COMMENTARY

Positional order and cellular handedness

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Introduction

A major problem in thinking about asymmetrical patterns in organisms is that of obtaining 'big hands from little hands' (Harrison, 1979). The big hands are our own, whereas the little hands are asymmetrical organic molecules. Since the molecules that make up our left and right hands are of identical asymmetry, the different handedness of these big hands must be specified at some more complex level of organization. For Harrison (1979) and, more recently, Brown and Wolpert (1990), the link between the levels is a tethered macromolecule or macromolecular aggregate, implying a pre-existing structural system within which the tethering takes place.

Ciliates are a particularly favorable system for studying organismic handedness. These organisms are pervasively asymmetrical, avoiding the complications resulting from superimposition of asymmetries on a bilaterally symmetrical body plan (see Brown and Wolpert, 1990, and references cited therein). Ciliates are also unicellular, thus reducing the gap between the big and little hands. Among unicellular systems, ciliates have the unique advantage that a specific handedness can be perpetuated across cell generations, making it possible to study the two organizational enantiomorphs separately yet also to analyze the occasional transitions (both genic and non-genic) between them.

What I attempt to show here is that *reversible* intracellular handedness is an emergent property, based neither on local molecular asymmetries nor on local information repositories but rather on more global spatial relations. These relations can be modelled successfully using field equations that suggest a system of interactions of at least two molecular species.

The ciliate cortex: structural lattice and positional order

The surface layer or cortex of ciliates includes a complex structural lattice upon which a positional order is superimposed. I will here present a schematic account of both, using *Tetrahymena* as my example. A parallel description can be made for other ciliates, notably hypotrichs such as *Stylonychia* (Shi and Frankel, 1990).

The principal components of the structural lattice of the *Tetrahymena* cortex are ciliary units that are arrayed longitudinally to make up ciliary rows (Fig. 1A, CR). The ciliary units, centered around one or two basal bodies, are themselves complex and asymmetrical. New ciliary units form immediately anterior to old ones and acquire the same orientation as the neighboring 'parental' units; thus it is not surprising that all ciliary units within a ciliary row have both identical internal asymmetry and uniform orientation with respect to the anteroposterior and circumferential cell axes. The ensemble of ciliary units that makes up a ciliary row therefore acts as a structural supertemplate. Therefore, inverted (180°-rotated) ciliary rows can propagate their inverted orientation (Beisson and Sonneborn, 1965; Ng and Frankel, 1977). Note, however, that an inverted ciliary row is made up of internally normal ciliary units (and rows) with altered spatial orientation relative to the cell as a whole, *not* of ciliary units with reversed asymmetry. Reversals of asymmetry of ciliary units have never been observed.

The major ciliary organelle complex in *Tetrahymena* is the oral apparatus (Fig. 1, OA), made up of four compound ciliary structures, three membranelles (M) and one undulating membrane (UM), each containing many basal bodies. The basal bodies within these structures are organized in an intricate and precise arrangement, unique for each structure (Bakowska *et al.* 1982), which is crudely symbolized in Fig. 1 as a difference in size and tapering of the three membranelles. In addition, two sets of nonciliary structures are located at unique sites, close to particular ciliary rows. One is the cell-anus, or cytoproct (Fig. 1, Cyp); the other is a pair of outlet pores for the contractile vacuole (Fig. 1, CVP).

The positional order is the arrangement in space of these local structural elements. This order is seen most clearly if we contrast normal, 'right-handed' (RH) cells (Fig. 1A) with reversed, 'left-handed' (LH) cells (Fig. 1C), particularly in their polar projections (Fig. 1B,D). The CVPs are situated to the cell's right (clockwise) of the longitude of the oral apparatus (the oral meridian) in RH cells, and to the cell's left (counter-clockwise) of the oral meridian in LH cells. These CVPs are positioned at a nearly constant proportional distance (relative to the cell circumference) rather than at an absolute distance from the oral meridian, in both RH cells (Nanney, 1966*a*) and LH cells (Nelsen and Frankel, 1989). The proportional distance is the same in RH and LH cells, indicating that quantitative spatial relations remain unaltered when handedness changes. The Cyp is located along the right-most of the two post-oral ciliary rows (rows that abut anteriorly on the OA) in RH cells, and along the left-most of these two rows in LH cells. The anterior crown (AC) of basal body couplets is located at the anterior ends of the ciliary rows to the left

