unexcavated ruins remain in the park. This is fortunate, for they constitute a unique archeological reserve. Even though a great many sites of Mesa Verde culture exist outside the park, not many of them have escaped complete or partial destruction from agricultural or range expansion or from the activities of pothunters. However, the ruins in the park have been protected for over 60 years and will continue to be hereafter. In the future, sites will be excavated that promise to contribute to a particular scientific or interpretive problem or when new techniques of excavation or analysis are devised that will allow a fuller recovery of useful data from the investigations.

There still are problems to be solved about the peoples of Mesa Verde and their culture. Their relationships to other prehistoric developments elsewhere in the Southwest, and even in Mesoamerica, need to be considered; better knowledge of their adjustment to and control of their environment will be investigated; and, archeologists hope, a fuller understanding of the social, economic, and religious aspects of their lives will be obtained. As new finds are made by archeologists and their coworkers they will be reported to fellow scientists and incorporated into the interpretive programs and exhibits at Mesa Verde by the National Park Service for the benefit of park visitors.

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Cortical Patterns in Cellular Morphogenesis

Differences in cortical patterns in ciliates may be hereditary, but independent of genic differences.

David L. Nanney

An essential advance in our understanding of cellular differentiation came with the recognition that the activities of genes are capable of systematic and programmed regulation. The earlier view was that genic activities, like genic structures, are largely and necessarily immune from extranuclear interference. The experimental basis for this interpretation was tenuous, but its influence was pervasive. Cellular differentiation was conceived in terms of interaction of "gene products," occurring in the cytoplasm and conditioned by a variety of intra- and extracellular environmental circumstances. Because the gene products themselves were many and ill defined, because many of the significant environmental variables could only be guessed, and because the essential variability in the kinds and numbers of gene products was not grasped, interpretations of developmental events tended to be formalistic, untestable, and essentially sterile.

Perhaps the transition era began in the 1940's with a recognition that the usual gene product is a protein, coupled with an appreciation that the protein compositions (and particularly the enzymic capabilities) of cells change in the course of development. But the reality of functional nuclear modification was established more directly through studies in the 1950's on nuclear transplantation in amphibia, through cytological and cytochemical observations on polytene chromosomes in insects, and most convincingly by an explication of genetic regulatory elements in bacteria. Not only is the fact of nuclear regulation established beyond any reasonable doubt and in a variety of biological systems, but basic mechanisms responsible for the regulation of genic functions have been identified, and experimental procedures for discriminating among them have been developed. We now speak confidently of transcriptional control and translational control, depending upon whether regulation occurs at the level of synthesis of messenger RNA or during the fabrication of polypeptide chains. We do not yet understand sufficiently well the mechanisms whereby the qualities and quantities of gene products are controlled, but the fact of such control is compellingly established and has become the cornerstone of any synthetic edifice in developmental biology.

The question I discuss here, however, is not the validity of nuclear modification as a factor in cellular differentiation, but its sufficiency. Genic regulation is a beautiful truth, but it is not all we know or all we need to know. The attempt to interpret the interactions of gene products was sterile a quarter century ago, and the time may not yet be ripe for a fruitful analysis. But the problem itself is not obsolete. Eventually cell biologists must rationalize the interactions of gene products which lead to the integrated structural and functional state.

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A modest start has been made in this direction. We know that the functional state of an enzyme may depend upon the presence of specific compounds of low molecular weight—as in feedback inhibition. In some cases metabolites condition the association of protein subunits, which in multimeric assemblies have distinctive properties. Studies are available on the in vitro assembly of fibrous components such as collagen or bacterial flagella. Intensive efforts are being directed toward understanding how protein subunits are assembled into heads, tails, and coats of virus particles. These efforts are modest neither in their sophistication nor in their accomplishments, but only in their restriction to the lowest limits of biological organization and to the interaction of one or a few molecular types. The constraints on such studies favor the discovery of certain kinds of mechanisms, and the principles the studies elucidate may not be entirely adequate at higher levels of association. More particularly, these studies gain incisiveness to the degree that the system components are purified and isolated; a prime experimental objective is to gain simplicity and to dispense with the cell. Obviously those processes which require the integrative functions of supramolecular structures are not to be discovered in this way. And equally obviously, the exploration of processes in intact cells is a far more difficult task than exploration of processes in isolated systems. I do not want to argue against a reductionist procedure, or to denigrate the products of those procedures. I do want to suggest that the whole truth may not be obtained in this way.

