief account of the life cycle of *Dictyostelium discoideum*, at the stage of the vegetative myxamoebae, which grow indefinitely in the presence of sufficient food (see Fig. 1). Under starvation conditions, however, growth ceases, and differentiation is initiated. All the amoebae present aggregate to form a pseudoplasmodium composed of many thousands of cells already differentiated in many respects. The apical one-third of this multicellular body becomes the stalk, and the rear two-thirds become the spores of a final fruiting body, the sorocarp. The latter is constructed dur-

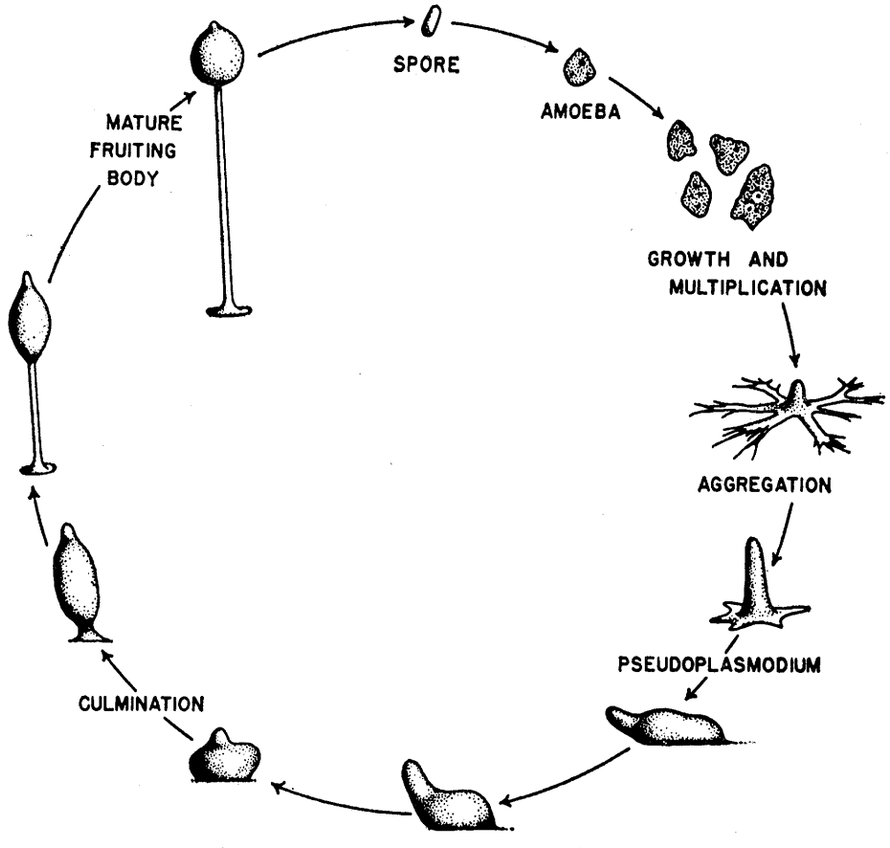

Fig. 1. Life cycle of *Dictyostelium discoideum*.

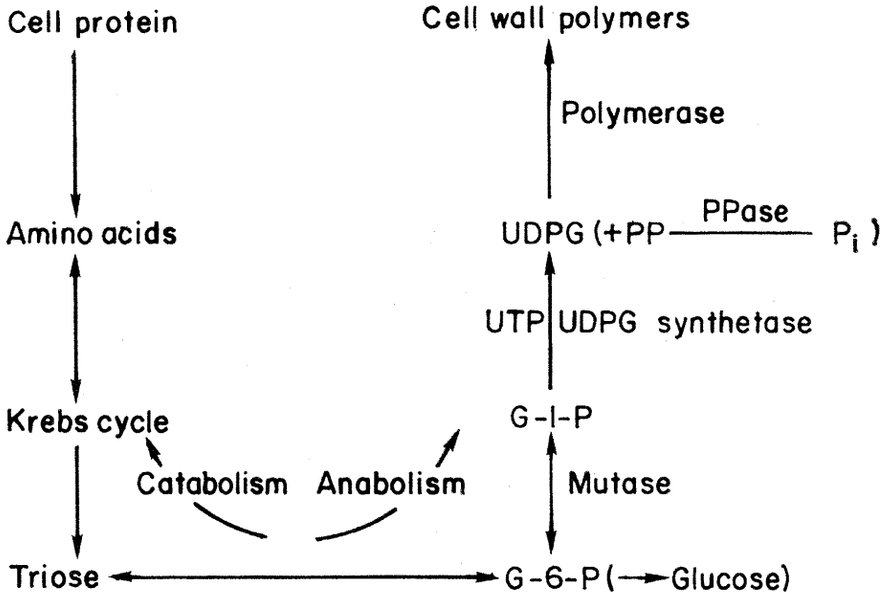

Fig. 2. Endogenous metabolic changes during differentiation which contribute to cell wall synthesis. *PP*, pyrophosphate; *PPase*, pyrophosphatase.

