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Development of the Male Reproductive System and Sexual Transformation in the Nematode *Caenorhabditis elegans*

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The small free-living nematode *Caenorhabditis elegans* is usually found as a hermaphrodite, but occasionally true males appear in the population. This study provides an account of gonadogenesis in the normal male and in a mutant that is a temperature-sensitive sex transformer.

Male and hermaphrodite gonads develop from morphologically identical primordia. The small primordial gonad lies on the ventral side of the worm in the coelomic cavity. The gonadial primordium contains four nuclei at parturition. As this primordium develops in a hermaphrodite, it produces a double-armed, mirror symmetrical gonad that produces first sperm and then eggs. In the male, however, this primordium develops into an asymmetrical structure composed of a ventrally located testis, a loop region, a seminal vesicle, and a vas deferens. The male gonad presents a linear sequence of nuclei in successive stages of spermatogenesis beginning with a mitotic region in the testis, followed by clearly distinguishable stages of meiosis throughout the loop region to the seminal vesicle.

A temperature-sensitive sex transformer mutant, *tsB202*, has been isolated. *tsB202* carries an autosomal recessive mutation in linkage group II that at restrictive temperature transforms an XX hermaphrodite into a phenotypic male, complete with a normal male gonad and vestigial external genitalia. These transformed males are classified as pseudomales because they do not exhibit mating behavior. Temperature shift experiments have determined the specific temporal sequences of gonadogenesis, oogenesis, and spermatogenesis. Proper manipulation of the temperature regimen causes the production of intersexes. In one intersex, a male gonad complete with sperm, seminal vesicle, and vas deferens also contains oocytes. In another intersex produced by the complementary temperature shift, a hermaphrodite-shaped gonad develops that produces only sperm and no oocytes.

INTRODUCTION

Genetic control of sexual differentiation is a problem basic to both cellular and developmental biology. The study of mutations affecting sexual dimorphism may provide insight into genetic mechanisms controlling cellular differentiation. Recently, we have begun to study the mechanisms of gonadogenesis during development of the small nematode *Caenorhabditis elegans* (1-3). This free-living soil nematode has the advantages of: (a) a relatively simple morphology with a fixed number of somatic cells; (b) a genetically manipulable system complete with a variety of characterized markers for genetic analysis (4); (c) easily obtainable mutants; (d) a simple and extensively mapped nervous system (5, 6); and (e) a well-characterized gonadogenesis (1, 7-11).

We have studied gonadogenesis in *C. elegans* by isolating temperature sensitive mutants blocked in various stages of development and characterizing them morphologically (2, 3). Previous work analyzed gonadogenesis in the hermaphrodite of *C. elegans*. However, equally important to the study of sexual dimorphism is the analysis of gonadogenesis in the male and the characterization of genes that affect sexual differentiation. The present study provides an account of gonadogenesis in the normal male and in a temperature-sensitive sex transformer mutant. This

mutant develops as a male at high temperature although genotypically it is a hermaphrodite.

MATERIALS AND METHODS

Nematode strains. Caenorhabditis elegans var. Bristol, is from the University of Colorado, Boulder, stock and was originally obtained from Brenner in Cambridge, England. The mutants *lon-2* X E678 and *tra-1* III E1099 were provided by Brenner (4). The temperature-sensitive mutant *tsB202* was isolated in our laboratory after ethylmethanesulfonate mutagenesis. The culture techniques were identical to those reported earlier (1, 4). Briefly, nematode cultures were maintained on agar plates seeded with *Escherichia coli* strain OP50. For analysis of temperature-sensitive mutants, the permissive temperature was 16°C and the restrictive temperature was 25°C.

Synchronous cultures. Synchronous cultures of nematodes were obtained as described previously (4). Briefly, adult and larval worms were washed off agar plates leaving only unhatched eggs, which, because of their adhesive nature, remain on the agar surface. The eggs were then allowed to hatch for 2 hr and all of the newly hatched larvae were collected. In all experiments zero time was the time of hatching.

When it was necessary to obtain synchronous cultures prior to hatching, adult nematodes were dissected and eggs at specific cleavage stages were collected using a micropipet under the dissecting microscope.

EMS mutagenesis. The technique for obtaining temperature-sensitive mutants after mutagenesis with ethylmethanesulfonate was described in detail elsewhere (4).

Microscopy. Routine observations were made with a Wild M5 dissecting microscope equipped with a KG-1 heat-absorbing filter. Observations at higher magnifications were made using a Zeiss Universal Photomicroscope equipped with Zeiss–Nomarski differential interference contrast optics.

Observations on developing gonads were performed on live worms using a technique developed by Sulston (personal communication). Briefly, newly hatched worms were placed on small agar disks (5% agar, 0.5-mm thick) on microscope slides. A slurry of *E. coli* (OP50) was added to the agar disk, followed by a no. 1 coverslip. The edges of the coverslip were sealed with Vaseline to prevent drying. This technique allows continuous observation of gonadogenesis during development of the worm into a mature, fertile adult.

Feulgen preparation. Male gonads were dissected on gelatinized slides in *C. elegans* Ringer's solution by amputating the anterior 1/3 of the worm (1). After dissection, gonads were fixed in Carnoy's fixative and flattened by the addition of a coverslip (12). After the coverslips were removed by dipping in liquid nitrogen, slides were passed through 100, 95, and 75% ethanol solutions and subsequently into distilled water. The dissected gonads were then hydrolized in 1 *N* HCl (60°C) for 9 min and placed in Schiff's reagent for 15 min. After bleaching in 0.5% sulfurous acid the slides were rinsed in distilled water, dehydrated, and mounted in Euparol (GBI Labs Limited).

Scanning electron microscopy. Synchronized worms were washed four times in M9 salt solution at room temperature, followed by fixation in 3% gluteraldehyde in 0.1 *M* sodium cacodylate (pH 7.4) for 24 hr (1). After fixation, the worms were washed twice in cacodylate buffer and postfixed in 1% OsO_4 in 0.1 *M* cacodylate buffer for 15 min. The worms were subsequently dehydrated in 30, 50, 70, 90, and 100% acetone. After dehydration the worms were transferred directly from 100% acetone into liquid CO_2 in a critical point drying apparatus according to the procedure of Porter *et*

al. (13). After coating the specimen with gold in a Denton DV-50 vacuum evaporator, the worms were observed using a Cambridge Stere-o-scan Model S4 scanning electron microscope.

RESULTS

Adult Male Gonad

Although *C. elegans* is usually a hermaphrodite, males occur spontaneously in populations with a frequency of about 1 per 700 worms (11). Males are XO in karyotype and are most easily distinguished from hermaphrodites by the copulatory bursae on their tails. Hermaphrodites are XX. Male stocks are made by crossing spontaneously appearing males with hermaphrodites, and are maintained by repeated backcrossing of male progeny. Newly hatched males, like hermaphrodites, undergo four larval stages (L1 through L4) punctuated by molting. Worms reach maturity after the fourth larval molt. The adult male is approximately 1000-μm long and 50 μm in diameter (Fig. 1). The male nematode, like the hermaphrodite, is encased in a proteinaceous cuticle (14, 15). The nematode feeds through an oral cavity and a bilobed muscular pharynx pumps bacteria into the intestine. In the male both the intestine and the vas deferens empty into a cloaca.

Sensory receptors are located at the front of the animal. Six symmetrically located papillae contain proprioreceptors, mechanoreceptors, and a pair of amphids, which are probably the chemoreceptors. The male has two extra pairs of papillae associated with the outer cephalic papillae (5).

From Fig. 1 it is obvious that the male nematode is a specialized mating vehicle whose main function is fertilizing the hermaphrodite. As many as 900 progeny have been counted from the mating of a single wild-type male (16).

The adult male gonad is an asymmetrical structure located in the coelomic cavity of the worm (Figs. 1 and 2). The testis is found on the ventral side of the worm (Fig. 1). Feulgen staining reveals many mitotic figures in the testis (Fig. 3A and B). Nuclei in the characteristic pachytene stage of meiosis are present through and beyond the loop (Fig. 3A and C). There follows a continuous progression of nuclei in stages of pachytene, diplotene, and diakinesis (Fig. 3D). Between the nuclei in diakinesis and the condensed nuclei of mature sperm there is a small region where one can see meiotic metaphase figures. This is the only place in which meiotic metaphase is seen. Therefore, we presume that both meiotic divisions occur in this small region. Mature sperm are in the seminal vesicle immediately anterior to the vas deferens (Fig. 2). Thus, the male gonad presents a linear sequence of nuclei in successive stages of spermatogenesis. Unlike the protandrous hermaphrodite, the male appears to be capable of continuously producing sperm.

