MINIREVIEW

What Do Genic Mutations Tell Us about the Structural Patterning of a Complex Single-Celled Organism?\(^\dagger\)

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PROLOGUE: FROM THE CYTOPLASM TO THE NUCLEUS

Structural inheritance in the ciliate cortex. Ciliates make up a distinctive group of unicellular organisms characterized by dualism of germ line and somatic nuclei, sex by reciprocal exchange of gametic nuclei with subsequent replacement of the somatic nucleus, and an extraordinarily complex organization of the cell surface layer. This organization is dominated by cytoskeletal structures, including rows of basal bodies, cilia, and accessory fibrillar structures, and is perpetuated by longitudinal extension of these structural ensembles during clonal growth.

This mode of perpetuation provides an ideal opportunity for the demonstration and analysis of cellular heredity at a non-genic level. This opportunity was seized by three gifted biologists, the comparative zoologist Emmanuel Fauré-Fremiet, the experimental embryologist Vance Tartar, and the geneticist Tracy Sonneborn, who together led the way in establishing the demonstration and analysis of cellular heredity at a non-genic level. This opportunity was seized by three gifted biologists, the comparative zoologist Emmanuel Fauré-Fremiet, the experimental embryologist Vance Tartar, and the geneticist Tracy Sonneborn, who together led the way in establishing the demonstration and analysis of cellular heredity at a non-genic level. This opportunity was seized by three gifted biologists, the comparative zoologist Emmanuel Fauré-Fremiet, the experimental embryologist Vance Tartar, and the geneticist Tracy Sonneborn, who together led the way in establishing the demonstration and analysis of cellular heredity at a non-genic level. This opportunity was seized by three gifted biologists, the comparative zoologist Emmanuel Fauré-Fremiet, the experimental embryologist Vance Tartar, and the geneticist Tracy Sonneborn, who together led the way in establishing the demonstration and analysis of cellular heredity at a non-genic level. This opportunity was seized by three gifted biologists, the comparative zoologist Emmanuel Fauré-Fremiet, the experimental embryologist Vance Tartar, and the geneticist Tracy Sonneborn, who together led the way in establishing the demonstration and analysis of cellular heredity at a non-genic level. This opportunity was seized by three gifted biologists, the comparative zoologist Emmanuel Fauré-Fremiet, the experimental embryologist Vance Tartar, and the geneticist Tracy Sonneborn, who together led the way in establishing the demonstration and analysis of cellular heredity at a non-genic level. This opportunity was seized by three gifted biologists, the comparative zoologist Emmanuel Fauré-Fremiet, the experimental embryologist Vance Tartar, and the geneticist Tracy Sonneborn, who together led the way in establishing

Although this review is primarily devoted to the contribution of genic mutations to the understanding of the ciliate cortex, the interpretation of some of the more interesting of these mutations becomes more meaningful against a background of the three major arenas of nongenic structural inheritance in the ciliate cortex, all of which were solidly established before the genetic approach was initiated.

The best-known form of structural inheritance is the organization of the longitudinal ciliary rows that cover the surfaces of most ciliates. This was first demonstrated by Beisson and Sonneborn (12), who showed that an inversion (180° rotation) of one or more ciliary rows of Paramecium tetraurelia (then Paramecium aurelia, syngen 4) (Fig. 1A) could be nongenically inherited; Sonneborn gave the name “cytaxis” to “this ordering and arranging of new structures under the influence of pre-existing cell structure” (137). This demonstration was later repeated for Tetrahymena thermophila (then Tetrahymena pyriformis, syngen 1) (118) and for other ciliates (62, 63, 71). It was extended by Nanney’s observation that the preexisting number of ciliary rows in T. thermophila tended to be conserved (103, 104), presumably due to the same structural constraints that conserve the geometrical organization of these rows.

A second form of structural inheritance is demonstrated by the propagation of the number of complete sets of cortical structures. This was investigated in detail by Fauré-Fremiet (31, 32), who noted that ciliates that became fused side by side, as a consequence of blockage of division followed by anterior sliding of the presumptive posterior daughter cell, could propagate their duality. Later, Vance Tartar (147) demonstrated that microsurgically constructed Siamese-twin doublets in the large ciliate Stentor coeruleus could perpetuate their doublet condition. In both cases, the way in which the doublets were created virtually rules out the hypothesis that this condition had arisen from a genic mutation; instead, it was due to a “contrainte structurale” (32). Sonneborn (136) completed the demonstration in Paramecium tetraurelia (Fig. 1B) by proving with results of appropriate crosses that the difference between the singlet and doublet conditions was not caused by differences either in nuclear genes or in exchangeable internal cytoplasm and hence had to reside in the cortical layer. There is good reason to believe that the same conclusion applies to doublets induced in other ciliates, including Tetrahymena thermophila (103, 109). This structural constraint within the cell cortex does not influence the number of macronuclei in doublets; these cells typically reverted from possession of two macronuclei to one, while the cortex continued to propagate its duality (14, 109).

The third form of structural inheritance was discovered by Fauré-Fremiet (31). He noted that whereas the great majority of the doublets that he studied manifested a twofold rotational symmetry in the organization of their two normal sets of cortical structures, one exceptional clone of a ciliate named Urostyla trichogaster displayed a mirror image symmetry in the arrangement of its two sets of cortical structures. This initial discovery was followed up by a Chinese group (153) and then was confirmed and extended by investigators worldwide for a variety of ciliates (44, 64, 70, 142, 155, 166). In these doublets, the arrangement of cortical structures in the “reversed” partner was close to a mirror image of that in the “normal” partner (Fig. 1C), but the internal organization of each individual ciliary structure in the “reversed” component was normal, though sometimes rotationally permuted relative to the cellular axes (13, 64, 70, 131, 142). The mirror image arrangement could be reliably generated by certain microsurgical operations (132, 153) and was not caused by any relevant genic difference (114, 151). When such mirror image doublets were bisected midlon-

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FIG. 1. Examples of forms of nongenic structural inheritance. (A) Inversion of a ciliary row. Shown are three adjacent ciliary rows on the
surface of *Paramecium tetraurelia*, with basal bodies (BB), coated pits (CP), and striated rootlets (SR) indicated. N indicates normally oriented
rows; I indicates an inverted row. (B) Siamese-twin doublet of *Paramecium tetraurelia*, indicating the locations of the two oral apparatuses (OA1
and OA2), the two cytoprocts (Cyp1 and Cyp2), and the two sets of contractile vacuole pores (CVP1 and CVP2). Dashed lines indicate structures
behind the plane of view. (C) Ventral surface of a mirror image doublet of a spirotrich (formerly hypotrich) ciliate such as *Oxytricha ot Stylochocia*,
with the normal and reversed oral apparatuses (nOA and rOA, respectively) labeled. The vertical dashed line indicates the approximate plane of
the mirror image symmetry in the arrangement of structures. Panels A, B, and C were modified from Fig. 4.3a, 4.8b, and 8.3, respectively, from
reference 38 with permission of the publisher.

itudinally to yield two nucleated moieties, the normally ar-
ranged moiety produced a clone of normal singlet cells, whereas the reversed moiety was unable to propagate itself
despite repeated attempts to do so. This failure was caused by
an inability to form food vacuoles, because the cilia of the
rotationally permuted oral structures beat in the wrong direc-
tion, away from the mouth (31, 61, 133, 152). Reverse-singlet
cells in the less complex hymenostome ciliates *Glaucoma
scintillans* (143) and *Tetrahymena thermophila* (112, 114) could
partially overcome this difficulty and produced slow-growing
clones that inherited a reversed configuration of their major
cortical landmarks.

While no investigator denied that genes could influence the
structural organization of ciliates, the dominant paradigm be-
fore about 1970 had been the “pattern and substance” view of
Vance Tartar (147, 148), namely, that the nucleus supplies the
“building blocks” for structural patterns, but “where and how
the building blocks are put together in formed organelles is
probably the work of the ectoplasm and its pre-formed struc-
ture” (148). Sonneborn’s conclusion a decade later was more
even-handed: “There is no evident escape from the conclusion
that essential aspects of development in *Paramecium* are en-
coded partly by cortical geography, not solely by DNA” (138).

**Genes and structural patterns in ciliates.** In the middle of
this period, Sonneborn’s long-time associate, Geoffrey Beale,
pointed out that something important was largely missing from
the study of the inheritance of ciliate structural patterns. So
Beale proposed a research program: “In order to find and
study gene-controlled surface variants, the same procedures as
are used conventionally in genetics should be tried. A search
among wild-races and varieties could be made to see if there
are sufficiently definite and permanent variants, or changes
induced by mutagenic agents could be studied” (9).

The first part of Beale’s research program was impeded by
the circumstance that ciliates tend to evolve reproductive is-

lating mechanisms that prevent genetic exchange (108, 135)
and interspecific incompatibilities between nucleus and cyto-
plasm (147) more rapidly than they evolve morphological dif-
fferences. This part of Beale’s program nonetheless achieved
some success with two subspecies of the marine ciliate *Euplotes
minuta*, where a difference in the maximum number of ciliary
rows was shown to be under long-term genic control (66).
Other genically controlled structural variants have been dis-
covered in aberrant clones isolated from nature (28, 72, 73, 74)
or derived from inbreeding (34, 89, 122). Despite these suc-
cesses, it became clear that the breeding of natural variants
could not provide a sufficient foundation for the genetic anal-
ysis of structural pattern in ciliates.

The second part of Beale’s program, the study of “changes
induced by mutagenic agents,” was pursued intensively with
the two best-understood genetic models among ciliates, *Para-
meceum tetraurelia* and *Tetrahymena thermophila*. The results of
this research program first appeared in the 1970s (27, 139, 157)
and were comprehensively reviewed in 1990 by Jerka-Dziadosz
and Beisson (75). In the present review, I will focus on the
large number of mutations affecting structural organization in
the cell cortex of *Tetrahymena*. This review has three main
objectives: first, to provide an inventory of genes known to
affect structural organization in the ciliate cortex and of aspects
of this organization influenced by gene products; second, to
employ the altered phenotypes generated by genic mutations
as analytical tools to improve our understanding of ciliate
cortical organization; and third, to relate the genically con-
trolled variants to the three forms of structural inheritance
outlined above.