At the risk of sounding unfashionably "holistic," I want to dwell on this matter a little longer. An extreme reductionist might view the assembly of a cell as beginning with a coded message in the nucleus. With the assistance of a proper set of "Ur" machinery (the molecular equipment for transcription and translation), this message is decoded into specific sets of polypeptide chains. Because of the unique distribution of charges and bond angles among the amino acid components, the chains fold into characteristic secondary and tertiary structures. The shapes and charge distributions of the folded proteins determine further interactions which generate specific homo- and heteromultimers. Monomers and multimers with enzymatic activity act upon appropriate substrates to develop specific populations of other organic compounds—including carbohydrates and lipids. These in turn interact with each other and with structural proteins to form the granules, fibers, and membrane systems which compose the finished cell.

This interpretation leaves many questions unanswered, but it holds no uncomfortable mysteries. The entire cell, with all its structural and functional specialization, is encapsulated in essence in the nucleic blueprint. All that is required in order to comprehend cellular morphogenesis is a better and better understanding of the interactional properties of the molecular components. The chief difficulty with this interpretation is that cells are not made this way. Cells are made by preexisting cells. This dictum was one of the grandest generalizations of the 19th-century biology—of sufficient prestige to be expressed in a Latin aphorism, *omnis cellula e cellula.* What, if anything, is its significance to current concerns? It suggests perhaps that some of the information required for the generation of a cell, and for the perpetuation of cellular specificity, is contained in supramolecular assemblies, and not uniquely in any one molecular component. We are accustomed to think of all cellular properties as derived from nucleic reservoirs, and to think of evolutionary history in terms of the unbroken continuity of nucleic acid molecules replicating semiconservatively. But we forget that the nucleic acids are transmitted as parts of cells, that nucleic continuity is only a part of the unbroken protoplasmic bridge through biological time. Of course, cellular bridges may be only a convenience, and I would not argue on such grounds alone for some special property of supramolecular templates. I do wish to open the door to consideration of the possibility that essential biological information is encoded and transmitted by materials other than nucleic acids and by means other than linear templates. In an extreme polar interpretation, one might postulate that nucleic acids specify only protein, which must be appropriate for cellular design, but not decisive. In this case the cellular architects (that is, preexisting structures) might be required to determine whether the eventual edifice constructed of the building blocks would be a railroad station or a cathedral. I doubt the value of this extreme analogy, but some intermediate position may be more consonant with larger biological realities than either extreme.

The Ciliate Cortex

I want to discuss some efforts to approach the problem of pattern at the supramolecular level in the ciliated protozoan *Tetrahymena pyriformis.* One may quibble about whether a *Tetrahymena* is a cell or an organism, and one may wonder whether ciliates speak authoritatively to general problems of cell biology. A ciliate is certainly not a generalized cell, but we can probably agree that the generalized cell is a fiction and that *Tetrahymena* is a real live thing. We can probably also agree that mechanisms discovered in any living system have some applicability in other forms. Ciliates manifest much structural detail. They may not be more highly organized in any fundamental sense than amoebas or leukocytes, but their patterns of organization are more clearly visible on casual observation, and more amenable to analysis.

The most familiar features of the ciliate cortex is the cilium, hundreds or thousands of which project from the surface of an animal like *Paramecium* or *Tetrahymena.* These cilia are similar in their organization to those observed throughout the plant and animal kingdoms, and require little discussion. I only want to emphasize at this point that the cilium is itself a complex and highly organized association of many kinds of molecules, arranged in a variety of supramolecular patterns. The cilium, however, is only a part of a larger cortical unit (*1*). At its base is the kinetosome—a cylinder of fibrous elements very similar to the centrioles associated with spindle apparatuses in other cells. And like centrioles, the kinetosomes are associated with other fibrous elements, including (in addition to the cilia) the kinetodesmata, the fibers which arise on the anterior edges of the kinetosomes, run anteriorly beneath the surface of the cell, and compose an organized fibrous system. The membranous cell surface is also highly differentiated, taking the form of vesicles surrounding the base of the cilium, and possessing a number of characteristic features, such as the poorly understood parasomal sac. Some ciliates have still other cortical elements—such as mucocysts and trichocysts—distributed in regular arrays within the cortex. Their functions, whether defensive, offensive, or simply structural, are poorly understood.

