Temperature-sensitive mutations in *Caenorhabditis elegans*.

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SUMMARY

A temperature sensitive sterile mutant of *C. elegans* (Sis2) was studied. This monofactorial, Mendelian, autosomic, semi-dominant mutation was thermosensitive between the beginning of the pachytene stage and that of the dialnucleic stage. Light and electron microscopy demonstrated the lack of membranes between newly formed oocytes, and between these oocytes and the rachis. These cellular membranes appear again towards the end of the pachytene stage. They delimit anucleated or multinucleated cytoplasmic islets of varying sizes rather than a normal anucleated rachis. Beyond the ootestis loop, during cytoplasmic growth, oocytes are individualized but contain a large number of pseudo-pachytene nuclei. Autoradiography showed incorporation of tritiated uridine in pachytene cells but not in multinucleated growing oocytes of S,ts mutants, although the nuclei remained in pseudo-pachytene nuclei. The regulation of membrane synthesis necessary to the maturation of pachytene cells (associated to the rachis) appears to be affected by this mutation.

RÉSUMÉ

Mutations thermosensibles chez *Caenorhabditis elegans*:

Un mutant thermosensible stérile de *Caenorhabditis elegans* (S,ts), induit par une solution d'EMS 0,1 M, est étudié du point de vue génétique, physiologique, ultrastructural et autoradiographique. La période thermosensible de cette mutation monofactorielle, mendelienne, autosomique et semi-dominante s'étend entre les débuts des stades pachytène et diacinèse. L'observation en microscopie photonique et électronique montre une complète disparition des membranes entre les ovocytes nouvellement formés et entre ces ovocytes et le rachis. À la fin du stade pachytkne, les membranes cellulaires réapparaissent. Elles ne délimitent pas un rachis axial anucléé normal mais des Plots cytoplasmiques de taille variable, anucléés ou plurinucléés. Après le coude gonadique, pendant l'accroissement cytoplasmique, les ovocytes sont alors individualisés mais contiennent un grand nombre de noyaux à un stade 

B. L'étude autoradiographique montre que ce mutant S,ts incorpore de l'uridine tritiée dans les cellules pachytknes mais il n'y a pas d'incorporation dans les ovocytes plurinucléés en accroissement malgré la persistance d'un nucléole dans chaque noyau pseudo-pachytkène. La fonction biologique affectée par cette mutation thermosensible semble être le système de régulation de la synthèse membranaire nécessaire au passage normal de l'ovocyte (associé au rachis) du stade pachytkne à l'ovocyte diacinétique individualisé.

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In multicellular organisms, ts mutation have proved very useful for studies of the specific phenomenon of gametogenesis. In Drosophila melanogaster, such work has resulted in the elucidation of the role of the Y chromosome in spermatogenesis and spermiogenesis processes (Ayles et al., 1973) and the role of the X chromosome in gametogenesis. In Drosophila (S. melanogaster), such work has resulted in the elucidation of the role of the Y chromosome in spermatogenesis and spermiogenesis processes (Gans, Audit & Masson, 1975; Zalokar, Audit & Erk, 1975; Cline, 1976).

Caenorhabditis elegans is an autogamic, protandric hermaphroditic nematode, and its two ovarian tubes show an extremely simple organization, with a linear progression of the oocytes without any follicular or nutrient cells (Nigon, 1949; Nigon & Brun, 1955; Abi Rached, 1974). These ovaries are well suited to investigate and understand the cytodifferentiation of germ cells.

In the Bergerac strain, different ts sterile mutants have been obtained and studied (Abdulkader & Brun, 1976, 1978; Abdulkader, 1977). The present work, concerns a ts sterile mutation affecting the racorial and cytoplasmic membranes during the differentiation of the female germinal lineage.

Materials and methods

General characteristics of the sterile mutant: culture conditions

The ts sterile mutant gon2 F102 (designated S₂ts) was obtained from the wild-type Bergerac strain of C. elegans, following a 7 hr 0.1 M EMS treatment. It was maintained in individual xenic cultures on drops of agar on histological slides (Abdulkader & Brun, 1978).

At 24° (restrictive temperature), this hermaphroditic mutant did not produce any egg, while wild-type hermaphrodites produced normal eggs. Reversely, at 18° (permissive temperature), the females produced 110 eggs instead of 150.

Experiment I (Fig. 1). Ts homozygous hermaphrodites (S₂ts/S₂ts) were crossed, at 18°, with wild-type +/+ males. After 1.5 days, the parents were discarded and the remaining larval and embryonic stages, were transferred to 24°. Two days later, the F₁ heterozygous hermaphroditic adults, recognized by the presence of eggs in the uterus, were placed on new drops of nutritive medium. Their F₂ eggs were collected and incubated for 3 days at 24° before observation.

