

Temperature-sensitive mutations in *Caenorhabditis elegans* : a sterile mutation affecting oocyte I core relations

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SUMMARY

A temperature sensitive sterile mutant of *C. elegans* (S_2^{ts}) was studied. This monofactorial, Mendelian, autosomic, semi-dominant mutation was thermosensitive between the beginning of the pachytene stage and that of the diakineti-
c 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_2^{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) into
diakinesis oocytes I, appears to be affected by this mutation.

RÉSUMÉ

Mutations thermosensibles chez Caenorhabditis elegans :
mutation stérile affectant les relations ovocyte I — rachis

Un mutant thermosensible stérile de *Caenorhabditis elegans* (S_2^{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, mendélienne, autosomique et semi-dominante s'étend entre les débuts des stades
pachytène et diacinese. 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. A la fin du stade pachytène,
les membranes cellulaires réapparaissent. Elles ne délimitent pas un rachis axial anucléé normal mais des flots
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
« pseudo-pachytène ». L'étude autoradiographique montre que ce mutant S_2^{ts} incorpore de l'uridine tritiée dans les
cellules pachytènes mais il n'y a pas d'incorporation dans les ovocytes plurinucléés en accroissement malgré la per-
sistance d'un nucléole dans chaque noyau pseudo-pachytène. 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 I (associé au rachis) du stade pachytène à l'ovocyte diacinéti-
que individualisé.

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 oogenesis and maternal effect phenomena (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 germs 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 rachial 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 S_2^{ts}) 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 hermaphroditic nematode reached sexual maturity at the age of 2.5 days, and laid about 50 eggs in 1.5 to 2 days.

At 18° (permissive temperature), the fecundity of the hermaphroditic mutant was slightly lower than that of the wild-type (approximately 110 eggs were produced instead of 150).

GENETICS, PHYSIOLOGY, CYTOLOGY AND ULTRASTRUCTURE OF THE STERILE MUTANT (S_2^{ts})

Genetics

Experiment I (Fig. 1). *Ts* homozygous hermaphrodites (S_2^{ts}/S_2^{ts}) 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 F_1 heterozygous hermaphroditic adults, recognized by the presence of eggs in the uterus, were placed on new drops of nutritive medium. Their F_2 eggs were collected and incubated for 3 days at 24° before observation.

Experiment II. Heterozygous F_1 males produced from a cross of homozygous S_2^{ts}/S_2^{ts} hermaphrodites with wild $+/+$ males at 18°, were test-crossed at this temperature with S_2^{ts}/S_2^{ts} hermaphrodites (Fig. 1). From successful cultures (where males were present among the F_2 progeny), the F_2 adult hermaphrodites were transferred to fresh culture medium. Their F_3 eggs were incubated at 24° for 3 to 3.5 days. The number of surviving F_3 adults and fertile individuals was then determined.

Experiment III. Homozygous S_2^{ts}/S_2^{ts} hermaphrodites were crossed with heterozygous, ($Qbl_3^{ts}/+$) male mutants (Fig. 1) [The *ts* lethal mutant let3 F101 designated Qbl_3^{ts} (Abdulkader, 1977) was used as a genetic marker (Qb = semi-dominant mutation affecting hermaphrodite tail form)]. The doubly heterozygous F_1 hermaphrodites produced from this cross could easily be recognized by their intermediate tail form. They were collected and incubated at 18°. Their F_2 eggs were cultured in batches of 150 and incubated either at 24°, killing the F_2 Qbl_3^{ts}/Qbl_3^{ts} abnormal tail animals, or at 18° for one day then at 24°, thus allowing the development of F_2 Qbl_3^{ts}/Qbl_3^{ts} abnormal tail animals.

Numbers of fertile and sterile surviving adults were determined from these cultures after 3 to 3.5 days. The so-called "fertile class" included animals with eggs in the uterus, corresponding to the wild-type $+/+$ homozygote or $S_2^{ts}/+$ heterozygote. The sterile class consisted only of animals with no eggs in the uterus, thus sterile