I should state at the outset that the analysis of mutations
affecting structural organization in ciliates is still mostly in the
premolecular era. In this respect *Paramecium* is somewhat
ahead of *Tetrahymena*, owing to the discovery for the former
organism of a practical means of cloning by complementation
genes previously identified only by classical genetic methods (65, 92). One gene with a mutation known to have pleiotropic effects on cortical as well as nuclear organization, kin241 (80), has been cloned and found to encode a “cyclophilin-RNA interacting protein” (94); simultaneously, the small-19 gene, which controls cell size and the number of basal bodies (127), was found to encode a member of a new class of tubulin, eta-tubulin (126). Unfortunately, differences in the mechanisms of processing of macronuclear DNA (30) make the specific method of complementation cloning employed for Paramecium ineffective for Tetrahymena, and the available methods of DNA-mediated transformation for Tetrahymena (167) have not been suitable or of sufficiently high efficiency to allow cloning by complementation yet. Nonetheless, recent technological advances in the cloning and transformation of larger Tetrahymena DNA inserts, subsequent to the sequencing of the macronuclear genome (29), suggest that cloning by complementation of the more diverse array of “classical” genes affecting cortical morphogenesis in Tetrahymena may be feasible (R. Coyne and E. Orias, personal communication), inviting a molecular genetic analysis of intracellular patterning in this model organism.

THE ORGANISM: ANATOMY AND TOPOLOGY

Before describing mutations affecting cortical structure in Tetrahymena, I must first introduce the “wild type” organism, as seen from a cortical perspective.

The cortical anatomy of Tetrahymena thermophila is schematically illustrated in ventral (Fig. 2A) and polar (Fig. 2B) views. This cell, which is about 40 to 50 μM long, is typically covered by 18 to 21 longitudinally oriented ciliary rows (Fig. 2A). All but the two postoral ciliary rows originate anteriorly, near the anterior pole of the cell (Fig. 2B). Unlike the situation in Paramecium (Fig. 1A), the structural units of the ciliary rows of Tetrahymena are all made up of single basal bodies and their associated structures (“monokinetids” [99]), except for an asymmetrical crown of paired basal bodies (“dikinetids”) at the anterior ends of the rows from the fifth row to the right of the oral apparatus (OA) to the second row to its left (Fig. 2B). (Throughout this review, “right” and “left” are used to mean the cell’s right and left, as seen by an imaginary observer standing inside the cell, aligned with its anteroposterior axis, and looking outward toward the surface. The cell’s right side is the actual viewer’s left side, and vice versa.)

Each ciliary unit (“kinetid”) of the ciliary rows has multiple accessory structures, including striated rootlets extending to the anterior-right of basal bodies (shown in Fig. 1A for Paramecium), as well as microtubular transverse and postciliary microtubule bands (not shown here) (1; reviewed in reference 40). Longitudinal microtubule bands, not connected to the basal bodies, extend just under the plasma membrane parallel and to the right of the basal bodies.

The ciliary units are embedded within a structurally continuous membrane-skeletal layer, originally called the “epiplasm” (1). Subsequent analyses revealed that this layer has a complex substructure (162), with structurally and chemically distinctive basal-body domains (161) embedded within a continuous layer that itself exhibits a complex spatial distribution of its three major molecular components (163).

The ciliary rows do not meet and join at their anterior ends (Fig. 2B) or at their posterior ends (data not shown). Thus, while Tetrahymena is topologically a sphere from the perspective of the plasma membrane and the membrane-skeletal layer below it, cytoskeletally it is more like a barrel bounded by curved staves.

Three major structural landmarks are superimposed on the relatively uniform ciliary rows. The most prominent by far is the OA, a complex structure that includes four compound ciliary elements, the three membranelles (Fig. 2A and 6A) and an undulating membrane (UM), as well as several other microtubular and fibrillar structures, such as the ribbed wall and the deep fiber (see Fig. 6A). It is this organization that gives Tetrahymena (58) its name. The UM, on the right of the OA, consists of a single row of cilia (whose bases are seen in Fig. 8A) underlain by a double row of basal bodies (8, 111, 159). The three membranelles, on the left of the OA, each consist of three rows of ciliated basal bodies modified into a “sculptured” pattern generated during late stages of oral development (see Fig. 8A) (8, 159).

The other two major structural landmarks are located near the posterior end of the cell. A slit-like cytoproct (Cyp) (Fig. 2), the site of defecation from spent food vacuoles (3), is located immediately to the left of the posterior end of the right postoral ciliary row. One or (more typically) two contractile vacuole pores (CVPs) (Fig. 2) are situated immediately to the left of the posterior ends of (typically) the fourth and fifth ciliary rows to the right of the right-postoral ciliary row, which is designated the oral meridian. Nanney (102) demonstrated that the distance between the oral meridian and the midpoint of the CVP meridians (measured in ciliary-row intervals) is not fixed; rather, it is somewhat less than one-quarter of the circumference of a cell (or of a “semicell” in a Siamese-twin doublet).

The geometry of the Tetrahymena cortex can be defined by two orthogonal axes: an anteroposterior axis (Fig. 2A) and a circumferential axis (Fig. 2B). Both typically remain unchanged during clonal growth (see below). However, under
FIG. 3. Structural features of wild-type *Tetrahymena thermophila* at sequential developmental stages. Micronuclei (Mic) and macronuclei (Mac) are shown in red. (A) Nondividing cell, with the OA, the cytoproct (Cyp), and one of two CVPs labeled. (B) An oral primordium (OP) forms in the midregion of the cell’s right postoral ciliary row, and the micronucleus has moved to the cell periphery and starts to divide. (C) Membranelles and the UM have become differentiated within the OP, the micronucleus has divided, and a fission zone (FZ) appears at the cell equator as a ring of gaps in the ciliary rows. New CVPs (nCVP) form anteriorly to the FZ. The macronucleus begins to elongate. (D) The division furrow (DF) constricts along the FZ; a new cytoproct (Cyp), and one of two CVPs labeled. (B) An oral primordium (OP) forms in the midregion of the cell’s right postoral ciliary row, at the same time that the OP, the micronucleus has divided, and a fission zone (FZ) appears at the cell equator as a ring of gaps in the ciliary rows. New CVPs (nCVP) form anteriorly to the FZ. The macronucleus begins to elongate. (D) The division furrow (DF) constricts along the FZ; a new cytoproct (nCyp) appears anteriorly to the FZ; and the macronucleus has completed its division.

some unusual circumstances, the direction of the circumferential axis may be reversed, so that the cell’s left-postoral ciliary row bears the cytoproct, and the CVPs become located four to five rows to the left of that oral meridian.

The dynamics of cortical structures through the cell cycle is shown schematically in Fig. 3 (see also the photographs in Fig. 15A to E), with the two types of nuclei, the smaller micronucleus and the larger macronucleus, indicated. The ciliary rows elongate by the addition of new ciliary units anterior to old ones in the middle and posterior regions of cells that are preparing to divide (91, 107, 113). These rows are then subdivided transversely just before cytokinesis begins (Fig. 3C). The old OA is retained, and a new OA develops from an oral primordium (OP) located to the left of a subequatorial portion of the right-postoral ciliary row, at the same time that the micronuclei divides mitotically (Fig. 3B). New CVPs (Fig. 3C) and a new cytoproct (Fig. 3D) appear along the appropriate ciliary rows just anterior to the fission zone and the division furrow (Fig. 3D), which subsequently constricts along that zone. The macronucleus divides nonmitotically at the same time that the cell divides. The two daughter cells are similar in size (see Fig. 15A to E) (27).

The asymmetry of the developing fission zone has also been documented at the molecular level by the finding that one of the three major membrane-skeletal proteins, the EpIB protein, discovered by Williams and coworkers (162, 164), is asymmetrically distributed on opposite sides of the fission zone in predividing and early-dividing cells (84, 87), as are other cortical markers (84, 85, 116).

As Vance Tartar first pointed out for *Stentor* (150), cell division in a ciliate is really a process of segmentation. In striking contrast to yeast and animal cells, in which the direction of the cellular polar axis may be respecified within daughter cells at each cell division, in ciliates this direction remains unaltered; what changes is only the positional value of each point of the axis (think of a 1-2-3-4-5—1-2-3-4-5 sequence transforming itself into 1-2-3-4-5—1-2-3-4-5 before the cell divides), while this is happening, the ciliary rows perpetuate themselves and the positional values around the circumferential axis remain constant. As Tartar originally pointed out (149), a ciliate clone can be thought of as a cylinder engaged in indefinite longitudinal extension with periodic transverse subdivision.

**THE MUTATIONS**

Generation and detection of mutations affecting structural patterning. This review will attempt to cover all of the known single-gene variants affecting the spatial patterning of the cortex of *Tetrahymena* (summarized in Table 1). One of these variants is a segregant originally isolated from an inbred strain (89), whereas all but one or two of the remainder are nearly or fully recessive mutations, virtually all of them induced by nitrosoguanidine, mostly within the B inbred strain of *T. thermophila* (for the strain history, see reference 4). These mutations were brought to expression after a single cross by one of two genetic tricks. The first method, used prior to 1979, was allelic assortment, in which random segregation of the noncentromeric macronuclear chromosomes of a heterozygous macronuclear eventually results in some macronuclei in which all of the macronuclear chromosomes carry only one of the two alleles (4, 101, 165). The second method, employed from 1979 onward, is the process of induced self-fertilization (“cyogamy”), in which two sister gametic nuclei derived from the same product of the second meiotic division of the germinal macronucleus are induced to fuse with each other during conjugation instead of undergoing reciprocal exchange (125).

Once thus effectively rendered fully homozygous, progeny derived from mutagenized cells were screened for putative mutants of interest. Since screening by direct visualization of cortical structures involved an impractical amount of labor, surrogate phenotypes were utilized. For most of the mutants, that phenotype was abnormality of cell shape, a method that had been first applied by Whittle and Chen-Shan (157) to search for morphological mutations in *Paramecium*. In *Tetrahymena*, this approach was carried out with cells grown overnight at 39.5°C, a temperature that is close to the upper limit for continuous exponential growth of this organism (46). This method succeeded in detecting most of the cortical-pattern mutations to be described below, including many that are also expressed at lower temperatures. This protocol also selects a large number of mutations that prevent the completion of cytokinesis at a restrictive temperature and thereby generate tandem chains and irregular monsters (49, 52, 82, 157).

