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A *Caenorhabditis elegans* RNA Polymerase II Gene, *ama-1 IV*, and Nearby Essential Genes

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ABSTRACT

The amanitin-binding subunit of RNA polymerase II in *Caenorhabditis elegans* is encoded by the *ama-1* gene, located approximately 0.05 map unit to the right of *dpy-13 IV*. Using the amanitin-resistant *ama-1(m118)* strain as a parent, we have isolated amanitin-sensitive mutants that carry recessive-lethal *ama-1* alleles. Of the six ethyl methanesulfonate-induced mutants examined, two are arrested late in embryogenesis. One of these is a large deficiency, *mDf9*, but the second may be a novel point mutation. The four other mutants are hypomorphs, and presumably produce altered RNA polymerase II enzymes with some residual function. Two of these mutants develop into sterile adults at 20° but are arrested as larvae at 25°, and two others are fertile at 20° and sterile at 25°. Temperature-shift experiments performed with the adult sterile mutant, *ama-1(m118m238ts)*, have revealed a temperature-sensitive period that begins late in gonadogenesis and is centered around the initiation of egg-laying. Postembryonic development at 25° is slowed by 30%. By contrast, the amanitin-resistant allele of *ama-1* has very little effect on developmental rate or fertility. We have identified 15 essential genes in an interval of 4.5 map units surrounding *ama-1*, as well as four γ-ray-induced deficiencies and two duplications that include the *ama-1* gene. The larger duplication, *mDp1*, may include the entire left arm of chromosome IV, and it recombines with the normal homologue at a low frequency. The smallest deficiency, *mDf10*, complements all but three identified genes: *let-278*, *dpy-13* and *ama-1*, which define an interval of only 0.1 map unit. The terminal phenotype of *mDf10* homozygotes is developmental arrest during the first larval stage, suggesting that there is sufficient maternal RNA polymerase II to complete embryonic development.

RNA polymerase II is the enzyme responsible for mRNA synthesis in eukaryotic cells. It seems likely that a clear understanding of RNA polymerase II function will be needed before the mechanisms that control gene transcription during development can be understood. The development of the nematode *Caenorhabditis elegans* has been described, and this animal is also well suited to a genetic analysis of RNA polymerase II structure and function. By selecting mutant strains resistant to the fungal toxin, α-amanitin (amanitin), SANFORD, GOLOMB and RIDDLE (1983) identified a gene (*ama-1*) that appears to encode a subunit of RNA polymerase II. The *ama-1* gene was initially defined by the dominant mutation, *m118*, and was mapped close to *dpy-13* on linkage group (LG) IV. Strains carrying *m118* produce an RNA polymerase II that is 150-fold less sensitive to amanitin *in vitro* than the wild-type enzyme. RNA polymerase II from *C. elegans* has been purified (SANFORD, PRENGER and GOLOMB 1985) and found to be similar to RNA polymerase II from other eukaryotes (LEWIS and BURGESS 1982; SENTENAC 1985). It consists of two large subunits with apparent molecular weights of 200,000 and 135,000, and eight smaller subunits ranging in size from 29,000 to 9,500.

A genetic approach to the study of RNA polymerase II structure and function involves the isolation of mutations that affect enzyme function, and the identification of genes encoding the various subunits that constitute polymerase activity. The mutant enzymes obtained can be analyzed biochemically, and the mutations themselves can be characterized by genetic and molecular methods. The value of the genetic approach has been demonstrated by GREENLEAF *et al.* (1979), who isolated an amanitin-resistant mutant of *Drosophila melanogaster* that was shown to carry a mutation in an *X*-linked gene (*RpII215*) encoding a subunit of RNA polymerase II. Lethal and conditional-lethal alleles of *RpII215* have been identified, and the developmental effects of several of these mutations studied (GREENLEAF *et al.* 1980; COULTER and GREENLEAF 1982; MORTIN and LEFEVRE 1981; MORTIN and KAUFMAN 1982, 1984). The *RpII215* locus was cloned using *P*-element transposon tagging (SEARLES *et al.* 1982), allowing its identification as the gene encoding the largest subunit of RNA polymerase II (GREENLEAF 1983). The cloned *RpII215* DNA was shown to be homologous to the gene that mutates to confer amanitin-resistance in mammalian cells (INGLES *et al.* 1983). By analogy with these results, the *ama-1*

gene in *C. elegans* should encode the largest subunit of nematode RNA polymerase II.

This paper describes an analysis of the *C. elegans ama-1 IV* gene and the adjacent chromosomal region. We have isolated lethal mutations in *ama-1*, including temperature-sensitive (*ts*) alleles, and we have characterized the mutant phenotypes. Also, we have identified 15 essential genes in a 4.5 map units region surrounding *ama-1*. Chromosomal deficiencies and duplications have been characterized for use in genetic and physical mapping, and in gene dosage studies.