ing a process called culmination, initiated about 24 hours after the onset of starvation at 23°C. The stalk and spore coats of the sorocarp are both composed of an insoluble polysaccharide complex of cellulose (a β-1, 4-linked, linear glucose polymer) and glycogen (an α-1,4- and α-1,6-linked branched glucose polymer). It is with the synthesis of this material at the terminal stages of development that we are concerned. The formation of this insoluble polysaccharide complex represents an excellent index of differentiation, as the complex accounts for some 5 percent of the dry weight of the sorocarps and is present at insignificant concentrations in the myxamoebae at the beginning of differentiation. Some overall metabolic changes occurring during starvation are briefly outlined in Fig. 2. Endogenous protein is degraded as a major source of both chemical energy and precursors for gluconeogenic activity. Since this is a closed system, operating in the absence of exogenous nutrients, a competition exists during differentiation for intermediates common to catabolic and anabolic pathways. With cell-free preparations of the slime mold, it has been shown that uridine diphosphoglucose (UDPG) is a precursor of cell wall polysaccharides (*12*). Before these polymers accumulate during sorocarp construction, precursors such as glucose-6-phosphate (G-6-P) (*13*), uridine triphosphate (UTP) (*14*), and UDPG (*7*), as well as the enzyme UDPG synthetase, rise to peak concentrations and then drop to lower levels as cell wall material is rapidly formed. Some of these changes are indicated schematically in Fig. 3. Comparable changes have been observed in other organisms as a result of starvation, though they have been unaccompanied by differentiation. For example, the initial phases of starvation in bacteria can induce the accumulation of adenosine triphosphate (ATP) (*15*) or UDPG (*16*).

In this description of the process of cell wall synthesis in the slime mold, certain unresolved problems are avoided and certain simplifying assumptions made (for the original data, see *12, 13, 17*). The cellulose and glycogen polymers of the cell wall cannot be separated by treatment with alkali (30 percent at 100°C), urea, concentrated salt solutions, and so on. Their association may be more than physical; this is not yet known. Only by using reagents which dissolve cellulose can the polymer complex be brought into solution and resolved into its constituent polysaccharides. By weight, the cellulose and glycogen fractions of cell wall material are present in equal amounts. I call the enzyme of this discussion a polymerase; it is responsible for catalyzing the synthesis of the glycogen moiety of cell wall material from UDPG. (Synthesis of the cellulose fraction is not discussed.) Polymerase activity can be found in two "compartments" of the cell: bound to the insoluble cell wall as it forms in the terminal stages of development, and bound to the glycogen in the 100,000g pellet fraction at all stages of development. We have good but indirect evidence that the cell wall enzyme originates from the pellet fraction during differentiation. The glycogen-bound polymerase, from the cytoplasm of the cell, is identical with the enzyme that synthesizes ordinary (water-soluble) glycogen from UDPG. This polymerase, which decreases in concentration during differentiation, will catalyze in vitro the incorporation of glucose (from UDPG) into soluble glycogen or into the insoluble glycogen moiety of the cell wall, depending upon which of these materials is present as primer. Each type of primer competitively inhibits synthesis of the other in vitro. Some of these relationships are diagramed in Fig. 4, which is largely derived from in vitro experiments. The data suggest a competition for the polymerase both with respect to its location in the cell and with respect to primers, since each primer inhibits the synthesis of the other. The dual function of the polymerase, demonstrated in a model system in vitro, should be expected, in vivo, to result in a competition, at the enzyme level, between the synthesis of soluble glycogen on the one hand and of insoluble cellulose-bound glycogen on the other.

Competition at the Substrate Level

The concentration in differentiating cells of UDPG and G-6-P was determined (Table 1) and found to be limiting for the synthesis of both soluble glycogen and insoluble cell wall glycogen (Table 2). As may be seen by comparing the data of Tables 1 and 2, the concentrations of UDPG and G-6-P required for maximum in vitro activity are in excess of the concentrations in intact cells. Trehalose and Mg^{++} stimulate cell wall synthesis, but only in the presence of G-6-P. There is evidence that both Mg^{++} and trehalose are limiting as stimulants of cell wall synthesis at culmination (*13*). The availability of cell wall primer must limit cell wall synthesis at the time of its initiation during culmination, since this material is present in insignificant amounts at the earlier stages of differentiation. In contrast, a com-

parison of Tables 1 and 2 suggests that glycogen is not limiting as a primer for the in vivo synthesis of free glycogen (*18*). Uridine triphosphate and ATP, at the concentrations at which they are present in differentiating cells, inhibit the synthesis of cell-wall-bound glycogen and, to a lesser extent, that of free glycogen; toward culmination, their concentration tends to fall (*14*). We do not know the extent to which the concentration of substrates determined for extracts of cells corresponds to their concentration in the cytoplasm of the living cell. There is undoubtedly some localization of substrates within a cell, and especially with respect to one cell type as compared to the other (stalk relative to spore). We do know that we can arrive at a more realistic analysis of the control of cell wall synthesis during development by taking such data into account rather than by ignoring them altogether! Let us now see how changing levels of such metabolites as UDPG and G-6-P could influence the competition between the synthesis of free glycogen and the synthesis of cell wall material.