The vas deferens is composed of about 20 cells and is the channel connecting the seminal vesicle to the cloaca and eventually to the exterior. The copulatory bursa is located at the posterior end of the male and opens to the ventral side (Fig. 4, 5a and b). The bursa is composed of a membranelike fan supported by nine rays on each side. A pair of retractable copulatory spicules are located next to the small cloaca. The male inserts these spicules into the vagina of the hermaphrodite during copulation.

Male Gonadogenesis

In a newly hatched male or hermaphrodite larva, the gonadial primordium lies on the ventral side of the coelomic cavity, midway along the length of the worm (Figs. 6a and 7a). There are four nuclei in the primordium, and they begin dividing posteriorly and anteriorly along the longitudinal axis of the worm. Four-

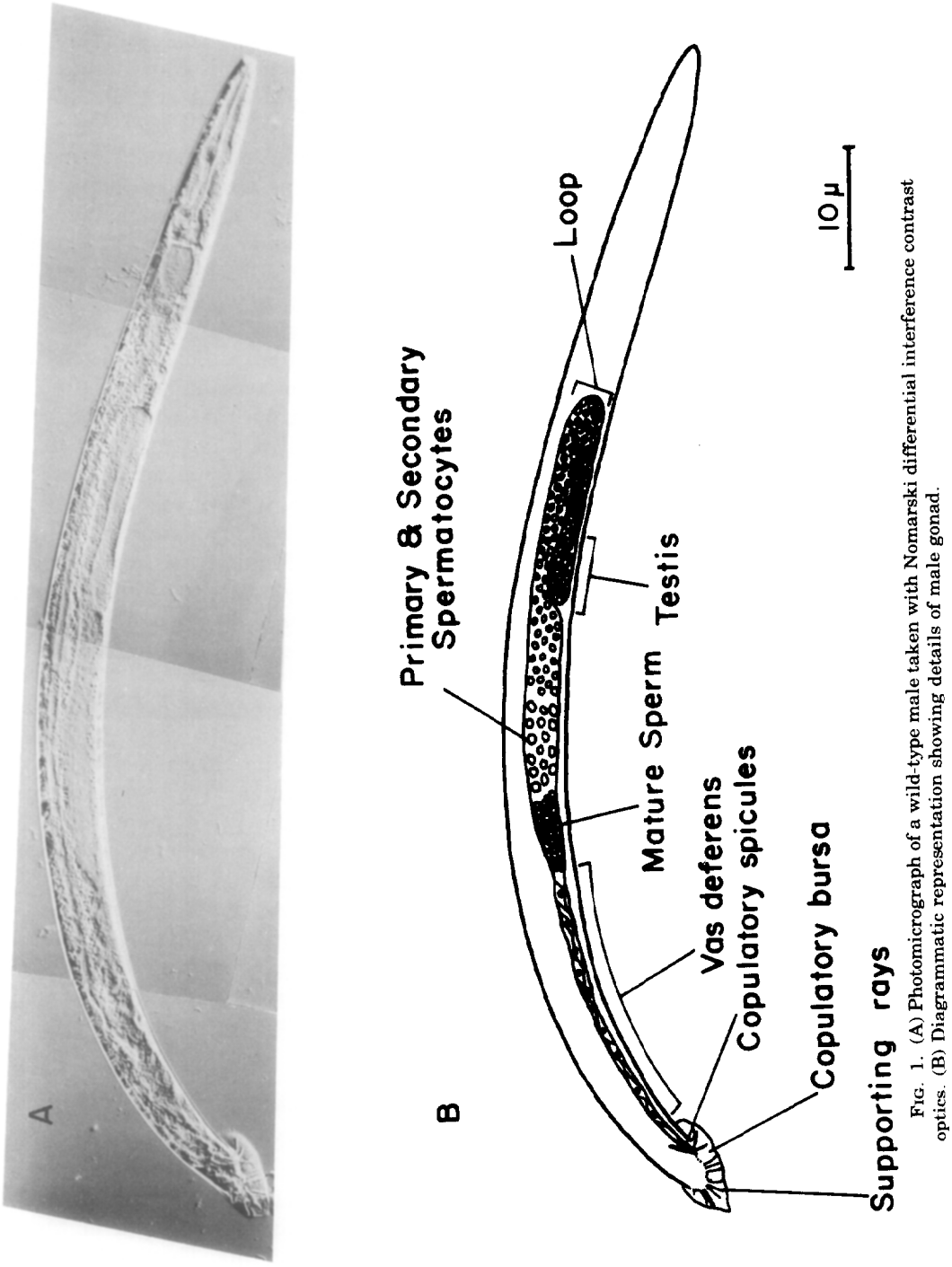


FIG. 1. (A) Photomicrograph of a wild-type male taken with Nomarski differential interference contrast optics. (B) Diagrammatic representation showing details of male gonad.

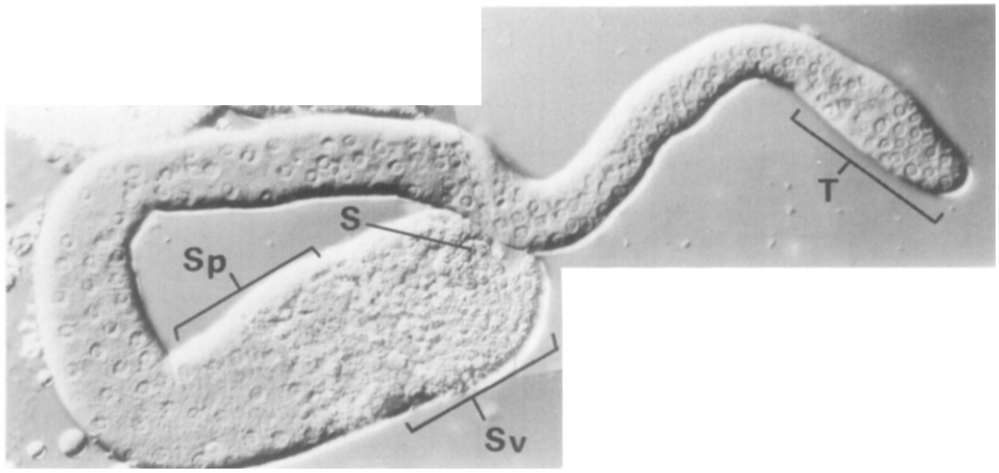


FIG. 2. Photomicrograph (Nomarski optics) of dissected male gonad. Vas deferens is not shown. Sp, spermatocytes; Sv, seminal vesicle; S, mature sperm (magnification 440 ×).