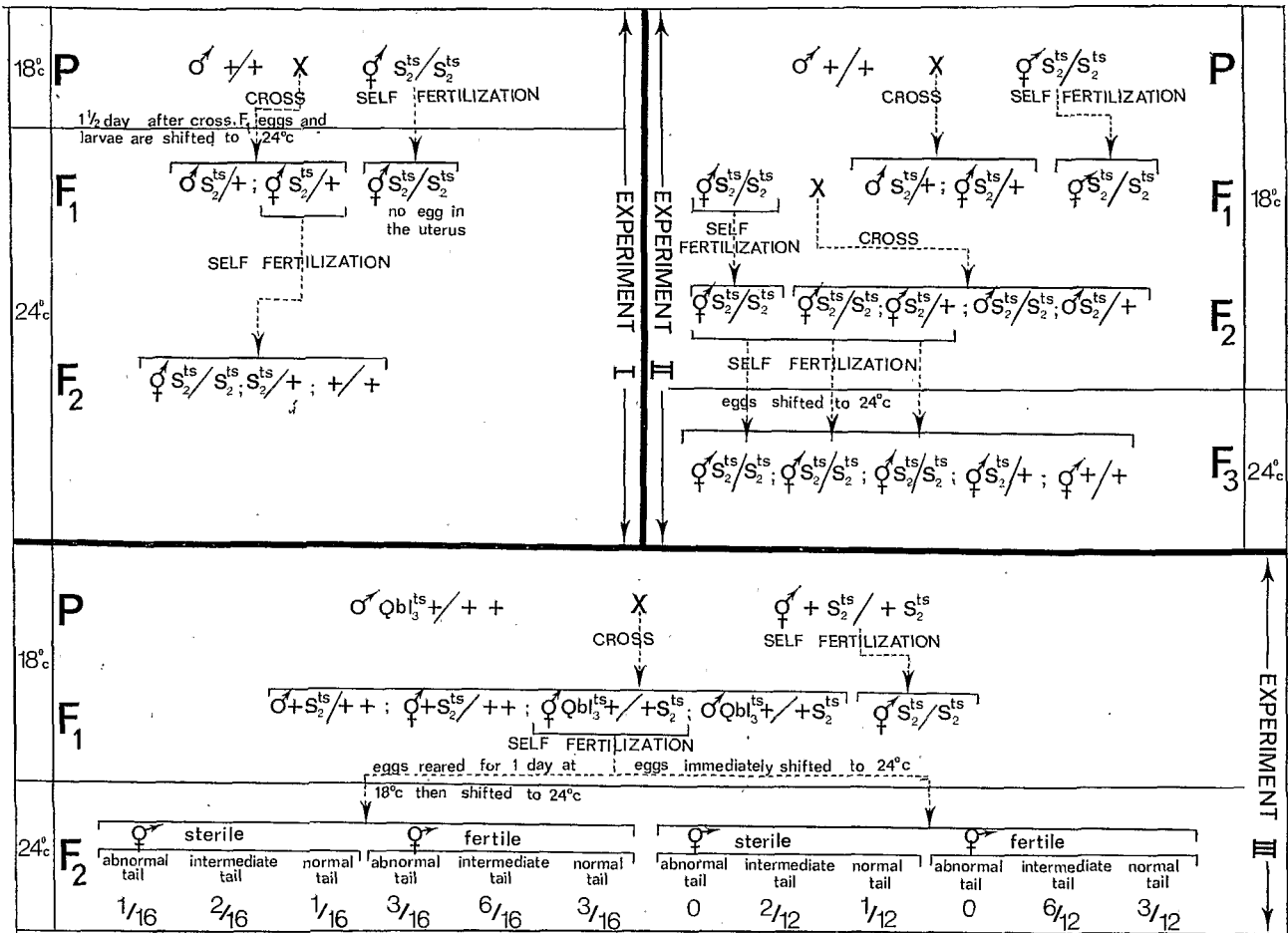


Fig. 1. Genetic studies of the *ts* sterile mutant S_2^{ts} — Qb_{13}^{ts} is a *ts* lethal mutation affecting tail form and embryonic development ; Qb (= queue en boule) is semi-dominant ; l_3^{ts} (= temperature sensitive lethal) is recessive.

S_2^{ts}/S_2^{ts} 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° by young S_2^{ts}/S_2^{ts} adults, were incubated at 18°, in batches of 150 for 1.5, 2.5, 3, 3.5 or 4 days, then transferred to 24°.

Shift down: Batches of 150 eggs in the same conditions as above, were incubated at 24° for 2, 2.5 or 3 days, the transferred to 18°.

From each treatment at least 40 young adult hermaphrodites at female sexual maturity were cultured singly at 24° for the shift up and 18° for the shift down. After 1.5 days, they were transferred to fresh medium drops.

After 5 days at 18° or 3 days at 24°, the number of adult descendants (i.e. fecundity), were determined from these two sets of individual cultures.

The results were compared to those of S_2^{ts}/S_2^{ts} control animals raised at 18° or at 24° after transfer from 18° at the earliest embryonic developmental stages.