Experiment II. Heterozygous F₁ males produced from a cross of homozygous S₂ts/S₂ts hermaphrodites with wild +/+ males at 18°, were test-crossed at this temperature with S₂ts/S₂ts hermaphrodites (Fig. 1). From successful cultures (where males were present among the F₂ progeny), the F₂ adult hermaphrodites were transferred to fresh culture medium. Their F₃ eggs were incubated at 24° for 3 to 3.5 days. The number of surviving F₃ adults and fertile individuals was then determined.

Experiment III. Homozygous S₂ts/S₂ts hermaphrodites were crossed with heterozygous, (Qb₁3ts/+ ) male mutants (Fig. 1). The ts lethal mutant let3 F101 designated Qb₁3ts (Abdulkader, 1977) was used as a genetic marker (Qb = semidominant mutation affecting hermaphroditic tail form). The doubly heterozygous F₂ hermaphrodites produced from this cross could easily be recognized by their intermediate tail form. They were collected and incubated at 18°. Their F₃ eggs were cultured in batches of 150 and incubated either at 24°, killing the F₃ Qb₁3ts/Qb₁3ts abnormal tail animals, or at 18° for one day then at 24°, thus allowing the development of F₃ Qb₁3ts/Qb₁3ts abnormal tail animals.
Mutation of Caenorhabditis elegans affecting oocyte I core relations

Fig. 1. Genetic studies of the ts sterile mutant S2ts-Qb,ts is a ts lethal mutation affecting tail form and embryonic development; Qb (queue en boule) is semi-dominant; lgts (temperature sensitive lethal) is recessive. S2ts/S2ts homozygotes. Moreover, the tail form phenotypes could be easily distinguished from each other. Animals with the Qb/Qb genotype, whose tail showed the typical ball-like swelling are referred to as "abnormal tail" while those with the Qb/+ genotype and with the wild-type +/+ genotype, are referred to as "intermediate tail" and "normal tail" respectively.

Physiology

Determination of the ts period (Fig. 2)

Shift up: Slightly segmented eggs (with approximately eight blastomeres), laid at 18°C by young S2ts/S2ts adults, were incubated at 18°C, in batches of 150 for 1.5, 2.5, 3, 3.5 or 4 days, then transferred to 24°C. Batches of 150 eggs in the same conditions as above, were incubated at 24°C for 2, 2.5 or 3 days, the transferred to 18°C. From each treatment at least 40 young adult hermaphrodites at female sexual maturity were cultured singly at 24°C for the shift up and 18°C for the shift down. After 1.5 days, they were transferred to fresh medium drops. After 5 days at 18°C or 3 days at 24°C, the number of adult descendants (i.e. fecundity), were determined from these two sets of individual cultures. The results were compared to those of S2ts/S2ts control animals raised at 18°C or at 24°C after transfer from 18°C at the earliest embryonic developmental stages.
Fig. 2. Determination of the ts sterile period of the $S_{24}^{18}$ mutation. Average fecundity of $S_{24}^{18}$ animals subjected to shift and heat shock experiments.
Heat shocks (Fig. 2)

Batches of 150 eggs, similar to those of the shift experiments, were first cultured at 18° for 3 to 3.5 days, transferred to 24° for 12 or 24 hr then returned to 18°.

The fecundity of these nematodes was determined and compared with that of 24° controls.

Cytology

Two groups of sterile S^ts^ts/S^ts^ts mutants were dissected after incubation from the earliest embryonic developmental stages, for 2 to 3 days at 24°, or for 3 days at 18°, then for 7 hr at 24°. After fixation with Carnoy, their ovotestes were stained with Feulgen (Nigon & Brun, 1955). They were compared under a light microscope to those of wild-type animals treated in the same way.

Ultrastructure

The dissected gonads of 2 or 3 day-old S^ts^ts/S^ts^ts mutants, raised at 24° from the earliest embryonic developmental stages, were fixed in 1.5% glutaraldehyde with 0.1 M sodium cacodylate-3% CaCl₂ buffer at pH 7.4.

After rinsing in 0.12 M saccharose-0.16 M sodium cacodylate-3% CaCl₂, they were post-fixed in buffered osmium tetroxide OsO₄ 1% sodium cacodylate 0.1 M-CaCl₂ 3%-saccharose, 0.15 M. The gonads were embedded in gelose then in Epon-Araldite®.

Transversal sections 600 Å thick, cut with a Reichert ultramicrotome were stained with uranyl acetate and lead citrate, and observed under a Philips EM 300 (80 KV) electron microscope.