Glucose-6-phosphate is known to lower the K_m for UDPG in a number of systems in which glycogen synthesis has been studied (*19*). This effector, at saturation concentrations, has a striking influence on the affinity of the slime-mold-amoebae enzyme for UDPG in the synthesis of free glycogen, lowering the K_m from about 10^{-3} to $10^{-4}M$ (*18*). In the synthesis of cell wall glycogen, the K_m for UDPG is changed very little, but the reaction is stimulated about threefold at the lower concentrations of UDPG sometimes found in the cell (Table 2). With respect to glycogen synthesis, experiments have shown that increasing the concentrations of G-6-P lowers the K_m for both glycogen and UDPG; conversely, increasing the concentrations of glycogen or UDPG lowers the requirement for G-6-P. For unknown reasons, intracellular concentrations of UDPG vary significantly from one study to another—that is, they may be unusually high or low throughout differentiation of a particular batch of cells, although the peak concentration always occurs at culmination. The very nonpredictability of concentrations of UDPG (G-6-P, glucose, UTP, ATP, and so on) at given stages of differentiation is in itself evidence of the multiple dependencies involved. As an example of the complex relationships of one critical metabolite, in vitro data indicate that G-6-P exerts a stronger stimulating influence on the synthesis of both free and cell wall glycogen in cells with low concentrations of UDPG than in cells not so limited with respect to UDPG. In the presence of G-6-P, the K_m for UDPG in the synthesis of free glycogen (catalyzed by the pellet enzyme from amoebae) is lower by a factor of 10 than the K_m for UDPG in the synthesis of cell wall glycogen (10^{-4} as compared to $10^{-3}M$). Thus, the rising concentrations of G-6-P prior to culmination might be one factor favoring free glycogen synthesis. In the presence of G-6-P and the very limiting UDPG concentrations found in earlier stages of differentiation, glycogen synthesis could compete favorably with cell wall synthesis. Later in differentiation, however, both G-6-P and UDPG fall to low concentrations, and competition for the UDPG might favor synthesis of the cell wall material, which is less dependent on the presence of G-6-P. Furthermore, less polymerase is then associated with the soluble glycogen pellet, and an increasing amount of enzyme is becoming bound to the cell wall fraction. Concentrations of ATP and UTP also fall at this time, and it has been found that these triphosphates, at levels present in vivo, inhibits cell wall synthesis. Thus, changing concentrations of substrates, primers, effectors, inhibitors, and enzymes

