Genetic analysis of morphogenetic processes in \textit{Paramecium}

I. A mutation affecting trichocyst formation and nuclear division

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\textbf{SUMMARY}

Mutation \textit{tam38} of \textit{Paramecium tetraurelia} is a nuclear recessive mutation with a pleiotropic effect on both trichocyst morphogenesis and nuclear processes. The analysis of the defective nuclear processes (micronuclear and macronuclear divisions, nuclear reorganization at autogamy) shows that these defects result from an abnormal localization of the nuclei. Phenocopies of \textit{tam38} abnormalities can be obtained by vinblastine treatment of wild-type cells at late stages of division. Taking into account the similarity between \textit{tam38} and a series of other mutations which also prevent trichocyst attachment to the cell surface and disturb nuclear divisions, the following interpretation is proposed: the absence of attached trichocyst induces structural changes in the plasma membrane or in the cortical region which disturb the normal cortical control of the localization of nuclei.

1. INTRODUCTION

At the cellular level, morphogenesis takes place either during division, when two new individuals develop from the mother cell, or during the differentiation of new structural and physiological properties. In both cases it involves a cycle of changes in the organization and physico-chemical properties of the surface and in the internal structure, i.e. changes in location, shape and size of organelles and in particular of the nucleus. An increasing number of data and speculations (Pardee, 1971; Bennett, Boyse & Old, 1972; Beisson, 1972; Burger, 1973; De Terra, 1974; Mazia, 1974), draw attention to this correlation and suggest that it might be the changes in the surface, in contact with the ‘environment’, which trigger and control the internal changes. As pointed out by Bennett \textit{et al.} (1972), one should consequently find evidence that ‘genes which are known to perform critical functions in embryogenesis in fact have products located on the cell surface’. It is also conceivable that genes whose products are not located on the surface but interfere with its organization play an important role in morphogenesis. However, even if the cell surface controls some key steps of morphogenesis, it is still necessary to analyse how the surface communicates with the interior and what mechanisms accomplish the ordered structural changes.

Some aspects of this multiple problem can be studied using mutations affecting morphogenesis in the unicellular organism \textit{Paramecium}. The favourable features
of this system, often stressed by Sonneborn (1970, 1974a), are threefold: (1) the surface of *Paramecium* displays thousands of repetitive landmarks (cilia and associated structures, trichocyst attachment sites), arranged in a specific pattern, and the cell’s interior contains a variety of organelles (macronucleus, micronuclei, trichocysts, etc.); (2) morphogenetic processes in *Paramecium* take place not only at fission but also during sexual phenomena (conjugation and autogamy) which involve in particular a complete reorganization of the nuclear apparatus (see Sonneborn, 1974b); (3) mutations affecting cell morphology and morphogenesis are relatively easy to obtain (Whittle & Chen-Shan, 1972) and many are already available (see Sonneborn, 1974b for a list).

In this paper we describe a nuclear pleiotropic mutation affecting three aspects of morphogenesis: cell shape, trichocyst formation and nuclear division. Our analysis of the nuclear defects shows that the mutation affects the localization of nuclei. The similarity between the phenotype of this mutant and of various other independent ‘trichocyst’ mutants previously described (Beisson & Rossignol, 1975; Sonneborn, 1974b) suggests that the observed defective localization of nuclei results from modification of the surface or subcortical organization due to the absence of attached trichocysts.

2. MATERIAL AND METHODS

The methods used were basically those described by Sonneborn (1970).

(i) *Strains and culture procedures*

The reference wild-type strain from which all the mutants were obtained was a line of stock d4-2 of *Paramecium tetraurelia*, according to the new nomenclature (Sonneborn, 1975); formerly this was *P. aurelia*, syngen 4. The following mutants were used or will be referred to: *kin241, tam6, tam8, tam38, ptA2, nd9, tsm21, t33, ts111, ts401*. A description of most of these mutants is given in Sonneborn (1974b); for *tam6* and *tam8*, see also Beisson and Rossignol (1975), and for *kin241*, Beisson et al. (in preparation). The mitochondrial marker $E_{102}^{6}$ (Adoutte & Beisson, 1970) was also used.

Cells were grown in Scotch Grass infusion bacterized by *Aerobacter aerogenes* generally at 28 °C, or at 18, 32 or 36 °C for particular purposes.