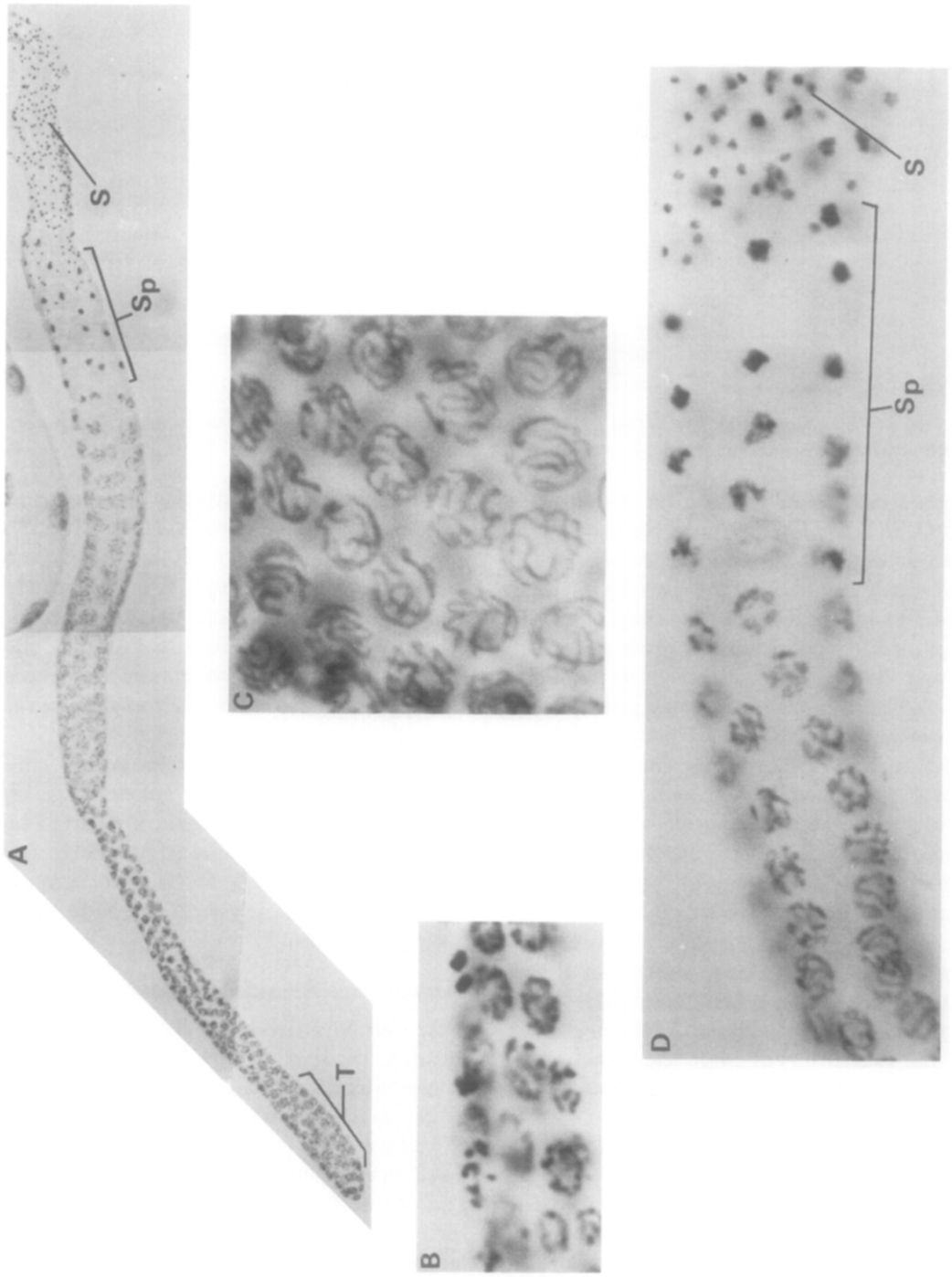
teen to eighteen hours after hatching at 25°C, the developing gonad contains about 30 nuclei (Figs. 6b and 7b). At this stage, the posterior end of the developing male gonad ceases to elongate while the anterior portion continues to grow anteriorly along the ventral side. By the time the gonad contains 80 nuclei, the proliferating anterior end of the gonad begins to make a 180° turn (Fig. 6c). After completing this turn, it continues growing posteriorly along the dorsal side until it passes the nongrowing end on the ventral side (Fig. 6d and e). The proliferating end than flares out and takes a 45° path to the ventral side (Fig. 6f and g). It continues to grow posteriorly and forms a cone-shaped structure, the seminal vesicle, that eventually tapers down to the vas deferens. At the beginning of the fourth larval stage the growing end of the gonad sends out a single line of cells that continues to grow toward the tail. This line of 20 cells joins the cloaca at the same time that the lumen forms down the length of these cells (Fig. 6h). This structure is the vas deferens. During the fourth larval stage sperm maturation takes place. By the fourth larval molt, mature sperm can be seen in the seminal vesicle (Fig. 4).

Unlike the developing hermaphroditic gonad in which both anterior and posterior ends proliferate and reflex 180° (Fig. 7), only the anterior end of the developing male gonad proliferates, first anteriorly and then, after making a 180° dorsal turn, posteriorly until it finally reaches the cloaca. A similar developmental scheme was reported for males of *Rhabditis maupasi* (18).

The adult hermaphroditic gonad contains approximately 150 sperm in each proximal arm. These sperm appear to be derived from approximately 35 to 40 meiotic nuclei which are visible in each arm of the developing gonad by 25 hr after hatching at 25°C.

Temperature-Sensitive Transformer

A temperature-sensitive transformer mutant, designated *tsB202*, was isolated in our laboratory. Microscopy reveals that all of the mutant worms, when reared synchronously at restrictive temperature, have male gonads complete with sperm and vas deferens. The developmental pattern of the gonad of *tsB202* at restrictive temperature is identical to that described above for normal wild-type (XO) males (Fig. 6). The *tsB202* transformed worms are referred to as pseudomales because they exhibit no mating behavior. They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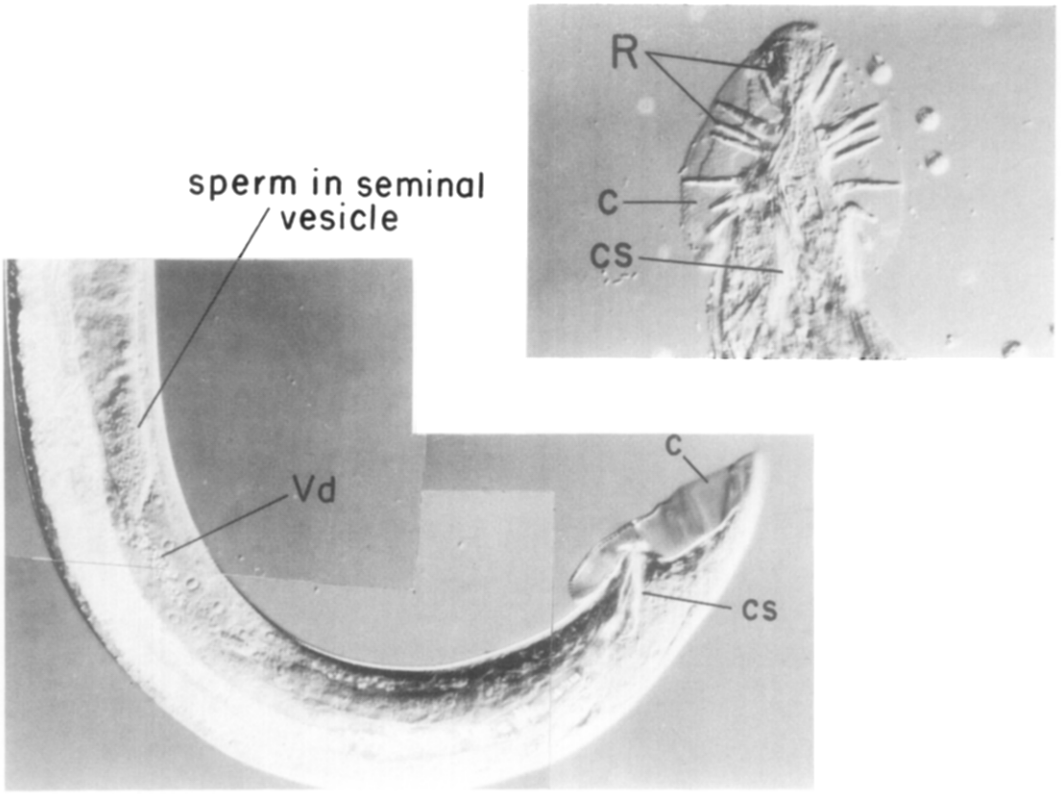

FIG. 4. Nomarski photomicrograph of wild-type male tail (magnification 440 ×). Vd, vas deferens; c, copulatory bursa; cs, copulatory spicules, R, supporting rays.

never attempt to mate with hermaphrodites nor do they display any of the locomotory behaviors peculiar to normal XO males (16).