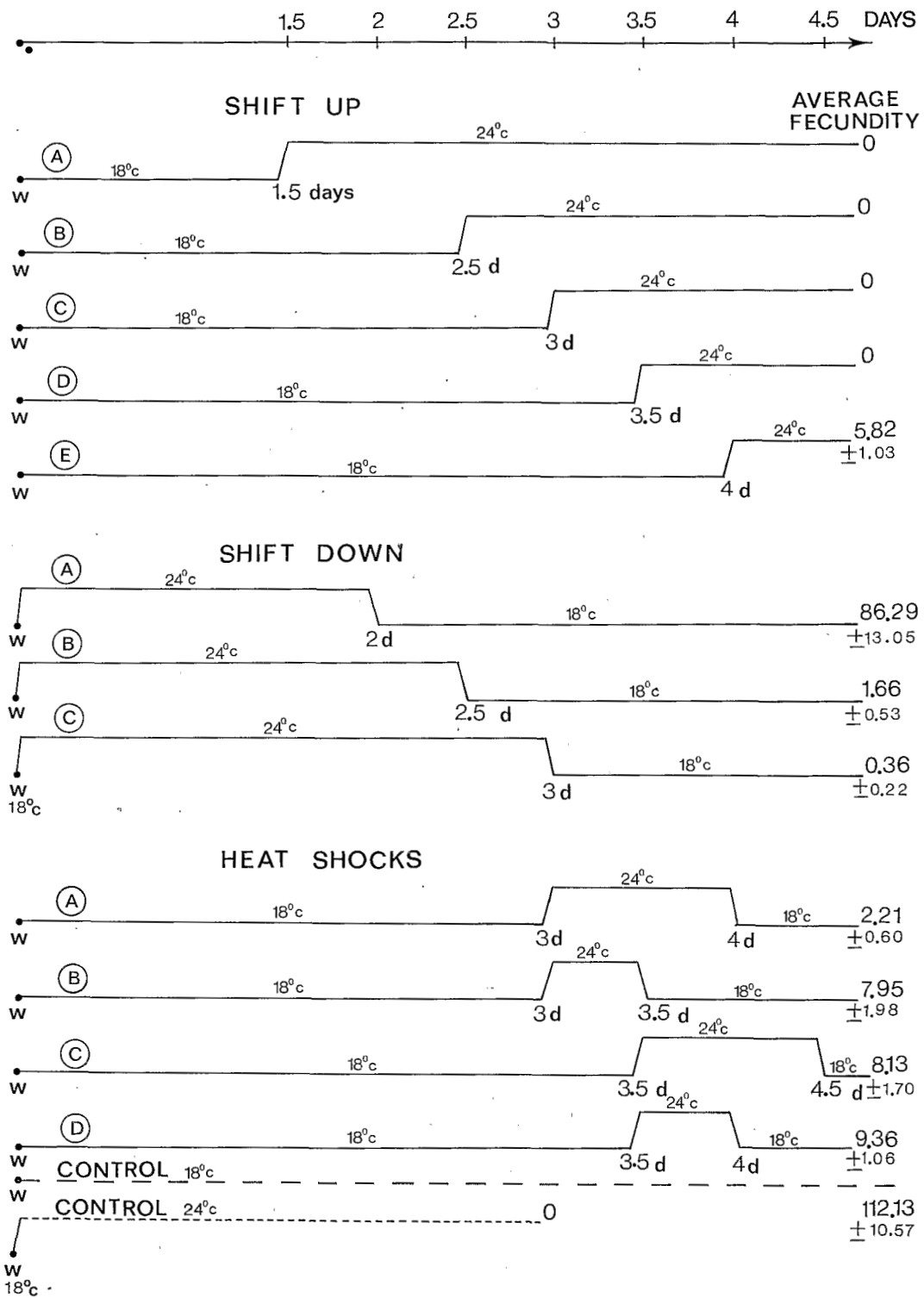


Fig. 2. Determination of the ts sterile period of the S_2^{ts} mutation. Average fecundity of S_2^{ts} animals subjected to shift and heat shock experiments.

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_2^{ts}/S_2^{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_2^{ts}/S_2^{ts} mutants, raised at 24° from the earliest embryonic developmental stages, were fixed in 1.5% glutaraldehyde with 0.1 M sodium cacodylate-3% $CaCl_2$ buffer at pH 7.4.

After rinsing in 0.12 M saccharose-0.16 M sodium cacodylate-3% $CaCl_2$, they were post-fixed in buffered osmium tetroxide OsO_4 1%-sodium cacodylate 0.1 M- $CaCl_2$ 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_2^{ts}/S_2^{ts} mutants, raised at 24° from eggs (8 cells stage) were incubated at 24° *in vitro* for one or two hours at 24°, with 20 $\mu Ci/ml$ 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_2^{ts} .