A very different selection protocol was based on an inability to form food vacuoles at a restrictive temperature (134, 144). This protocol was designed to select mutants affected in phagocytosis but could also detect mutants with defects in the construction of the OA. This protocol succeeded in detecting mutant phenotypes that might have been overlooked by the cell shape-based protocol but, conversely, could not have detected oral-patterning mutants that produced fully functional oral structures. The fact that these two different surrogate selection protocols produced nonoverlapping sets of mutant phenotypes indicates that the array of morphological mutant phenotypes to be described below does not exhaust all of the possibilities.

Extensive complementation crosses were carried out to test
for allelism. Clones carrying independently isolated noncomplementing mutations almost invariably expressed the same phenotypes, though sometimes with differences in penetrance and expressivity at the same or different temperatures. Such noncomplementing mutations were considered to be allelic.

Descriptive conventions: nomenclature, techniques, and organization. The organism under review is *Tetrahymena thermophila*, so named by Nanney and McCoy (108). (In papers published in 1977 or earlier, this species was known as *Tetrahymena pyriformis*.) The nomenclature of genotypes in this species was originally unsystematic and has undergone two major revisions. The first, originating in 1977 but not formally published, required that gene loci be represented by an abbreviated gene name followed by a letter to indicate the locus, then a hyphen, and then a second allele. The second revision, in 1998 (5), restricted the abbreviation of the numbered alleles now separated from the lettered loci with the hyphen represents an allele. Thus, a mutation that was originally published, will now be numbered, according to the newest rules should be renamed “cda1-1.”

TABLE 1. Abbreviated summary of structural-pattern mutants of *Tetrahymena thermophila*

| Location and type of defect | Mutated gene(s) | Figure(s) | Publication(s) |
|----------------------------|-----------------|-----------|----------------|
| Ciliary rows               |                 |           |                |
| Few and variable           | *lkn1*          | 4B        |                |
| Twisted                    | *twi1, twi2*    | 5A        |                |
| Disorganized               | *disA, disB, disC, disD, disE* | 5B | 6, 35, 79 |
| Oral apparatus             |                 |           |                |
| U1                         | *num1*          | 6B        | 96             |
| Membranelles               | *mpA, mpC, mpD, mpG, mpH, cdaI* | 7, 8 | 37, 38, 51, 57, 89, 90 |
| Both                       | *mpB, mpF, doa1, doa2* | 13C and D |                |
| Neither                    | *vacA*          | 9B and C, 18 | 124, 134, 154, 160 |
| Anterior-posterior subdivision |               |           |                |
| Fails                      | *psmA, psmB, psmC, psmD* | 10 | 35, 37, 57 |
| Aborts                     | *cdaA, cdaH*    | 11, 12    | 17–20, 48, 49, 52, 54, 56, 59, 60, 81, 83, 86, 87, 119–121, 156 |
| Is displaced anteriorly     | *cdaI, cdaK*    | 13, 14    | 95             |
| Is displaced posteriorly    | *con1, elo1*    | 15, 16    | 27, 98, 129    |
| Circumferential positioning |               |           |                |
| Broadened domains          | *bcd1, IISG (phg*) | 17B, 18 | 21, 23, 24, 25 |
| Displaced landmarks        | *hpo*           | 17C       | 55             |
| Reversed domain            | *IISG (phg*)*   | 18        | 88, 154        |
| Doublet former             | *janA, janB, janC* | 17D, 19A | 22, 25, 42, 43, 45, 47, 50, 53, 68, 69, 76, 141 |

* Mutations that are expressed only at high temperatures, or whose expression is greatly enhanced at high temperatures, are boldfaced. Gene designations are as follows: *bcd*, broadened cortical domains; *cda*, cell division arrest; *con*, conical; *dis*, disorganized; *dbf*, doublet former; *elo*, elongated; *hpo*, hypoangular; *jan*, janus; *lkn*, low kinetics (ciliary row) number; *mp*, membranelar pattern; *num*, misaligned undulating membrane; *NP*, nonphagocytosis; *phg*, phagocytosis; *twi*, twisty; *vac*, (food) vacuole.

* No defects were detectable by light microscopy.

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following an order similar to that employed in the sole earlier review exclusively devoted to this topic (75): category 1, mutations affecting the organization of basal bodies in the ciliary rows; category 2, mutations affecting the organization of the OA; category 3, mutations affecting subdivision along the anteroposterior polar axis; category 4, mutations affecting organization along the circumferential axis. Categories 3 and 4 correspond to the “body-plan” mutations of Jerka-Dziadosz and Beisson (75). Of course, some mutations fit into two or even three categories; these will be described within a single category and briefly mentioned in other categories where they also fit.

**Mutations affecting the organization of basal bodies in the ciliary rows.** As mentioned above, cells of *T. thermophila* typically have 18 to 21 longitudinal ciliary rows, and this number is preserved during vegetative growth and through conjugation (103, 104). Both the number of ciliary rows and their conservation during vegetative growth were affected, in a non-temperature-sensitive manner, in cells homozygous for the *lkn1-1* mutation. The number of ciliary rows in the mutant homozygotes ranged from 11 to 17 (mode, 13 to 14), whereas the cell size and the estimated total number of basal bodies remained approximately normal, so that ciliary rows were more widely spaced in cells of homozygous *lkn1-1* clones than in their wild-type sister clones but basal bodies were more numerous, and hence more crowded, within each row (Fig. 4). This is in striking agreement with Nanney’s observations of constancy in the overall number of basal bodies in cells with differing numbers of ciliary rows (106).

Incomplete ciliary rows, which are rare in wild-type cells, were common in *lkn1-1* homozygotes (Fig. 4B). In agreement with this, the fidelity of propagation of the preexisting number of ciliary rows in subclones (103, 104) was low in *lkn1-1* homozygous clones and subclones (J. Frankel, unpublished observations). The *lkn1-1* mutation thus severely modifies the normally strict cytotic control of the propagation of ciliary rows.

A second allele, *lkn1-2*, expressed this phenotype to a somewhat milder degree than *lkn1-1* and was not studied in detail. In addition, the *conical* (*con1-1*, originally *co*) mutant clones commonly expressed a somewhat lower mean and higher variance in the ciliary row number than parallel wild-type clones (27).

**FIG. 4.** Silver preparations of wild-type (w.t.) (A) and *lkn1-1* (low kinety number) mutant (B) cells, both maintained at 29°C, with the OAs and OPs of both cells in the final stage of development. FZ indicates the fission zone. The arrow in panel B marks an incomplete ciliary row. This figure and all subsequent figures are printed so that the viewer’s left corresponds to the cell’s right. The scale bar in this and all subsequent figures of light microscopic images indicates 10 μM. This and subsequent photographic images were uniformly processed in Adobe Photoshop to improve contrast and visibility.

Are there mutations that increase the number of ciliary rows? The majority of the mutants to be described below had a normal range of ciliary row numbers, from 18 to 21. However, there were occasional exceptions, such as the original *janA-1* mutant clone, which maintained a stable number of 21 to 25 ciliary rows (76) and the *big1-1* mutant, which, unsurprisingly, was not only larger than normal but also had more ciliary rows (57; Frankel, unpublished). This makes it probable that in *Tetrahymena*, as in *Euplotes* (66), the “stability center” (36, 103) of ciliary row numbers is itself under genic control. The same is likely to be true for *Paramecium*, in which both *kin241* and *crochu* mutant cells are larger than wild-type cells and have increased numbers of ciliary rows (77, 80).

The *twisty* (*twi1-1*) mutation brings about a pronounced helical twist of the ciliary rows over the surface of the cell. This
phenotype was expressed shortly after the transfer of cells to a high temperature (39°C) and did not prevent cells from developing and dividing normally (Fig. 5A). The direction of the twist generally was clockwise as viewed from the anterior end of the cell (Fig. 5A) but occasionally was counterclockwise. Interestingly, the locations of the newly developing cortical landmarks, such as the OP (Fig. 5A) and the new CVPs, were completely normal with reference to the ciliary rows. However, the fission zone, which normally bisects the ciliary rows equally and perpendicularly at the cell equator (Fig. 4A), in *twi*-*l*-*l* cells bisected the ciliary rows approximately equally but not perpendicularly (Fig. 5A) (J. Frankel and L. M. Jenkins, unpublished observations); this provides a hint that what is being bisected is the anteroposterior axis of the cell as a whole. These phenotypic effects were not novel, since similar observations were reported 36 years ago for five nonallelic mutations of *Paramecium tetraurelia* (157). The best characterized of these mutated genes was later named *scrawly1 (sc1)* (139)—now *scr1*, with five available alleles (http://paramecium.cgm.cnrs-gif.fr/).

The conclusion that the plane of bisection of the cell does not depend on the geometry of the ciliary row was demonstrated more clearly by cells expressing one of several nonallelic *disorganized* mutations. The most highly expressed and most thoroughly investigated of these is the *disA*-1 mutation (6, 35, 79). This mutation is located on chromosome 5 (15). It was expressed moderately at normal growth temperatures and more highly at 39°C; it brought about a disorganization of the arrangement and spatial orientation of ciliary units (Fig. 5B), without severe disruption of the internal organization of each individual unit (6, 79). Remarkably, not only could *disorganized* mutant cells continue to grow and divide for some time even at restrictive temperatures; they could also develop normal new OAs at their normal locations (Fig. 5B) and place the CVPs at correct intracellular latitudes and approximately correct longitudes (79). This, therefore, clearly demonstrates the dissociability of the large-scale patterning of the major cortical landmarks and of the distinctive mechanisms that are responsible for the development of oral structures (39, 115) from the spatial order of the ciliary rows (reviewed in reference 41).

Similar mutations at four other loci bring about disorganization of ciliary rows. All of them except *disD*-1 (79) were more weakly expressed than *disA*-1, and none have been studied in detail. *disB*-1, *disC*-1, and *disD*-1 were all fully temperature sensitive, whereas *disE*-1 was only minimally temperature sensitive. *disC*-1 expression was weak even after prolonged maintenance at 39°C (Frankel, unpublished). Most of these mutations also express other phenotypic effects, such as oral abnormality in *disB*-1 and reduced numbers of ciliary rows in *disD*-1 and *disE*-1.