Treatment of dividing wild-type cells by anti-microtubule drugs was done using either vinblastine sulphate (E. Lilly & Co.) or colchicine (G. T. Gurr L.T.D.). These were kept at −20 °C as stock solutions of 1000 µg/ml and 10 mg/ml, respectively, and diluted to the required concentration just prior to use.

(ii) *Origin of the mutants*

Mutant *tam38* was obtained after UV mutagenesis (4000 ergs/mm²; 80% survival) of a line of stock d4-2, mating type 7, harbouring the $E_{102}^{6}$ mitochondrial marker which confers resistance to erythromycin (Adoutte & Beisson, 1970). The other mutants from this laboratory cited were obtained either after UV mutagenesis (*ts111, ts401, kin241*) or after nitrosguanidine (50 µg/ml, 30 min).
Morphogenetic processes in Paramecium

111

Morphogenetic processes in Paramecium (tam6, tam8, ptA2, tsm21, t33). The origin of mutations nd6 and nd9 is unknown. In all cases mutagenesis was carried out on wild-type populations in exponential growth but competent for autogamy. About two fissions after treatment, autogamy was induced, autogamous cells isolated, and mutant clones selected by various simple criteria depending on the experiments: abnormal morphology, thermosensitivity, slow growth, defect in trichocyst extrusion, etc.

(iii) Techniques of observation

Only light microscopy techniques were used.

(a) Cortical pattern. Paramecia were stained according to the Chatton-Lwoff silver impregnation technique (cf. Corliss, 1953).

(b) Trichocysts. Picric acid was used to test whether the cells were able to discharge their trichocysts (Pollack, 1974). Phase-contrast observation of living cells slightly compressed between slide and coverslip was used to observe trichocyst phenotype and especially their attachment to the cell membrane.

(c) Nuclei. Macronuclear division was observed at low magnification using Dippell's (1955) stain. The numbers of micronuclei and macronuclear anlagen were counted using phase-contrast in stained cells flattened under a coverslip. This is a rapid and reliable technique for the observation of large numbers of cells.

(iv) Genetical procedures

Crosses were performed according to the classical methods developed by Sonneborn (see Sonneborn, 1970). The presence of a mitochondrial marker in strain tam38 facilitated the identification of the cytoplasmic origin of ex-conjugant clones (Adoutte & Beisson, 1970).

3. RESULTS

(i) Phenotypic analysis of mutant tam38

Mutant tam38 was isolated as a strain showing slow and irregular growth after UV mutagenesis (see Material and Methods). Mutant cells appear slightly but systematically rounder than wild-type cells and incomplete divisions and monsters are occasionally observed; however, no particular defect in the cortical pattern was detected. The growth rate is heterogeneous: when ‘healthy’, tam38 cells undergo 2–3 fissions per day at 27 °C, but in the course of continued vegetative growth, very slow-growing clones with big slow-swimming cells appear. Healthy clones are generally obtained by reisolating cells from stock cultures or after autogamy. The cells of tam38 are thermosensitive: they die in 24 h at 36 °C.

The two other striking abnormalities of tam38 cells concern their trichocysts and nuclei. Trichocysts are of the football-type described by Pollack (1974): scarce, ovoid, tipless, consequently unattached and unable to discharge even when free in squashes. A variable number of micronuclei are present instead of the normal two, and the macronucleus is of variable size and shape. Abnormal reorganization at autogamy is also observed. The three types of nuclear defects were analysed as follows.
(a) Micronuclear division

Table 1(b) shows the intraclonal variation of the number of micronuclei, counted in clones 7–8 divisions old, derived in each case from a cell which contained two micronuclei. It can be seen that: (1) about 20% of the cells deviate from the normal two micronuclei; (2) the number of cells with one micronucleus equals that of cells with 3, while rare cells have 0 or 4 micronuclei; (3) the distribution remains centred around the modal number of two in each clone.

Since the frequency of deviations is not very high, the modal number for a given clone may be taken to represent the number of micronuclei present in the mother cell of that clone. Making this assumption, Table 1(a), (c) and (d) shows the distribution of the number of micronuclei per cell in clones derived from cells containing presumably one, three or four micronuclei respectively. The higher the initial number of micronuclei, the broader the distribution tends to be. Cells containing very high numbers of micronuclei (up to 24) were repeatedly obtained: conversely, practically no variation was observed in clones with one micronucleus.