In experiment I (Fig. 1), the S_2^{ts} mutation appears semi-dominant. Indeed, although 965 F_1 heterozygous hermaphrodites did not give a single adult F_2 descendant at 24°, they all formed eggs which developed to the end of segmentation, while the F_1 S_2^{ts}/S_2^{ts} homozygotes did not produce a single egg.

Experiment III (Fig. 1) gave supplementary information on the genetic determination: the ratio of fertile F_2 to sterile F_2 was 284-99 (Tab. 1). Statistical analysis of this ratio shows that it is compatible only with a monofactorial determinism of the S_2^{ts} mutation ($\chi^2 = 0.149$ is less than 3.841, the 5% critical value with one degree of freedom). Moreover in Table 2, the ratio of fertile F_2 to sterile $F_2 = 776/253$ is again incompatible with anything but the monofactorial proportions 3 : 1 ($\chi^2 = 0.099$).

Table 1

Genetic determinism of the ts sterile S_2^{ts} mutation and of an eventual linkage with the Obl_3^{ts} mutation

	Abnormal tail	Intermediate tail	Normal tail	Total
Sterile ♀	32	47	20	99
Fertile ♀	76	144	64	284
♂	—	—	—	2
Total	108	191	84	385

F_2 animals obtained, by self-fertilization, from F_1 double heterozygous $Obl_3^{ts}+/+S_2^{ts}$ 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 F_2 adults descended by self-fertilization from the F_1 hermaphrodites, 91 were fertile. This fecundity proves that the S_2^{ts} mutation is autosomic, since linkage with the X chromosome would imply the sterility of all the 447 adults obtained.

Non-linkage between S₂^{ts} locus and the autosomic Qbl₃^{ts} mutation

Results of experiment III (Tabl. 2) lead to the conclusion that the F₂ 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 S₂^{ts} mutation and of an eventual linkage with the Qbl₃^{ts} mutation

	Abnormal tail	Intermediate tail	Normal tail	Total
Sterile ♂	0	164	89	253
Fertile ♀	0	494	282	776
♂	—	—	—	4
Total	0	658	371	1,033

F₂ animals obtained by self-fertilization, from F₁ double heterozygous Qbl₃^s+/+S₂^s hermaphrodites (Exp. III, Fig. 1).

Laid at 18°, the eggs (1,500) were immediately shifted to 24 °C.

The non-linkage of S₂^{ts} and Qbl₃^{ts} is confirmed by the results from experiment III (Tab. 1). Animals with either abnormal, intermediate or normal tails, and either sterile or fertile, occurred in the proportions 1/16, 2/16, 1/16 and 3/16, 6/16, 3/16 respectively. These proportions are expected in the case of non-linkage : $\chi^2 = 4.474$ is less than 7.815, the critical value with three degrees of freedom.

PHYSIOLOGICAL STUDIES

Beginning of the ts period (Fig. 2 and 3)

Two day-old mutants subjected to a shift down (Exp. A, Fig. 2) showed a fecundity of 86, close to the 112 of 18° control animals. However 2.5 or 3 day-old animals shifted down (Exp. B and C, Fig. 2) showed a considerably reduced fecundity of 2 and 0.4. Therefore, we conclude that the ts period begins around 2 days at 24°.

Spermatozoa are absent in the ovotestis of a 2 day-old S₂^{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 spermatogenic cells.

In the ovotestis of a 2 day-old S₂^{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 between 3.5 and 4 days at 18°.

In the ovotestis of 3.5 day-old S₂^{ts}/S₂^{ts} mutants, raised at 18°, one can usually see the appearance of the first diakinetoc oocyte in the proximal arm. Therefore the ts period ends when the female germinal cell enters the diakinetoc phase of its evolution and the effect of the altered S₂^{ts} genic product should be obvious in the oocytes only during the pachytene and diplotene stages of the meiotic prophase.

Variations in the phenotypic expression of the ts mutation S₂^{ts}

The results of the heat shock experiments (Fig. 2) show that although the phenotypic expression of sterility increases with exposure to 24°, the fecundity of mutants shocked at 3 days (Exp. A and B, Fig. 2) is always lower than that of animals treated at 3.5 days (Exp. C and D, Fig. 2).

Thus pachytene cells appear less temperature-sensitive at advanced stages of the meiotic phase than at its beginning.