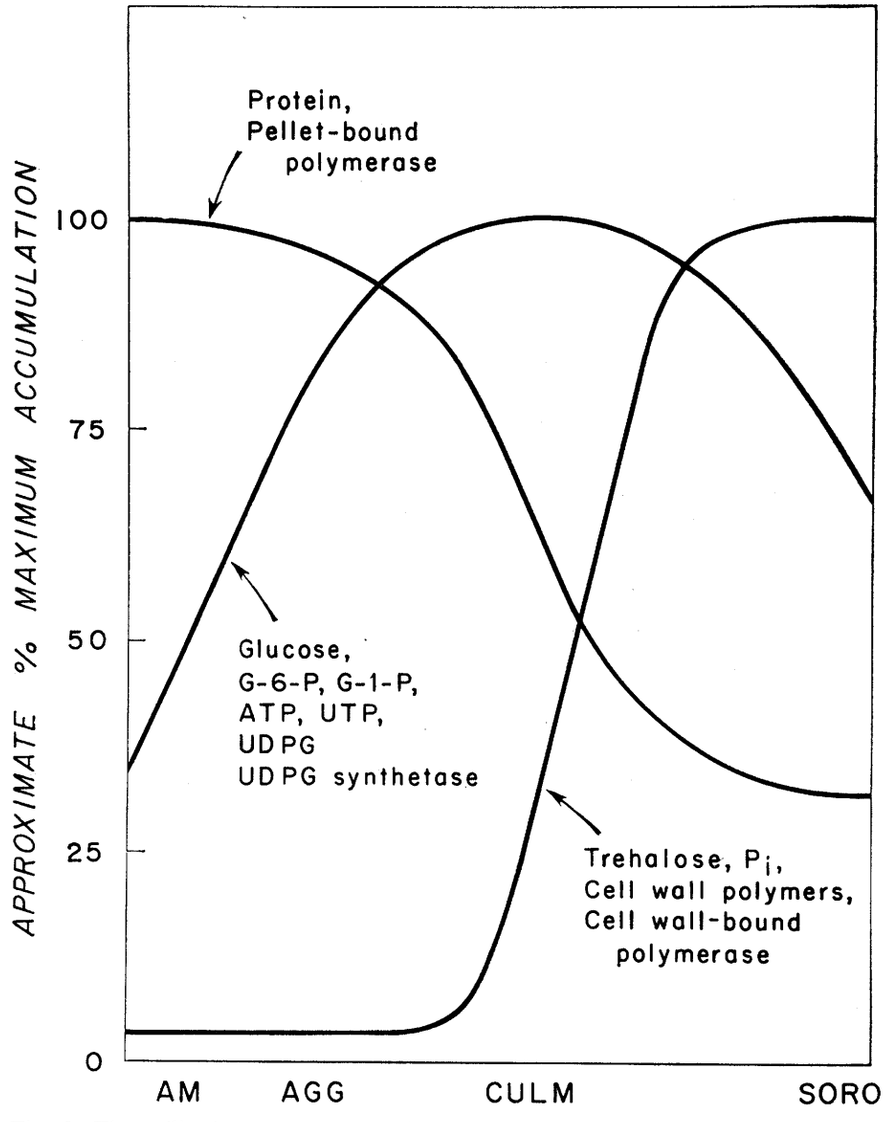

Fig. 3. The utilization and accumulation of various cellular constituents, including three enzymes, during differentiation. The stages indicated are: *AM*, amoebas; *AGG*, aggregation; *CULM*, culmination; and *SORO*, sorocarp.

Table 1. Approximate concentrations in vivo, based on packed cell volume, of metabolites affecting glycogen and cell wall synthesis.

Compound	Concentration
Glycogen	4 mg/ml
UDPG	$1 \times 10^{-4}M$
G-6-P	$1 \times 10^{-4}M$
ATP	$5 \times 10^{-4}M$
UTP	$1 \times 10^{-4}M$

regulate the synthesis of both free and cell wall glycogen. Some of these materials are shared in the formation of the two types of polysaccharides, and some are differentially inhibitory (for example, primers and UTP). Changes affecting the synthesis of one polymer will thus automatically be reflected in the synthesis of the other.

These are undoubtedly only a few of the changes occurring at culmination which favor cell wall synthesis; the discovery of additional factors affecting this process directly or indirectly (for example, phosphorylase activity) will surely modify and further complicate this picture. An excellent model system illustrating the influence of multiple effectors and inhibitors on the activity of an enzyme (phosphofructokinase) has recently been developed (*20*). The large number of activator and inactivator molecules involved allows relatively sharp activation and cutoff points. At these points, the enzyme activity will change sharply with only a moderate change in conditions. Thus, although the sudden appearance of cell wall material suggests a drastic metabolic change (such as the appearance of a new enzyme), it may instead be the culminating effect of many minor, interacting changes in metabolism.

Aside from reactions directly related to polysaccharide synthesis, the competition for intermediates common to this process and to catabolism must eventually be understood (see Fig. 2). Hexose phosphates are not only precursors for polysaccharide synthesis but are also important intermediate products in the oxidative degradation of sugar, which provides the cell with chemical energy. We know that in this latter role, too, their endogenous level is limiting: exogenous glucose supplied to differentiating cells stimulates respiration, especially during and after the culmination process (*21*). Furthermore, it has been shown that a change in hexose concentrations in vivo actually influences the manner in which hexose is catabolized (*22*). The interaction of this area of metabolism with biosynthetic pathways necessary to morphogenesis may be referred to as "catabolic competition."

General Characteristics of Differentiating Systems

A glance at some recent reviews on the regulation of metabolic pathways in homeostasis leaves one with a sense of awe at the ingenuity displayed, through the use of various types of regulation, by organisms in coping with the complex requirements of different metabolic states: concerted feedback inhibition, cumulative feedback inhibition, multivalent repression, enzyme multiplicity, and coordinate repression, to name but a few. As Atkinson has recently pointed out (*23*), others will surely be discovered. To try to envision how such intricate mechanisms are elaborated during differentiation, particularly in more complex organisms subject also to regulation by nervous and circulatory systems, hormone action, and so on, staggers the imagination. But if we try to understand the consequences of certain metabolic characteristics common to some of the simpler differentiating systems, it may be possible to find clues as to their significance, at least for the elaboration of some of the less complex types of control.

The fact that the endogenous concentrations of a number of substrates and effectors limit various reactions in the differentiating slime mold is not at all surprising. There is a good deal of evidence in the literature that, owing to substrate limitation, the in vivo activity of many enzymes in fully differentiated (*24*) and differentiating systems (*4, 5, 25*) is far below their potential maximum. During morphogenesis, such a state may result from a dependence upon endogenous reserve materials as a source of both energy and precursors for synthetic processes. Such a dependence upon endogenous metabolism can, in turn, result from (i) an insufficient supply of exogenous nutrients and (ii) permeability barriers, which help to prevent the entry of exogenous foodstuffs as well as the exit of essential endogenous materials. The slime mold offers a striking example of the conflict between a dependence upon exogenous nutrients for cell multiplication on the one hand and morphogenesis on the other, since the latter is initiated only during starvation. The transformation of a vegetatively multiplying cell into a differentiating cell frequently occurs under nutritionally poor conditions, necessitating a dependence upon endogenous material as an energy source (*26*). Even cells incapable of differentiation utilize their macromolecules under such conditions, and the more efficient this utilization is, the longer they survive (*27*). In analyzing the stimulation of spore germination by alanine, it was found that most of the alanine degraded was actually of endogenous origin (*28*). Proteases in the sea urchin egg become activated upon fertilization (*29*), and the sequential utilization of endogenous material, followed by the appearance of oxidizable substrates, is well

Table 2. Stimulation in vitro of glycogen and cell wall synthesis by UDPG, G-6-P, and glycogen. These data represent a summary of a number of experiments in which either glycogen or cell wall synthesis was studied. In the case of glycogen synthesis, the enzyme source was the 100,000*g* pellet fraction; in the case of cell wall synthesis, a cell wall preparation was the source of both enzyme and primer. Experimental details for the in vitro synthesis of these two polysaccharides are given elsewhere (*12, 17, 18*).