Temperature shift experiments. The following experiments were performed with *tsB202* worms to determine the critical times of temperature sensitivity *(tcrits)* for expression of the transformer phenotype (Fig. 8 and 9). There are several ways to measure the expression of the transformer phenotype. One measure is the inability of the worms to produce progeny by self-fertilization. The *tcrit* for the inability to produce offspring by self-fertilization extends from at least 6 hr before hatching to 65 hr after hatching (Fig. 8a). Another measure of the transformer phenotype is the presence of a morphologically distinct male gonad. Using this criterion for transfomer expression, the *tcrit* is from 6 hr before hatching to 12 hr after hatching (Fig. 8b). Worms shifted to restrictive temperature after 12 hr have gonads with the basic hermaphroditic morphology. However, if these worms are shifted up prior to 35 hr posthatching, their hermaphroditic gonads lack oocytes and contain excess

FIG. 3. Male gonads were dissected and stained by the Feulgen reaction as described in Materials and Methods. (A) Dissected male gonad; T, testis; Sp, spermatocytes; S, mature sperm (magnification 378 ×). (B) Enlargement of the mitotic region of the testis (magnification 3060 ×). (C) Enlargement of the loop region showing chromosomes in the characteristic pachytene stage (magnification 2430 ×). (D) Enlargement of the region preceding the seminal vesicle showing nuclei in a linear progression of pachytene, diplotene, and diakinesis. Sp, spermatocytes; S, mature sperm (magnification 1530 ×).

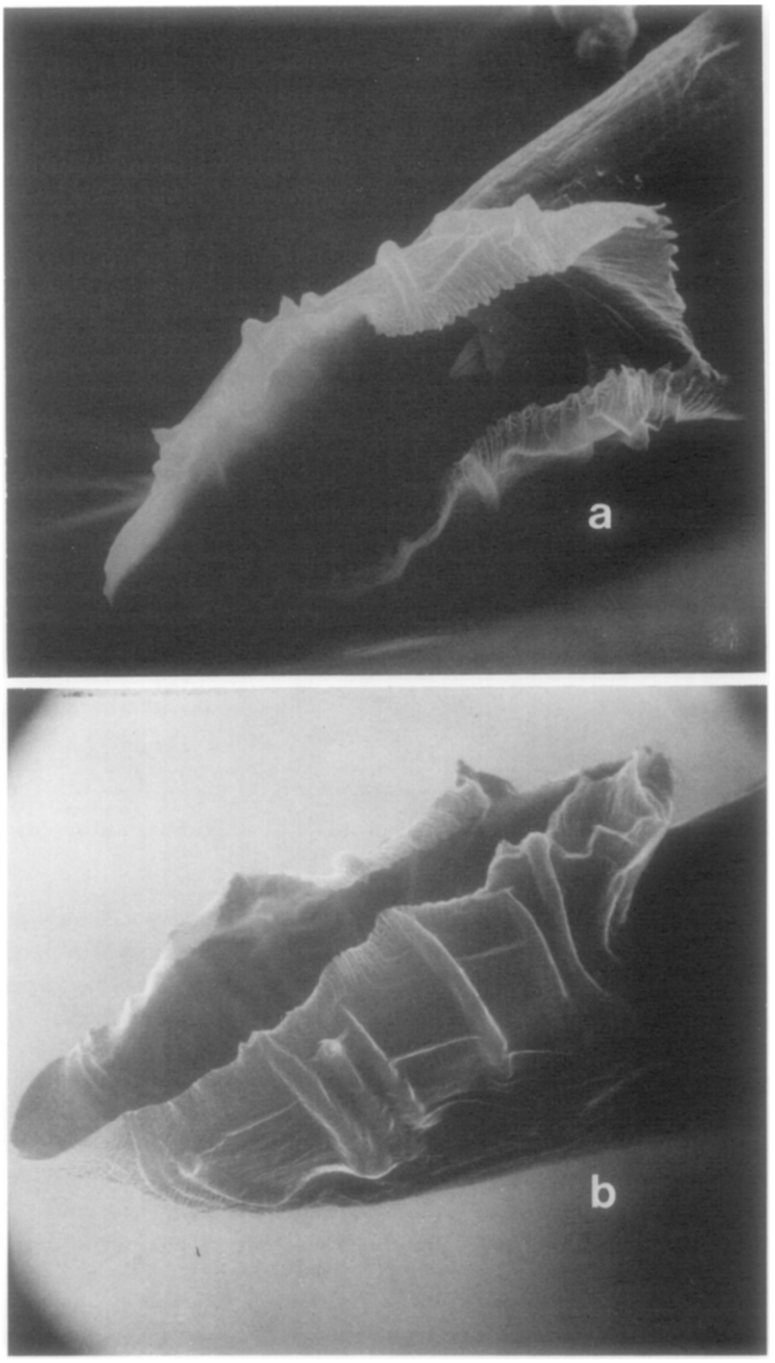

FIG. 5. (a) and (b) Frontal and side views of normal wild-type male tail as seen in the scanning electron microscope (magnification 1900 × and 2000 ×, respectively). (c) Male tail of a *tsB202* pseudomale reared completely at restrictive temperature (magnification 1300 ×). (d) Stunted male tail of a *tsB202* worm reared at restrictive temperature until 6 hr after hatching, then shifted to permissive temperature (magnification 1900 ×).

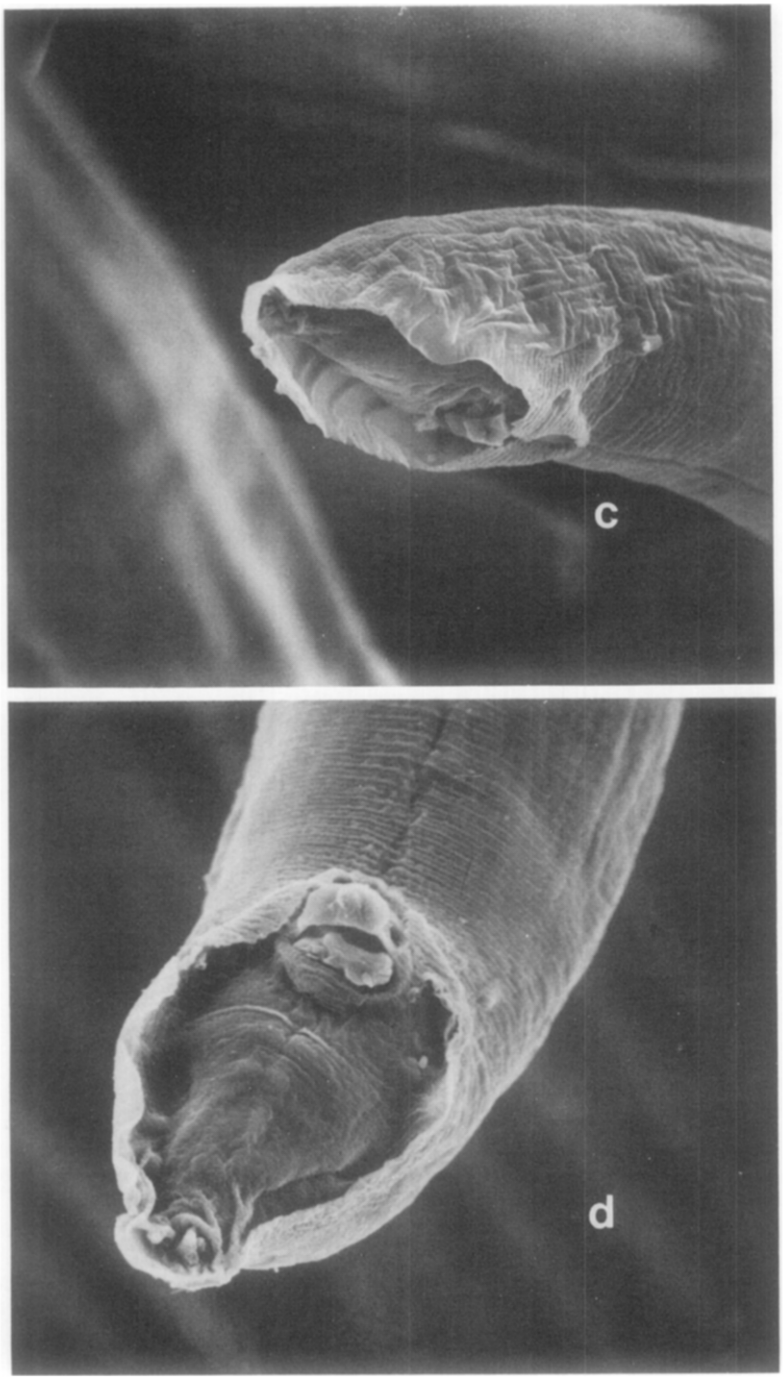

FIG. 5—*Continued*

sperm in each proximal arm (>500 sperm per arm, compared to 150 per arm in wild-type hermaphrodites). The sperm never become incorporated into the spermathecae in these *tsB202* mutant hermaphrodites shifted up prior to 35 hr posthatching. Oocytes appear in animals shifted to restrictive temperature between 35 and 65 hr, but few progeny are produced, suggesting either that the gametes in these ani-