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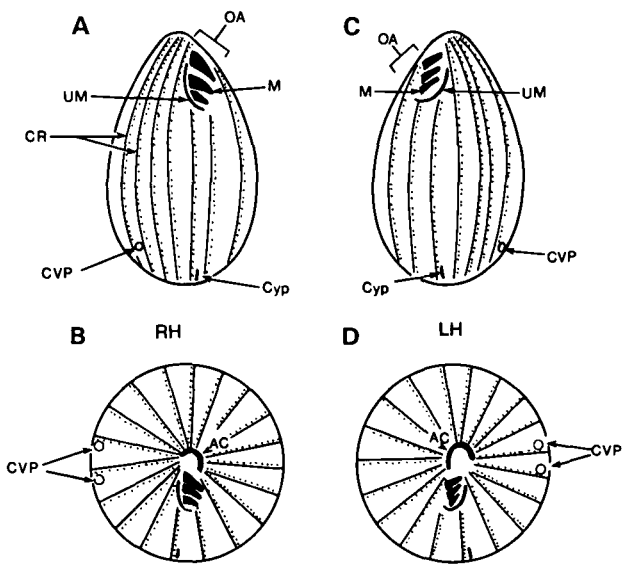

Fig. 1. The cell surface organization of (A,B) right-handed (RH) and (C,D) left-handed (LH) cells of *Tetrahymena thermophila*, drawn as schematic ventral views (A,C) and as polar projections (B,D). The ciliary rows (CR) are shown schematically, with lines representing longitudinal microtubule bands and dots indicating basal bodies. The oral apparatus (OA) includes three membranelles (M) and one undulating membrane (UM). The other cortical features shown are the cytoproct (Cyp), contractile vacuole pores (CVP), and the location of the anterior crown (AC) of paired basal bodies. Slightly modified from Fig. 1 of Nelsen *et al.* (1989*a*).

of the OA in RH cells, and to the right of the OA in LH cells.

This enantiomorphic arrangement of cell structures is, however, superimposed on a lattice of ciliary units that is similar in both RH and LH cells. This is symbolized in Fig. 1 by the identical relations of the longitudinal microtubule bands to basal bodies (Nelsen and Frankel, 1989). Further, the Cyp and CVPs are located on the same side (the cell's left) of the ciliary rows in both RH and LH cells. Thus, the local aspects of positioning of these structures (called 'fine positioning' by Ng and Frankel, 1977) remain normal even when the large-scale aspects are reversed.

The oral apparatus is a meeting ground of local and global influences. Its spatial organization is highly variable in LH cells, with some of this variation shown in Fig. 3B. The most extreme arrangement, sketched in Fig. 1C, is a superficial mirror-image of the OA in Fig. 1A. Close examination, however, revealed that each membranelle of such an LH-OA appears as a 90° (counterclockwise) rotational permutation of a membranelle in an RH-OA, whereas the undulating membrane (UM) appears as if transposed from the right to the left side without alteration of its internal organization (Nelsen *et al.* 1989*b*). Such partial inversions occur in oral structures and not in ciliary rows of LH cells, perhaps because new oral membranelles are organized *de novo* far from old OAs, and thus can be influenced more readily by components of global fields.