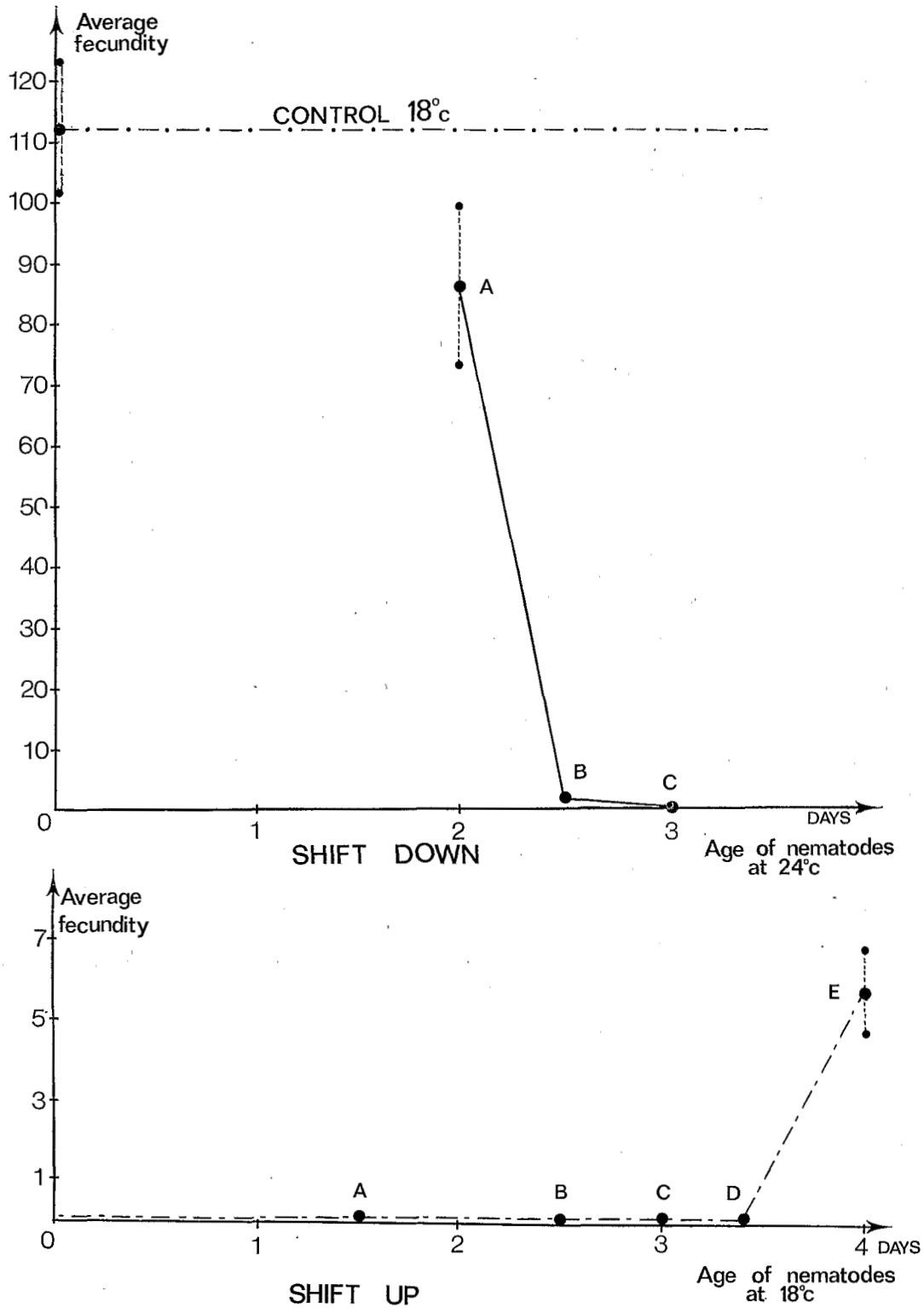


Fig. 3. Fecundity of S_2^{ts} nematodes in shift experiments.