Glycogen* (mg/ml)	UDPG (molarity)	G-6-P (molarity)	Glucose incorporated (10^{-3} μmole)	
			Glycogen	Cell wall
2	1×10^{-4}	None	1.3	0.3
2	1×10^{-4}	1×10^{-4}	4.0	.6
2	1×10^{-4}	5×10^{-3}	10.0	1.1
2	2×10^{-4}	1×10^{-4}	0.5	0.3
2	1×10^{-3}	1×10^{-4}	4.0	1.4
2	2×10^{-3}	1×10^{-4}	7.0	4.0
0.5	1×10^{-3}	1×10^{-4}	0.7	
1.0	1×10^{-3}	1×10^{-4}	1.0	
2.0	1×10^{-3}	1×10^{-4}	1.0	

* Relevant only to glycogen synthesis data.

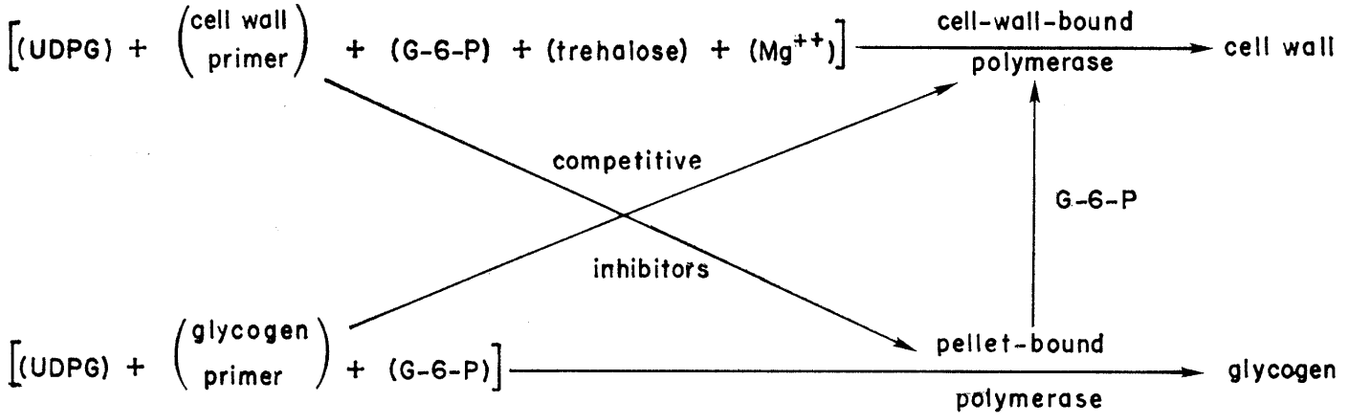

Fig. 4. Diagrammatic representation of factors influencing competition between the synthesis of free and cell wall glycogen.

documented in seed (*30*), slime mold (*16*), and amphibian (*31*) development. Permeability barriers have been reported in studies of development in slime molds (*7*), sporulating bacteria (*32*), amphibia (*8*), and sea urchins (*9*). The eggs of reptiles and birds are of course quite well protected from environmental influences as differentiation proceeds, as are insects undergoing metamorphosis (*33*).

Perhaps this dependence of differentiating systems on an endogenous metabolism has proved the best insurance for the safe outcome of a delicate operation in the midst of a cruel and ever-changing environment! Use, by the organism, of an exogenous source of nutrients for cell multiplication is of course necessary. It is not even precarious, in that a variety of energy sources are both available and adequate for the purposes of growth. Furthermore, while growth can afford to be regulated by external factors, since it may be either fast or slow, this is *not* true of differentiation. This complex process must be precisely regulated in time; specific metabolic events must be interlocked in a particular order. Synchronization of these events may well require such delicate regulation as to necessitate a dependence upon the reproducible milieu of endogenous metabolism.

To pursue this thought further, What might be some other consequences and advantages during development of a self-imposed dependence upon multiple limiting factors, and of interdependent competing reactions? One consequence of this circumstance would be that unusual deficiencies or abundances of precursor material in the cell or its environment would be unlikely to upset the process of differentiation. A simple model to illustrate this point may be taken from recent work on hexokinase, which catalyzes the formation of G-6-P from glucose and ATP (*34*). The data simply demonstrate that, when the concentrations of both glucose and ATP limit the reaction, increasing the concentration of either one increases the rate of the reaction. Were either substrate present in excess, formation of G-6-P would depend entirely on changes in concentration of the other substrate. In this sense, the system would be less *flexible* than it would be if both substrates were limiting (*13*). Thus, if this situation prevailed in the differentiating cell, somewhat low levels of one substrate could be compensated for by high levels of the other.