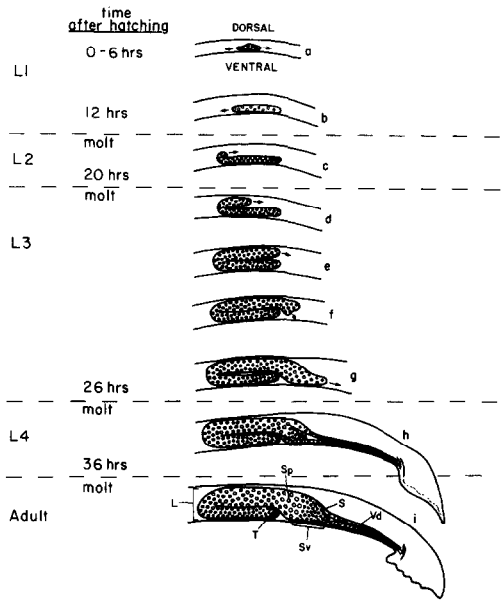

FIG. 6. Diagrammatic representation of the development of a normal male gonad at various times after hatching. 0 hr = time of hatching; T, testis; L, loop; Sp, spermatocytes; Sv, seminal vesicle; S, mature sperm; Vd, vas deferens. All time has been standardized to 25°C time.

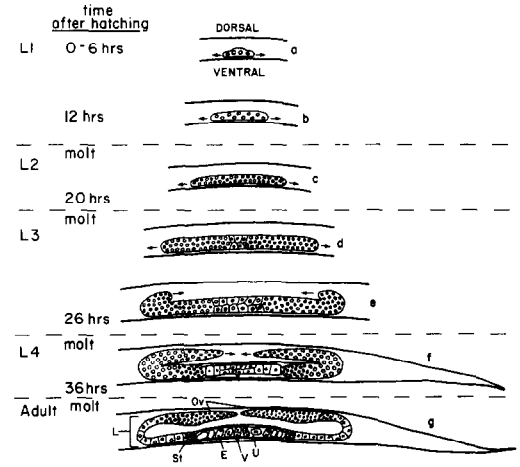

FIG. 7. Diagrammatic representation of the development of a normal hermaphrodite gonad at various times after hatching (time at 25°C). 0 hr = time of hatching. Ov, ovary; L, loop; St, spermateca; U, uterus, V, vulva; E, fertilized egg.

mals are defective or that the oocytes and sperm cannot reach each other.

Therefore, the overall *tcrit* for progeny production in *tsB202* can be divided into three distinct periods. If worms are shifted to restrictive temperature during the first period, a male gonad is produced. If they are shifted up during the second period, a hermaphroditic gonad is produced but oocytes are not made. If worms are shifted up during the third period, both sperm and oocytes are present in the hermaphroditic gonad but few progeny are produced.

Formation of the vulva and the male copulatory bursa also are temperature sensitive. The *tcrit* for vulva formation is between 3 hr before hatching and 24 hr after hatching (Figs. 8e and 9e). Even in *tsB202* adults reared completely at permissive temperature, the vulva often appears abnormal. The formation of the male tail and copulatory bursa is induced by high temperature from 6 hr before hatching to 6 hr after hatching (Figs. 8c and 9c). However, the male copulatory bursa is stunted in *tsB202* worms reared completely at restrictive temperature from fertilization (obtained by placing *tsB202* larvae at 25° just prior to egg formation) (Fig.

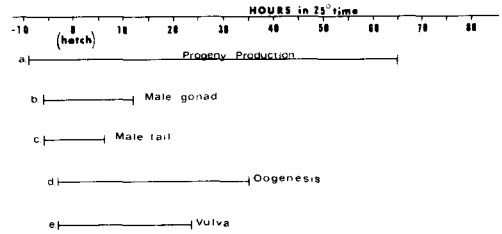

FIG. 8. Summary of critical times of temperature sensitivity *(tcrits)* represented in Fig. 9. The *tcrits* are defined by the earliest time of shift down from 25 to 16°C at which the mutant phenotype is observed and the latest time of shift up from 16 to 25°C that yields the mutant phenotype (2). (a) *tcrit* for progeny production. Restrictive temperature during the indicated period inhibits progeny production. (b) *tcrit* for male gonad development. Restrictive temperature during the indicated period causes the production of a male gonad. (c) *tcrit* for male tail production. Restrictive temperature during the indicated period results in the formation of a male tail. (d) *tcrit* for oogenesis. Restrictive temperature during the indicated period inhibits oocyte production. (e) *tcrit* for vulva development. Restrictive temperature during the indicated period inhibits vulva formation.

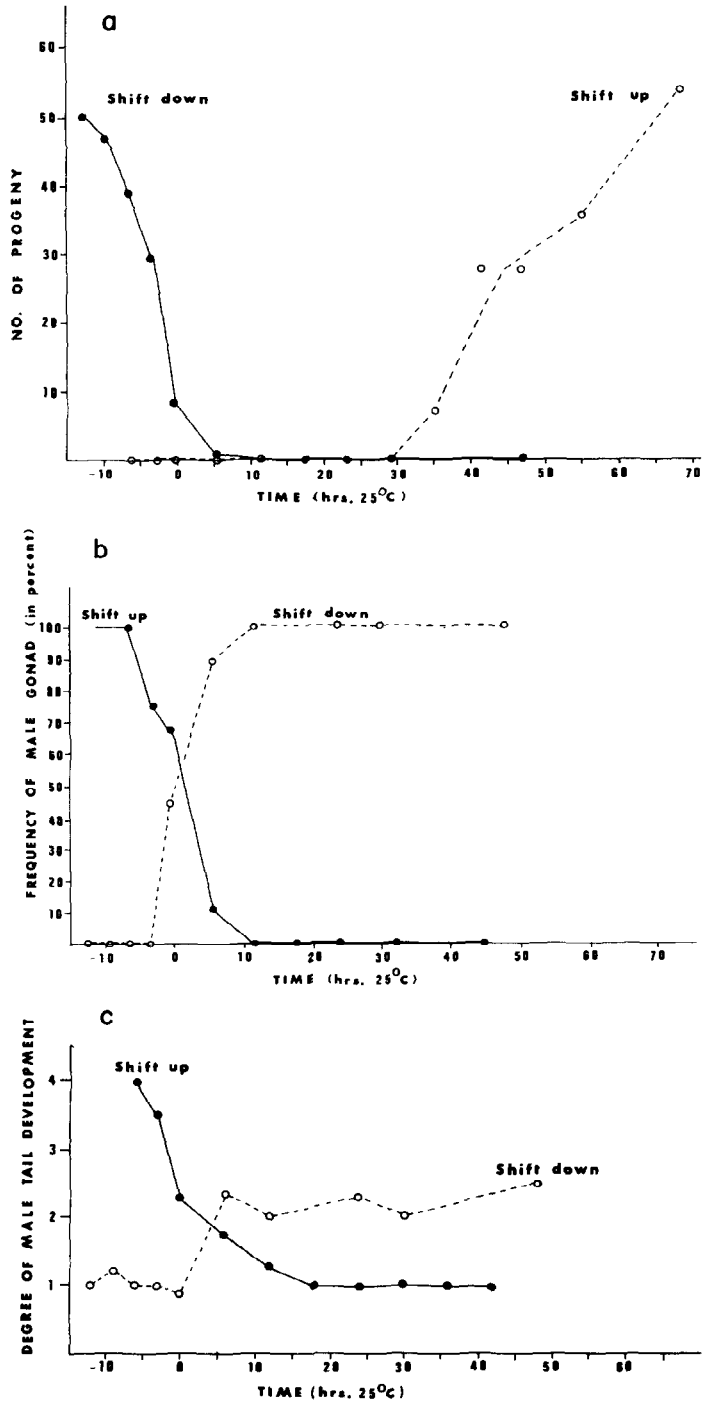

FIG. 9. Temperature shift experiments were done as reported earlier (2). All time is standardized to 25°C time. Each point represents observations on 10 individual worms. 0 hr = time of hatching. (a) Total number of progeny produced by *tsB202* worms subjected to restrictive temperature at various time was recorded. The mean number of progeny from *tsB202* worms reared at permissive temperature was 54 ± 20 (±95% C.I.M.). (b) *tsB202* worms were subjected to restrictive temperature at various times and the frequency of worms having a male gonad was recorded. (c) The degree of male tail development was scored on a scale of 1–4 with 4 being the most malelike and 1 being the most hermaphroditelike. (d) The degree of vulva development was scored on a scale of 0–4 with 4 being a normal hermaphrodite vulva and 0 indicating no vulva.