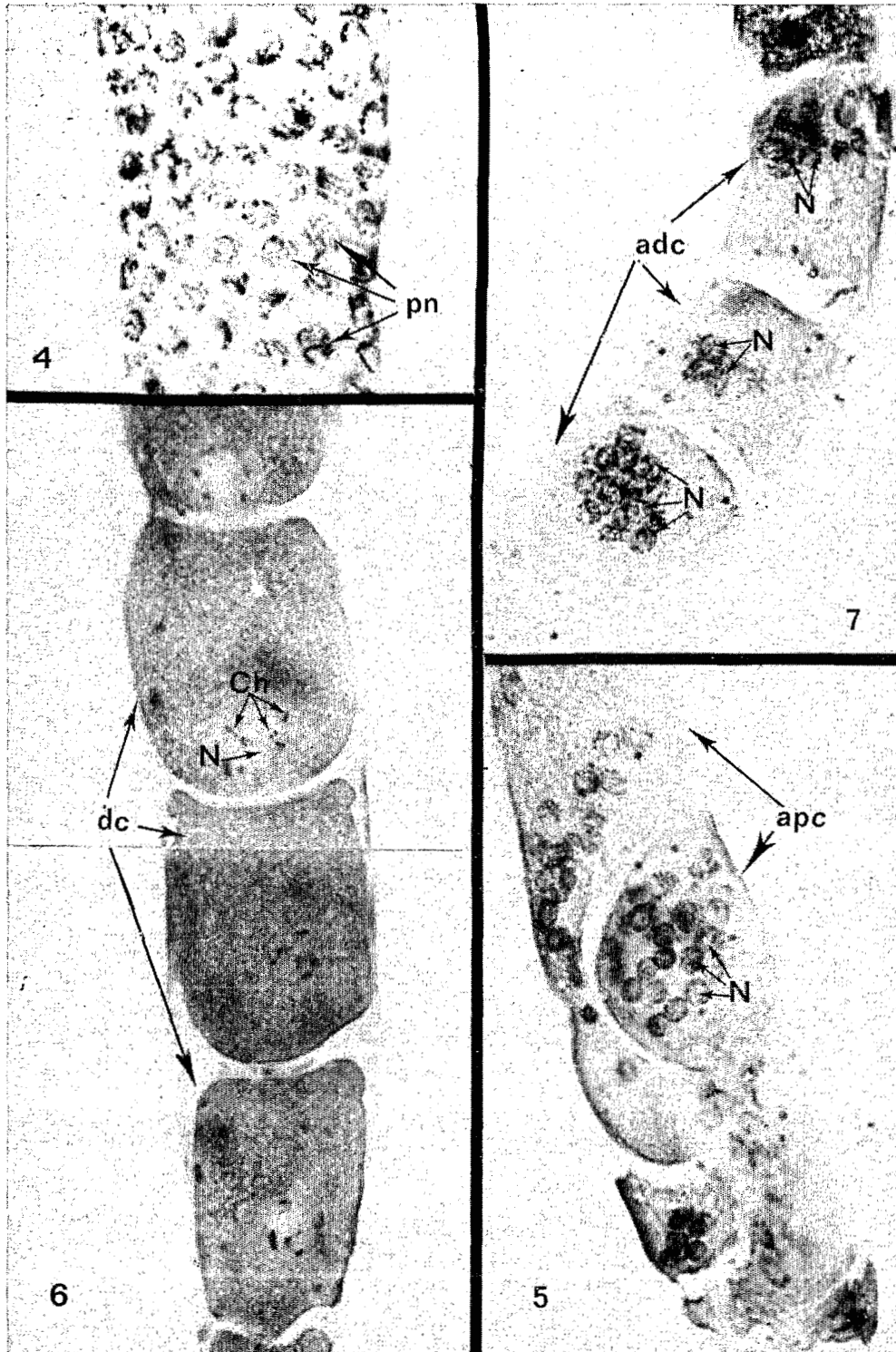


Fig. 4 to 7 ; 4 : Wild-type strain female pachytene germ-line cells at 24° (X 1,000) ; 5 : S₂^{ts} female pachytene germ-line cells at 24° (X 750) ; 6 : Wild-type female diakinetes germ-line cells at 24° (X 750) ; 7 : S₂^{ts} female diakinetes germ-line cells at 24° (X 700).

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

CYTOLOGICAL DISTURBANCES IN THE GERMINAL CELLS

Light microscope observations

The ovotestis of 2 day-old S_2^{ts}/S_2^{ts} 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 one 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 S_2^{ts} mutants (at 24°) incorporated tritiated uridine in the oogonia and in pachytene cells, while 3 day-old S^{ts} (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 S_2^{ts} 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 diakinetical condensation of the chromosomal material. But in the S_2^{ts} 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 S_2^{ts} mutation lies between the beginning of the pachytene and the beginning of the diakinesis stages.

