Selective mirror-image reversal of ciliary patterns in Tetrahymena thermophila homozygous for a janus mutation

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Summary. The development of the oral apparatus (OA) and of neighboring ciliary structures of Tetrahymena thermophila was analyzed in cells homozygous for a janus (janA) mutation plus a recessive enhancer of janA (eja). Such cells frequently possess two OAs located on opposite sides of the cell, a primary (1°) OA previously reported to be normal, and a secondary (2°) OA previously reported to express a mirror-reversal of right-left asymmetry. This study confirms the reality of a reversal in the gross orientation of membranelles in most developing 2° OAs. It also shows that there is a reversal of asymmetry in the pattern of resorption of basal bodies of ciliary rows adjacent to the 2° OA, and in the arrangement of basal-body couplets making up the portion of the apical “crown” of the cell situated close to the 2° OA. However, the locations at which membranelles of the 2° OA become modified during late phases of oral development remain normal, so that membranelles of 2° OAs are superimposable on those of 1° OAs. In addition, the membranelles of 2° OAs frequently undergo a rotation during the final phases of oral development, so that even their spatial orientation becomes normal. This mixture of reversed and normal features can be accounted for by postulating a superimposition of a reversed large-scale asymmetry on a normal local asymmetry of ciliary units. This postulate predicts that no single mutation can bring about a complete mirror-image reversal of ciliary patterns.

1° OAs appear normal by light microscopy. However, detailed analysis of SEM preparations of isolated 1° OAs indicate subtle abnormalities of basal body arrangement in some of these OAs.

Key words: Cellular asymmetry reversal – Cell surface patterns – Mutations affecting patterns – Ciliate development – Tetrahymena thermophila

Introduction

One of the most intriguing categories of pattern mutants converts an asymmetrical structure into a symmetrical one by replacement of approximately one-half of the normal pattern by a mirror-image duplicate of the other half. Such transformations are well known in Drosophila, and include leg and wing duplications brought about by engrailed (Tokunaga 1961; Garcia-Bellido and Santamaria 1972), mesothoracic duplications engendered by wingless (Morata and Lawrence 1977; James and Bryant 1981), and larval segmental duplications resulting from the action of wingless as well as a number of other mutations (Nüsslein-Volhard and Wieschaus 1980). The janus mutation of Tetrahymena thermophila (Frankel and Jenkins 1979) is a unicellular equivalent of these, in which the normal dorsal pattern is converted into a mirror-image duplicate of the ventral pattern (Jerka-Dziadosz and Frankel 1979). However, in multicellular organisms the duplicated entities are structures (such as bristles) produced by whole specialized cells; in ciliates, they are assemblies of intracellular structures, in particular of ciliary units. The ciliary units themselves have a characteristic organization (reviewed by Lynn 1981) with a definite polarity and asymmetry. These units can readily be rotated within the plane of the cell surface, as in inverted ciliary rows (Beisson and Sonneborn 1965; Ng and Frankel 1977) but are not known to undergo mirror-image reversal. It is therefore interesting to find out how a large-scale pattern reversal can make use of elements that are probably incapable of such reversal. This problem was first recognized and addressed by Grimes et al. (1980), who discovered that in a microsurgically engendered mirror-image doublet biotype of certain hypotrich ciliates, the internal organization of the elements of the reversed pattern was either normal or inverted (rotated 180°), a finding that was confirmed ultrastructurally by Jerka-Dziadosz (1983). Grimes (1982) concluded “...that the global patterning of the ciliature is determined independently of the individual assembly events.”

The initial description of ciliary patterning of Tetrahymena thermophila cells homozygous for the original janus mutation (janA) had concluded that the pattern reversal involves a true situs inversus, such that the abnormal ciliary structures (components of oral apparatuses) could not be superimposed on the corresponding normal structures (Jerka-Dziadosz and Frankel 1979). However, it was even then realized that the local organization of all of the ciliary rows was normal, and a subsequent ultrastructural analysis confirmed that ciliary units were organized no differently in janus cells than in wild-type cells (Jerka-Dziadosz 1981 b). Since then, the spatial organization both of the ciliary rows (Frankel et al. 1981) and of the oral apparatuses (Nelsen 1981; Williams and Bakowska 1982; Bakowska et al. 1982)
1982a, b) of wild type cells has been described in much greater detail, and a recessive enhancer of janus (eja) was characterized that increases the penetrance of the mirror-image duplication without noticeably changing the nature of the phenotype. We were thus in a position to carry out a detailed reanalysis of the ciliary pattern of janus cells, with a view to resolving the issue of whether or not there exists a mirror-reversal at any level of structural organization. We found that while a reversal does exist at the level of geometrical orientation of compound ciliary elements (oral membranelles), it does not at the level of the internal organization of these elements. The previously recognized dichotomy between two hierarchical levels of spatial pattern-control can thus be applied to a mutationally generated intracellular mirror-image duplication.

In addition to a modification of our interpretation of the nature of the pattern-reversal on the “duplicated” side of janus cells, refined methods for analyzing oral patterning permitted a wholly unanticipated discovery of subtle but unmistakable abnormalities in the oral pattern of the non-reversed side of some of these cells.