Thus, the cilium with its attendant structures may be considered a basic

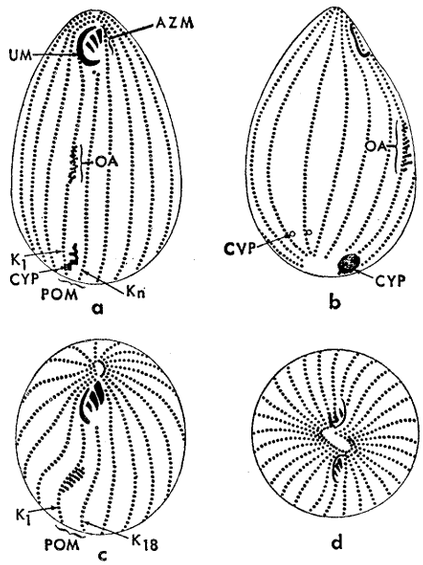

Fig. 1. Semidiagrammatic presentation of silver-staining structures in *Tetrahymena*: (a) ventral view, (b) right lateral view, (c) apical view of singlet, and (d) apical view of doublet cell. *AZM*, adoral zone of membranelles; *OA*, oral anlagen; *CYP*, cytoproct; *CVP*, contractile vacuole pore; *POM*, postoral meridians; K_1, kinety 1; K_n, kinety *n*. [D. L. Nanney (*4*)]

unit of cortical structure, and this unit is fundamentally asymmetric, with a clearly defined anterior-posterior and left-right organization. The ciliary units are themselves organized into still larger patterns which also manifest this asymmetry. The most general of the larger patterns is the ciliary row or kinety—the usually longitudinal array of cilia, vesicles, and kinetodesmata. The kineties have common periodicities and packing patterns; adjacent ciliary rows are commonly aligned to form characteristic two-dimensional sheets of organized cortex within which, again, the anterior-posterior and left-right patterns are readily evident.

The organizational details thus far considered account for much of the pattern of cortical ciliation; considered alone, they would provide a cylinder with a distinctive anterior-posterior axis and a left-right bias. But most ciliates are not simple cylinders; they have closed ends and they have a distinctive dorsoventral differentiation, reflected in the distribution of modified clusters of cortical elements and in the localization of specialized organelles. The oral apparatus, for example, is not usually terminal, but is subterminal or even shifted posteriorly into the caudal half of the cell. Its membranelles consist of elements similar to those composing the somatic ciliature, but their packing arrangements are different, and the cilia themselves may be associated into superorganelles. In many ciliates the somatic ciliature itself is modified into complexes with specialized functions, located at precise positions on the surface. Other cellular organelles, not so readily identified with ciliary units, are also observed. Chief among these are the pores of the contractile vacuoles and the openings of the egestatory apparatus (designated the cell anus, the cytoproct, or the cytopyge by different schools of protozoan proctology).

This brief survey of the ciliate cortex is intended to emphasize the hierarchy of organizational complexity which must be encompassed in a consideration of gross cortical patterns. We are far removed from the genes. We have passed through several levels of organization and must consider structures which incorporate the products of many genes, acted upon by forces which cannot be individually assessed, but which are integrated to yield consistent patterns.

Cortical Patterns in Tetrahymena

Tetrahymena is a familiar enough laboratory inhabitant. The silver-staining procedure developed by Chatton and Lwoff (*2*) makes visualization of its cortical features simple and reliable. One might imagine that one would encounter little difficulty in describing its pattern of organization. Indeed, this description at a qualitative level is quickly accomplished (Fig. 1). The oral apparatus, with the four membranelles which give the genus its name, is located subterminally. The ciliary rows extend from the posterior to the anterior end, except where they are interrupted by the mouth, and form a junction at the anterior apex. By convention the row terminating upon the right side of the cell's mouth (the observer's left) is designated row number 1, and the remaining rows are numbered consecutively to the cell's right. This first row is the normal site of appearance of the new oral primordium at the time of cell division, and for this reason it is sometimes designated the stomatogenic kinety. Near its base is located the opening of the cytoproct. The only other distinctive surface features are the contractile vacuole pores, which open to the outside near the posterior end of the cell, about halfway around the side of the cell. This qualitative description is accurate, so far as it goes, for nearly any cell within a culture, for any strain of *Tetrahymena pyriformis*, for any species of *Tetrahymena*, and for any of several presumably related genera (*3*).

Obviously, if one is interested in specific patterns, one must describe these in sufficient detail so that distinctions can be made. This is a little more diffi-