In addition, the *big1*-1 mutation, which was selected and characterized primarily on the basis of the unusually large size of mutant cells (57), turns out upon reexamination to bring about considerable disorganization of ciliary rows. Were it not for its large size, it could easily have been classified as a *disorganized* mutant. The same is true for *cda1*-1, a cytokinesis arrest mutant. Further, a recent reexamination of *janA*-1 with a sensitive monoclonal antibody revealed previously unrecognized irregularities in ciliary rows (141). These examples make it clear that (i) dramatic mutant phenotypes can lead us to overlook less prominent ones and (ii) irregularities in ciliary rows, which are rare in wild-type cells, are probably much more common in mutants.

**Mutations affecting the development of the OA.** Mutations affecting oral development have been selected in three different laboratories utilizing different protocols. I will review these mutations according to the ciliary structures affected, beginning with the one mutated gene locus for which a structural abnormality is unknown.

Suhr-Jessen and Orias isolated several allelic recessive *vacA* mutations that make cells unable to form food vacuoles after growth at 39°C while retaining all of the structures of the OA that are visible under the light microscope (144). Only OAs that were newly formed after the temperature upshift were unable to form food vacuoles (145). The nature of the oral defect in these mutants is unknown. One might expect it to be a malformation in the elaborate microtubular and fibrillar substructure of the OA, but this normally regresses and is re-formed during cell division (see reference 40 for a brief account and references); if the *vacA* defect were localized in these transient structures, one would expect old as well as new OAs to lose their capacity to form food vacuoles during culture growth in an enriched medium (123) at a restrictive temperature.

A single mutation, *misaligned undulating membrane* (*mum1*-1), affects the development of the UM selectively, with no apparent effects on the oral membranelles or any other structure. *mum1*-1 cells produced an excess of basal bodies in the portion of the OP destined to become the UM, followed by the formation of overlapping UM segments where the single UM normally appears (Fig. 6B) (96). This abnormality may reduce the efficiency of feeding, since *mum1*-1 cells grew at a rate 40% lower than that of parallel wild-type controls, even under optimal conditions (Jenkins and Frankel, unpublished).

The development of membranelles is specifically affected by mutations in five genes. Mutations at two of these gene loci (*mpG*-1 [Fig. 7A] and *mpH*-1 [data not shown]) frequently brought about the formation of OAs with two membranelles at both 28 and 39°C, whereas mutations at two other loci commonly resulted in the formation of OAs with four membranelles at both 28 and 39°C (Fig. 7B) or of OAs with four to five membranelles at elevated tempera-
tured only (mpD-1 and mpD-2 [Fig. 7C]) (51). The MPC locus was mapped on chromosome 3 (but not 3R) (51), MPD on chromosome 3R (57), and MPG on chromosome 4 (Jenkins and Frankel, unpublished). These mutations had few if any additional phenotypic effects apart from the moderate changes in cell size and shape by which they were originally selected. All of the mp mutant clones exhibited normal or nearly normal culture growth rates at temperatures at which the oral phenotypes were expressed in a large proportion of the cells. This suggests that an alteration in the number of membranelles does not by itself interfere with the ability of cells to grow and divide, at least in rich axenic media.

The extra-membranelle phenotype could also be engendered by mutations that have other, more-prominent effects, such as the *big1-1* mutation mentioned above, the *psmD-1* “pseudomacrostome” mutation, and the *cdaI-1* fission-zone-displacement mutation (see Fig. 13D), which will be described more fully below.

The “extra-membranelle” mutations proved useful in revealing that the patterning of the membranelles is spatially coordinated. In wild-type cells grown in nutrient media, the spatial “signature” of each fully formed membranelle is distinctive and remarkably uniform, so that one can easily distinguish the “M-1,” “M-2,” and “M-3” patterns of basal-body positions (159) (Fig. 8A). This might tempt one to surmise that there is a unique genically determined “blueprint” that specifies the spatial organization of each of the three membranelles. However, for mutants with an increased number of membranelles—notably mpD-1—the spatial “signature” of each membranelle depended on its relative position within the set of membranelles (51). Thus, in an OA with five membranelles, the first membranelle had an “M-1” signature, the third had an “M-2” signature, and the fifth had an “M-3” signature. The second and fourth membranelles had intermediate structural signatures (“M-1/2” and “M-2/3,” respectively), such as are not seen in normal three-membranelle OAs (8). This implies the existence of a spatially graded field, perhaps a field of forces responsible for displacing basal bodies as the oral cavity is sculptured late during oral development (see Fig. 28 in reference 7).

Two subsequently derived mutations that reduced the number of membranelles, *mpG-1* and *mpH-1*, allowed for a clear test of the published conclusions summarized above (51). In *mpG-1* mutants, approximately one-half of the cells had three membranelles, and these had “pattern signatures” very similar to those of wild-type cells (Fig. 8, compare panels A and C). In the other half of the *mpG-1* cells, which expressed two membranelles, both membranelles were almost invariably abnormal. The second membranelle had an M-2/3 pattern very sim-
ilar to that seen in the fourth membranelle of mpD-1 cells that have five membranelles (Fig. 8, compare panels B and D). The first membranelle had a somewhat hypertrophied pattern that can be interpreted as an M-1/2 intermediate (hence it is labeled “M-1+” in Fig. 8D) (38; Frankel, unpublished). The M-2/3 pattern is also observed in the second membranelles of severely starved wild-type cells that have only two membranelles in their OAs (8). The dependence of the membranelle pattern signature on the number of membranelles in mpG-1 cells is clearly more consistent with the “unified-membranellar-field” model than with the “membranellar-blueprint” alternative.

Finally, the mpA-1 genetic variant is the only one in Tetrahymena that conforms to the first part of Beale’s proposal for finding gene-controlled structural variants, by “a search among wild-races and varieties.” In inbred strain D of T. thermophila, about 10% of dividing cells and 80% of cells undergoing oral replacement expressed a unique abnormality in oral development that brought about a juxtaposition of two partial sets of membranelles (Fig. 7D), which could later fuse to form variable membranellar patterns (90). After outbreeding of strain D to another inbred strain (A) that did not show this abnormality, Kaczanowski (89) could demonstrate that this abnormality was based on a single recessive allelic variant that happened to be homozygous in inbred strain D. He called it mp, and I am here renaming it mpA-1.

A residual but highly miscellaneous category of mutations affecting oral development comprises those in which oral development is abnormal in more than the limited manner of the oral mutations considered thus far. These are illustrated in Fig. 9 in the order of severity of effect but will be described here in a different order.

I will begin with two pairs of superficially similar yet in reality highly contrasting mutations. One of these pairs, mpB-1 and mpF-1, was initially misdiagnosed as affecting only membranelles. Of these, the mpB-1 mutation (data not shown) had relatively low penetrance (~10% abnormal cells at 29°C, rising to 30 to 40% after prolonged maintenance at 39°C). However, it had high expressivity, with variable abnormalities affecting both the UM and the membranelles; culture growth was slow and essentially ceased after 6 h at 39°C (Frankel and Jenkins, unpublished).

In contrast, the oral phenotype of the mpF-1 mutation was entirely temperature sensitive, and this mutation was both more penetrant and more uniformly expressed at 39°C than mpB-1. Membranelles were increased in number and reduced in size, whereas the UM was characterizedly shorter than normal (compare Fig. 9A to Fig. 6A and 7). Culture growth remained normal for 7 h at 39°C (Frankel and Jenkins, unpublished). In my view, mpF-1 might well repay further study.

A second pair of nonallelic temperature-sensitive mutations have the same name—defective oral apparatus—but very different phenotypes. Cells expressing the doa2-1 mutation (data not shown) exhibited a rather general degeneration at 39°C, with progressive loss of oral structures, frequent oral replacement, rounding of the cell, signs of division blockage, and very slow growth after 2 h at 39°C (Frankel, unpublished).

Cells homozygous for the doa1-1 mutation resembled doa2-1 cells only in that they underwent gradual dismantling of OAs at 39°C, with intermediate stages characterized by variable and disordered membranelle fragments (Fig. 9D). However, doa1-1 cells grown at 39°C underwent very little oral replacement and maintained a normal shape while becoming progressively smaller. Culture growth continued at a rate similar to that for wild-type controls for 9 h at 39°C before dropping off afterwards. Examination of silver-stained slides strongly suggested that doa1-1 cells that had completely lost their OAs could still divide, sometimes after forming abnormal oral primordia, in some cases along unexpected longitudes (Frankel and Jenkins, unpublished). The doa1-1 mutant deserves more than the superficial examination it has received thus far.

It remains to describe two mutations that were obtained, like vacA, from a functional screen for the inability to form food vacuoles but that, unlike vacA, brought about severe morphological abnormalities. One of these, NP1 (nonphagocytosis 1), was like vacA in that it was temperature sensitive, and only OAs formed at the restrictive temperature were unable to form food vacuoles. It differed, however, in two ways. First, although the condition was vegetatively stable, it could not be transmitted to sexual progeny upon conjugation, suggesting that it was caused by a mutation in the macronucleus, which is lost during conjugation (134). Second, only rudimentary, nonfunctional oral structures were produced at restrictive temperatures (124) (Fig. 9C). At these temperatures, this mutant could grow only in a rich medium that was specially devised to allow sufficient entry of critical nutrients in the absence of food vacuoles (123).

The oral structures present in the NP1 macronuclear mutant were analyzed in detail by Williams and Honts (160), who
found that the cytoskeletal elements within the OA (basal bodies and associated structures) were normal but their higher-order arrangement was abnormal. Furthermore, the anterior OA was capable of carrying out the disassembly and reassembly of the complex microtubular and filamentous substructure of the anterior OA, which normally takes place during cell division. These investigators therefore concluded, “The primary lesion of NP1 may be in some early event required for the correct positioning of basal bodies within the oral apparatus as a whole” (160).