The onset of the abnormal distribution was studied in short-range pedigrees in two ways: (a) by isolating dividing cells, letting them undergo one more fission and counting the micronuclei in the four resulting cells or (b) by separating the two daughter cells and allowing them to undergo a few fissions before counting the nuclei. These experiments were carried out using a culture in which a relatively high frequency of errors was observed. The detailed results of method (b) experiments are given in Table 2 together with the conclusions of method (a) experiments which gave comparable results. They show that when different numbers of micronuclei are observed in sister cells, these numbers are complementary: 0:4, 1:3 etc. Therefore the variation observed results from errors in the distribution of the nuclei between sister cells and not from under- or over-replication of some nuclei. Furthermore, the greater the number of micronuclei present in a cell, the greater the probability that an error in distribution will occur at division. This accounts for the broad distribution observed in clones derived from a cell containing four micronuclei.

During the course of this experiment, one amicronucleate clone was obtained. Although some lethality was regularly noted among subclones, some viable ones have been maintained for over 60 generations. Abnormal, non-functional gullets were noted in some of these cells.

Finally no clear-cut modification of the cellular phenotype was observed as a function of the variations in number of micronuclei.

(b) Macronuclear divisions

In wild-type cells, prior to cell division, the macronucleus migrates from its normal position at the dorsal side of the buccal cavity to a position beneath the dorsal cortex of the cell; it then elongates into a rod parallel to the cortex, and is finally constricted as the fission furrow progresses (Kaneda & Hanson, 1974; Beisson & Rossignol, 1975) (Fig. 1a, b, c). In mutant tam38, the macronucleus is
Table 1. The pattern of intraclonal variation of the number of micronuclei in relation to the initial number

(The figures indicate the number of cells with 0, 1, 2, ... micronuclei found in clones obtained from a mother cell containing
1 micronucleus (2 clones), 2 micronuclei (4 clones), 3 and 4 micronuclei (2 clones in each case).)

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<td>(b)</td>
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<td>27</td>
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Table 2. The repartition of micronuclei between sister cells

(From isolated dividers, sister cells (represented by a and b in the table) were separated and grown into clones. After at least three divisions, once or repeatedly, samples from each clone were examined. The number of fissions undergone before staining is indicated under the heading 'age'. The figures indicate the number of amacronucleate or macronucleate cells containing 0, 1, 2, ... micronuclei. On the basis of the dispersion of the distribution and the modal number(s) of micronuclei, the number of micronuclei in the initial cell and their daughters is deduced as symbolized in the column 'Interpretation'. The total number of identical observed cases, in both type a and type b experiments (see text) is indicated in the last column.)

<table>
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<th>Macronucleate cells</th>
<th>Interpretation</th>
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<td>0 1 2 3 4 5 6 7 16 24</td>
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<tr>
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<td>3 2 1 1</td>
<td>26 4 . . . . . . . . . .</td>
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<td>b 8</td>
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<td>. 1 7 . . . . . . . . . .</td>
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never found in its dorsal subcortical position but remains central. It seems to elongate less than in wild type and can be passively constricted by the progression of the division furrow (Fig. 2d). Furthermore the macronucleus frequently ‘slips’, mainly towards the posterior pole, thus undergoing unequal partition. This slippage sometimes occurs after the macronucleus has started its constriction (Fig. 2a, b). In extreme cases, completely amacronucleate cells are formed (Fig. 2c). Immediately after completion of the constriction, the macronuclei frequently display a round shape (Fig. 2a). Finally, in non-dividing tam38 cells, the macronucleus appears to be less rigidly ‘anchored’ dorsal to the gullet and its shape is more irregular than in wild type. All these properties are similar to those of mutant tam8 described by Beisson and Rossignol (1975).

Amacronucleate cells are also formed in tam38 exconjugants during the first few fissions following conjugation with wild-type partners. They are easily spotted since the cells that lose their macronuclear anlagen or their macronucleus undergo macronuclear regeneration (Sonneborn, 1954a) and therefore display a tam38 phenotype clearly distinguishable from that of their heterozygous phenotypically wild-type sisters. Out of 34 tam38 ex-conjugants, 4 cases of macronuclear regeneration at the first post-conjugal division and 11 other cases at some later divisions were identified.

The possible correlation between errors in the distribution of micro- and macronuclei has not been extensively investigated: some correlation appears to exist but it is not systematic since the formation of amacronucleate cells was not always accompanied by unequal distribution of micronuclei.