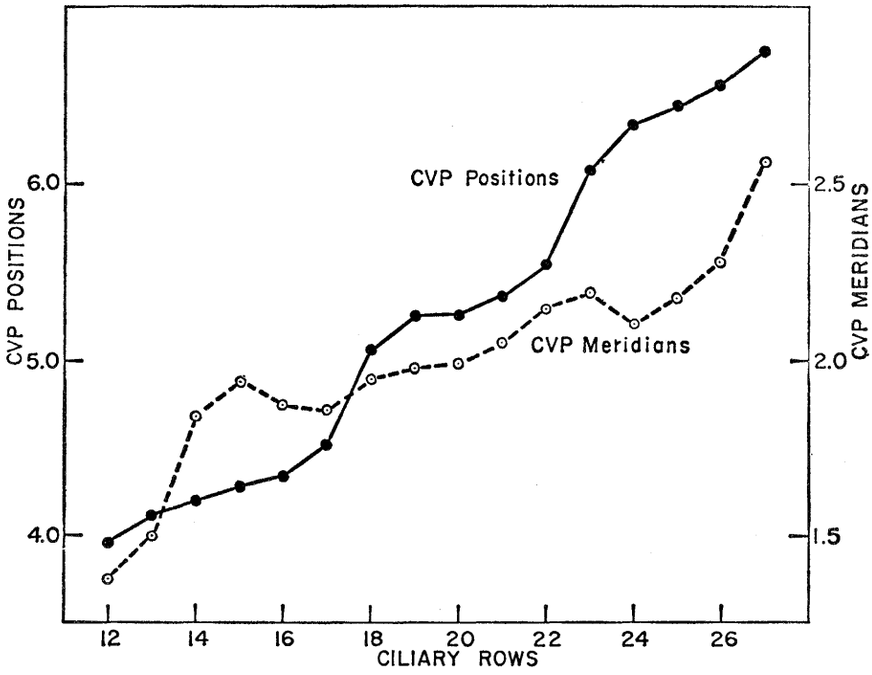

Fig. 2. Patterns of variation for contractile-vacuole-pore (CVP) positions and numbers of meridians with contractile vacuole pores in syngen 1 of *Tetrahymena pyriformis*. [D. L. Nanney (*4–9*)]

cult, for, even within a clonal culture, cells with distinctive properties can be identified. One cell may have 17 ciliary rows and another 18, or 19, or 22, or 28. One cell may have only one contractile vacuole pore while another will have two or three. Two cells with the same number of such pores may have them associated with different ciliary rows. Some cells will have two oral apparatuses, on opposite sides of the body; some may have two stomatogenic meridians, even when they have only one mouth. Every culture is in fact a polymorphic array which must be rationalized if the pattern is to be described accurately.

The resolution of this situation came with the recognition that the variations for the several cortical features were not independent and unrelated but were highly correlated (*4*). All the cortical features vary in a systematic and well-defined manner as the total number of ciliary rows changes (Fig. 2). The precise patterns of covariation are initially puzzling, but every little wiggle has a meaning all its own. More thorough analysis demonstrates an invariant geometric pattern underlying all the polymorphic variations (*5*). The major features of this pattern may be illustrated by the manner whereby the contractile vacuole pores shift with the number of ciliary meridians (Fig. 3). If the longitudinal axis of the cell is taken as one reference point and the first ciliary meridian as the second, the average position of the contractile vacuole pores can be expressed as an angle of approximately 90°. If this angle remains constant but the total number of meridians increases, the pores will move progressively from row 5 to row 6 to row 7 as the total number of meridians increases from 16 to 24.

The situation is slightly more complicated than this, because contractile vacuole pores are ordinarily located on two adjacent meridians, and the cortical area within which they are established must be conceived as a region rather than as a point. This region may be diagramed as an arc subtended by an angle which is capable of experimental definition. As the total number of rows increases, ciliary rows move into and out of the region in a predictable sequence. Because the region is approximately the width of two ciliary rows, one row is ordinarily lost at just the time another row enters the region. At this point in the meridional array one ordinarily finds an increased number of cells with only one contractile vacuole pore and also an increased number of cells with three. Apparently the angle defining the midpoint of the contractile vacuole pores (the "inductive angle") and that defining the width of the field (the "field angle") are constant for a given strain.

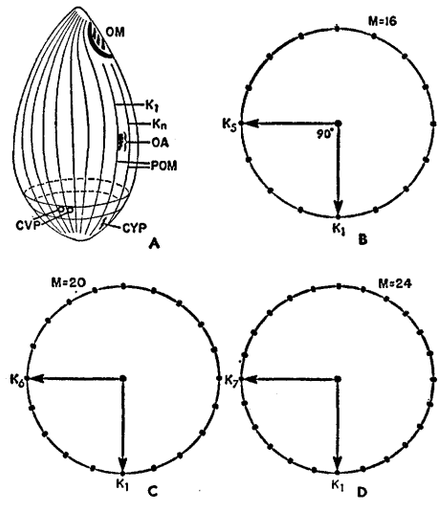

Fig. 3. The concept of the "inductive angle": schematic diagram illustrating the mode of shift of contractile-vacuole-pore (CVP) positions with increasing numbers of ciliary rows. *M*, ciliary meridian; *OM*, oral meridians; other designations as in Fig. 1. [D. L. Nanney (*5*)]

Observations of duplex cells—cells with double sets of all organelles—provide a test for these constructs, and some additional information. The simplest application of the model for single cells would predict the development of two contractile-vacuole-pore regions each having an extent of 36° and appearing at 90° from the two stomatogenic meridians. What is observed instead is two contractile-vacuole-pore regions each with a width of about 18° and located only 45° from the first meridian. A reconciliation of the two patterns is obtained by considering each half of the duplex cell to be a complete domain, redrawing the duplex configuration as externally tangential circles, and applying the standard geometry. Thus, the study of double animals demonstrates the applicability of the model even in unusual circumstances. It also shows that the geometry is in some ways an oversimplification; the cell does not employ a compass, or some structural analog thereof, in measuring relative distances.