Autoradiography

Ovotestes of 2 or 3 day-old S^ts^ts/S^ts^ts mutants, raised at 24° from eggs (8 cells stage) were incubated at 24° in vitro for one or two he at 24°, with 20 μCi/ml of tritiated Uridine (25 Ci/mM. C.E.A.), as described by Starck (1977). After a film exposure of 3 weeks, the gonads were stained with Unna.

Results

GENETICS

Monofactorial Mendelian genetic determinism of the autosomic semi-dominant ts sterile mutation S^ts^ts.

In experiment I (Fig. 1), the S^ts^ts mutation appears semi-dominant. Indeed, although 965 F₁ heterozygous hermaphrodites did not give a single adult F₂ descendant at 24°, they all formed eggs which developed to the end of segmentation, while the F₁ S^ts^ts/S^ts^ts homozygotes did not produce a single egg.

Experiment III (Fig. 1) gave supplementary information on the genetic determination of the S^ts^ts mutation: the ratio of fertile F₂ to sterile F₂ was 284-99 (Tab. 1). Statistical analysis of this ratio show that it is compatible only with a monofactorial determinism of the S^ts^ts mutation (x² = 0.149 is less than 3.841, the 5% critical value with one degree of freedom). Moreover in Table 2, the ratio of fertile F₂ to sterile F₂ = 776/253 is again incompatible with anything but the monofactorial proportions 3:1 (x² = 0.099).

Table 1

<table>
<thead>
<tr>
<th>Abnormal tail</th>
<th>Intermediate tail</th>
<th>Normal tail</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile ♀</td>
<td>32</td>
<td>47</td>
<td>20</td>
</tr>
<tr>
<td>Fertile ♀</td>
<td>76</td>
<td>144</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>191</td>
<td>84</td>
</tr>
</tbody>
</table>

F₁ animals obtained, by self-fertilization, from F₁ double heterozygous Qbl^ts+^/+S^ts^ts hermaphrodites (Exp. III, Fig. 1).

450 eggs reared for 1 day at 18 °C then shifted to 24 °C.

Experiment II (Fig. 1) shows that out of the 447 F₂ adults descended by self-fertilization from the F₁ hermaphrodites, 91 were fertile. This fecundity proves that the S^ts^ts mutation is autosomic, since linkage with the X chromosome would imply the sterility of all the 447 adults obtained.
Non-linkage between $S_{ts}$ locus and the autosomic $Qb13ts$ mutation

Results of experiment III (Tabl. 2) lead to the conclusion that the $F_2$ segregation of the intermediate and normal tail phenotypes do not give significantly different ratios from those expected in the case of non-linkage with respect to sterility and fertility ($2/12, 1/12, 6/12, 3/12$). Indeed, $\chi^2 = 3.649$ is less than 7.815, the 5% critical value with 3 degrees of freedom.

### Table 2
Genetic determinism of the $ts$ sterile $S_{ts}$ mutation and of an eventual linkage with the $Qb13ts$ mutation

<table>
<thead>
<tr>
<th>Abnormal tail</th>
<th>Intermediate tail</th>
<th>Normal tail</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile $\varphi$</td>
<td>0</td>
<td>164</td>
<td>89</td>
</tr>
<tr>
<td>Fertile $\varphi$</td>
<td>0</td>
<td>494</td>
<td>282</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>658</td>
<td>371</td>
</tr>
</tbody>
</table>

$F_2$ animals obtained by self-fertilization, from

Spermatozoa are absent in the ovotestis of a 2 day-old $S_{ts}$ mutant at 24° while there are about 200 spermatozoa in each spermatheca of the Bergerac wild-type hermaphrodite (Nigon, 1949; Delavault, 1958) coming from 50 pachytene spermatogenetic cells.

In the ovotestis of a 2 day-old $S_{ts}$ mutant at 24°, there are only about 50 cells no further developed than the pachytene stage. One can say that these normal cells eventually differentiate into spermatozoa: therefore, beyond 2 days at 24°, germ cells which enter the pachytene stage are oogenetic. We conclude that the ts period begins with the entry of the germinal cells into oogenetic pachytene.

### End of the ts period (Fig. 2 and 3)

The shift up experiments (Fig. 2) with animals less than 4 day-old (Exp. A, B, C and D, Fig. 2) brought about complete sterility in all cases. However, transfer at 4 days (Exp. E) allowed a low fecundity of 6. Since such fecundity is significantly higher than that of 24° controls (equal to zero), the end of the ts period lies...
Fig. 3. Fecundity of $S_{24}^{is}$ nematodes in shift experiments.
Pig.