All these properties are quite similar to those of mutant am of Paramecium (Sonneborn, 1954a; Berger, 1973).

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**Plate 1**

Fig. 1. Stages of macronuclear division in wild-type cells. (a) The macronucleus is elongated but not yet constricted; (b) the macronucleus and the cell are well constricted; (c) the end of the division. x 300.

Fig. 2. Macronuclear divisions in mutant tam38. (a) The macronucleus is unequally partitioned between the daughter cells and it is passively constricted by the fission furrow. Note the round shape of the macronucleus in the newly divided cell. (b) More pronounced case of unequal partition of the macronucleus. (c) Formation of an amacronucleate fission product by slippage of the macronucleus towards the posterior pole. (d) Defective elongation and passive constriction of the macronucleus. x 300.

Fig. 3. Macronuclear divisions in vinblastine (25 µg/ml) treated wild-type cells. (a)–(c) Cells stained after less than 3 min of treatment. Note in all three cases the displacement of already well-elongated macronuclei. (d) Cell stained after 10–15 min of treatment: defective elongation and passive constriction of the macronucleus are observed as well as some inhibition of growth of the proter. x 300.

Fig. 4. Abnormal nuclear reorganization in mutant tam38. (a) Four macronuclear anlagen (a) and one micronucleus (m) are visible as well as macronuclear fragments (f); three other micronuclei were in different focal planes. (b) Autogamous cell with six macronuclear anlagen (a) and two micronuclei (m).
(c) **Nuclear reorganization**

It is often difficult to obtain 100% autogamy in a clone and homogeneously starved populations are rarely observed. However, when the clones are healthy, autogamy normally occurs after 20–25 vegetative divisions. Nuclear reorganization at autogamy was studied in clones composed of cells with 1, 2, 3 or 4 nuclei (Table 3).

**Table 3. Abnormal nuclear reorganization in autogamous tam38 cells**

((1), (2), (3), (4) depict the number of macronuclear anlagen (a) and micronuclei (m) respectively observed in autogamous cells derived from clones in which the modal number of micronuclei before autogamy was 1, 2, 3 or 4. The figures correspond to the number of cells of each category and the framed figure to the normal 2a/2m situations.)

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</table>

The significance of the 0a/0m autogamies is doubtful: they most likely represent ‘young’ autogamous cells in which the nuclei were not yet detectable by the technique used.

(1) corresponds to one of the two studied clones which showed a modal number of micronuclei of 1; in (2), (3) and (4) the observation from respectively 9, 2 and 3 clones were pooled.

Cells with 2 or 3 micronuclei (Table 3, nos 2 and 3) give a majority of normal autogamies with 2 anlagen and 2 micronuclei (symbolized 2a/2m), not taking into account cells with 0a/0m or 0a/2m (see legend of Table 3); when autogamous cells depart from the normal situation of 2a/2m, the majority of the abnormalities consist of an excess of one or both types of nuclei (Fig. 4). Cells containing up to 6 micronuclei and 6 anlagen were observed. Furthermore in several instances, cells were isolated from such autogamous clones, grown into clones and observed: the number of micronuclei found was in agreement with the observations made on the autogamous cells. There is no systematic correlation between the excess (or deficiency) of each of the two types of nuclei, although the more frequent cases are 3a/3m and 4a/4m.

Cells with more than 3 micronuclei seem to yield a higher proportion of abnormalities (Table 3, no. 4).

Two clones with 1 micronucleus gave different results. One gave 10 normal cells and 1 abnormal, while the other (Table 3, no. 1) gave a majority of abnormal cells, among which 4a/4m cells were frequent.
(ii) Genetical analysis of mutant *tam38*

*tam38* was crossed with *ts401* and 60 pairs were isolated. 40 had undergone reciprocal exchange as could be deduced from the fact that both ex-conjugants became thermoresistant. All the mutant characteristics are recessive and the wild-type phenotype is rapidly expressed. The parental origin of the two ex-conjugant clones could be easily identified by a test in erythromycin-containing medium since the cells derived from the *tam38* conjugant remained $E^R$ and those derived from the *ts401* partner remained $E^s$.

Many cases of macronuclear regeneration were observed in the clone derived from the *tam38* ex-conjugant, as described above. Mitochondria were transferred normally between the conjugants: in all the pairs in which a cytoplasmic bridge was observed, the cells derived from the $E^s$ conjugant were transformed into $E^R$ cells after a few fissions in erythromycin-containing medium.