A much more complex example may be seen in Fig. 4. Increasing the number of factors contributing to cell wall synthesis increases the probability of its occurrence by decreasing the dependence on any one factor. The premature or delayed accumulation of one precursor or effector will neither trigger nor prevent the timely formation of cell wall material. In fact, we observe significant variability with respect to both time and concentration in the accumulation patterns of UDPG, G-6-P, ATP, and so on, during normal differentiation in the slime mold. Clearly, patterns of enzyme-substrate-effector interaction have evolved which confer enormous flexibility, since in general a variation in the concentration of any one metabolite merely changes the operating range of several others, rather than rigidly controlling the end result. Complex and interde-

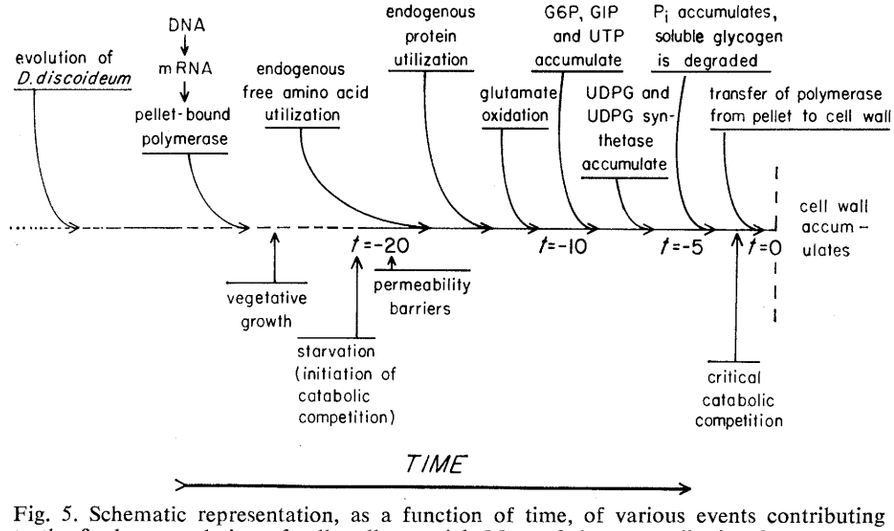

Fig. 5. Schematic representation, as a function of time, of various events contributing to the final accumulation of cell wall material. Most of these contributing factors occur continuously, but with a change in intensity. They are shown separated in time, for the sake of clarity.

pendent relations such as I have discussed are being described with increasing frequency as we gain understanding of the intricacies of intermediary metabolism.

At this point it may be instructive to consider regulation of synthetic pathways by multivalent repression. The synthesis of four critical metabolites (isoleucine, valine, leucine, and pantothenate) involves two different pathways which have four enzymes in common. All of the end products are required *together* to repress these enzymes, thus preventing an excess of any *one* of the amino acids from blocking the formation of the others (*35*). A somewhat analogous safety feature prevents failure of cell wall synthesis: a slight deficiency in the concentration of UDPG is compensated for by a greater stimulation by G-6-P, for example. Pathways made interdependent by virtue of a common substrate or enzyme would have a built-in mechanism by which to synchronize sequential steps leading eventually to visible morphological changes. For example, we have seen that free-glycogen synthesis competes favorably with cell wall synthesis at high concentrations of G-6-P. When the levels of G-6-P, UDPG, UTP, and ATP fall, cell wall synthesis is favored over glycogen synthesis. This may be advantageous, since a decreasing supply of these four compounds could mean that the endogenous energy supply is reaching a level such that the remainder must be used for the last act: cell wall synthesis.

These and many other (as yet unknown) changes set the stage and bring about the final steps of morphogenesis. Such multiple limiting factors and interdependent pathways insure links to the overall metabolic state of the cells, thus creating a delicate and necessary balance between the competing yet complementary needs for viability and morphogenesis.

"First Cause" of Morphogenesis

In conclusion, let us return to the quest for a single or major "first cause" of differentiation. Please recall that the cell wall polymerase of the slime mold catalyzes the synthesis only of the glycogen moiety of the cell wall. Ockham might have argued that, although the synthesis of the glycogen fraction of the cell wall does not involve a (template-dependent) synthesis of a new enzyme, the accumulation of cell wall material *really* depends upon the synthesis of the insoluble cellulose moiety. If the latter synthesis were to depend on the formation of an enzyme not yet present in the cell, then cell wall formation would be directly linked to a special mRNA, and thus could be traced to a genetic cause. But the synthesis of such an enzyme could not have mediated glycogen synthesis, which occurs even prior to the initiation of development (see Fig. 5). Thus, control of the synthesis of the two polymers must be interdependent, and the synthesis of glycogen is fully as critical to the eventual production of cell wall material as the synthesis of cellulose is.