The cytogeometric procedures thus developed identify a constant pattern of organellar relationships. This pattern takes on some of the character of a Platonic ideal; any single cell reveals the pattern only imperfectly, but the pattern can be determined readily if a sufficiently large number of individuals is examined. Two sets of questions are raised by such studies: What is the physical basis of the Platonic pattern? And what is the physical basis for the various manifestations of the pattern within a genetically uniform population? No detailed answers to either question are available, but qualitative answers are emerging with reasonable clarity. The patterns themselves are probably determined by conventional genetic determinants, while the permutations on the patterns reflect the activity of epigenetic mechanisms.

The evidence for genetic control of pattern in *Tetrahymena* is thus far circumstantial. Cortical patterns have been determined for a number of strains of various degrees of relationships. Strains within the same genetic species (syngen), as well as more distantly related strains, have been compared (*6–8*). The inbred strains of syngen 1, with a common ancestry in the laboratory, are indistinguishable. Strains of other syngens collected in widely separated places may be alike in some characteristics and different in others. Thus far the positions of the contractile vacuole pores appear to be constant within a syngen, but striking differences between syngens can be demonstrated. Unfortunately, the differences within syngens have thus far been found only in strains which have been maintained for long periods in the laboratory and which have become sterile. A proper breeding analysis for pattern difference must await the discovery of strain differences in fertile strains. Nevertheless, the presumption that the pattern details are controlled by conventional genetic elements is a reasonable one, and the evidence should be forthcoming eventually.

Pattern Permutations

Perhaps the more interesting questions concern, not the basic pattern itself, but the permutations of the pattern. Cells with precisely the same nuclear constitution, and manifesting the same basic pattern of cortical organization, may nevertheless be highly distinctive. At least 20 distinguishable classes can be demonstrated within strains of syngen 1, solely on the basis of the number of ciliary rows. The precise significance of this intraclonal polymorphism is not known, but its biological role may be similar to that of genetic

polymorphism in other organisms. Certainly *Tetrahymena,* a crossbreeding diploid with a long life cycle, is genetically conservative, and its highly polygenomic macronucleus is an effective genetic buffer. Adaptation through mutation may have to be supplemented through diversifying mechanisms of other kinds. In any case, different corticotypes certainly arise—or accumulate —under different cultural conditions.

The analogy between genetic and epigenetic polymorphism, or that between gene mutation and corticotypic modulation, is not as farfetched as one might imagine, because corticotype is in fact a hereditary property of the cell. This conclusion derives from a series of studies on corticotypic stability (*8, 9*). The standard procedure in these studies is to isolate individual cells from a diversified culture, allow each to produce a 20-fission clone, and then sample the population for corticotypes. Although many of the clones have more than one corticotype, all have a restricted number of types, and many are monotypic. Moreover, the variation within a clone is clearly related to the average number of ciliary rows. If one plots the clonal variance for ciliary rows against the mean number, one obtains typically a U-shaped curve (*8*) (Fig. 4). This indicates that the strain has a point of maximum stability—a characteristic corticotype which is most stable under the growth conditions employed. Cells with fewer meridians tend to gain meridians, and cells with more meridians tend to lose them, at rates proportional to the extent to which the number of meridians differs from the number characteristic of the strain's most stable corticotype—or proportional to the cell's distance from what might be called the "stability sink." Presumably if one permitted growth to continue indefinitely under these conditions, nearly all of the cells would eventually come to lie in the sink. The location of the sink and the pattern of movement to the sink differ among strains and are probably controlled by genic determinants.