The last mutation in this group to be considered putatively exists in a strain, known as II8G, “derived from CU399 . . . a temperature-sensitive strain forming no food vacuoles at 37°C”; the phenotype is “most probably caused by a single gene mutation at a locus called phg” (154). This is in several ways the most enigmatic of all the mutants described in this review. According to the original authors, “The oral structures are missing often at 37°C, but present at 28°C” (154). More recently, this mutant was described as being “able to proliferate [in enriched medium at 37°C] yielding small cells with very variable phenotypes including some cells without oral apparatuses and some cells in OR [oral replacement] morphogenesis” (88). This strain was also sent to us, and we were unable to obtain progeny following crosses; we also found that the II8G cells produced silver- and protargol-stainable oral structures no matter how long the cells were kept at 39°C (Fig. 9B and 18A). Moreover, these oral structures were unlike those found in any other mutant; they consisted of a variable number of membranelles, all of which were arrested in their development at the stage of addition of a third ciliary row to two-row promembranelles (cf. reference 46), with few traces of a UM and no oral cavity. Even more remarkable is the way in which these oral structures were formed: not by the usual method of a single oral field produced next to the subequatorial region of a cell becomes transformed into juxtaposed membrane-skeletal proteins, EpiC (162, 164), brought about a tran-formation, generating two daughter cells in a tandem array with identical polarity (Fig. 3). An exemplary case is pseudomacrostome (pseudomacrostome) phenotype (110) and in exconjugants (22, 93). In psmA cells, (i) the OP extended for most of the length of the cell (Fig. 10A) rather than being confined to a small region just posterior to the old OA; (ii) oral replacement took place in nutrient medium; and (iii) it resulted in a large cell with an unusually large and prominent OA.

At the outset, however, the mutations that are being left out of this account should be noted. From a topological perspective, the process of cortical subdivision is already completed before division constriction begins. This distinction provides an excuse for leaving out of this account mutations that prevent constriction at some point after the formation of the fission zone. An exemplary case is cdaC (56), which has been shown by anatomical study (49) and temperature shift experiments (52, 146) to primarily affect the process of division furrow constriction; other examples are alleles of cdaD, cdaE, and cdaF (all described under earlier names in references 49 and 56), as well as cdaG and cdaJ (Jenkins and Frankel, unpublished) and the recently characterized cdaL mutation and possible cdaM mutation (E. Cole, personal communication).

The pseudomacrostome (psmA) mutations, which prevent anteroposterior subdivision, all cause a switch from cell division to a peculiar form of oral replacement that brings about the formation of OAs much larger than normal, with longer UM and membranelles (Fig. 10). The pseudomacrostome type of oral replacement differed in three respects from the typical oral replacement that normally takes place in starved cells (33, 90), especially in cells developing the “rapid swimmer” phenotype (110) and in exconjugants (22, 93). In psmA cells, (i) the OP extended for most of the length of the cell (Fig. 10A) rather than being confined to a small region just posterior to the old OA; (ii) oral replacement took place in nutrient medium; and (iii) it resulted in a large cell with an unusually large and prominent OA.

The pseudomacrostome phenotype has been analyzed most extensively in the first psmA mutation to be discovered, psmA-1, of the PSMA gene located on chromosome 5 (57; E. Hamilton, personal communication). It was severely temperature sensitive, with fairly strong expression at 25 to 28°C (22°C is “permissive” for this allele). Penetrance and expressivity were typically high (57) but were dependent on the culture medium in which the cells were grown and also differed in different ho-
mozygous psmA-1 clones, suggesting the presence of genetic modifiers that have not yet been analyzed.

The psmA mutations are excellent examples of the general rule that allelic mutations are invariably identical in qualitative phenotype but often differ greatly in penetrance: psmA-1 was expressed at all but extremely low temperatures (57), and psmA-2 was weakly expressed only after 2 generations of growth at a high restrictive temperature (39°C) (57), whereas the most recently discovered allele, psmA-3, had zero penetrance of the pseudomacrostome phenotype at 29°C and close to 100% penetrance of that phenotype a few hours after a shift to 39°C (Frankel, unpublished)—and therefore is the recommended allele for all users.

There are three additional pseudomacrostome loci, each represented by one allele. None of them had the degree of penetrance or expressivity of the pseudomacrostome phenotype shown by favorable stocks of psmA-1 and by psmA-3. However, whereas psmC-1 (also located on chromosome 5) looked and responded much like the “weak” psmA-2, the other two psm mutants each had certain peculiarities: in psmB-1 (located on chromosome 4L [15]), cells tended to have a highly tapered posterior region and commonly showed unequal division, with the resulting posterior daughter cell smaller than the anterior one (a phenotype occasionally seen in psmA-1 as well; see Fig. 6c of reference 35). psmd-1 (located on chromosome 3R) was highly pleiotropic, since in addition to its variably expressed pseudomacrostome phenotype at 39°C, it frequently formed OAs with four membranelles (57) and also produced oral primordia that shifted anteriorly and generated “hammerhead” phenotypes similar to those expressed by the cda-1 mutations, to be described below (Frankel, unpublished). Such pleiotropy, observed in some (but not all) of our cortical-pattern mutants, reveals the frequent arbitrariness of the naming of these mutations: psmB-2 could readily have been given new places within the mp or cda series.

Before leaving the psm mutations, I should briefly explain the name of this set of mutations: it refers to the macrostome OA of the obviously dimorphic Tetrahymena species such as Tetrahymena vorax, which is derived from the microstome form by a process of oral replacement (16) rather similar to that of a pseudomacrostome phenotype at 39°C. The focus on the cell surface. A normal set of CVPs is located near the posterior end of the cell (arrow), yet there are no CVPs, and no other indication of a fission zone, in the equatorial region. This figure was modified from Fig. 3 and 4 of reference 49 with permission of the publisher.

FIG. 11. Two focal levels of a single silver-stained cdaA-1 cell maintained at 39°C. The focus on the left side is on the OA and an OP that has completed its development. On the right side, the focus is on the cell surface. A normal set of CVPs is located near the posterior end of the cell (arrow), yet there are no CVPs, and no other indication of a fission zone, in the equatorial region. This figure was modified from Fig. 3 and 4 of reference 49 with permission of the publisher.

normal position but prevents all other known structural (49) (Fig. 11) and molecular (81, 87, 121) processes of segmental subdivision from taking place. The temperature-sensitive period of cdaA-1 at 36°C was an interval of about 10 min during the middle of oral development, prior to the appearance of the fission zone (52). Micronuclear division, which accompanies oral development (Fig. 3), occurred normally in cdaA-1 cells at a restrictive temperature, but macronuclear division, which normally occurs after the fission zone forms, failed to take place under these conditions (54); periodic DNA synthesis continued within the undivided macronucleus (20, 54). The cdaA-1 cells grew, went through multiple cell cycles and additional rounds of oral development (81, 86) without dividing, and eventually became gigantic, irregular monsters (49). Thus, the CDA4 gene is required for a critical step in the subdivision of the anteroposterior axis, in the absence of which cells cannot begin to divide.

This mutation is unique among our entire collection in that it has been the subject of an extensive molecular inquiry. A protein was found that migrated differently in 2-dimensional gels of homogenates from cdaA-1 cultures grown at a restrictive temperature, and this protein (called p85 because of its molecular weight) was localized near basal-body couples that normally are situated at the anterior ends of most ciliary rows (Fig. 2) (119, 121). At a restrictive temperature, these couples did not form at their normal sites just posterior to the fission zone, and p85 was not localized at these sites (121). These authors presumed that there is a causal connection between the localization of p85 and the capacity to form a fission zone, a line of analysis that was pursued further by the Nomura group (60, 119). One problem with this analysis is that both apical basal-body couples and p85 are absent between the fourth ciliary row to the right and the first row to the left of the OA
and of the OP (60, 79), yet a fission zone develops normally there in wild-type cells as well as in cdaA-1 cells at permissive temperatures. When the cDNA that coded for p85 was cloned and sequenced, it was found that “there was no difference in the predicted amino acid sequences of wild-type and cdaA1 p85” (59). Thus, the true gene product of CDAA is still unknown. This CDAA product was presumed by Gonda et al. (59) to be a molecule involved in the posttranslational modification of p85. The meaning of these analyses is still unclear.

The other mutation that prevents cortical subdivision, cdaH-1, located on chromosome 5 (15), is also 100% penetrant at 39°C, but it has been much less studied than cdaA-1. In cdaH-1 mutant cells grown at 39°C, oral development was initiated normally (Fig. 12A) and continued more or less normally up to the stage when the fission zone would normally appear (Fig. 12B). As with cdaA-1, no fission zone formed, but in the cdaH-1 mutant the new posterior OA sank into a subsurface vesicle and migrated anteriorly (Fig. 12C) to end up just posterior to the old anterior OA (Fig. 12D). At the same time, there was considerable posterior extension of the cell, perhaps exaggerating the impression of a forward migration of the OP. As can be seen in the “inner” focal planes of the topmost cells in Fig. 12C and D, the micronucleus divided whereas the macronucleus did not, consistent with similar but far more detailed observations of cdaA-1 (Frankel, unpublished).

The temperature-sensitive period for cdaH-1 at 36°C (a temperature at which the phenotype is still 100% penetrant but expression is less extreme than that at 39°C) was longer and somewhat later than that of cdaA-1: it occurred during a 25- to 30-min interval around the time when the fission zone is normally formed (52).

Mutations at four additional gene loci permit cortical subdivision to occur but alter its location along the anteroposterior axis. Two of these, cdaI and cdaK, bring about anterior displacement of the fission zone at restrictive temperatures. In cells expressing all three cdaI mutations, the OP first appeared at its normal subequatorial location (Fig. 13A), but at 39°C it then slid anteriorly on the cell surface. At the stage when the fission zone normally appears, the OP had already reached a location just posterior to the old OA, and the fission zone concomitantly was radically displaced anteriorly (Fig. 13C), while new CVPs sometimes did and sometimes did not arise anterior of it. There was then a varying amount of furrowing, ranging from total absence (typical of the “strongest” allele, cdaI-3) to successful cell separation (common in the “weakest” allele, cdaI-2) (Frankel and Jenkins, unpublished). Most commonly, an incomplete and unilateral furrow formed, leading to a distinctive “hammerhead” phenotype (Fig. 13D). The incompleteness of the process of subdivision might be a by-product of the extreme anterior displacement of the fission zone.