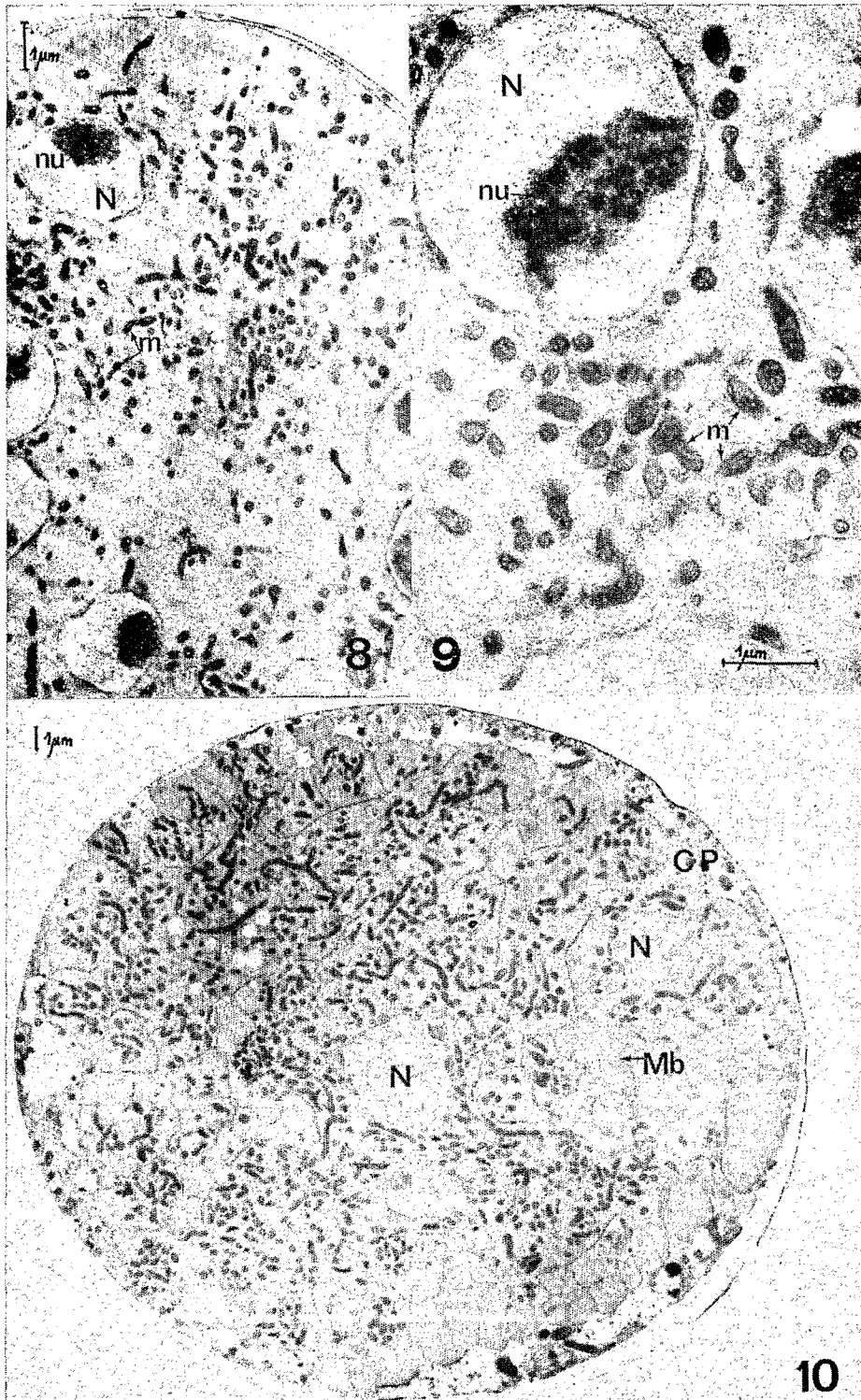


Fig. 8 to 10 ; 8 : Transverse section of gonad through cells at the "Synapsis" stage. Nucleoli are next to the nuclear membranes. The oocytes are not delimited by membranes. (X 6,400) ; 9 : Cross section of gonad at the synapsis stage. There are no membranes between the oocytes. (X 13,300) ; 10 : Cross section of gonad during pachytene. Membranes are formed throughout the section delimiting cytoplasmic islets of varying sizes with or without nucleus. (X 3,000).

CP : parietal cells ; m : mitochondria ; Mb : membranes ; N : nucleus ; nu : nucléolus.