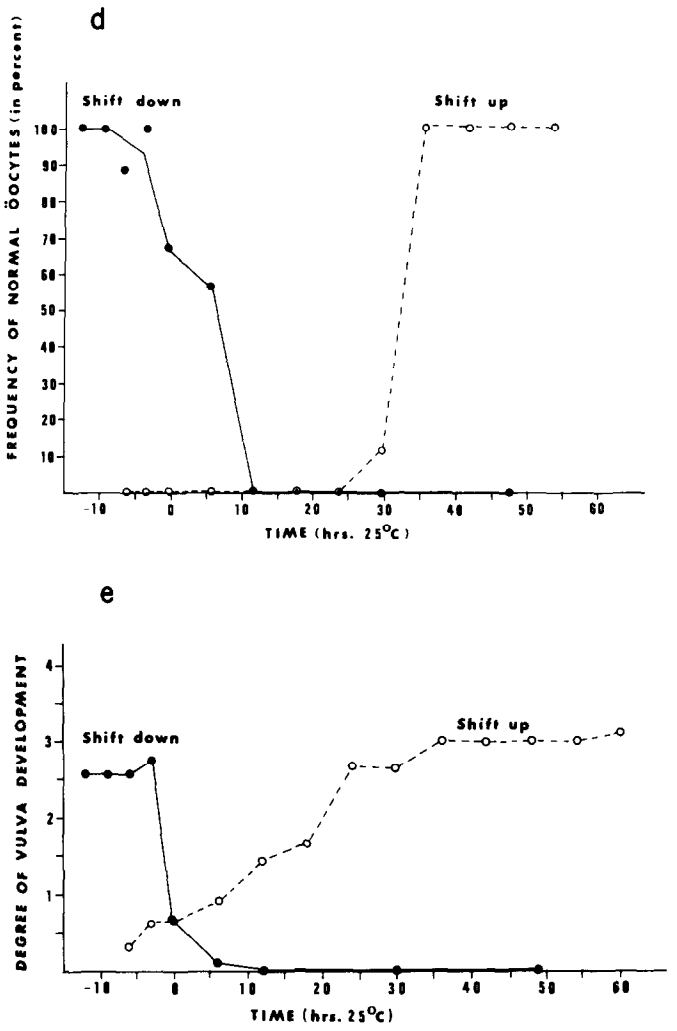

FIG. 9—*Continued*

5c). Shifts to permissive temperature during the *tcrit* for expression of the male tail cause further reduction in the development of the male tail (Fig. 5d).

Intersex. Since *tcrits* for male gonad formation and oocyte formation overlap, it should be possible to obtain intersexes by manipulation of the temperature regimen. Indeed, if *tsB202* embryos are reared at restrictive temperature until 6 hr after hatching and then shifted to permissive temperature for the completion of development, the adult worm has a male-shaped gonad with sperm and oocytes (Fig. 11A). We have not been able to determine the viability of these oocytes. The sperm in the gonad of an intersex animal never fertilize the oocytes of the same gonad. As previously mentioned, the complementary intersex also can be obtained. Rearing *tsB202* at permissive temperature until 10 hr after hatching and then shifting to restrictive temperature for completion of development produces a hermaphrodite-shaped gonad with excess sperm in the proximal arms, but the gonad has no oocytes (Fig. 11B).

Inheritance. Normally, *C. elegans* males are XO and hermaphrodites are XX (4). Genetic crosses were done to deter-

mine whether the *tsB202* mutation is recessive, whether the mutation is autosomal or sex-linked, and whether the transformed pseudomales produced at restrictive temperature are XX rather than XO as in wild-type males. True males as opposed to pseudomales occur spontaneously in the population of *tsB202* worms. These spontaneous males exhibit normal mating behavior regardless of temperature and are XO. All male progeny from a cross between true *tsB202* males and hermaphrodites homozygous for recessive morphological markers display the phenotype of the mother. These XO males of the *tsB202* stock were used as vectors for the *tsB202* mutation in the genetic experiments that are diagrammed in Fig. 10.

tsB202 (XO) males were crossed with *lon* hermaphrodites at high temperature (*lon* is a sex-linked recessive mutation causing abnormally long worms). This cross produced hermaphrodites with wild phenotype. These hermaphrodites are the double heterozygotes from cross fertilization, and their wild phenotype shows that the *tsB202* mutation is recessive.

The *tsB202* mutation was shown to be autosomal in the following way. The double heterozygotes from the previous cross were allowed to undergo self-fertilization at permissive temperature. The long segregants from these hermaphrodites were individually plated and allowed to undergo self-fertilization at restrictive temperature. Twenty-seven of the 114 long worms or one-fourth of the long worms yielded only long pseudomales. One-fourth yielded only long hermaphrodites. The remaining one-half of the long worms yielded both long hermaphrodites and long pseudomales. This segregation ratio of longs and pseudomales shows that the *tsB202* and the *lon* mutation segregate independently. Therefore, the *tsB202* mutation is not sex-linked.

The following analysis shows that the *tsB202* pseudomales produced at restrictive temperature are genotypically XX.

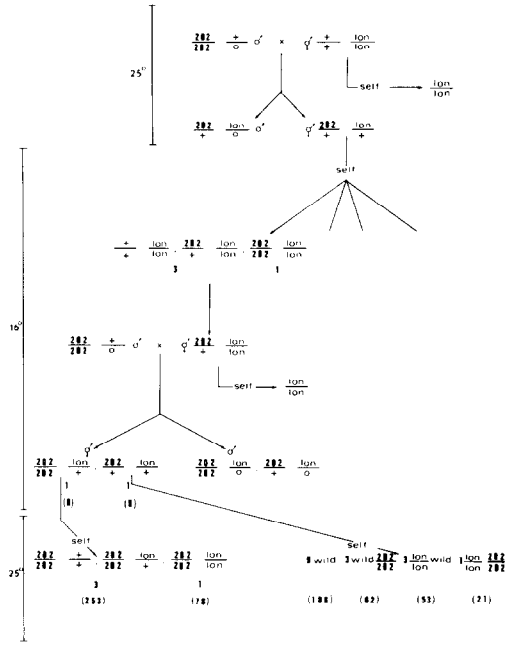

FIG. 10. Diagram of genetic crosses involving *tsB202*. 25°C is restrictive temperature; 16°C is permissive temperature; *lon*, is the marker *lon-2* X E678, a sex-linked recessive mutation causing abnormally long worms; ♂, male; ♀, hermaphrodite; *self*, indicates that hermaphrodites were allowed to undergo self-fertilization. Numbers in parentheses indicate actual number of individuals in that class.