The clonal cylinder and perpetuation of pre-existing positional order

The unique geometry of cell growth and division in ciliates

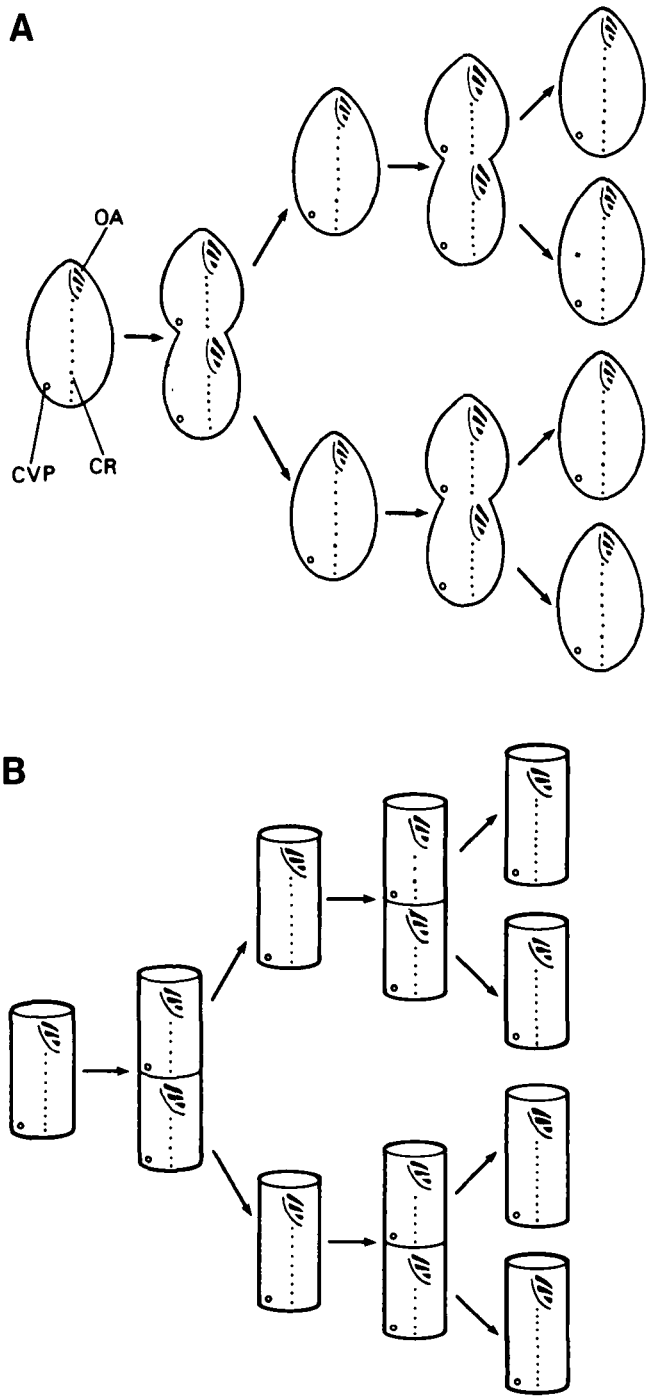

Fig. 2. Clonal growth during two division cycles in normal (right-handed) *Tetrahymena*; (A) showing cell shape realistically; and (B) in a cylindrical distortion. The structures shown in each diagram are the oral apparatus (OA), one ciliary row (CR), and one contractile vacuole pore (CVP). Slightly modified from Fig. 1 of Frankel *et al.* (1987) (*Genetic Regulation of Development*, ed. W. F. Loomis, copyright © 1987 Alan R. Liss, Inc.).

facilitates the detection of anomalies of surface patterning. Growth is longitudinal, whereas division is transverse (Fig. 2A). This geometry is topologically cylindrical (Fig. 2B) (Tartar, 1962). It permits any structure or arrangement that can grow longitudinally to propagate its pre-existing order across cell generations. This capacity

for clonal perpetuation of pre-existing structural configurations contrasts with the lack of opportunity for such perpetuation in other cells, even in other unicellular organisms with parallel rows of ciliary or microtubular structures. In these other cells, growth is transverse and division longitudinal, so that cell division segregates complete structural ensembles and each daughter cell receives half of its ensembles from the parent cell and forms the other half anew (Euglenoids: Sommer and Blum, 1965; Hoffman and Bouck, 1976; Hypermastigids: Cleveland, 1960).

The cylindrical topology of the growth and division combined with stability of cortical cytoskeletal organization explains a ciliate's capacity to perpetuate an inversion of a ciliary row: as the clone grows, the local organization of the ciliary row is simply extended along the clonal cylinder and then subdivided at cell division. This combination of longitudinal extension and periodic subdivision makes it possible for an inversion of even a fragment of a ciliary row to become extended over the entire length of a daughter (or grand-daughter) cell and to be propagated in its progeny (Beisson and Sonneborn, 1965).

Longitudinal perpetuation of global organization does not require direct structural continuity. The new OAs and the new CVPs and Cyps usually appear along the same longitudes as the corresponding pre-existing structures, but at a considerable distance from these structures. Since these new structures develop far from old ones of the same kind, something other than direct templating must be responsible for maintaining the longitudinal continuity of these structures. This guidance can not be supplied by the individual ciliary rows, because although both oral primordia and new CVPs develop next to ciliary rows, there is nothing inherent in *particular* ciliary rows to favor one row over another as a site of oral or CVP development. Nanney (1967) has shown that in certain *Tetrahymena* strains the location of the oral primordium is displaced a consistent distance and direction from the expected postoral ciliary row. He pointed out that such 'cortical slippage' implies that every ciliary row can take its turn as the stomatogenic (OA-generating) row. In other situations, the site of development of OAs jumps to distant longitudes (Fauré-Fremiet, 1948; Nelsen and Frankel, 1986; Frankel and Nelsen, 1986*a*; see below). Hence, large-scale patterning is not determined by inherent characteristics of particular ciliary rows.