Just as important as glycogen synthesis are the breakdown of endogenous protein upon starvation; the oxidation of amino acids such as glutamate; the accumulation of inorganic phosphate (Pi), G-6-P, UDPG; and the role of catabolic competition. Is starvation, then, perhaps the "first cause?" Hardly, since the complex series of events initiated by starvation are, in turn, completely determined by the unique characteristics of a slime mold cell, for these characteristics allow the cell to remain viable while utilizing endogenous reserve material in such a way that a specific, characteristic type of morphogenesis ensues. Countless microbes do not undergo morphogenesis during starvation. In this sense, then, all changes occurring during differentiation depend upon the particular genetic and cytoplasmic composition of the cell prior to the initiation of morphogenesis—just as this composition, in turn, is dependent upon all the changes that occurred during the evolution of the organism. Some of the factors contributing to cell wall accumulation are indicated in Fig. 5.

We are faced with an endless search for the first cause, a term which has now become so inclusive as to lose its meaning and its usefulness. Since definitions are meant to be useful, I will (and may) take the liberty in this context of changing the meaning of *cause* by including the element of *time*. For example, when we use our modified definition in analyzing a specific differentiation process, a very meaningful question to ask is, What types of control are strictly correlated with, and therefore immediately cause, the morphogenic change? The induction of puffing in chromosomes can be prevented by inhibitors of nucleic acid metabolism—a fact which suggests that control at the nucleic acid level is directly responsible (in time) for this differentiation process (*3*). On the other hand, in other systems, although the potential for all enzyme formation ultimately resides at the genetic level, the "message" therein may have been transmitted to the RNA level (as in the amphibian) or even to the enzyme level (as in the slime mold) long before the initiation of the differentiation process under consideration. In the latter case, the existence of the template responsible for the formation of a particular enzyme is of course fully as important (as an *early* cause) to the outcome of the differentiation process as are the factors immediately in control. Thus, the answer to the question "Which comes first, the enzyme or the substrate?" is, obviously, "Neither"; the enzyme and the substrate and their unique interdependence evolved together. Their influence in time with respect to the control of a given differentiation process may vary from one system to another. Specification of the element of time in relation to the "causes" of differentiation should help to clarify the role of various levels of control and place a more balanced emphasis on the mutual importance of the many interdependent forces resulting in morphogenesis. Thus, the results of an analysis of morphogenesis on the molecular level complements the conclusions of an analysis on a much more complex level in higher organisms: induction is clearly not a trigger event, and it is nearly impossible to say just where it begins (*36*).

Summary

In this article the process of differentiation is analyzed, cell wall synthesis in a cellular slime mold being used as a model system. The causes and levels of control responsible for morphogenesis are many and complex; their interdependence must be understood in order to interpret their individual roles in the regulation of morphogenesis.

References and Notes

1. S. C. Shen, in *Biological Specificity and Growth*, E. G. Butler, Ed. (Princeton Univ. Press, Princeton, N.J., 1955), p. 73; G. W. Brown, W. R. Brown, P. P. Cohen, *J. Biol. Chem.* **234**, 1775 (1959).