Two pairs were analysed in $F_2$ and a total of 167 $F_2$ clones studied. A 1:1 (82:85) segregation of mutant: wild-type phenotypes was obtained. In all 82 mutant $F_2$ clones, all the mutant properties remained associated: slow growth, round cell shape, trichocyst abnormality, thermosensitivity and abnormal nuclear divisions and reorganization. The *tam38* and *ts401* mutations are independent and the *tam38-ts401* double mutants cannot be distinguished from *tam38* cells.

Mutant *tam38* was also crossed to mutants *t33*, *tam6*, *tam8*, and *kin241* and was found to be genetically independent.

(iii) Phenocopies of mutant *tam38*

Abnormal nuclear divisions similar to those observed in *tam38* were induced in wild-type cells by adding vinblastine or colchicine to an exponentially growing population, to final concentrations of 25 $\mu$g/ml or 5 mg/ml respectively. Cells in mid- or late division stages were picked up and stained for observation of the macronucleus. Dividing cells examined within the first 2–3 min of treatment generally showed no alteration. As early as the 3rd–5th min of treatment, a variety of macronuclear abnormalities appeared in all treated cells (Fig. 3a, b, c): first, defective location was observed, as if the macronucleus were no longer anchored and slid within the cells, and then defective elongation or constriction (Fig. 3d).

This was quite similar to observations of untreated dividing cells of mutant *tam38*, as well as of *tam6* and *tam8* (Beisson & Rossignol, 1975). Similar effects of colchicine treatment have been previously reported in *Tetrahymena* (Tamura, Tsuruhara & Watanabe, 1969).

The effect of vinblastine on the distribution of micronuclei was also studied in sister clones derived from cells treated at an early stage of division or just before the onset of visible division and retransferred to normal medium when constriction was nearly completed. A total of 209 dividing cells were treated with vinblastine at various concentrations (25–75 $\mu$g/ml) in five independent experiments which gave comparable results. These are pooled in Table 4. It is clear that vinblastine, like mutation *tam38*, induces errors in the distribution of micronuclei: of special
interest are the couples of sister clones with complementary numbers of micronuclei.

From the above experiments, 22 clones or subclones with 1 micronucleus, 19 with 2 micronuclei, 17 with 3 micronuclei and 12 with 4 micronuclei were observed at their next autogamy. All of them showed normal autogamy (2a/2m). The only exception was a clone composed of cells with 1 micronucleus which yielded a few autogamous cells with either 0a/0m or 1a/1m. Therefore wild-type cells practically always recover 2a/2m at autogamy regardless of their number of micronuclei before autogamy.

Table 4. The effect of vinblastine on the repartition of micronuclei between sister cells
(The results from five different experiments are pooled (see Text). The unviable dividers were those which could not complete division and died.)

<table>
<thead>
<tr>
<th>No. of dividers isolated</th>
<th>No. of viable clones</th>
<th>No. of clones studied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>209</td>
<td>195</td>
<td>123</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of clones with micronuclei</th>
<th>0m</th>
<th>1m</th>
<th>2m</th>
<th>3m</th>
<th>4m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>4*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>6†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>88</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In four cases the treated divider yielded one cell with 0m, and one cell with 4m.
† In six cases the treated divider produced one cell with 1m and one cell with 3m. For the other abnormal clones, the sister clone was missing.

The eight amicronucleate clones obtained in these experiments (plus 14 others obtained in other experiments) all died within less than 8 fissions and all showed a progressive degeneracy of the gullet.

4. DISCUSSION

tam38 is a pleiotropic mutant displaying two salient phenotypic properties: errors in nuclear processes (at division and reorganization) and defective trichocyst morphogenesis. The genetical analysis of the mutant indicates that all the mutant features are probably due to a single gene mutation since no disjunction was observed between them in crosses. Although the genetic data do not exclude the possibility that more than one gene (i.e. two or more closely linked genes) is responsible for the mutant phenotype, this seems unlikely in view of the fact that several other genetically independent mutations, isolated after various mutagenic treatments, also show a similar dual effect on trichocysts and nuclei (Table 5). It is improbable that in all these cases two closely linked genes were mutated simultaneously. Therefore some link seems to exist between trichocyst morphogenesis and nuclear behaviour.