These observations do not suggest that corticotypes are hereditary variants until one considers the rates of change with which we are concerned. The rates for particular corticotypes can be estimated from the clonal dispersion patterns (*9*). The striking feature of the results from the syngen-1 strains is the very low rate of change for a series of corticotypes from 16 to 21. These values

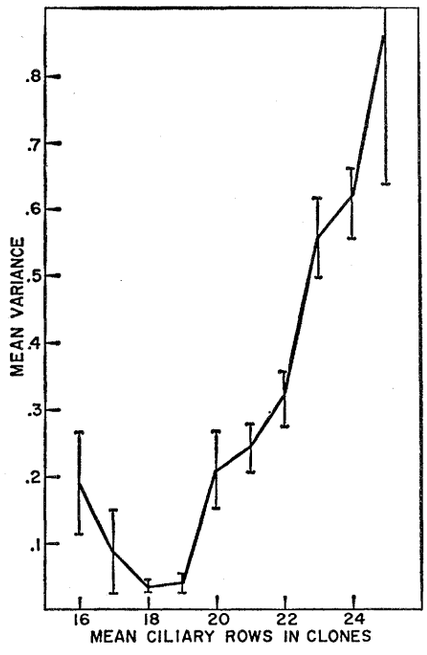

Fig. 4. The "stability pattern" of syngen 1, defined by a plot of clonal variance against the mean number of ciliary rows in the clones. [D. L. Nanney (*8*)]

range from about one to eight changes per thousand cell divisions. This fact is even more impressive when one considers that observational error (in counting ciliary meridians in silver-stained slides) probably accounts for a significant fraction of the changes scored at these frequencies.

Perhaps the most generally applicable (but still imperfect) operational definition for "hereditary differences" is based on a dilution test. If cells with distinctive properties are cultivated in identical environments, they should become alike if their distinctions are not hereditary. If hereditary, the distinctions should persist. In either case, however, one must place some limits on the time required before a judgment is rendered. A commonly accepted limit for cell cultures is 40 cell generations, because the number of molecules in a cell is probably of the order of 2^{40}. After 40 cell generations the original molecules of the parent would be distributed to progeny equal in number to these molecules, and many of the progeny would be without any material remnant of the initial parent. According to this reasoning, and on the basis of this operational test, the corticotypes of *Tetrahymena* are clearly "hereditary" variants. Certainly two clones initiated on opposite sides of the stability sink would require far more than the prescribed 40 fissions before they became alike, or even before they would begin to overlap appreciably.

If one dislikes the conclusion of this analysis, one may of course quarrel with the definition. Let us examine the assumptions behind the dilution test. The chief assumption is that the molecular composition of a cell determines its properties and is the consequence of two factors—the environment and the genes. By deliberately eliminating environmental variables, one restricts molecular variation to a single source—differences in the genetic reservoir. However, molecular differences accumulated through previous cultural history would still be present, and would have to be diluted out before the intrinsic genetic differences are manifested. The existing molecular differences might be imagined to be of many sorts, including messages or low-molecular-weight inducers of genic activity, but the simplistic approach requires them all to be diluted out in the time specified. One may reject the applicability of the dilution test and conclude that corticotypes are not hereditary. But to do so, one must reject the assumptions underlying the test. Either the properties of the cell are not determined solely by the molecules which compose it, or its molecular composition is regulated by something besides the genes and the environment. Thus, whether one concludes that corticotypic differences are hereditary or that the assumptions are faulty is immaterial. In either case one is required to abandon a stereotype of the significance of "hereditary differences."

Clearly the cortical patterns are maintained by mechanisms which require further exploration. And the most powerful device for elucidating hereditary mechanisms is the breeding test. Conjugation is the chief device for genetic analysis in ciliates. Because two cells come together, exchange nuclei, and become genically alike in this process, each conjugating pair constitutes in effect a reciprocal cross. Although the nuclei are alike with respect to all conventional genic markers, they are housed in cytoplasmic structures derived from different sources. The expected consequence of a reciprocal cross is that differences caused by genic differences will disappear, and that progeny, regardless of their cytoplasmic housing, will become alike. If, in contrast, the differences persist for an appropriate number of generations after

conjugation, it is clear that they are not determined by differences in nuclear genes but must be related in some way to cytoplasmic properties. This is in fact the result obtained when crosses are made between different corticotypes in *Tetrahymena* (*9*). When a mating is arranged between a cell of corticotype 16 and another of corticotype 20, for example, the type-16 cell remains type-16 and produces a clone of predominantly type-16 progeny—with no more drift toward the stability sink than is expected of any other type-16 cell. Meanwhile the original type-20 cell produces a clone of predominantly type-20 progeny. The fact that the cells have conjugated, and have exchanged nuclei, has no detectable effect on their subsequent behavior.