Long worms, heterozygous for *tsB202*, were crossed with *tsB202* (XO) males at permissive temperature. Seventeen hermaphrodite progeny of normal length were obtained from cross-fertilization. Eight of these progeny of normal length produced 253 pseudomales of normal length and 78 long pseudomales at restrictive temperature. The fact that the parents of these progeny are nonlong but segregate both long and nonlong progeny shows that the parents were XX. The 3:1 ratio of nonlong pseudomales to long pseudomales indicates that these progeny pseudomales also must be XX. If the pseudomales produced at restrictive temperature had been XO, then a 1:1 segregation of nonlong to long pseudomales would have been expected. The hermaphrodites heterozygous for both *tsB202* and *lon* were allowed to undergo self-fertilization at restrictive tempera-

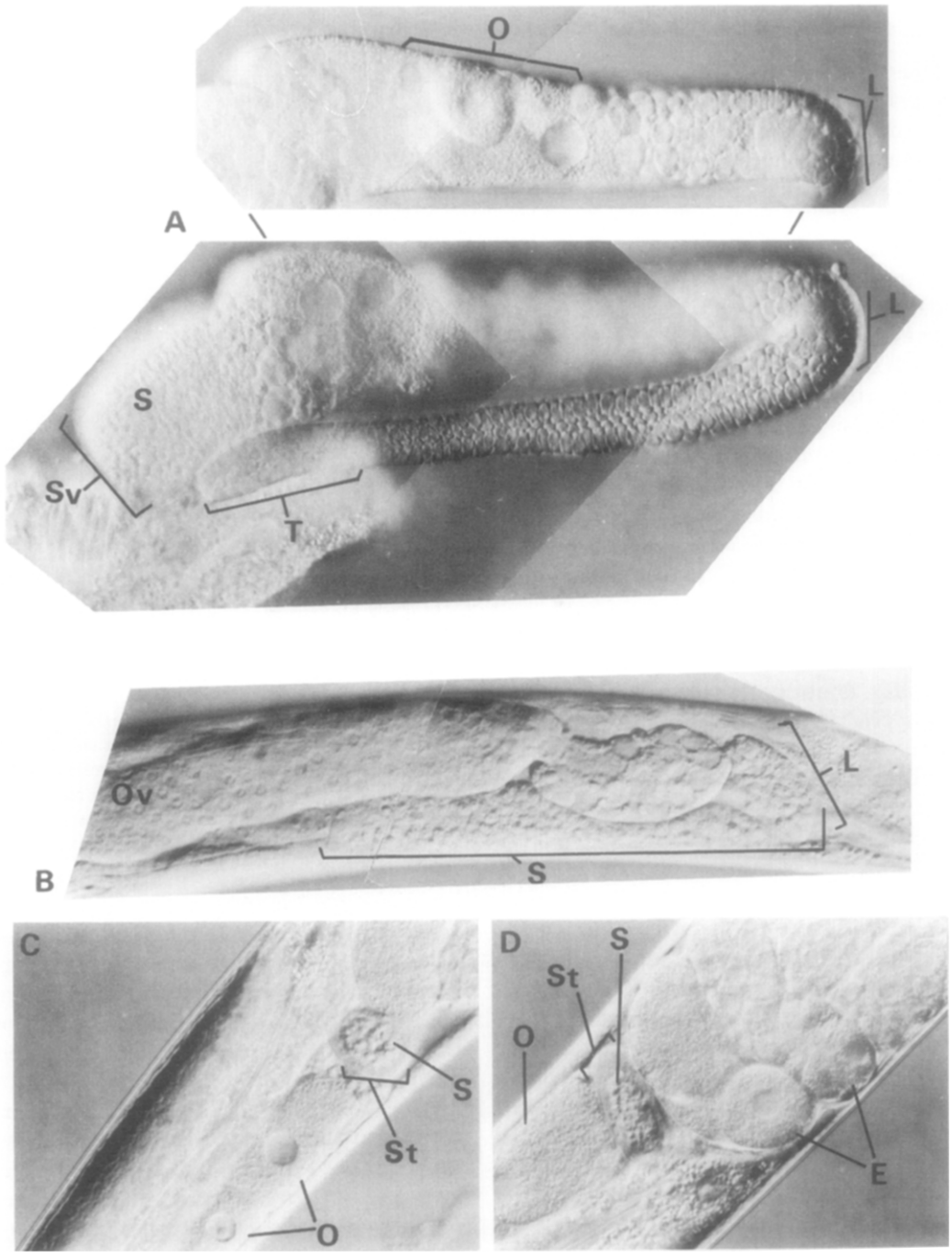

FIG. 11. (A) Dissected malelike gonad from an intersex *tsB202* reared at restrictive temperature until 6 hr after hatching, then shifted to permissive temperature for the remainder of the developmental period. Two focal planes of the same gonad are shown. (B) Photomicrograph of an intersex *tsB202* reared at permissive temperature until 10 hr after hatching, then shifted to restrictive temperature for the remainder of the developmental period. Figure shows only the anterior half of the hermaphrodite-shaped gonad. Sperm completely fill the proximal arms in both halves of the hermaphrodite gonad. (C), (D) *tsB202* reared completely at permissive temperature. (C) Portion of gonad posterior to vulva; (D) portion of gonad anterior to vulva. O, oocytes; L, loop region; S, mature sperm; Sv, seminal vesicle; T, testis; Ov, ovary; St, spermatheca; E, fertilized eggs (magnification 440 ×).

ture. These hermaphrodites produced 186 wild-type progeny, 62 pseudomales of normal length, 53 long hermaphrodites, and 21 long pseudomales. This 9:3:3:1 segregation shows that *lon* and *tsB202* segregate independently and that the *lon* marker segregates in a diploid pattern. The pseudomales produced at restrictive temperature are therefore XX.

tsB202 complements another transformer that is not temperature sensitive, *tra-1* III E1099 described by Hodgkin (16). *tsB202* fails to complement an allele of a second nontemperature-sensitive transformer, *tra-2* II, also described by Hodgkin (16) (S. Brenner, personal communication).

It seemed that *tsB202* might show a maternal effect because of the early *tcrit* for male gonad development (Fig. 8b). To test this possibility, homozygous *tsB202* hermaphrodites were mated with wild-type males at restrictive temperature. The resulting heterozygous progeny had wild phenotype even though they were conceived in homozygous *tsB202* mothers at restrictive temperature. Therefore, *tsB202* does not display a maternal effect.

DISCUSSION

The hermaphrodite gonad of *C. elegans* is a bilateral, mirror symmetrical structure possessing a definite polarity delineated by nuclei in successive mitotic to meiotic stages (1). This polarity of nuclear stages begins in the dorsally located ovaries and progresses through the loops to the ventral oviducts. In contrast, the male gonad is asymmetrical and the polarity begins in a single ventrally located testis, progresses anteriorly through the loop region, and continues posteriorly to the ventral side of the tail to the vas deferens. Thus, the heramphrodite reproductive system develops as two mirror symmetrical gonads and the male reproductive system develops as one asymmetrical gonad. Furthermore, the polarity established by the zones of mitotic and meiotic neuclei is opposite in the two sexes. These differences in symmetry and polarity raise questions about the morphogenesis and differentiation of the reproductive system. The identification of the transformer genes and in particular the temperature-sensitive transformer mutant indicates that the action of a single gene can determine the polarity and symmetry of the developing gonad. The primary gene action need not reside in the gonadial cells themselves. Distant cells could exert hormonal effects on the gonadial cells, or neighboring somatic cells, such as neurons or hypodermal cells, could exert contiguous effects on the differentiation of the gonadial cells. Interaction between somatic and germ cells seems essential for the organization of testicular structure in mammals (17). The resultant differences in symmetry and polarity could arise from alterations in the orientations and/or number of mitoses of the developing gonadial cells.

Mutations that lead to sexual transformation have been described in Drosophila (19), mice (20), and nematodes (16). The temperature-sensitive transformer, *tsB202*, aids in elucidating the temporal nature of gene expression for sexual determination. The information from *tsB202* infers that a decision as to whether a male or hermaphordite gonad will be made occurs very early (6 hr before hatching to 12 hr after hatching), much earlier than any sexual differentiation can be observed microscopically in the gonad itself. The developing gonad has only 10 cells by the end of this critical time of temperature sensitivity, yet these cells have been predetermined to a set developmental plan that cannot be changed by further manipulation of the temperature. A decision as to whether or not oocytes will be produced is made independently and at a different time, between hatching and 35 hr after hatching. In the *tsB202* mutant exposed to the proper temperature regimen, oocyte development can be induced in a male gonad.

In the normal hermaphrodite, oocytes are derived from nuclei in the late stages of diplotene and the early stages of diakinesis. These nuclei are located throughout the loop region (1). In one type of intersex produced by manipulation of the temperature regimen, a gonad with the basic male morphology is observed. This malelike gonad possesses primary and secondary spermatocytes and a seminal vesicle filled with mature sperm. However, anterior to the primary spermatocytes, nuclei in late diplotene and early diakinesis are located in cells that are mrophologically distinguishable as oocytes. Therefore, the specific stage of meiosis is important for oogenesis rather than the specific location of nuclei in a hermaphrodite gonad. One suspects that the nuclei in pachytene and early diplotene are in an indeterminate state and that these nuclei can produce either sperm or oocytes.