Our data show that the mutant is defective in the distribution of nuclei at division. For the macronucleus, the defects are clearly correlated with its abnormal position at fission: it remains in the centre of the dividing cell instead of reaching a subcortical location. Proper interaction between the macronucleus and the cortex,
Morphogenetic processes in Paramecium 119

which would normally provide the guidelines for elongation and equal partition may be lacking. The importance of such an interaction has already been demonstrated in Stentor (De Terra, 1971, 1973). In the case of micronuclei, we have shown

Table 5. Relationships between trichocyst defects and abnormalities in nuclear processes

(This table is a compilation of data obtained at Gif-sur-Yvette (G) and Bloomington (B). All the Bloomington trichocyst mutants listed (except nd3 and nd6) were isolated by Pollack (1974) and their nuclear defects characterized by M. Schneller (cited in Sonneborn, 1974a). More details can be found in Sonneborn (1974b).)

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Nuclei</th>
<th>Trichocysts</th>
<th>Cell</th>
<th>Cortical</th>
<th>Genetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>R</td>
<td>Morphology</td>
<td>A</td>
</tr>
<tr>
<td>tam6</td>
<td>(G)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>tam8</td>
<td>(G)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>tam38</td>
<td>(G)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Football</td>
</tr>
<tr>
<td>ptA²</td>
<td>(G)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Pointless</td>
</tr>
<tr>
<td>t33</td>
<td>(G)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Football</td>
</tr>
<tr>
<td>tsm21</td>
<td>(G)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Football</td>
</tr>
<tr>
<td>ftA</td>
<td>(B)</td>
<td>.</td>
<td>-</td>
<td>.</td>
<td>Football</td>
</tr>
<tr>
<td>ndA</td>
<td>(B)</td>
<td>.</td>
<td>-</td>
<td>.</td>
<td>Reduced number</td>
</tr>
<tr>
<td>ptA</td>
<td>(B)</td>
<td>.</td>
<td>-</td>
<td>.</td>
<td>Pointless</td>
</tr>
<tr>
<td>stA</td>
<td>(B)</td>
<td>.</td>
<td>-</td>
<td>.</td>
<td>Stubby</td>
</tr>
<tr>
<td>stB</td>
<td>(B)</td>
<td>.</td>
<td>-</td>
<td>.</td>
<td>Stubby</td>
</tr>
<tr>
<td>t1</td>
<td>(B)</td>
<td>.</td>
<td>-</td>
<td>.</td>
<td>Trich-less</td>
</tr>
<tr>
<td>pt2</td>
<td>(G)</td>
<td>+</td>
<td>.</td>
<td>.</td>
<td>Pointless but normal when starved</td>
</tr>
<tr>
<td>nd6</td>
<td>(B)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Normal</td>
</tr>
<tr>
<td>nd9, nd3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Normal</td>
<td>+</td>
</tr>
<tr>
<td>(G)</td>
<td>+</td>
<td>+</td>
<td>.</td>
<td>Normal</td>
<td>+</td>
</tr>
</tbody>
</table>

' + ' and '- ', Normal and abnormal characters, respectively. A point indicates absence of information. Abnormalities in nuclear divisions refer to unequal distribution of micronuclei (m) or macronuclei (M) at binary fission. Abnormalities at nuclear reorganization (R) refer to numbers of micronuclei and of macronuclear anlagen departing from the normal situation (2a/2m). Mutants t33 and tsm21 display two alternative relatively stable phenotypes: either normal or abnormal for both trichocyst properties and nuclear divisions. Mutants nd3 and nd9 are probably identical since they were isolated independently at Gif and Bloomington as mutations preexisting in the same stock, d4-84. Abnormalities in cell shape refer to a tendency of the cells to round up or to be bigger. For trichocysts, abnormalities concern morphology, attachment (A) and discharge (D). The genetic independence data listed in the last column concern only the relationships between the listed mutants.

that tam38 causes errors in their distribution. This could be due to a defective elongation of the mitotic spindle and/or mislocalization with respect to the division furrow. In any case, both types of nuclei replicate and daughter nuclei separate: it
is the forces or structures involved in the control of their spatial distribution to daughter cells that are defective.