The demonstration that RH and LH *Tetrahymena* cells (Fig. 1A,C) do not differ in relevant nuclear genes (Nelsen *et al.* 1989*a*) is consistent with the idea that positional order can be propagated directly along the clonal cylinder. This is illustrated in Fig. 3, in which 'positional values' are assigned around the circumference and along the length of the cell-lineage, here represented topologically as a cylinder. Whereas latitudinal values, represented by letters, must be reorganized in every cell cycle, longitudinal values, represented by numbers, are continuous across cell borders and can be perpetuated indefinitely. It cannot be overemphasized that the numbers *do not* represent structures such as ciliary rows, but rather represent contours of some underlying positional gradation. In the style of positional information (Wolpert, 1971), coordinates of the positional grid specify the location of cortical structures; thus the pair of coordinates 5A might specify the location of an OA, whereas 7F would specify the location of CVPs. Although these structures normally form next to ciliary rows, the positional system specifies

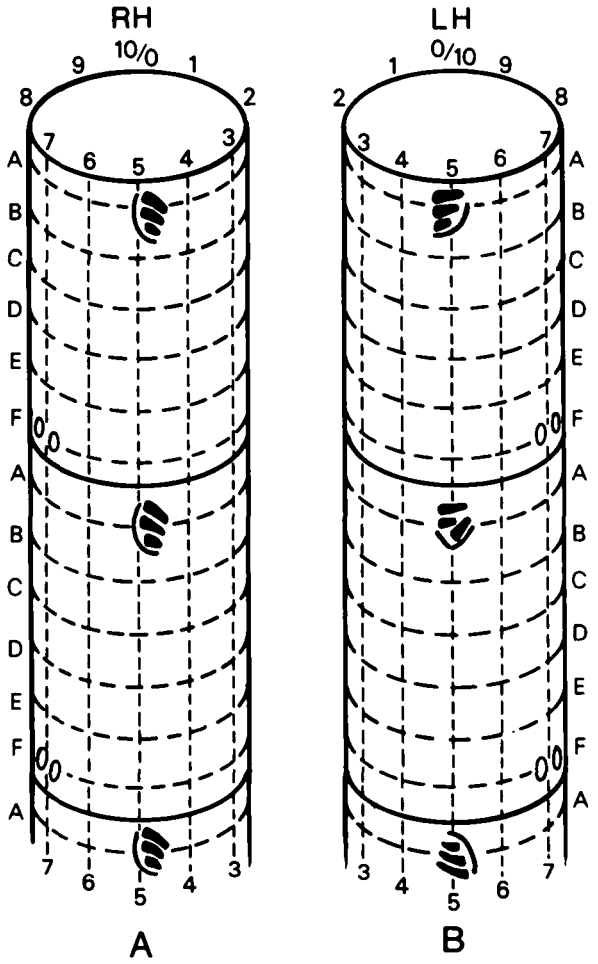

Fig. 3. Orthogonal coordinates superimposed on the clonal cylinder: (A) for right-handed (RH); and (B) for left-handed (LH) *Tetrahymena* cells. The numerical coordinates are numbered in the manner analogous to a clock-face, with 10 and 0 identical. The oral configurations shown in B are a sample of those observed in LH cells. For further explanation, see the text. From Fig. 10.2 of Frankel (1989).

which ciliary rows are chosen and in which part of the chosen ciliary row the structure develops.

The coordinates of the longitudinal system are wound around the circumference of the cell, and the direction of winding is perpetuated as the clonal cylinder elongates. As long as the polarity of the anteroposterior axis remains fixed, the 'positive' winding of the RH clonal cylinder (Fig. 3A) can be converted into the 'negative' winding of the LH cylinder (Fig. 3B) only by breaking into the cylinder itself, deleting a subset of positional values, and inserting a complementary subset in reverse order; in fact, this must be done twice to convert an RH cylinder (with a 'winding number' of +1) to an LH cylinder (with a winding number of −1). Given the cylindrical mode of ciliate growth, vegetative propagation of pre-existing cellular handedness would be the expected norm, and changes of handedness should be exceptional.

The existence of cells with opposite structural handedness is not unique to ciliates. In both fibroblasts and neuroblastoma cells, daughter cells frequently manifest roughly opposite structural handedness (Albrecht-Buehler, 1977; Solomon, 1979). However, this situation differs from that encountered in ciliates in three respects:

first, the forms of the mammalian cells within which opposite handedness is observed are highly variable, so that it is not possible to recognize dual enantiomorphic forms in large cell populations; second, even in daughter cell pairs, the two daughters are often but not always mirror images; third, the way in which these mirror-image configurations are formed at division makes them non-heritable. The crucial advantage of ciliates lies in the combination of structural complexity, stability, and, above all, the unique cylindrical topology of clonal growth. Nonetheless, the existence of mirror-image configurations in cells other than ciliates (see also below) indicates that ciliates may be a particularly favorable system for study of a phenomenon that is more general.