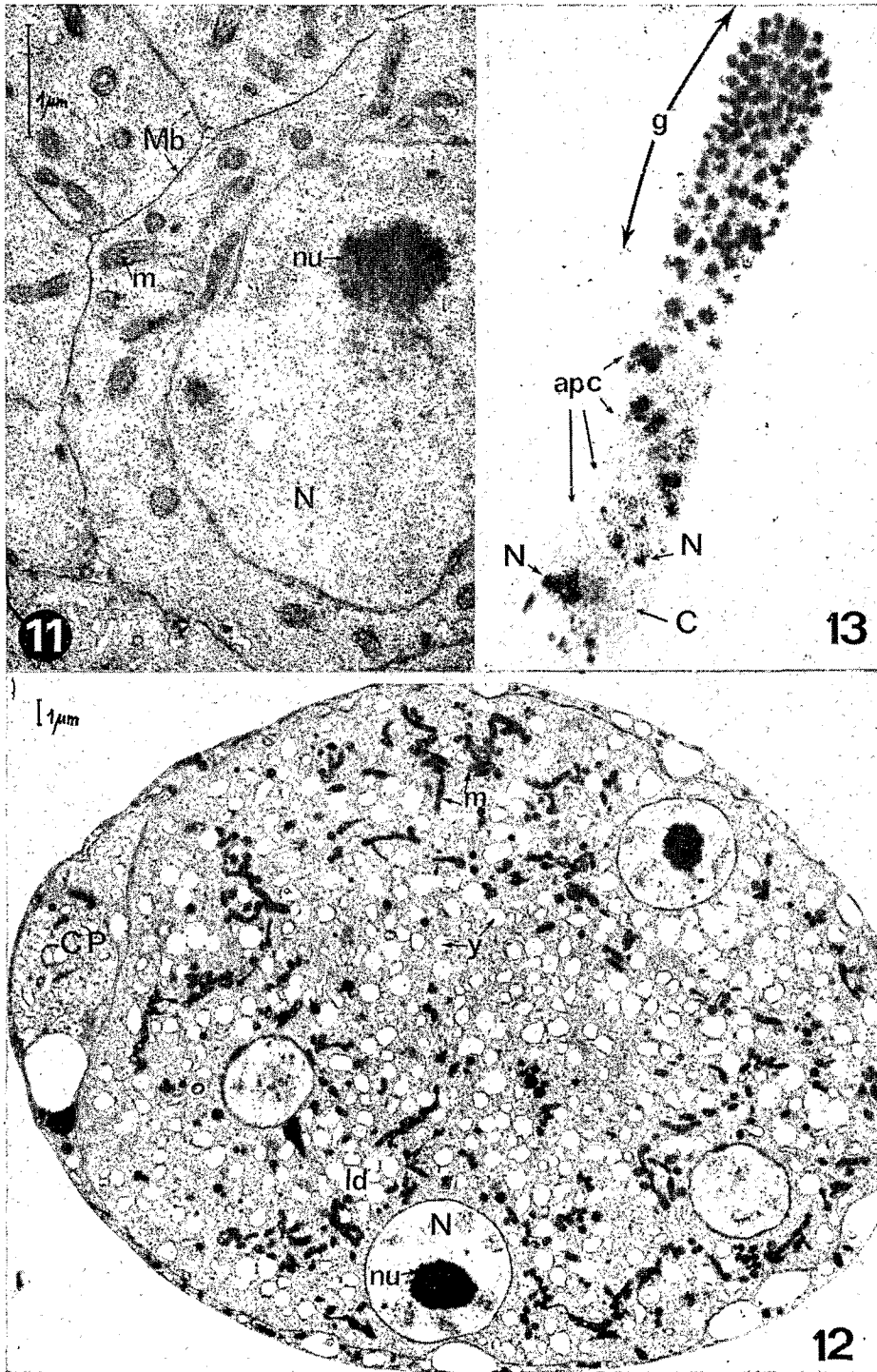


Fig. 11 to 13; 11 : Cross section of gonad through pachytene cells. New membranes surround small cytoplasmic islets, containing mitochondria, endoplasmic reticulum and a few lipidic droplets, and a larger one which also contains one or several oocyte nuclei. (X 15,400); 12 : Transverse section of gonad at the cytoplasmic full growth level. The section shows four nuclei. Note also numerous mitochondria, lipidic droplets and yolk. (X 3,600); 13 : Incorporation of H³ uridine (20 μ Ci/ml) in a gonad incubated for 1 hr in Mg buffer with 3% sucrose. Oogonia and abnormal pachytene cells are labelled in the distal arm at the nuclear and cytoplasmic levels. (X 360).

apc : abnormal pachytene cells ; G : cytoplasm ; CP : parietal cells ; g : oogonia ; ld : lipidic droplets ; m : mitochondria ; Mb : membranes ; N : nucleus ; nu : nucleolus ; y : yolk.

Complete phenotypic expression requires exposure to 24° 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 normal membranes, but contain a large number of "pseudo-pachytene" type nuclei.

Hirsh, Oppenheim and Klass (1976) have suggested that in the loop region, there is normally a remodelling of the membrane as the oocytes pass from the diplotene to the diakinesis stage. At this level, the oocyte and core membranes would be lysed and new oocyte membranes, embodying the oocyte cytoplasm and part of that of the core, would be formed.

In the S_2^{ts} mutant one can envisage a membrane lysis occurring at the beginning of meiotic I prophase, as a result of perturbation of the repression mechanisms of the structural gene(s) responsible for membrane lysis

The blocked meiotic I evolution in oocyte nuclei in full growth could be due to disturbances in the nucleus-cytoplasm relations.

The hypothesis of the intervention of S_2 gene in the regulation mechanisms is strengthened by the incomplete dominance of this mutation (Sadler & Novick, 1965 ; Suzuki, 1976), and by the gradually varying intervention of the gene as a function of the time of exposure to non-permissive temperature.

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