These results establish unequivocally that cortical differences are not the consequence of conventional genic differences. They *suggest* cytoplasmic mechanisms for the maintenance of cortical states. Other studies on ciliates have shown, however, that nuclei can differentiate and maintain their differentiations; the manner of differentiation may, moreover, be markedly influenced by the cytoplasm to which they are exposed immediately after conjugation (*10*). Whether cellular traits are dependent upon nuclear differentiation or upon constituents of the fluid cytoplasm, the systematic differences between exconjugants disappear when massive cytoplasmic exchange occurs between conjugants. The cytoplasmic exchange test, therefore, does not adequately distinguish between hereditary bases in the nucleus and in the fluid cytoplasm, but it does distinguish *anchored* cytoplasmic elements from both of the other two. What then are the consequences of cytoplasmic exchange for corticotypic characters? The answer is reasonably clear, for McDonald (*11*) has provided evidence suggesting that in *Tetrahymena,* unlike the case for many other ciliates, massive cytoplasmic exchange occurs regularly at conjugation. Both labeled proteins and labeled nucleic acids, previously limited to one conjugating cell, are equally distributed in the cytoplasm of the conjugants by the end of the mating act. For this reason one must conclude that the hereditary differences in corticotypes are associated with cytoplasmic structures which are not capable of exchange. The most obvious elements of this sort are those embedded in the gelated cortex.

Knowing the geography of a hereditary system is not enough to characterize it, regardless of whether it is in the nucleus or in the cytoplasm. A reasonable assumption might be that genic elements associated with the cortex are responsible for its specific properties. Several reports suggesting that DNA occurs in the ciliate cortex lend plausibility to this interpretation (*12*). However, difficulties arise when one tries to specify exactly how such genic elements are distributed and how they control the cell's corticotype. Do cells of different corticotypes have different kinds of elements? Or is the only difference a difference in organization of the elements? Can one reasonably postulate that each of 20 or more cortical types is characterized by a unique kind of corticogene? And that the entire population of corticogenes is modified when corticotypic transition occurs? A much more tenable interpretation would appear to be that the corticogenic elements do not differ in kind among corticotypes, but differ only in their organization or proportions. Differences in proportions would require that differences in kinds of corticogenes exist, but that these differences occur even among the corticogenes of each kind of cell.

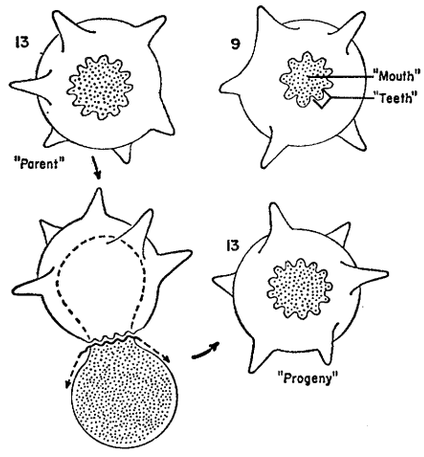

Fig. 5. Schematic representation of "sand templates" in *Difflugia corona* responsible for hereditary differences in numbers of denticles. [After H. S. Jennings (*19*)]

On this question considerable information is available from a number of ciliates. One interpretation of the different structures on different cortical segments of a ciliate is that different determinative elements are associated with specialized organelles. Oral determinants might be localized near the oral membranelles, for example, or arrayed along the "stomatogenic" meridian. This interpretation arose from a consideration of the events occurring when new structures arise; very often new organelles arise in association with old organelles of like kind, or at least on the same ciliary rows. Despite the circumstantial evidence provided by such observations, all attempts to verify the interpretation have given either ambiguous or negative results. Lwoff (*13*), for example, after considering the cortical modifications occurring during the complex life cycles of parasitic apostome ciliates, concluded that the cortical elements must be at least pluripotent. Tartar's (*14*) microsurgical studies on *Stentor* demonstrated that removal of cortical segments resulted in the appearance of their characteristic organelles in novel locations. Both Tartar and Suzuki (*15*), working with *Blepharisma,* found that surgically reconstructed cells often produced organelles in areas which would not ordinarily be expected to develop them.

A study on a genetic variant in *Tetrahymena* permits particular application of this general conclusion to this form (*16*). In one strain of *Tetrahymena* the new oral apparatus develops in the "normal" position on the postoral meridian on the cell's right in only about 50 percent of the cells. In the other cases primordia appear either on both postoral meridians or only on the left one. Other posterior organelles of the cell are also systematically shifted to the cell's left. This pattern of shift can be rationalized as due to a relative displacement of cortical elements which respond to morphogenetic stimuli. The ciliary rows may have undergone a torsion relative to the central cellular axis. Regardless of the precise geometric interpretation of this strain, the simple fact is that the new oral apparatus "slips" one ciliary row to the cell's left approximately half of the time, and that all the other cortical features shift in a corresponding fashion. The special morphogenetic processes and the specialized cortical features will presumably shift progressively one row at a time until they have completely circumambulated the cell. Each and every cortical structure will have developed on lineal descendants of each and every ciliary meridian within approximately 40 cell generations. The cortical elements cannot, therefore, be considered to be different in their fundamental capacities. If corticogenic elements exist, they are not only pluripotent but totipotent. A similar conclusion derives from the ambulatory patches of inverted cortex in *Paramecium* reported by Beisson and

Sonneborn (*17*). While such analyses do not categorically answer the question of corticogenic differences between corticotypes, they indicate no corticogenic differences in gross morphogenetic capacities.