How changes in cellular handedness occur

In ciliates cellular handedness has been changed either through reversal of the anteroposterior axis provoked by surgical manipulation or by circumferential reorganization following geometrical or genic changes (see Frankel, 1989, chapter 9, for details and references). Here I will concentrate on circumferential reorganization as observed in *Tetrahymena*, summarizing the nongenic and genic transitions, in that order.

All known nongenic routes to reversal of handedness in *Tetrahymena* proceed through a doublet intermediate. Such a doublet is a ciliate Siamese twin: two normal cells become fused back to back as a consequence of a previous arrest in cell division or conjugation. Doublet cells start out with a doubled cell circumference (as measured in number of ciliary rows), but tend to regulate slowly back to a normal circumference (Fauré-Fremiet, 1948; Nanney, 1966*b*). During the course of this regulation, unusual configurations may appear that include longitudinal sectors of reversed asymmetry. The most common of these forms is the 'triplet' form, in which a new LH oral apparatus develops transiently between two RH oral apparatuses (Fauré-Fremiet, 1948; Nelsen and Frankel, 1986). These triplets are intermediates in regulation from a Siamese-twin doublet state to a singlet condition of the same handedness as the original doublet. A rarer and more complicated type of reversal involves the transformation of one longitudinal half-cell of a doublet into a half-cell of the opposite asymmetry, converting a Siamese-twin to a mirror-image doublet (Frankel and Nelsen, 1986*a*; Nelsen and Frankel, 1989). Such a mirror-image doublet is the likely precursor of a singlet with handedness opposite to that of the original Siamese-twin doublet. Reversed singlets can be generated from mirror-image doublets either by microsurgical transection (in *Glaucoma*, a close relative of *Tetrahymena*; Suhama, 1985), or by rare endogenous regulation (in *Tetrahymena*; Nelsen and Frankel, 1989).

The stimulus for nongenic reversal of asymmetry is geometric, not metabolic. Much previous research on effects of inhibitors of metabolism and macromolecular synthesis on cortical development in *Tetrahymena* has not yielded a single case of reversal of asymmetry. Reversals come about under completely normal growth conditions, and what matters is that the antecedent cell is a regulating doublet in which the number of ciliary rows is about midway between that of a newly formed doublet and a normal singlet. We have accounted for this by assuming that the circumferential positional values depicted in Fig. 3 must maintain continuity and, further, have an optimal spacing. In a Siamese-twin doublet, there are two sets of values in tandem. As the doublet decreases its circumference, the positional values become excessively closely spaced, stimulating a reorganization in which a large subset of positional values is replaced by the complementary smaller subset, necessarily in reversed order (Nelsen and Frankel, 1986). This idea, inspired by the polar-coordinate model of French *et al.* (1976), was subsequently extended to other situations and to other ciliates (Frankel, 1989, chapter 10). Most recently, Brandts and Trainor (1990*a*,*b*) have replaced this provisional model with a more precise physical model, which I will take up again near the end of this essay.

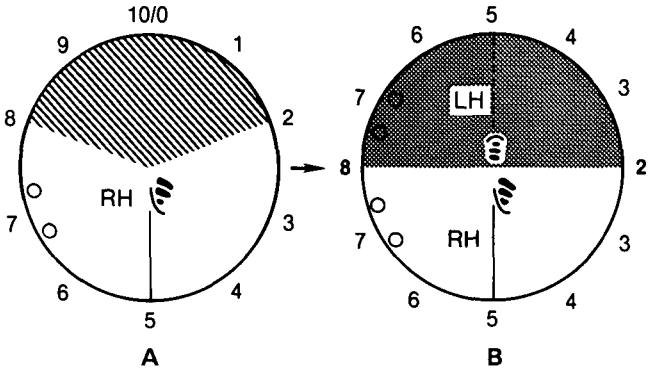

Fig. 4. Schematic polar projections of cells before (A) and after (B) expression of a *janus* mutation. Oral apparatuses, contractile vacuole pores, and one postoral ciliary meridian are shown. The numbers represent circumferential positional values, as in Fig. 3. The hatched region of the right-handed (RH) cell (in A) represents the subset of positional values that cannot be maintained by *janus* mutant alleles. This region then is replaced by a left-handed (LH) zone (shaded in B). Slightly modified from Fig. 9 of Frankel *et al.* (1987) (*Genetic Regulation of Development*, ed. W. F. Loomis, copyright © 1987 Alan R. Liss, Inc.).