4 to 7

Wild-type strain female pachytene germ-line cells at 240 (X 1,000);

SZts female pachytene germ-line cells at 240 (X 750);

Wild-type female diakinetic germ-line cells at 240 (X 750);

SZts female diakinetic germ-line cells at 240 (X 700).

adc: abnormal diakinesis cells;
apc: abnormal pachytene cells;
Ch: chromosome;
N: nuclei;
pn: pachytene nuclei.

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Cytological disturbances in the germinal cells

Light microscope observations

The oovotestis of 2 day-old S2ts/S2ts mutants at 24° did not show any noticeable disturbance or abnormality. In contrast to wild-type adults, those of adult animals — 3 day-old at 24° — were lacked normal pachytene oocytes replaced by multinucleated cytoplasmic masses in the distal arm of the gonad (Fig. 4 and 5). In the proximal arm of the ovotestis, at the cytoplasmic full growth level, were individual cells with several nuclei in the cytoplasm. They were small and scattered or heaped up against the other. At this level, as in the preceding zone the chromatin distribution in the nuclei resembled that of the pachytene of meiotic prophase. Normally shaped spermatozoa were present in the spermatheca of these abnormal adult gonads.

The gonads of animals transferred from 18° to 24° at 3.5 days and fixed after a 7 hr exposure to 24° differed from those described previously. The loop region sometimes contained 2 to 4 normal pachytene cells preceding a number of abnormal multinucleated oocytes in full growth, containing up to 50 small nuclei of the type described above, designated “pseudo-pachytene”.

The presence of a small number of normal pachytene cells can be explained if exposure of 3.5 day-old animals to non-permissive temperature for only 7 hr is insufficient to bring about the complete sequence of perturbations to all pachytene cells.

Electron microscope observations

The electron microscope revealed no abnormalities in the germinal cells, of 2 day-old mutants at 24°. However, abnormalities were detected in gonads of 3-day old adult mutants at 24° when compared with those of wild-type individuals.

At the beginning of the meiotic prophase (Fig 8 and 9), about fifteen nuclei were randomly scattered in the cytoplasm. No oocyte or core membranes could be seen except at the edge of the gonad. The axial core was missing at the pachytene stage (Fig. 10 and 11) and large cytoplasmic masses containing one or several nuclei were found next to smaller anucleic masses particularly at the edge of the gonad. The structure of the cellular membranes which surrounded them, more or less completely, seemed normal.

At the full growth level (Fig. 12), multinucleated oocytes were present, with normal nuclei and a normal cell membrane. But the large nucleoli resembling that of pachytene nuclei, even in size, was unexpected at this stage.

Autoradiography

Two day-old S2ts mutants (at 24°) incorporated tritiated uridine in the oogonia and in pachytene cells, while 3 day-old S2ts (at 24°), incorporation occurred in the oogonia and in the abnormal pachytene zone filled with multinucleated cytoplasmic masses at the gonadic loop (fig. 13). However, there was no incorporation in multinucleated oocytes in full growth before the spermatheca.

An increase in the incubation time lead to intensification and intracellular evolution of the label, from the nucleus towards the cytoplasm.

Thus the S2ts mutant synthesized RNA in the oogonia and in normal pachytene cells (animals raised for 2 days at 24°), and abnormal oocytes (3 days at 24°).

As with the wild-type strain (Starck, 1977), this synthesis decreased in the germinal cells lying in the proximal arm of the gonad. Usually, a drop in the rate of synthesis is correlated with the progressive disappearance of the nucleolus and the important diakinetic condensation of the chromosomal material. But in the S2ts mutant, this drop occurs, in spite of the persistence of the nucleolus in the “pseudo-pachytene” nuclei at a stage of low chromosomal condensation, favourable for transcription.

Discussion

The modifications in fecundity and gametogenesis resulting from the various temperature-shift experiments lead to the conclusion that the ts period of the ts sterile autosomic monofactorial incompletely dominant S2ts mutation lies between the beginning of the pachytene and the beginning of the diakinesis stages.
Complete phenotypic expression requires exposure to 24°C for slightly more than seven hours. Observations of the mutated ovotestis under electron and light microscopes show that this expression corresponds to a defect in the formation of membrane delimiting pachytene cells, and between them and the axial core (the germinal tissue appears like a syncytium). This state of disturbance develops all along the gonad:

- during the pachytene stage, cell membranes appear but do not demarcate a normal anucleated axial core; instead the surround anucleated or multinucleated cytoplasmic islets of varying sizes;
- beyond the ootestis loop, the female germinal cells are proper individual cells with...