In cells homozygous for the cdaK-1 mutation, the OP did not
shift, even at restrictive temperatures (36°C to 39°C). It developed at its normal midbody position (Fig. 14A) and appeared to stay there. A fission zone was then formed in a highly asymmetric manner, with an anteriorly directed tilt to the left of the OP (Fig. 14C) and a sharp anterior displacement (a “cliff”) to its right (not shown here; see Fig. 1G in reference 95). Some ciliary rows remained continuous across the fission zone, preventing division from going to completion (Fig. 14D). A ventral bulge formed (Fig. 14C) and became prominent in the presumptive anterior daughter cell (Fig. 14D). A “hammerhead” phenotype was thus generated, but in a manner entirely different from that observed with the \textit{cdaI} mutant cells.

For a more detailed description and analysis of the complex phenotype of this mutant, see reference 95.

The mutations that bring about a posterior displacement of the fission zone are quite different and generate less dramatic phenotypes. In these mutant cells, displacement was not accompanied by any blockage of cell division. Typically, the OP began to be formed too far back, sometimes near the posterior end of the cell, followed by a partial equalization of the sizes of the two presumptive daughter cells.

The first example of this appeared in a mutation affecting cell shape, \textit{conical} (\textit{con1-1}, originally \textit{co} [27]) (Fig. 15). In \textit{con1-1} mutant cells, the OP appeared to form near the posterior end of the cell (Fig. 15F), followed by an apparent anterior shift in its relative position, although how much of this was attributable to genuine posterior growth and how much to a change of cell shape from conical to ovoid (Fig. 15G) is not entirely clear (27). After cell division was successfully completed, the posterior daughter cell started out smaller, and also grew more slowly and divided later, than the anterior daughter cell (27, 129).

A more recent and unequivocal example of posterior localization followed by partial equalization was found in the “elongated” \textit{elo1-1} mutant (Fig. 16), in which the OP was initiated

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**FIG. 13.** Silver preparations of \textit{cdaI-2} cells maintained at 39°C, in sequential developmental stages. (A) Cell with an OA, mostly out of focus, and an OP that is at an early stage of development. (B) Cell with a later-stage OP, beginning to migrate anteriorly on the cell surface. (C) Cell in which the migration of the OP has been completed, and a fission zone is beginning to form anterior to it (arrowhead). The OP has four membranelles (small arrow). (D) Cell that is attempting to divide, with a unilateral furrow (arrowhead). The completely developed OP has a displaced fourth membranelle (small arrow), similar to the one in the \textit{mpC} cell shown in Fig. 7B.

**FIG. 14.** Silver preparations of \textit{cdaK-1} cells maintained at 36°C, in sequential developmental stages. (A) Cell with a normal OA, out of focus, and an OP at an early stage of development. (B) Cell with a late-stage OP, with a tilted fission zone (arrowheads) beginning to form. (C) The development of the OP is completed, and the cell is furrowing (arrowheads) along the tilted fission zone. The “X” marks a bulge along the oral meridian just anterior to the fission zone. (D) Cell attempting to divide. The “X” marks the region corresponding to the bulge in panel C, and the small arrow indicates some continuous ciliary rows that mark the region where cytokinesis is failing.

close to the posterior end of the cell (Fig. 16A). At later developmental stages, the portion of the cell posterior to the OP seemed to grow out without much change in overall cell shape (Fig. 16B to D). It is possible that the OP might also have been sliding anteriorly to reduce the distance between the OA and OP, but this point has not been conclusively established. The change in overall proportions during oral development was most extreme in *elo1*-1 cells grown at 39°C (Fig. 16) but was also evident in this mutant at 29°C (Frankel, unpublished). As briefly mentioned above, a similar phenotype was sometimes observed in *psmA-1* cells, and more commonly in *psmB-1* cells.

The fact that similar disproportions in the sizes of daughter cells were observed for cells of widely different shapes conflicts with an ingenious earlier hypothesis that proposed a common cause for the alteration of the shape of *con1*-1 cells and the disproportion in the sizes of their division products (98). A new nonallelic conical mutation, *con2*-1, produced daughter cells.

**FIG. 15.** Silver preparations of wild-type (w.t.) strain D (A to E) and *con1*-1 (F to J) cells, both maintained at 29°C, in sequential developmental stages. The focus is primarily on the oral structures (anterior OA and subequatorial OP). Thus, the fission zone gaps in the ciliary rows, which appear anterior to the OPs in the cells shown in panels C and H, are mostly out of focus. This figure is reprinted from Fig. 3 to 12 of reference 27 with permission of the author and the publisher.

**FIG. 16.** Silver preparations of *elo1*-1 cells maintained at 39°C, in sequential developmental stages. (A) Cell with a normal OA and an early-stage OP situated near the posterior end of the cell. (B) Cell at an intermediate developmental stage of the OP, which is no longer at the posterior end. (C) Cell at the stage of an incipient fission zone. (D) Cell starting to furrow. (E) Cell completing furrowing. Note the relative sizes of the daughter cells.
that upon casual examination appeared to be close to equal in size (Frankel, unpublished). Thus, cell shape and cell subdivision appear to be dissociable phenotypes.

**Mutations affecting organization along the circumferential axis.** The mutations affecting circumferential positioning are quite diverse. Three phenotypic sets (the broadened cortical domains, hypoangular, and janus loci) have received considerable attention in publications with supporting photographs, so their complex geometry will be illustrated mostly by schematic diagrams in this review.

We begin with the broadened cortical domains condition, represented by two mutations, bcd1-1 and bcd1-2, of the BCD1 gene, located on chromosome 3 (24). As the name suggests, the bcd1 mutant cells are characterized by a lateral broadening of the regions within which the major cortical landmarks of the cell, the OAs and the CVPs, develop (24) (Fig. 17B). Unlike the situation already mentioned for the IISG variant, the parallel oral primordia developed to completion and sometimes fused to form a single enlarged OA; otherwise, they formed two or more variably truncated OAs (24). Most other abnormalities in bcd1-1 vegetative cells were mild: the cell cycle length was extended by about 25%, and the relative location of the midpoint of the CVP sets increased somewhat, from a mean of 23% of the cell circumference, characteristic of wild-type cells (102), to a mean of 26% (24). The number of ciliary rows remained normal.

These mutations were originally believed to be non-temperature sensitive, but an “extreme oral phenotype” with an additional oral domain appeared in some bcd1-1 cells after prolonged maintenance at 39°C (25).

Two unexpected abnormalities turned up upon further study of bcd1-1: first, a great overabundance of corticaly docked dense-core vacuoles (mucocytes) was found in bcd1-1 cells (23); second, bcd1-1 cells were found to be unable to complete conjugation (21). When two mating types of bcd1-1 homoygotes were mixed with each other under conditions conducive to conjugation, they paired and went through meiosis but arrested the process just prior to the exchange of pronuclei. Since this phenotype has been partially phenocopied by drugs known to affect microtubules and was in some respects comparable to that of a mutant of the kinesin motor protein (Kar3) in budding yeast, Cole concluded that “[i]t seems quite possible that bcd represents a defective-microtubule-based organelle motor similar to kinesin” (21).

No fewer than four independent mutations of the hypoangular (HPO1) gene, located on chromosome 3, have been selected (55; Jenkins and Frankel, unpublished). All four share the same distinctive and highly pleiotropic suite of defects, differing only in their expression at different temperatures, ranging from mildly (hpo1-2 and hpo1-3) to severely (hpo1-1 and hpo1-4) temperature sensitive. The number of ciliary rows was normal in cells expressing all four alleles, and the culture growth rate was generally close to normal at 29°C. The defect that gives these mutations their name is the reduction relative to the wild type of the “cortical angle” between the oral meridian and an imaginary line extending from the cell apex to the midpoint of the CVP set (compare Fig. 17C to 17A) (cf. reference 102). In hypoangular cells, the CVP midpoint was typically closer to the oral meridian than in wild-type cells (55). This reduction of the “cortical angle” varied with the allele and the culture conditions, with the angle tending to become more acute, and even negative (see below), as the level of expression was increased with prolonged maintenance at a high temperature (55).

Other phenotypic manifestations of hpo1 mutations included, first, a tendency to increase the width of the oral domain (with two or three side-by-side oral primordia) and to reduce the width of the CVP domain (to a modal number of one rather than two CVPs) and, second, a “slippage” (cf. reference 105) of the sites of OPs toward the right, generating a progressive rightward (clockwise) shift in the oral meridian (55). These phenotypes were all present even at the moderate levels of expression of the hpo1 mutations observed at 29°C.

At a restrictive temperature (39°C), culture growth slowed and strange things happened in the cortex, as observed in preparations of hpo1-1 and hpo1-2 cells. As a result of differential rates of rightward slippage of oral and CVP meridians, faster for the former, the position of the oral meridian came progressively closer to the CVP midpoint, and finally the oral meridian crossed the CVP midpoint so as to end up to the right of the CVP midpoint; therefore, CVPs came to be located to the left, rather than the usual right, of the oral meridian. Then the direction of “slippage” of OPs reversed, so that OPs now developed to the left rather than the right of the OA (see Fig. 17)....
The cells thus effectively switched their cortical handedness at the moment when the CVP and oral meridians exchanged relative positions (55). When returned to a permissive temperature, \( hpo1^- \) cells became either globally normal or globally reversed (Nelsen and Frankel, unpublished), presumably depending on their geometry at the time of return to a lower temperature.

After prolonged maintenance of hypoangular cells at a restrictive temperature, patterning along the anteroposterior axis also became affected: OPs appeared near the posterior end of the cell (see Fig. 11D in reference 55), while fission zones remained equatorial, and astomatous cells resulted that nevertheless could continue to produce OPs (which has also been seen in doa1^- cells). Thus, under extreme conditions, a single mutant allele could affect both the anteroposterior and circumferential dimensions of the ciliate “body plan,” a phenomenon already noted for the \( \text{kin241} \) mutant of \( \text{Paramecium tetraurelia} \) (80).