The genic route to reversal of cellular handedness (Fig. 4) involves expression of *janus* mutants in *Tetrahymena thermophila* (Frankel and Jenkins, 1979; Frankel *et al.* 1987). A homozygous *janus* cell (Fig. 4B) mimics a mirror-image doublet, with an OA and a CVP set in reverse arrangement on what should be the dorsal surface of the cell. A detailed study of the phenotypic conversion of normal to *janus* cells after the macronuclear genetic transition from $janA^+/janA^+$ to $janA/janA$ showed that the conversion took place while the number and configuration of ciliary rows remained unchanged. The CVP domain broadened and eventually split into two domains, and new oral primordia of the characteristic LH type (see section on the ciliate cortex, above) later appeared within what previously was the mid-dorsal surface of the cell (Frankel and Nelsen, 1986*b*). The positional order changed profoundly while there was no apparent change in the structural lattice of the cell. In formal terms, one could imagine that products of the jan^+ alleles are required for maintenance of a dorsal subset of the positional values (8–9–10/0–1–2) (Fig. 4A). Following the withdrawal of a jan^+ gene product, the dorsal positional values would be lost, altering the spacing of values and also creating a potential major discontinuity at the former dorsal midline. To restore continuity plus near-normal spacing of positional values, the *janus* cell would then be forced to intercalate the permitted (ventral) subset of positional values, necessarily in reverse order (Fig. 4B).

The distinctiveness of positional order

In all cases in which RH cells transform to LH cells, the arrangement of the structures on the cell surface becomes reversed, but the internal asymmetry of these structures remains normal. This fact, first clearly recognized by Grimes *et al.* (1980) in the hypotrich ciliate *Stylonychia mytilus*, is now known to apply without exception to all cases of reversal of handedness in ciliates (see Frankel, 1989, chapter 8). Thus the arrangement of cell-surface organelles must have some mechanistic basis separate from that which specifies the internal organization of these organelles. Positional order is superimposed on, yet independent of, the structural lattice of the cell in the same logical sense that the national government of the United States is superimposed on, yet (in theory) independent of, state and local governments.

A superimposition of different patterning mechanisms relating to asymmetry, while clearest in ciliates, is also manifested in zooflagellates of the Class Diplomonadida (Vickerman, 1990), which includes the well-known intestinal parasite *Giardia*. In these organisms, a striking bilaterality of form as well as positioning of certain structures (such as basal-body complexes, nuclei and adhesive discs) is superimposed on the identical asymmetry of arrangement of basal bodies and accessory structures within the two ordered clusters (mastigonts) found in these cells (Brugerolle, 1975; Kulda and Nohýnková, 1978). The dynamics by which this superimposition is generated in *Giardia* and its relatives has been little investigated, though a reasonable guess would be that a mirror-image cell division mechanism (old left half generating new right half, old right half generating new left half) is superimposed on local templating mechanisms for the duplication of the basal-body apparatus (cf. Gould, 1975, for *Chlamydomonas*). The Diplomonads deserve more investigation from the perspective of intracellular patterning.

Can basal-body DNA (or RNA) explain positional order?

The spectacular recent claim that a large chromosome of the unicellular alga *Chlamydomonas*, the *uni* linkage group, is located in the basal body of that cell (Hall *et al.* 1989) reopens the broad questions of whether DNA is present in basal bodies of cells in general and, if so, what it does. Here we ask, do the basal bodies of ciliates contain their own DNA, and, if so, can this account for the phenomena of cellular handedness?

The question of the existence of basal-body DNA in ciliates has a long history (reviewed by Fulton, 1971). Positive claims were based on yellow-green fluorescence with Acridine Orange and on labelling with tritiated thymidine, presented most compellingly for *Paramecium* by Smith-Sonneborn and Plaut (1967, 1969). However, both claims met with skepticism (Fulton, 1971; Hartman *et al.* 1974). The molecular technology employed in the discovery of DNA in basal bodies of *Chlamydomonas* may render these older methods obsolete. Nonetheless, there is one conclusion drawn by Hall *et al.* (1989) for which the older evidence is relevant. These authors note that for the 6–9 megabase *uni* chromosome to reside inside a basal body, it must be packed very densely. They further point out that '...a candidate structure for an internal DNA can be identified in published electron micrographs of *Tetrahymena* basal bodies...' (Hall *et al.* 1989, p. 129). A similar dense internal structure is found inside basal bodies of *Paramecium*, and was shown by Ruth Dippell to be removed by RNase but not by DNase (Dippell, 1976).