2. H. Laufer, Y. Nakase, J. Vanderberg, *Develop. Biol.* **9**, 367 (1964).
3. W. Beermann and V. Clever, *Sci. Amer.* **210**, 50 (1964).
4. K. Aketa, R. Bianchetti, E. Marre, A. Monroy, *Biochim. Biophys. Acta* **86**, 211 (1964).
5. B. E. Wright, *Bacteriol. Rev.* **27**, 273 (1963).
6. K. Gezelius and B. E. Wright, *J. Gen. Microbiol.* **38**, 309 (1965).
7. B. E. Wright, in *Biochemistry and Physiology of Protozoa*, S. H. Hutner, Ed. (Academic Press, New York, 1964), p. 341.
8. D. D. Brown and E. Littna, *J. Mol. Biol.* **8**, 669 (1964).
9. P. R. Gross, L. I. Malkin, W. A. Moyer, *Proc. Nat. Acad. Sci. U.S.* **51**, 407 (1964).
10. R. J. Martinez, *Bacteriol. Proc.* **1965**, 87 (1965).
11. J. T. Bonner, *The Cellular Slime Molds* (Princeton Univ. Press, Princeton, N.J., 1959).
12. C. Ward and B. E. Wright, *Biochemistry* **4**, 2021 (1965).
13. B. E. Wright, in *Developmental and Metabolic Control Mechanism in Neoplasia*, D. N. Ward, Ed. (Williams and Wilkins, Baltimore, Md., 1966), p. 296.
14. R. G. Pannbacker, *Bacteriol. Proc.* **1966**, 93 (1966).
15. R. E. Strange, H. E. Wade, F. A. Dark, *Nature* **199**, 55 (1963).
16. N. B. Madsen, *Can. J. Biochem. Physiol.* **41**, 561 (1963).
17. B. E. Wright, C. Ward, D. Dahlberg, *Biochem. Biophys. Res. Commun.* **22**, 352 (1966).
18. B. E. Wright and D. Dahlberg, unpublished data.
19. L. Leloir and C. Cardini, in *The Enzymes*, P. D. Boyer, H. Lardy, K. Myrbäck, Eds. (Academic Press, rev. ed. 2, New York, 1962), vol. 6, p. 317.
20. D. Garfinkel, *J. Biol. Chem.* **241**, 286 (1966).
21. J. U. Liddel and B. E. Wright, *Develop. Biol.* **3**, 265 (1961).
22. B. E. Wright, M. Bruhmuller, C. Ward, *ibid.* **9**, 287 (1964).
23. D. E. Atkinson, *Science* **150**, 851 (1965).
24. A. Vetgosky and E. Frieden, *Enzymologia* **19**, 143 (1958); O. Lowery and J. V. Passonneau, *J. Biol. Chem.* **239**, 31 (1964).
25. M. E. Krahl, A. K. Keltch, C. P. Walters, G. H. A. Clowes, *J. Gen. Physiol.* **38**, 431 (1955).
26. B. E. Wright, in *Comparative Biochemistry*, M. Florkin and H. S. Mason, Eds. (Academic Press, New York, 1964), vol. 6, p. 1.
27. A. P. Harrison, Jr., and F. R. Lawrence, *J. Bacteriol.* **85**, 742 (1963).
28. R. O'Connor and H. Halvorson, *ibid.* **78**, 844 (1959).
29. W. E. Berg, *Biol. Bull.* **98**, 128 (1950).
30. W. O. James and A. L. James, *New Phytol.* **39**, 145 (1940).
31. S. Løvtrup, *J. Exp. Zool.* **140**, 231 (1959).
32. R. W. Bernlohr, in *Spores*, L. L. Campbell and H. O. Halvorson, Eds. (American Society of Microbiology, Ann Arbor, Mich., 1965), vol. 3, p. 751.
33. P. Karlson and C. E. Sekeris, in *Comparative Biochemistry*, M. Florkin and H. S. Mason, Eds. (Academic Press, New York, 1964), vol. 6, p. 221.
34. H. J. Fromm, E. Silverstein, P. D. Boyer, *J. Biol. Chem.* **239**, 3645 (1964).
35. M. Freundlich, R. O. Burns, H. E. Umbarger, *Proc. Nat. Acad. Sci. U.S.* **48**, 1804 (1962).
36. A. G. Jacobson, *Science* **152**, 25 (1966).
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Interpretation of Some Organic Photochemistry

Unusual reactions lead to understanding the behavior of electronically excited molecules.

Howard E. Zimmerman

Organic photochemistry is now at an evolutionary stage where almost every species isolated is new and nearly every reaction uncovered is a major advance. Relatively little is known with certainty, and the undiscovered measures immense relative to the discovered. Consider, for each of the multitudinous molecules already described in *Beilstein* and *Chemical Abstracts* that there exists at least one electronically excited state formed by light absorption, and a reasonable fraction of these excited species will undergo new transformations when put to test. Each of these excited states has the gross skeleton of the parent, ground-state molecule but differs in electron distribution and chemical reactivity.

However, research in photochemistry does face impediments, and two of these are of particular consequence. First, the structures of the electronically excited states still can be determined only approximately, and often only by methods not easily accessible to the organic chemist. Second, even when the excited-state structure is known, it is uncertain what criteria control the course of the excited-state reaction.

Controlling Factors in Photochemical Transformations

I have taken the view (*1–4, 5*) that photochemical processes are selective. Bonds are not broken indiscriminately although, frequently, more than enough electronic energy (that is, about 100 kilocalories per mole at 285 millimicrons) is absorbed to break carbon-to-carbon bonds. Rather, photochemical processes seem to be subject to the requirement of "continuous electron redistribution," defined (*1–4*) as a molecular transformation proceeding with minimum electron localization. Such a requirement is implicit in current treatment of the mechanisms of ground-state molecule reactions and is satisfied by mechanisms in which "arrow notation" is used properly. A discontinuous process is exemplified by the type mechanism occasionally unintentionally written by beginning students in organic chemistry when an arrow may be missing or improperly drawn.

This argues that it is fairly certain the excited-state molecules do not climb "energy mountain tops," especially when low-energy routes are available. It is not clear whether the lowest-energy route is invariably followed. A factor, cited by Hammond (*6*), controlling some photochemical reactions is the ease with which an excited-state species, having undergone geometric change, can form its unexcited, ground-state counterpart. Another suggestion occasionally made is that loss of electronic excitation without gross molecular change generates a reactant molecule with excess vibrational energy; and subsequently there is a transformation of the vibrationally excited but electronically unexcited (that is, "hot") species. This would be much like a pyrolysis. Such a mechanism becomes less likely in solution than in the vapor phase because of collisional deactivation. Also, the probability of such a "hot molecule" process occurring diminishes as the molecular size and the number of bonds that can interact with, and absorb, the vibrational energy increases. Except where especially low activation energies are needed for reaction and where the molecule is small, "hot molecule" ground-state processes seem unlikely to compete with solvent deactivation (*7*).

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