Discussion

These studies indicate that the structural organization of a cell possesses considerable homeostatic capacity; different patterns of organellar association tend to be perpetuated for extremely long periods in the apparent absence of structural differences in nuclear genes, functional differences in nuclear genes, or differences in the kinds or activities of possible cytoplasmic genes. This conclusion does not stand solely on the studies described. Indeed, it was enunciated and abundantly documented first in Sonneborn's (*17*, *18*) studies on experimentally reconstructed paramecia, to which this presentation must be considered an appendix. The *Tetrahymena* studies constitute an extension, in that they are concerned with another organism, and in that they treat naturally occurring cortical variants, but the central conclusions are unmodified. Preformed structures play an essential role in determining the organization of new structures.

The validity of the conclusion is not restricted to ciliates. One of the earliest studies pointing to the same conclusion is that of Jennings (*19*) on the sarcodinid protozoan *Difflugia corona*. This organism constructs a shell by cementing sand grains together with a cellular secretion (Fig. 5). The ventral surface of the shell possesses an opening, the "mouth," through which the cell communicates with the outside world. The edges of the openings are surrounded by a symmetrical array of "teeth." Jennings observed that the numbers of teeth varied among individuals, and he explored the question of their heredity by the only means available; he isolated individuals, allowed clones to develop, and inquired into clonal uniformity. Tooth number remained constant within a clone; differences in tooth number were hereditary. He was not able to conduct a breeding analysis, but he noted that, when the cell body divided, one of the daughter cells was extruded naked through the mouth and, while still in contact with its sister, began to construct its own sand castle, beginning in the region of contact. The new mouth structures were therefore constructed in direct contact with structures of the old mouth. This observation suggested to him that the old mouth might serve as a template to guide the organization of the new one—that new teeth were initiated in the interstices between the old teeth. This curious speculation might have remained just that had Jennings not tried his hand at oral surgery. He broke out denticles with a glass needle and examined the consequences of mutilating the parental template. Modified parents produced modified progeny, and new lineages were established with new tooth numbers. A few generations were required for symmetry to be achieved, but once that had been accomplished the tooth number stabilized and a new hereditary state was achieved. After considering these studies, one of my colleagues concluded ruefully that genetic specificity may be based on two different structural foundations—nucleic acid and sand. In view of the notorious instability of structures built upon the sand, this conclusion is peculiarly disturbing.

A more appropriate conclusion would ignore the differences in the materials involved and would focus on mechanisms. What is demonstrated is that biological specificity may be maintained and transmitted by mechanisms other than those which employ information encoded in linear molecular sequences. Perhaps we are unduly biased in favor of linear information sources, not only because of the successes of molecular biology but also because of our historical dependence on the written word. Now that we are encountering a cultural revolution in which the medium is becoming the message, our academic progeny may be more susceptible to a broader view of informational structure. Certainly biological information can be stored and transmitted by supramolecular mechanisms.

I suspect that most biologists who have examined the evidence will be willing to accept the reality of information storage and transmission by more complex structural assemblies. The more difficult question is the larger significance of such mechanisms. One can imagine the adaptive value of a series of metastable structural states, which are permitted by incomplete specifications in the nucleic blueprints or which are perhaps even specified as optional alternatives. Even this limited role, which is perhaps as much as a conservative evaluation would claim, may be of profound significance in an understanding of cellular differentiation.

Once one concedes this possibility, however, he must consider whether such mechanisms are involved in even more important processes. When the gene theory was in its infancy, some individuals who could not deny the evidence for genic control of organismic characteristics were willing to consider the idea that genes control the relatively unimportant details of biological specificity, but asserted that the more fundamental issues of heredity were untouched. We have reached the antithesis of this position now, and only reluctantly concede that heredity involves anything beyond the genes. Perhaps we are ready to begin moving toward a synthesis, which places a balanced emphasis on the various kinds of mechanisms. At least a recognition of the existence of other mechanisms is the first step toward understanding and evaluating them.

I have surveyed the studies bearing on the determination of cortical patterns in *Tetrahymena*. A variety of pattern permutations can be established on a common genic basis, and these permutations have sufficient stability to be designated *hereditary* variants. The mechanisms of hereditary maintenance apparently do not involve genic differences—either nuclear or cytoplasmic, either structural or functional—but involve rather, a multidimensional information storage and transmission system whereby the pattern, in a sense, maintains itself.

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