The strangeness of some of the hypoangular phenotypes should prepare the reader for a brief return to the enigmatic II8G (\( \text{phg}^- \)) clone. This clone, too, showed signs of reversal of cortical handedness, though in a manner entirely different from that of either \( \text{hypoangular} \) cells or the nongenic “left-handed” cells described in reference 112. As mentioned earlier, at a restrictive temperature II8G cells formed multiple parallel oral primordia, none of which completed their development normally (Fig. 18B to F). Further, these OPs were typically shifted one to two rows to the left relative to the incomplete anterior membranelles (Fig. 18E and F). In addition, the rudimentary oral membranelles were generally close to vertical in their orientation but sometimes had a reversed tilt (Fig. 18A). The domain of development of new CVPs at the cell equator was also broadened, but many of the extra new CVPs were resorbed, so that the completed anterior CVP domain was reduced to a nearly normal width. More surprisingly, the midpoint of the CVP set became progressively shifted rightward toward the mid-dorsal plane, directly opposite the oral meridian (Nelsen and Frankel, unpublished). In other words, this may be considered a “hyperangular” mutant, of indeterminate cortical handedness.

This, finally, brings us to \( \text{janus} \). This gene is the runner-up to \( \text{cdaA} \) in the number of papers published, 13 in this case (22, 25, 41, 42, 43, 45, 47, 50, 53, 68, 69, 76, 141). Unlike the situation with the circumferential-pattern mutations considered thus far, alleles at three different gene loci generate the \( \text{janus} \) phenotype—\( \text{JANA} \) on chromosome 3R (two mutant alleles), \( \text{JANB} \) on chromosome 2 (one mutant allele), and \( \text{JANC} \), probably on chromosome 1R (four mutant alleles) (47, 50; Jenkins and Frankel, unpublished). Of these, only \( \text{janB-1} \) is temperature sensitive. \( \text{janA-1} \) cells had a somewhat lower growth rate and often a higher number of ciliary rows than wild-type cells, whereas the \( \text{janB-1} \) and \( \text{janC} \) cells that we have studied were normal in both of these respects. All of these mutations are completely recessive, except that \( \text{janB-1} \) heterozygotes showed limited expression at 39°C (50; Jenkins and Frankel, unpublished).

The principal phenotype that distinguishes all of the \( \text{janus} \) mutant alleles is the conversion of the cortical pattern of the dorsal surface of the cell into a global mirror image of the ventral pattern (Fig. 17D). This was manifested by the frequent formation of a partially reversed OA (called the “secondary” OA [sOA]) in the center of the reversed region and the appearance of a second set of CVPs to the left of the sOA (25, 42, 53, 76). The distribution of basal-body couplets at the anterior ends of ciliary rows, along with the underlying apical filamentous band (68), was modified in a consistent manner within such a reversed region (53, 141). Nonetheless, the internal organization of the ciliary rows remained normal in every other
respect (53, 76), and the ultrastructure of the basal bodies and associated cytoskeletal elements of the OA (68, 69) was completely normal.

The primary OA (pOA) of janus cells sometimes expressed modest abnormalities (53, 141), whereas the sOA was usually highly abnormal. The sOA typically developed in a reversed manner (53, 76) and often later rotated to achieve a more normal orientation of the oral membranelles, but with their unique specializations (“sculpturing”) at the wrong end (53).

In general, OAs that developed within globally reversed domains (whatever the origin of these domains) were variable structural compromises engendered by a conflict between the normal intrinsic handedness of the basal bodies and the pervasive influence of the reversed global left-right polarity (39, 115). These two influences typically ended up specifying opposite directions of alignment of basal-body couplings into promembranelles, often eventually resulting either in a compromise (inverted oral structures) or in chaos (fragmented structures).

The frequent absence of a second set of oral structures in janus cells could be partially accounted for by a failure of sOAs to develop to completion as a consequence of having to respond to conflicting patterning instructions; because a normal or nearly normal pOA was present on the opposite surface, these cells could feed, survive, and grow. The question then arises: does a reversed cortical domain still exist in the mid-dorsal region of janus cells, even when no sOA is present? Two lines of evidence suggest that it does. First, a detailed analysis of the placement of the CVP sets revealed that (i) the midpoint of the two CVP sets was halfway between the two sets of oral structures when both were present and (ii) the positions of these CVP sets were the same irrespective of whether a sOA was present or not (42). More direct evidence followed, with the discovery of a longitudinally oriented “postoral meridional filament” (pmf) in cells stained by the highly informative monoclonal antibody 12G9 (78). In wild-type cells, the pmf marked the oral meridian and was not found elsewhere. In janA-1 and janC-2 cells, the pmf was commonly also present along the mid-dorsal axis, even when secondary oral structures were not present there (141). This then made an “invisible” secondary axis visible and strongly suggests that the reversed domain was present even when it was not expressed in any highly obvious manner.

Originally, the phenotypes resulting from mutations at all of the janus loci were deemed nearly identical, except that high penetrance of the secondary oral structures in janA mutants depended on the presence of a separate janA-specific enhancer. However, two differences between janA and janC mutant clones were discovered more recently (22, 141). One was the mysterious failure of the apical band of janA-1 (but not of janC-2) cells to be immunostained with 12G9 or anti-centrin antibodies (141). The other was the inability of janA (but not janB or janC) mutant stocks to complete conjugation when mated with each other (22). The point of blockage was later and more heterogeneous in the janA-1 × janA-1 matings than in the bcd1-1 × bcd1-1 matings described above. This blockage may supply a useful means for selecting revertants in attempts to clone the \textit{JANA} gene by complementation, with the hope that reversal of the conjugation block will simultaneously reverse the cortical anomaly.

Finally, it is instructive to compare \textit{janus} mutants with true Siamese-twin doublets, of the type used by Fauré-Fremiet, Tartar, and Sonneborn to demonstrate the cortical inheritance of global cell organization. Although the \textit{janus} mutants often have two sets of oral structures (Fig. 19A) and other relevant cortical landmarks, they still have a number of ciliary rows characteristic of singlet cells; when the \textit{janA-1} mutation came to expression synchronously, the number of ciliary rows did not change while the two CVP sets bifurcated and the sOA made its later mid-dorsal appearance (43). Thus, \textit{janus} mutant cells are not true doublet cells; rather, they are singlet cells in which a broad domain has undergone a reversal of circumferential polarity.

Interestingly, our mutant hunt did turn up a true Siamese-twin doublet-forming mutation (Fig. 19B). The mutation was aptly named ‘doublet-former’ (\textit{dbf1-1}), and when homozygous it produced Siamese-twin doublets with a variable penetrance at restrictive temperatures, 75% in one sample and not much above zero in another. These were perfectly normal Siamese-twin doublets with twofold rotational symmetry, like the doublets studied by Nanney et al. (109). The modal ciliary row number of doublets was 28, which was near the lower limit of the expected row number for doublet cells; this fit with the observation that doublet cells tend to lose rows rapidly shortly after their formation but maintain the doublet condition until the number of ciliary rows falls into the range of 25 to 28 rows (103, 109). The only unusual aspect of the \textit{dbf1-1} doublet clones was that they were selected as recessive homozygotes following mutagenesis.
**Double mutants.** The analysis of double mutants is especially useful for working out metabolic or developmental pathways, in which epistasis can sometimes allow one to infer the order of successive steps. However, when we employed this method with our structural mutations, we typically found joint expression of phenotypes, notably in our analysis of cells bearing mutations of different fission arrest genes (49).

Double-mutant analysis was carried out for *janus* mutations combined with a variety of other mutations. The phenotype of double homozygotes of *janA-1* and *disA-1* was, unsurprisingly, totally additive: *janus* primary and secondary OAs in approximately normal opposite positions were superimposed on a highly disorganized cortical landscape (53). The *janA-1–psmB-1* combination was more interesting because it provided a clear example of epistasis; enlarged pseudomacrostome-type OAs were produced in the “primary” location, whereas sOAs remained either small or absent (53). The severe local-global polarity incompatibility in globally reversed domains seems to have interfered with the formation of large pseudomacrostome-type secondary oral structures.

Double homozygotes of mutations at all three *janus* loci were made with *bcd1-1* and were thoroughly analyzed (25). The details were complex, but three relatively straightforward observations were made. First, most of the phenotypes of the *janus-bcd* combinations were additive, including “both the broadened oral and CVP domains characteristic of the bcd mutants, and the mirror image arrangements of oral structures and CVP sets characteristic of *janus* mutations” (25). Second, two significant aspects of mutual enhancement were found in all three *jan-bcd* combinations: the penetrance of secondary oral structures (as shown in the center of the shaded region in Fig. 17D) was dramatically enhanced, and the location of the secondary oral meridian was shifted further to the right, so that it was no longer almost directly opposite the primary set of oral structures but came to be located at approximately 1 o’clock (if we place the primary oral meridian at 6 o’clock). In these cases, the *bcd1* mutation appeared to be influencing the expression of the *janus* phenotype. Third, an opposite influence was found only in the *janB-1–bcd1-1* combination: *janB-1* suppressed broadening of the oral domain of *bcd1-1* at the permissive temperature and attenuated it at the restrictive temperature, providing a second example of epistasis (25).

Our fairly extensive analysis of the *janC-1–hpo1-1* combination remains unpublished. The main finding was, again, that there is phenotypic additivity. Yet a priori, there are two different ways in which a *jan–hpo* cell might express such additivity: either (i) the normal and reversed cortical domains could remain opposite to one another (the primary oral meridian at 6 o’clock and the secondary oral meridian at ∼12 o’clock on a polar projection), with the CVP sets arrayed in a double hypoangular mode, one a short distance to the right of the pOA and the other a short distance to the left of the sOA, or else (ii) the secondary oral meridian might be shifted closer to the primary oral meridian (the primary at 6 o’clock and the secondary at ∼10 o’clock) with the two CVP sets in between. The latter was observed, so that one can speak of the *janus* phenotype being superimposed on a *hypoangular* body plan, rather than the reverse. In addition, the slippage was made with *bcd1-1* and *hpo1-1* double homozygote. Slippage was directed toward the right for the primary oral meridian (as in *hypoangular* mutants alone) and occasionally toward the left for the secondary oral meridian. Just how this could prevent the two oral meridians from ultimately joining and possibly annihilating each other is not clear at the moment.

