

SOME CAUSES UNDERLYING CELLULAR DIFFERENTIATION¹

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In the stems of most plants we are likely to find large, as well as, small cells, short and elongate cells, cells with thick walls and those with thin walls. Some cell walls may be lignified while others are composed primarily of cellulose. Cells may be found whose walls show a pattern of pits or raticulae, or helices, or annular thickenings. Quiescent cells may lie beside those which are meristematic. Some cells contain no pigment while others may appear red, purple, or green. Cell shapes may vary from tissue to tissue and from cell to cell within each tissue. Dead cells lie along side of living cells. Why? How are we to explain these differences in form, chemistry, meristematic activity, and physiology?

We know, of course, that the gene compliment of a cell is one of the factors controlling its differentiation. If a corn plant lacks certain genes, it will be an albino, not green, regardless of the environment in which it is placed.

Environment also affects the course of differentiation. A corn plant kept in the dark during its entire life span will not be green, regardless of its genotype.

Either environment or genes may in some cases ultimately determine the course of differentiation. Furthermore, the rate and a degree of differentiation may be controlled either by environment or by heredity or by both. For instance, if the light intensity to which a plant is exposed is very low, its cells will produce very little chlorophyll and the plant will be light green rather than dark green. Similarly the cells of some genotypically different varieties of corn contain an abundance of chlorophyll and as a result, the plants are dark green. Corn plants of other varieties are never more than pale green, no matter to what environment they are exposed.

Many additional examples could be cited to illustrate the principle that the kinds of processes as well as the rates and/or duration of the processes are the results of differences in gene compliment or environment or both, and that these differences result in cells or groups of cells of invisibly different physiological states. It is, of course, these invisible differences in the complex of life processes of cells which precede and are responsible for visible morphological differences. In other words, invisible *physiological differentiation* precedes and is causally related to visible *morphological differentiation*.

Differentiation then is a process or, more precisely, it is a complex of processes which are ultimately controlled by the environment and by the gene compliment. Furthermore, it is a progressive process. That is, it does not occur instantaneously but does occur over a period of time. Morphological differentiation may be rapid, slow, or saltatory.

This may be an appropriate time to consider the concept of dedifferentiation. It is difficult to understand how this term may be applied appropriately to a cell or to a group of cells. If morphological differentiation is the result of successive physiological changes, then dedifferentiation must imply that there has been an "undoing" or a reverse cancellation of each preceding physiological state. Obviously, this is impossible. Time cannot be recalled nor can an event occurring during the period of time with its accompanying effects be eliminated. It would seem that the inventor of this term ignored the fact that physiological change precedes morphological differentiation.

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A cell in which a process has occurred is not the same cell, physiologically speaking, as before. Let us consider a colorless cell in a leaf. The process of chlorophyll synthesis commences and continues until the cell is green. Later in the growing season the cell becomes colorless. Now, even though the cell may appear to have returned to its original state, microchemical examination of it would prove that this is not so. End products of chlorophyll disintegration will be found in the cell late in the season but not early in the season. Products formed during respiration will be different if the cell is observed both in the spring and in the fall. Very likely these differences will be both quantitative and qualitative. The cell has not become dedifferentiated. Differentiation has simply continued. Of course, as a result of continued differentiation, the cell may finally appear, superficially, as it did earlier in the differentiation process.

In considering the problem of cell, tissue, or organ differentiation within an individual plant or animal of a species, we are apt to disregard certain aspects of the causal factor, *heredity*. Gene compliment is frequently ignored because of the widely accepted assumption that, barring mutation, all somatic or vegetative cells, of which the individual organism is composed, are identical in-so-far-as gene compliment is concerned. This may not be an admissible assumption. Quite a few instances of naturally occurring supernumerary chromosome compliments have been found in scattered cells throughout a tissue, in certain tissues of an organ, and in specific organs of an individual. Instances of polysomy are known from both the animal and plant kingdoms.

Therman and Timonen (1951) studied chromosome numbers in the cells of healthy humans. Cells of the skin, brain, liver, connective tissue, intestine, uterine epithelium, and other tissues were studied. They found that the chromosome number of the cells varied greatly, aneuploid numbers prevailing in all tissues. Furthermore, the most commonly encountered chromosome number for cells outside the sperm and egg line was not 48. They reported that the highest peak of frequency lies between 20 and 25 chromosomes and that there is a much lower frequency between 45 and 50 chromosomes.

Therman (1951) states "it can be regarded now as an established fact that differentiated tissues in insects represent various degrees of polyploidy and polyteny."