The errors observed at nuclear reorganization (extra numbers of micronuclei and macronuclear anlagen) can also be interpreted as resulting from a defective localization. Errors are not simply due to the fact that *tam38* cells contain abnormal numbers of micronuclei since this situation can always be corrected at autogamy – as demonstrated in clones derived from vinblastine treated wild-type cells. As shown previously, both in *Tetrahymena* (Nanney, 1953) and *Paramecium* (Sonneborn, 1954), the number of divisions of the zygotic nucleus and the differentiation of postzygotic nuclei into micro- and macronuclei seem to depend on their localization within the cell. In *tam38* cells, zygotic (or post-zygotic) nuclei might occasionally be misplaced in such a way that the signal controlling their division and differentiation loses its accuracy. This signal may either lie in the cytosol or in the cortex itself. Indeed the zygotic nuclei come into intimate contact with a very specific cortical region both in *Tetrahymena* and *Paramecium* (see Sonneborn, 1954).

Even if the abnormalities in the distribution of mutant *tam38* nuclei are caused by defective control of their localization, the link which correlates positioning of nuclei and trichocyst morphogenesis is not understood.

With regard to this question it is interesting to take into account a number of other mutations affecting trichocysts, isolated and analysed either in Gif-sur-Yvette or in Bloomington, and listed in Table 5. These mutants can be grouped into two classes: (1) the trichocysts are absent, abnormal or normal, depending on the mutation, but not attached to the cortex; (2) the trichocysts are apparently normal and attached, but cannot be extruded. Whenever the relevant observations have been made, mutants of the first type display abnormal nuclear divisions similar to those of *tam38*, while mutants of the second type have normal divisions. This correlation is particularly evident in *tam6* (Beisson & Rossignol, 1975), whose dual phenotype is temperature-dependent; in *tam6* cells grown at 18 °C, very few trichocysts are attached and 80% of dividers show abnormal macronuclear divisions; in cells grown at 27 °C, a significantly higher number of trichocysts are attached and only 25% of the cells have abnormal macronuclear divisions. We therefore suggest that the nuclear abnormalities observed in mutant *tam38* and in the other mutants of the same class (Table 5) are due to the absence of attached trichocysts, and that all mutants with unattached trichocysts should have abnormal nuclear divisions. This interpretation explains why so many different mutations blocking the trichocyst cycle at various levels all cause similar defects in nuclear division. Of course, mutations which affect nuclear division (for instance, by direct or indirect defects in spindle function) but do not prevent trichocyst attachment are also likely to be found.

If our hypothesis is valid, a likely explanation for the absence of attached trichocysts disturbing nuclear divisions, and for identical defects being induced in wild-type cells by short treatments with antimicrotubule drugs applied at late stages of division, is that both conditions cause some alteration of the cell surface
or of the cortical region and displace or disorganize some structural link (microtubules? microfilaments?) between the cortex and the nuclei which normally guide nuclear movements and position.

The attachment of trichocysts to the cortex induces two precise molecular rearrangements in the plasma membrane: a change in the configuration of the ‘outer ring’ of particles marking the trichocyst attachment site and the formation of a central ‘rosette’ of particles necessary for trichocyst exocytosis (Beisson et al. 1976). It is therefore reasonable to assume that trichocyst attachment triggers other changes in the organization of the plasma membrane or of the cortical region that are important for their interaction with nuclei.

As for the effects of vinblastine and colchicine, it is now known that these drugs – in addition to their more or less specific interaction with tubulin (see Olmsted & Borisy, 1973) – can induce a number of modifications of the cell surface, either through the interactions that may exist between the surface and intracellular microtubules (Berlin et al. 1974) or by a direct effect on the surface itself (Cheng & Katsoyannis, 1975). In our experiments, where the observed effects are obtained within minutes in cells already in the process of division, it is unlikely that the drugs act only by preventing or disrupting assembly of microtubules, and especially in the cases of ‘slippage’ of already elongated macronuclei, it is conceivable that it is the cortex and cortical control of nucleus localization that is affected.

In conclusion, our results are consistent with the hypothesis that the primary effect of mutation tam38 is on trichocyst morphogenesis and that attachment of trichocysts to the cortex is a prerequisite for the cortical control of nuclear division.

Finally it can be pointed out that the various nuclear alterations produced by this type of mutation provide a range of interesting situations to study the role of micronuclei, the interrelationships between micro- and macronuclei, the regulation of the amount of DNA in the cell, and the mechanisms of nuclear differentiation after reorganization.

REFERENCES