One outcome of the analyses of both the *jan–bcd* and *jan–hpo* double homozygotes is that the simple notion of some fixed dorsal domain that is uniquely subject to a global reversal (as shown in Fig. 11 of reference 41 and Fig. 9 of reference 50) does not agree with these observations. It appears as if mutations at any of three *janus* loci may create the essential preconditions for a reversal, whereas the genetic background in which these mutations are expressed would influence the locations at which these reversals may be expressed. These locations might even be dynamic within a mutant clone.

Finally, double homozygotes of *bcd1-1* and *hpo1-1* were constructed specifically to test whether the reduction in the number of ciliary rows bearing CVPs that is characteristic of the *hpo* mutants would also be exhibited in a genetic background of *bcd-1*, which by itself increases the number of CVP rows. Since the phenotypes of the two mutants are opposites, one-way epistasis might have been an outcome, but the actual result was a compromise: the numbers of CVP rows in the *bcd1-1–hpo1-1* and *bcd1-1–hpo1-2* double homozygotes were higher than those in wild-type cells but significantly lower than those in *bcd1* cells alone; further, the “stronger” *hpo1-2* allele was more effective in bringing down the number of CVP rows than the “weaker” *hpo1-1* allele (55).

**EPILOGUE: FROM THE NUCLEUS BACK TO THE CYTOPLASM**

**Complementarity of structural inheritance and genomic control.** My thesis here, which is not particularly novel (10), is that genic control and structural inheritance should be thought of as complementary rather than antagonistic. Genic mutations can generate phenotypes that then are structurally inherited; they can attenuate the transmission of preexisting structural phenotypes; and in a few cases, they may bring about major topological transitions previously known only from studies of nongenic cortical inheritance.

The classic structurally inherited phenotype is the ciliary-row inversion in *Paramecium* (see Fig. 1A), which was originally generated by a natural grafting operation carried out by conjugating paramecia that failed to separate after exchanging their genetic material (12). However, ciliary-row inversions, which then are propagated cytotactically, are also generated by cortical disturbances brought about by the *kin24* (80) and *crochu* (77) mutations.

Another possible example of the mutational generation of phenotypes that subsequently are structurally inherited is the *doublet-former* (*dbf1-1*) mutation in *T. thermophila*. This mutation resulted in the appearance of perfect doublets similar to those generated by the failure of conjugants to separate. In this case, the mode of origin of the doublets has not been pinpointed, but it is likely to resemble that which Faure´-Fremiet used to create his doublets: blockage of division followed by an anterior migration of the posterior daughter cell. Variations in the frequency of occurrence of this initiating event would ac-
count for the highly variable penetrance of this mutation, since doublets, once formed, propagate their condition for a while but tend to revert to singlets by loss of ciliary rows and possibly also become diluted by overgrowth by singlets in a mixed culture (109).

Two specific cases in which genic mutations attenuate cytotactic propagation are the single-gene-controlled basal-body-deficient (bbd) condition in Euplotes minuta (34) and the low kinezity number (lkn1-1) mutant in Tetrahymena, described above. In both, the fidelity of propagation of ciliary rows, which is normally high (34, 104), is seriously compromised. This condition is probably due to two factors: (i) incomplete propagation of ciliary rows, so that they eventually fail to extend across the fission zone and therefore become lost in one of the two products of cell division (Fig. 4B), and (ii) atypical formation of new basal bodies outside of the longitudinal axis of the ciliary rows, which could eventually generate new ciliary rows (34). These imperfections in no way contradict the general rule of spatially accurate structural guidance within ciliary rows but instead point out its likely dependence on the normally invariant propagation of new ciliary units anterior to old ones within ciliary rows (2, 26, 67).

The recently achieved capacity for molecular modification of crucial cytoskeletal components of basal bodies has, in some cases, led to relaxation of these structural constraints. As examples, an RNA interference-induced depletion of the basal-body centrin in Paramecium (128) and certain modifications of gamma-tubulin in Tetrahymena (130) have both brought about the formation of new basal bodies at abnormal locations outside of the longitudinal axis of existing ciliary rows. Since the fidelity of propagation of ciliary units within ciliary rows is likely to depend on the intrinsic polarity of basal bodies (11), I would expect that such modifications would attenuate the fidelity of cytotaxis.

Genic mutations that strengthen cytotactic propagation would be hard to select, given the already very high fidelity of longitudinal perpetuation of ciliary rows in wild-type P. tetraurelia (12). However, whereas cytotactic propagation of ciliary-row inversions and associated structural modifications exists in Tetrahymena (118), it is not as strong as it is in Paramecium (97, 117). One could perhaps imagine genetically specified molecular alterations that might strengthen cytotactic propagation in the former organism.

Finally, the janus mutations can be thought of as inducing expression of the widespread ciliate capacity to generate and propagate nongenic reversals in the global arrangement of cortical structures. Whereas in the dorsoventrally flattened spirotrich ciliates, such as Styloynchia or Oxytricha, this capacity is most strongly expressed in the propagation of stable mirror image doublets (Fig. 1C), in Tetrahymena a mirror image doublet is a brief intermediate stage in the transition from the “balanced” Siamese-twin doublet state to the singlet condition (44). This transitory mirror image doublet stage is geometrically remarkably similar to the phenotype of the janus mutant (44).

From a perspective dominated by genetic thinking, one would consider the transient mirror image configuration in wild-type cells as a “phenocopy” of the janus mutation. But this “phenocopy” did not arise from any unusual environmental insult that might have altered the expression of a particular gene; it was instead a topological intermediate in the process of regulation of doublets to singlets that took place under perfectly normal culture conditions; the “stress” that generated this condition was entirely geometrical. It is more appropriate, I believe, to consider the janus phenotype as a “genocopy” of the nongenic mirror image doublet phenotype. The fact that this configuration can be observed in genetically wild-type cells indicates that the janus mutations did not manufacture the pattern reversals but rather created conditions under which Tetrahymena cells were stimulated to express their normal capacity to generate such a reversal. This perhaps accounts for the surprising frequency of janus mutations, which have appeared in breedable form on seven different occasions at three widely separated gene loci, despite our inefficient means for selecting such mutations. If and when they are cloned and sequenced, I suspect that these janus genes will be found to encode widely differing molecules, the absence of any one of which could serve as a trigger to unleash a latent topological capability.

Modifying the rulebook. It is impossible to predict accurately which of the 27 mutant-genes whose phenotypic effects have been briefly described in this review will turn out to be the most interesting once they are cloned by complementation. I would nonetheless like to conclude this review by developing an argument for a special interest in three of them.

Basic to my argument is the notion that there is an existing patterning “rulebook” that is ultimately encoded by the cell’s genes. One obvious rule is that there is equatorial partitioning along the anteroposterior axis prior to division constriction. I have described above mutations in several genes that modify or even ignore that rule; the cloning and sequencing of these genes might lead to insights into the molecular basis of that partitioning. But here I would like to emphasize a more subtle empirical rule, which has held up remarkably well. This is the rule of relational CVP positioning around the cell circumference, discovered long ago by David Nanney (102). With certain refinements and complications, Tetrahymena cells position their CVP midpoint at just under one-quarter of the cell circumference to the right (or clockwise) of the oral meridian (102) (Fig. 17A). In Siamese-twin doublets, CVPs are positioned just under one-quarter of the distance between the two oral meridians (Fig. 19B, bottom panel). To account for refinements in the numbers of CVP rows and their relative positions, Nanney (102) also postulated an “inductive field” of a definite width around the CVP midpoint.

These rules, which in my view must reflect some fundamental global positional order around the cell circumference, are well obeyed in most mutants and nongenic variants, so much so that they have been little remarked upon in this review. Reversed singlet cells seem to flout the rules by placing their CVP sets to the left rather than the right of the oral meridian, but when one does the relevant counts, one finds that these cells still obey Nanney’s rules perfectly, only making their measurements in the opposite direction (112). The global dimensions in reversed and normal cells are as similar as our left and right hands (this does not apply to the OAs of the reversed cells, because they have unreversed basal bodies to contend with). Even janus cells do their utmost to obey Nanney’s rules, taking into account the existence of side-by-side normal and reversed cortical domains in the same cell (42, 76).
In my view, the most interesting mutants are those that violate these rules. The reasoning is this; if we wish to achieve an understanding of the molecular foundations of any set of cellular rules, we must find the genes that help to encode these foundations. When such genes are mutated, the rules are likely to be altered.

Which of the mutations that I have described can cause cells to flout Nanney's rules? I can immediately identify three. The first is broadened cortical domains (bcl1), which shifts the CVP set somewhat to the right and, above all, dramatically broadens the CVP field width. Interestingly, a fundamentally similar phenotype had been discovered earlier in the form of the “multi-left-marginal” mutants of Paraurorysta weissei (28, 72, 73, 74). The second is the enigmatic IIG clone, which broadens the CVP field angle and also causes a major shift of the CVP set to the right; it is not clear whether this strain can still produce viable progeny. Finally, there are the hypoangular (hpo1) mutations. They have been selected four times independently, are all allelic, and always produce the same phenotype, suggesting that (unlike janus) they are affecting a unique gene product that happens to specify a portion of the cell's positional rulebook. These mutants break just about every rule in the book, most notably by reducing the CVP distance variably depending on the degree of expression. So, possibly, the HPO1 gene encodes an indispensable part of the rule-setting machinery.

Availability of the Mutations

Some of the mutants described here (including IIG8) are available from the American Type Culture Collection (http://www.atcc.org), and an increasing number are available from the Tetrahymena Stock Center (http://tetrahymena.vet.cornell.edu/). I have limited time and resources available for distributing mutant stocks directly, though I will respond to requests by prioritizing shipments to the Tetrahymena Stock Center (if the stocks are not there already!). I feel no proprietary interest in any of the mutations selected in my laboratory and will be delighted if any or all of the responsible genes are cloned and sequenced by interested researchers. I will also be happy to share unpublished information about these mutations to the extent that it is available. Since it is doubtful that any of these mutants have commercial value, it is desirable that any interesting results obtained from the study of these mutations be published in the open literature, although it should be apparent from perusal of this review that the author himself has not always obeyed this injunction.

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