Polysomy occurs to a greater or lesser degree as a regular developmental process in at least 19 genera of plants. Published results indicate that periblem is the tissue whose cells are most frequently polysomatic although polysomatic cells have been reported for the plerome of hemp, muskmelon, and spinach roots, the root cap of *Bryonia verrucosa*, and for the tapetum of spinach, dandelion, tomato, and the giant summer hyacinth (Lorz, 1947; Brabec, 1953).

In *Xanthisma texanum* and *Sorghum purpureo-sericeum*, cells of the shoots regularly contain more chromosomes than those of the roots (Berger et al., 1955).

Summarizing then, there is a very considerable body of evidence indicating that polyploidy and aneuploidy commonly occur in the various cells, tissues, and organs of many plants and animals, including *Homo sapiens*. This well documented but largely ignored fact leads one to consider the possibility that these variations in chromosome numbers may be causally related to cell, tissue, and organ differentiation.

If we assume that cells of an organ or a tissue are identical in chromosome compliment or if we admit that differences in chromosome compliments have not yet been shown to be causally related to differentiation, then how are we to explain such simple differences as those of cell size and cell wall thickness? It can be stated unequivocally that the apical meristems of shoots and roots exert a powerful influence, possibly complete control, over differentiation in shoots and roots of plants (Wetmore, 1956). Such a general statement, however, is in no sense a satisfying explanation of differentiation. This is particularly true if we

recall that a great deal of differentiation precedes the organization of root and shoot apices in the embryo.

Assimilation and diffusion of water are, of course, the processes immediately responsible for increases in wall thickness and cell enlargement. The assimilation of protoplasm, that is, the formation of additional protoplasm, is a complex and incompletely understood process. But we do know that foods, such as proteins, fats, and carbohydrates, as well as enzyme systems, must be present in the cell at least in minimal quantities before the process will occur. The manufacture by the cytoplasm of additional cell wall materials, such as lignin, cellulose, and pectic compounds, and the incorporation of these materials into the cell wall may, presumably, follow either of two courses. Particles of cellulose and other wall materials may be deposited among the constituent particles of the existing wall (intususception) or the wall materials may be deposited as additional layers on the inner face of the existing wall (apposition). In either event thickening or the extension of cell walls is dependant upon adequate supplies of certain foods and enzyme systems. Environmental factors, such as light, are known to influence not only rate of assimilation but also the course of cell wall differentiation. For instance, as the cell walls of cotton fibers thicken, the pattern of deposition of cellulose particles may be altered by light (Anderson et al., 1937). If the cotton plant is continuously illuminated, the fiber walls appear homogeneous when cross sections are examined under the microscope. Cotton plants exposed to alternating periods of light and darkness, on the other hand, produce fibers whose walls are conspicuously laminated. One lamella appears for each light-dark cycle as long as the wall is growing thicker. The more dense portion of each lamella is deposited during the light phase of the cycle.

In short, the initiation of, as well as, the subsequent rate of assimilation of a given cell component is dependant upon an adequate supply of the proper kinds of foods, enzyme systems, other internal environmental conditions, and external environmental conditions. Could we reasonably expect this multiplicity of factors to be identically balanced in the cells of two different plants? In two different organs of the same plant? In different tissues of the same organ? Or in adjacent cells of the same tissue? Not at all. These are some of the factors which certainly vary from cell to cell and consequently, in as much as they affect the kinds and rates of the processes, they must be responsible in some measure, for differentiation. We should *expect* cells to be different from one another, some physiologically different and others both physiologically and morphologically different.

It has already been suggested that diffusion of water into the vacuole or vacuoles of a cell is just as important as assimilation in the process of differential cell enlargement. The diffusion of water into or out of a cell and the rate of diffusion are dependent upon many factors some of which are the availability of water around the cell, the concentration of water in the vacuole vs. the concentration of water around the cell, the permeability of the differentially permeable cytoplasmic membrane and the plasticity of the permeable cell wall. Even though plenty of water enters the roots of a plant only minimal quantities may be available around most cells. If the rate of transpiration is high, very large quantities of water (125 gal per day for a palm tree) may enter the roots, be quickly pulled through the water conducting vessels of the roots, stems, and leaves, and just as quickly evaporate from the leaves into the surrounding atmosphere. In fact, more water may evaporate from the plant than enters the plant, resulting in wilting. Such environmental factors as light per se, temperature, and relative humidity affect the rate of transpiration, hence, the availability of water in the plant, and consequently differentiation of cells by means of cell enlargement. The concentration of water within the vacuole rarely remains constant. Solutes diffuse in and out of the cell and are constantly being used, or converted into

insoluble substance, or both. Insoluble foods may be digested to soluble foods and soluble sugars may be synthesized from water and carbon dioxide. The complex cytoplasmic membrane constantly undergoes alterations in the kind, quantity, and distribution of molecules of which it is composed. As a consequence its permeability is continuously changing. A given kind of molecule may pass through in large numbers, scarcely at all, or not at all depending upon the momentary composition of the membrane. Instability is the essence of this intricate mosaic of interacting molecules. Thickness of the cell wall and the balance existing between the various kinds of molecules of which the wall is composed are not the only factors determining wall plasticity. Naturally occurring hormones (auxins) are without doubt causally related to plasticity of cell walls. Cell elongation occurs only in the presence of auxins. However, concentration of auxin and the degree of plasticity of the wall are not necessarily directly related in a quantitative sense. The same concentration of auxin may result in different degrees of plasticity in different cell walls and relatively high concentrations may result in decreased wall plasticity.