In a normal hermaphrodite approximately 150 sperm are produced in each arm of the gonad. By 25 hr after hatching, the precursor nuclei to the sperm have been laid down in the course of gonadogenesis. Nuclei laid down at later times develop into oocyte precursor nuclei and enter pachytene and early diplotene. In a second type of intersex produced by temperature shifts, a gonad with the basic hermaphroditic morphology is formed. The proximal arms of the gonad are completely filled with sperm but the gonad lacks oocytes. A shift to restrictive temperature 10 hr after hatching suppresses oocyte development, and many of the oocyte precursor nuclei appear to undergo spermatogenesis in the absence of an oocyte-producing stimulus. Again, one suspects that nuclei in late pachytene and early diplotene are in an indeterminate state and can produce either sperm or oocytes. Both these intersexes indicate that production of oocytes depends on a signal being received by nuclei in the late stages of pachytene and early diplotene and that this signal is temeprature sensitive in the mutant *tsB202*.

The normal production of oocytes in the hermaphrodite involves the presence of a core of cytoplasm in the center of the gonad. This core has been described in detail and has been hypothesized to be a necessary contributor of ooplasm to the developing oocytes (1). Our preliminary results from electron microscopy indicate that the gonad of a normal XO male also contains a cytoplasmic core. However, the core is less extensive than that found in a hermaphrodite. It would be important to establish by electron microscopy whether the core in the gonad of a pseudomale and an intersex resembles the core seen in the hermaphrodite gonad or that observed in the male gonad.

Other transformer mutations in *C. elegans* that are not temperature sensitive have been studied in detail by Hodgkin (16). These mutations transform genotypic XX hermaphrodites to males regardless of rearing temperature. Thus far two genes have been identified: *tra-1* in linkage group III and *tra-2* in linkage group II. *tsB202* is the only temperature-sensitive transformer isolated. Generally, *tra-1* XX males have normal male gonads with reduced numbers of sperm, normal copulatory bursae, and normal mating behavior; *tra-2* XX worms are pseudomales that have defective copulatory bursae although they have normal male gonads with sperm. *tra-2* Pseudomales never attempt to mate (16).

These findings indicate that the expression of at least two different genes is necessary for normal hermaphrodite development, and that mutations in either of these genes can transform a genotypic hermaphrodite into a male. One possible interpretation originally suggested by Hodgkin (16) is that the developing gonad has a propensity toward maleness unless otherwise determined by hermaphrodite-directing genes.

Normally, a genotypic XX gonad will develop according to the scheme A → B′ → C′ under the direction of genes *tra-1* and *tra-2*. However, in the case of *tra-1* mutant, the normal product of this gene is missing and a genotypic XX gonad develops according to the scheme A → B, to produce phenotypic males. When expression of *tra-2* is absent, the XX gonad develops according to the scheme A → B′ → C, producing a pseudomale with normal male gonad but vestigial genitalia and no mating behavior. In this scheme *tra-2* would be suppressed by *tra-1* because the decision has been made to go A → B and because *tra-2* acts only on pathway A → B′. Hodgkin has shown experimentally that indeed *tra-1* is epistatic to *tra-2* (16).

Since males are XO and hermaphrodites are XX, any explanation of sexual determination must involve dosage differences between the hemizygous males and XX hermaphrodites. One possible model is that an inducer on the X chromosome must be in a 2X concentration to induce the autosomal *tra-1* gene which in turn acts to induce the *tra-2* gene to produce a normal hermaphrodite. In normal XO males the single dose of inducer is insufficient to activate the *tra-1* gene.

The behavioral comparisons of the male nematode and the transformed males indicate that the same genes that determine morphology might also determine specialized mating behavior. The *tra-1* mutant males exhibit normal male mating behavior in contrast to *tra-2* pseudomales. Because *tra-1* mutant males exhibit normal male behavior, it is suggested that the normal allele of the *tra-1* gene suppresses male behavior in a genotypic hermaphrodite, whereas the wild-type allele of the *tra-2* gene is not involved in the determination of behavior. Therefore *tra-2* pseudomales are never sexually active because the normal allele of the *tra-1* gene is present to suppress male behavior.

The nervous system of normal males differs morphologically from that found in hermaphrodites (16). Therefore, the temperature-sensitive transformer *tsB202* also offers the possibility of probing the temporal expression of a gene affecting neurological development.

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REFERENCES

1. HIRSH, D., OPPENHEIM, D., and KLASS, M. (1976). Development of the reproductive system of *Caenorhabditis elegans*. *Develop. Biol.*, **49**, 200–219.
2. HIRSH, D., and VANDERSLICE, R. (1976). Temperature sensitive developmental mutants of *Caenorhabditis elegans*. *Develop. Biol.*, **49**, 220–235.
3. VANDERSLICE, R., and HIRSH, D. (1976). Zygote defective mutants of *Caenorhabditis elegans*. *Develop. Biol.*, **49**, 236–249.
4. BRENNER, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.
5. WARD, S., THOMPSON, N., WHITE, J., and BRENNER, S. (1975). Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J. Comp. Neurol.* **160**, 313–338.
6. WARE, R. W., CLARK,D., CROSSLAND, K., and RUSSELL, R. L. (1975). The nerve ring of the nematode *Caenorhabditis elegans:* Sensory input and motor output. *J. Comp. Neurol.* **162**, 71–110.
7. HONDA, H. (1925). Experimental and cytological studies on bisexual and hermaphrodite free-living nematodes, with special reference to problems of sex. *J. Morphol. Physiol.* **40**, 191–233.
8. NIGON, V. (1965). Développement et reprduction des nématodes. *In* "Traité de Zoologie" (P.-P. Grassé, ed.), Vol. 4(2), pp. 218–386. Masson et Cie., Paris.
9. NIGON, V., and BRUN, J. (1955). Evolution of nuclear structures during oogenesis in *Caenorhabditis elegans*. *Chromosoma* **1**, 129–169.
10. NIGON, V., GUERRIES, P., and MONIN, H. (1960). L'architecture de l'oeuf et les movements des

constituants cellulaires au cours des premièrs étapes du développement chez qulques nématodes. *Bull. Biol. Fr. Belg.* **93**, 131–202.

11. NIGON, V. (1949). Modalités de la reproduction et déterminisme du sexe chez quelques Nématodes libres. *Ann. Sci. Nat. Zool. (ser. II)*, **11**, 1–32.
12. HUMASON, G. G. (1967). "Animal Tissue Techniques." W. H. Freeman and Co., San Francisco.
13. PORTER, K. R., PRESCOTT, D., and FRYE, J. (1973). Changes in surface morphology of CHO cells during the cell cycle. *J. Cell Biol.* **57**, 815–836.
14. JOSSE, J., and HARRINGTON, W. F. (1964). Role of pyrolidine residues in the structure and stabilization of collagen. *J. Mol. Biol.* **9**, 269–287.
15. BIRD, A. F., and DEUTSCH, K. (1957). The structure of the cuticle of *Ascaris lumbricoides* var. Suis. *Parasitology* **47**, 319–328.
16. HODGKIN, J. A. (1974). Genetic and anatomical aspects of the *Caenorhabditis elegans* male. Ph.D. Thesis, Darwin College, Cambridge, England.
17. WACHTEL, S., OHNO, S., KOO, G. C., and BOYSE, E. A. (1975). Possible role of H-Y antigen in the primary determination of sex. *Nature (London)* **257**, 235.
18. COIFAIT, H. (1959). Enumeration Des Grottes Visiteés. *Arch. Zool. Exp. Gen.* **97**, 209–465.
19. STURTEVANT, A. H. (1945). A gene in *Drosophila melanogaster* that transforms females into males. *Genetics* **30**, 297–299.
20. CATTANACH, B. M., POLLARD, C. E., and HAWKES, S. G. (1971). Sex-reversed mice: XX and XO males. *Cytogenetics* **10**, 318–337.