Since RNA is known to be an informational macromolecule, I will assume for the sake of the argument that basal bodies in ciliates as well as other unicellular organisms do contain *either* DNA or RNA, and that the nucleic acid is transcribed (if DNA) and translated in the close neighborhood of the basal body in which it resides, allowing for possible regional differences in proteins coded for by these nucleic acids. Could this help to account for the properties of positional order described earlier in this essay?

I believe not. There are difficulties in attributing the control even of locally propagated patterns (such as the orientation of ciliary rows) to basal-body DNA (or RNA), and the difficulties become nearly insuperable when this attribution is extended to large-scale patterning.

Taking the local context first, consider the most interesting known gene locus of the *uni* linkage group of *Chlamydomonas*, *uni* itself. A mutation at this locus generates a peculiar asymmetry in the normally symmetrical biflagellate *Chlamydomonas* cell, as only one flagellum is present, and that flagellum is located on the side of the cell opposite to the unilaterally located eyespot (Huang *et al.* 1982). However, midsagittal sections of the flagellar apparatus of *uni* mutant cells revealed that both basal bodies are present, with one basal body flagellated and often sporting extra transition-zone structures at the base of the flagellum, the other basal body non-flagellated and lacking transition zone structures. The two basal bodies of the normal flagellar apparatus of *Chlamydomonas* are known to differ in age (Gould, 1975). If, as Huang *et al.* (1982) suggest, the flagellated basal body of *uni* cells is the older one and the non-flagellated basal body is the younger one, then the defect in *uni* mutants is likely to be a one-generation delay in maturation of a new basal body for flagellum formation, with a simultaneous misdirection of surplus transition-zone products to the older basal body. The *uni* mutant phenotype would thereby uncover, but not create, an asymmetry in positioning of the new basal body in *Chlamydomonas* (Huang *et al.* 1982, p. 762).

Considered in this light, there is no conflict between the claims made for the role of the *uni* linkage group in the formation of new flagella and possibly also basal bodies (e.g. see Ramanis and Luck, p. 426) and T. M. Sonneborn's earlier vehement denial of a role of basal-body DNA in inheritance of the orientation of ciliary units and rows in *Paramecium* (Sonneborn, 1970, p. 354). The *formation* of basal bodies could occur under the direction of an indigenous nucleic acid without involvement of other structures, whereas the *orientation* of basal bodies requires an external reference point, which must be supplied by other cellular structures. In ciliates, basal-body DNA (or RNA) may help to explain how a new basal body forms near a definite part of an old basal body, and therefore how a ciliary row can maintain a consistent orientation, but cannot account for the difference between a normally oriented and inverted ciliary row. In diplomonads such as *Giardia*, positioning with respect to an external reference structure, analogous to the eyespot of *Chlamydomonas*, could explain the bilaterally symmetric locations of the two internally asymmetrical basal-body clusters (mastigonts).

The difficulties are compounded if one invokes basal-

body DNA (or RNA) to explain the large-scale arrangement of multiple cell structures. There are two major objections to considering basal-body nucleic acid as a source of positional information in a ciliate. One is that mutations in DNA or RNA of individual basal bodies of a ciliate might, in several generations, become established in all of the basal bodies of a ciliary row; however, owing to the longitudinal continuity of these rows, it would take a very long time before any such mutant could spread to all ciliary rows. This necessarily slow intracellular spread of a basal-body mutation contrasts with the relative swiftness of the processes leading to changes in ciliate handedness, within a few cell generations in *Tetrahymena* (Nelsen and Frankel, 1986, 1989), and in the space of a few hours in the hypotrich *Stylonychia* (Shi *et al.* unpublished data).

The second difficulty lies in the independence of global positional order from basal body orientation (see above). Given this independence, it is hard to see how the global positional order could be affected by genes involved specifically in the assembly of basal bodies or cilia. This makes it highly unlikely that basal-body DNA (or RNA) could be the source of global patterning instructions. It is perhaps more plausible that basal-body DNA or RNA could act as a recipient or interpreter of instructions emanating from another source (e.g. a nucleus), although the other instructions would then actually specify the pattern (cf. Nanney, 1967, p. 167).