It is, of course, entirely unrealistic to expect that all of these factors affecting cell enlargement would be the same for any two cells, even though the cells are genetically identical and located side by side. Therefore, we should be rather surprised to find two cells of the *same* size, shape, and wall thickness lying side by side rather than being astonished that the adjacent cells are morphologically different from each other.

There is much more to be said regarding the role of hormones in cell differentiation in plants. Hormones which are synthesized in stem tips, root tips, and both young and older leaves are necessary for cell division. Hence, they are responsible for the differentiation of meristematic cells from quiescent cells. If the tops of young sunflower seedlings are left undisturbed or if urine or indole-3-acetic acid is applied to the surface of the seedling axis after removal of the tops, a cylinder of meristematic cells (the vascular cambium) differentiates basipetally through the axis, (Snow, 1935). If the cut surface of the seedling axis is left untreated, the meristem does not form.

Reactivation of the cambium in a tree and the subsequent formation of additional xylem and phloem cells is an annual affair. This renewal of meristematic activity in a cylinder of previously meristematic cells is triggered by the renewed production of hormones in the terminal bud (Avery et al., 1937). As the hormone moves downward through the trees, cells of the cambium again become meristematic. Renewal of cambial activity finally occurs in the small roots of the tree.

Differentiation of root primordia from cells at the cut end of a stem is initiated by hormones produced in the stem tip and leaves, (Went et al., 1937). The transformation of mitotically inactive cells of almost any tissue of the stem into meristematic cells which become organized into a pattern characteristic of root tips, not stem tips, is a truly remarkable phenomenon.

We have known for 50 years that when a vein in a stem of a plant such as *Coleus* is cut and a mica plate is inserted in the incision to prevent grafting, cells of the pith, which would ordinarily have remained pith cells until the plant died, begin to divide. The resultant cells eventually differentiate into tissues characteristic of those in the vein. In other words an extension of the uppermost cut end of the vein differentiates downward through the pith, around the incision, until a union is established with the portion of the vein below the incision. It was discovered only a very few years ago that this remarkable resumption of cell differentiation, that is the differentiation of xylem cells from pith cells, is initiated by a hormone produced in the shoot apex and blades of leaves above the incision, (Jacobs, 1952). If the natural sources of hormone are removed and a synthetic hormone, indole-3-acetic acid, is applied, differentiation of xylem cells from pith cells occurs as before.

If cells from the cambium zone of lilac stems are cultured in vitro, a homogeneous callus of thin-walled cells develops. No vascular tissue differentiates from the cells even though the callus may live and grow for more than six years. However, if a shoot apex of lilac is grafted into a V-shaped groove cut into the callus, several strands of xylem cells differentiate basipetally in the callus from the lower end of the shoot apex. If a similar groove cut into the callus is filled with agar, no differentiation of xylem occurs. But if the groove is filled with agar containing indole-3-acetic acid, strands of xylem cells differentiate basipetally through the callus beginning at varying distances from the agar depending upon the concentration of hormone in the agar (Wetmore, 1956).

We might now turn our attention to the question, "how long does a cell retain its ability to differentiate?" In considering this question, we must keep in mind that when a cell divides, that cell no longer exists, but instead, two new cells come into being. The so-called Big Trees (*Sequoia gigantea*) of the western slope of the Sierra Nevadas may attain the age of more than 4,000 years. Yet it is extremely doubtful that any living cell in the tree is 4,000 years old. It is conservatively estimated that more than 90 percent of all cells in these trees are dead (and most of these dead cells lived for only a few weeks at most). Nevertheless, it is true that a relatively few cells in a Big Tree remain alive for about a century (Mac Dougal et al., 1927). These are cells of the xylem rays. There is no evidence, however, that these centenarians continue to enlarge, divide, or otherwise continue to differentiate after the first few weeks of their life.