Materials and methods

Stocks. All stocks used in this study were Tetrahymena thermophila of the inbred B strain (Allen and Gibson 1973, Table 2) homozygous for the janA mutation (Frankel and Jenkins 1979). This mutation was originally designated jan, but now has been redesignated janA due to our recent discovery of a new mutation, not allelic with janA (hence designated janB) that is very similar genetically to janA (see Discussion). Stocks IA192 and IA220 were used for the detailed light microscopical study (although many other stocks were examined more casually), while stock IA220 was employed for scanning electron microscopical (SEM) analysis. IA192 is the stock designation for progeny clone #19 of Table 4 in Frankel and Jenkins (1979); it was derived by two generations of successive outcrossing and inbreeding of the original janus clone, CU127 (see Frankel and Jenkins 1979). IA192 differs from CU127 and from its sister clones in a consistently high expression of secondary (2rd) oral structures (Frankel and Jenkins 1979, Table 6). A breeding analysis of IA192 demonstrated that this high level of expression is due to the presence of an independently transmitted recessive enhancer of janus (eja), and hence the genotype of IA192 could be written as janA/janA eja/eja. This was demonstrated by (a) a manifestation of high expression of 2nd oral structures in one-half of the janus progeny obtained after janA/janA+ eja/eja clones derived from the outcrossing of janA/janA eja/eja clones (such as IA192) were carried through a genomic-exclusion (Allen 1967) testcross, (b) transmission of the state of high expression by one-half of the non-janus progeny clones of this testcross (i.e. janA+ janA eja/eja clones, demonstrated by a cross of these clones to low-expression janus (janA/janA eja+/eja+) clones followed by a genomic-exclusion testcross of the putative janA/janA+ eja/eja+ double heterozygotes, and (c) the observation that janA/janA eja/eja+ clones, derived from a cross between janA/janA+ eja+/eja+ clones and janA/janA eja/eja clones, were all low-expressing. Clone IA220, like clone IA192, was of a janA/janA+ eja/eja genotype, derived from clone IA192 by two generations of successive outcrossing to wild-type cells followed by inbreeding through genomic-exclusion crosses.

IA220 is genetically very closely related to the wild-type stocks (B1975 and B2079) that had been used to characterize wild-type ciliary patterns. All of the stocks trace a common origin to the stock B1868 (18th generation of inbreeding, derived in 1968) from Dr. D.L. Nannney’s laboratory; B1975 and B2079 are, respectively, the 19th and 20th inbred generations established in 1975 and 1979 in this laboratory. The original janA clone CU127 is a mutagenized derivative of B1868 (Bruns and Sanford 1978); furthermore, three outcrosses to B1975 followed by inbreeding intervened between CU127 and IA220. Hence, it is unlikely that morphological differences between IA220 and our current wild-type stocks can be attributed to differences in genetic background.

Two clones homozygous for janA (with eja) plus other mutations affecting cell-surface pattern were also investigated. Clone IA231 is of genotype janA/janA eja/eja disA/disA (dis = “disorganized”; see Frankel 1979, Fig. 5B). This was produced by a cross of IA220 (janA/janA eja/eja) to IA159 (disA/disA) to generate a janA/janA+ eja/eja+ disA/disA+ triple heterozygote, followed by a genomic exclusion cross with selection of progeny clones expressing disA and janA. The inferred janA/janA+ disA disA genotype of three such clones was verified by obtaining only disorganized progeny following a cross to disA disA homozygote, and by obtaining only janus progeny following a genomic exclusion cross (a janA×janA cross does not yield viable progeny). One of the three janA/janA+ disA disA double-homozygous clones manifested a high level of expression of secondary oral structures, suggesting that it was also homozygous for eja, although this conclusion was not tested further. The second combination, IA211, is of genotype janA/janA eja/eja psmB/psmB (psm = “pseudo-macrostome”; see Frankel et al. 1984a). It was generated by a cross of IA203 (a janA/janA eja/eja clone derived from IA192 by one generation of outcrossing followed by inbreeding) to IA156 (psmB/psmB), producing a janA/janA+ psmB psmB eja/eja+ progeny clone, followed by a genomic exclusion cross with selection of three progeny expressing both psmB and janA. The inferred janA/janA+ psmB psmB genotypes of these clones was verified by the same strategy as was used with the janA/janA+ disA disA joint homozygotes. In this case all three of the double-homozygous clones showed high expression of the janus phenotype, suggesting that all were homozygous for eja, but again this conclusion was not tested rigorously.

Media and growth conditions. Cells were grown in one of three different peptone-based culture media described by Nelsen et al. (1981), with the photographed preparations grown in the richest of these, PIPYGF. Conditions of culture were also as described by Nelsen et al. (1981); all cells examined were from exponentially-growing cultures, since the secondary oral apparatus of janus cells is most highly expressed under these conditions (Jerka-Dziadosz and Frankel 1979).

Cytological methods. Light microscopical preparations were made using the protein-silver (protargol) method of Ng and Nelsen (1977) as modified by Außerheide (1982), and using the Chatton-Lwoff silver-impregnation method of Frankel and Heckmann (1968) as modified by Nelsen and DeBault (1978). SEM preparations of oral apparatuses were made by the procedures of Williams and Bakowska (1982), in a few cases using the modification of Bakowska et al.