Thus, although genes within basal bodies were not formally ruled out in the demonstration of the non-genic nature of changes in cellular handedness of *Tetrahymena* (Nelsen *et al.* 1989*a*), such genes, even if they exist, are not likely candidates for the source of the positional order on which cellular handedness is based.

What is the basis of positional order?

The short answer is that we do not know. Rather than stop there, I would like to argue for a particular approach to the problem. The tendency of most cell and molecular biologists confronted with the problem of spatial patterning is to deal with it as a jigsaw-puzzle. An example of the success of this approach is the elucidation of bacteriophage assembly (Wood, 1980). At the cellular level, the jigsaw-puzzle spirit animated the proposal of 'nearest-neighbor interactions' first put forward by Sonneborn (1975) for intracellular patterning in ciliates (especially *Paramecium*) and later revived (under the same name!) by Martinez-Arias (1989) for cell-to-cell interactions during segmentation in *Drosophila*. I regard this style of thinking as inadequate for the spatial system considered here, whose properties cannot be extrapolated from those of any known smaller-scale structures (see above) and which regulates proportionally on a large scale (see above). We are dealing here with an intracellular version of a classical morphogenetic field (Huxley and DeBeer, 1934).

In seeking to understand the properties of morphogenetic fields, models devised by physicists who are accustomed to thinking about physical fields can be helpful. Even though the biological world is largely a product of evolutionary tinkering (Jacob, 1977; Gould, 1980) rather than of the uniform operation of natural laws, physical models can give us some insight into the *minimal* complexity that must be built into a spatial system that has properties such as those summarized in the previous paragraph (the actual complexity may, of course, be much greater). Such a model has been devised by Brandts and Trainor (1990*a*,*b*) for the system controlling cellular handedness in *Tetrahymena*.

The model begins with certain fundamental requirements, namely that positional information be *unique* (so that every point around the cell circumference has a distinct positional value), *continuous*, and *periodic* around the circumference. As pointed out earlier by others (Sibatini, 1981, p. 440; Totafurno and Trainor, 1987, p. 427) for geometrically comparable situations in other organisms, these three conditions require a minimum of *two* quantities varying around the circumference of the field. These quantities could be designated in various ways: examples include two offset periodic scalar functions (Goodwin, 1976, pp. 174–178) or the two components of a vector within some 'biological coordinate space' (Totafurno and Trainor, 1987). Brandts and Trainor (1990*a*) have chosen the latter designation for its convenience and simplicity, although an equivalent but perhaps more unwieldy model might have been constructed using the former. In either case, the existence of two separate components distinguishes the assumptions of this model from ideas based on a simple gradient of a single component, such as that reflected by the gradation of widths of pigment stripes in *Stentor* (Uhlig, 1960; Tartar, 1962; Frankel, 1989, pp. 120–128). A linked variation in two quantities permits both uniqueness and continuity at all points of the cell circumference, whereas a gradation of a single component necessarily sacrifices either uniqueness or continuity (the latter in the case of the *Stentor* striping system).

The essence of Brandts and Trainor's model is the idea of minimization of energy contained in a vector-field that is defined around the cell circumference. The total energy of the vector field is obtained by summing the energy density at each point around the circumference. This energy-density contains two terms, one reflecting the idea that there is an 'optimal gradient' of the vector-field (analogous to 'optimal spacing' of positional values), the second representing the idea that *changes* in magnitude or direction of that gradient cost energy. When the cell circumference is at a normal value, or at an integral multiple of that normal value (corresponding to a normal singlet cell or a newly formed Siamese-twin doublet cell, respectively), then the minimum-energy solution is one in which the gradient-field is constant; i.e. the entire cell-complex has the same handedness. However, when the cell circumference is abnormal, e.g. halfway between the normal singlet and doublet values, then a solution in which the sign of the gradient-field changes over a portion of the cell circumference has the minimum energy; i.e. reverse-intercalation is energetically favored. With this model, adjustment of two parameters, one that is the cell circumference and the other that specifies the relative importance of the two terms of the energy function, allows most of the detailed observations on 'triplets' by Nelsen and Frankel (1986) to be explained with remarkable accuracy (Brandts and Trainor, 1990*b*).

A model of this kind may sound abstruse to many biologists. In my view, it is helpful in revealing the requirements that must be met by a system made up of real molecules interacting in real cellular space. The mathematics of the model are of course far more precise than any concrete images that they convey to a biologist; to me, at least, they suggest two or more species of mobile molecules interacting in or under the cell membrane according to certain restrictive rules, with a positional

'interpretation' depending not on either one alone but on the ratio between the two. The challenge now is to identify such molecules and characterize the way in which they interact.

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