There is a plant however, that attains the age of 150 years or more, most of whose cells remain alive from the time of their formation until the plant dies and decays (Mac Dougal, 1926). The plant, composed mostly of parenchyma, is the Giant Cactus (*Cereus giganteus*). Not only do the pith cells remain alive for more than 100 years but so do the cells of the epidermis, including the guard cells (Molisch, 1938). Both morphological differentiation and physiological differentiation continue during the course of life of these cells. Pith cells increase in size, their walls thicken, the glucose content increases, and there is a steady decrease in quantities of mucilages, fatty substances, and pentosans in the cells (Mac Dougal, 1926). Xylem and phloem cells continue to differentiate from pith cells as long as the tree lives. However, if an incision is made into the pith, cells which have remained nonmeristematic for more than a century, and would have remained so, had not the incision been made, begin to divide very rapidly and form a cambium. Cells which are formed by the cambium differentiate into cork cells.

The facts just stated and the events described, although remarkable, should not be surprising or unexpected. If it is true that a cell retains all of its chromosomes and genes until death, then we should expect it to retain all of its potentialities for differentiation for life. If the cell is subjected to the proper environment, we should expect any of these potentialities to be expressed. There is abundant evidence that this latter inference is true.

A given kind of cell or tissue may differentiate from any one of a relatively large number of tissues composing a plant. Cork cambium, for instance, may differentiate from cells of the epidermis, hypodermis, cortical parenchyma, endodermis, pericycle, phloem, xylem or pith of stems as well as, from many, if not all, tissues of roots, hypocotyls, leaves, fruits, and parts of flowers (Popham, 1952).

Whole plants may differentiate from the descendant cells of any one of many tissues of the plant. The phenomenon of vegetative propagation is evidence of this. Hundreds of square miles of important crop plants, such as, Irish potato, sugar cane, and sweet potato are propagated vegetatively by planting pieces of stems or roots. Complete new plants arise by proliferation and differentiation of a few cells of the propagule (Popham, 1952).

Cells of the root tip may differentiate physiologically and become reorganized into a shoot apex (Allen, 1947). Bud scale primordia may differentiate into leaves

(Steeves et al., 1957; Al-Talib, 1957). Leaf primordia may differentiate into bud scales, bracts, sepals, petals, or into complete new plants (Gabriel et al., 1957). Complete plants have grown, in vitro, from a 0.5 mm piece taken from the tip of a sunflower stem. In fact, complete plants of several different genera have grown from 0.25 mm slices taken from tips of stems of older plants of the same kind (Wetmore, 1956).

It was stated earlier that differentiation of a cell may be saltatory. We have already mentioned some examples of sudden, unexpected differentiation of cells. Pith cells in the stems of most plants remain pith cells until death of the plant. However, if a vein of the stem is cut, a strand of pith cells differentiates into thick-walled, lignified, elongate xylem elements. Differentiation of cells which have been pith cells for 100 years or more and could be expected to remain pith cells for at least another 50 years suddenly differentiate into highly meristematic cells of a cork cambium when an incision is made into the center of the stem of the giant cactus. A less dramatic but a much more frequently occurring example of saltatory differentiation may be found in the stems of perennials such as sycamore maple (Elliott, 1935) and arbor vitae (Bannan, 1955). At the end of each growing season, meristematic cells newly formed by, and on the phloem side of the vascular cambium, differentiate from meristematic cambium cells. Differentiation is by enlargement, some elongation, and thickening of the cell walls. Morphological differentiation apparently stops at this point and the cells remain in this condition during the winter months. Early in the following spring these cells undergo drastic physiological differentiation which results in morphological changes. To be more specific, these cells differentiate into highly specialized cells of the phloem.

With these examples of saltatory differentiation in mind, it may be profitable to examine our concept of *maturity*. When does a cell become mature? Is cell maturity a physical state or is it a physiological state? Is it possible to recognize a mature cell by examining it through the microscope? Is a mature cell large or small, thick or thin walled?

It would certainly seem that pith cells which change little, if any, in size, wall thickness, or any other morphological characteristic from a time shortly after they are formed until the plant dies and decays, are mature cells. Cells which remain pith cells for a hundred years or more certainly would be referred to as mature cells. Yet we have just concluded that they retain all of their potentialities until death. Furthermore, they do sometimes further differentiate and some of the long latent potentialities find morphological expression.

It would seem then, that maturity could be thought of as a state of physiological quiescence or even death whereas differentiation is a state of physiological activity. In a physiologically quiescent cell (a mature cell), processes may be thought of as occurring at the same rate or with so little variation in rate as to leave the cell unchanged morphologically. It is possible, of course, that processes, such as photosynthesis, might periodically start and stop without resulting in morphological change. On the other hand, cells in which the rates of the processes continue to accelerate and/or in which new processes commence and continue, that is to say cells in which a physiological evolution or revolution is in progress, these are the cells which undergo morphological change and these are the ones which may be thought of as differentiating cells.

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