MATERIALS AND METHODS

Culture conditions: Nematodes were grown on NG agar plates spread with *E. coli* strain OP50 (BRENNER 1974). Adult hermaphrodites were tested for resistance to amanitin in microtiter wells (Costar 96-well dishes) containing 50 μl of a 1% (w/w) suspension of *E. coli* strain OP50 in S medium (SULSTON and BRENNER 1974), and 20 μg/ml amanitin (Boehringher Mannheim). Resistant hermaphrodites produced progeny that grew and reproduced in the presence of amanitin, whereas the progeny of sensitive hermaphrodites arrested development as first-stage (L1) larvae (SANFORD, GOLOMB and RIDDLE 1983). Several sensitive (wild-type) and resistant (*ama-1(m118)*) hermaphrodites were included in each test as controls.

Genetic markers: The nomenclature used in this paper conforms to the recommendations of HORVITZ *et al.* (1979). All mutant strains are derivatives of the wild-type Bristol strain, N2 (BRENNER 1974). The mutations used in this study are: *unc-13(e450)I*, *unc-4(e120)II*, *unc-32(e189)III*, *unc-25(e156)III*, *unc-45(e286)III*, *daf-7(e1372)III*, *dpy-9(e12)IV*, *unc-17(e113)IV*, *dpy-13(e184)IV*, *ama-1(m118)IV*, *unc-5(e53)IV*, *unc-8(e49)IV*, *unc-24(e138)IV*, *daf-15(m81)IV*, *daf-14(m77)IV*, *unc-43(e408)IV*, *unc-22(s7)IV*, *let-52(s42)IV*, *unc-31(e169)IV*, *unc-30(e191)IV*, *dpy-4(e1166)IV*, *unc-42(e270)V* and *sup-7(st5)X*. The *ama-1(m118)* mutation confers dominant resistance to amanitin, *dpy-13(e184)* is semidominant, and the remaining mutations are recessive to their wild-type alleles. A lethal mutation, such as *m236*, derived from an *ama-1(m118)* parent was designated *ama-1(m118m236)* when the secondary mutation was within the *ama-1* gene, as determined by loss of resistance to amanitin and by complementation testing.

Three-factor recombination mapping of *ama-1*: Two strains, one of genotype *unc-17 dpy-13* +/+ + *ama-1(m118)* and the other of genotype *dpy-13* + *unc-5*/+ *ama-1(m118)* +, were constructed to position *ama-1* relative to *dpy-13*. All of the progeny of hermaphrodites from these two strains were screened for dumpy (Dpy) recombinants which, when found, were placed in microtiter wells containing amanitin to identify those carrying *ama-1*. Resistant Dpys would be found among the progeny of heterozygotes carrying *unc-17* if *ama-1* were located to the left of *dpy-13*, and conversely, among the progeny of heterozygotes carrying *unc-5*, if *ama-1* were to the right of *dpy-13*. A homozygous *dpy-13 ama-1(m118)* strain was established from a *dpy-13 ama-1(m118)* +/*dpy-13* + *unc-5* recombinant.

Isolation of EMS-induced mutants: Hermaphrodites of genotype *dpy-13 ama-1(m118)* were treated with EMS (ethyl methanesulfonate, from Sigma) as described by BRENNER (1974), except that 0.025 M EMS was used instead of 0.05 M. The mutagenized hermaphrodites were mated with wild-type (N2) males. A total of 3200 semi-Dpy F_1 hermaphrodites were selected as fourth-stage (L4) larvae, placed singly onto 60 × 15 mm Petri plates and incubated at 25°. After 4 days, the plates were screened for the presence or absence of fertile, adult Dpy progeny. Fertility was assessed by observing eggs within the uteri, and by testing for production of self-progeny. The absence of Dpy progeny indicated that a lethal mutation had been induced on the *dpy-13 ama-1* chromosome. Strains heterozygous for lethal mutations were crossed with N2 males and balanced after confirmation of their genotypes.

To identify mutations affecting the *ama-1* gene, the lethal strains obtained in this screen and the screen for γ-ray-induced mutants (see below) were tested for loss of resistance to amanitin. Hermaphrodites heterozygous for a lethal or sterile mutation linked to *dpy-13 ama-1* were placed in each of two microtiter wells (two hermaphrodites per well) containing amanitin, and incubated at either 15°, 20° or 25°. The wells were screened at the appropriate time for the absence of adult progeny. The *ama-1* lethal mutations were balanced over *nT1*, a reciprocal translocation between chromosomes *IV* and *V* (FERGUSON and HORVITZ 1985), whereas the remaining lethal and sterile mutations were balanced over a chromosome carrying *unc-17* and *unc-5*. These procedures involved two crosses with unmutagenized genetic backgrounds.

Isolation of γ-ray-induced mutants: Hermaphrodites of genotype + *dpy-13 ama-1(m118)* + / *unc-17* + + *unc-5* were exposed to 1500 rad of γ-radiation from a cobalt-60 source (ROSENBLUTH, CUDDEFORD and BAILLIE 1983). Their heterozygous self-progeny were selected as L4 larvae or young adults, and were placed individually onto 60-mm plates. The progeny of these hermaphrodites were screened as described above. Confirmed lethal or sterile mutations were crossed with N2 males, and semi-Dpy F_1 male progeny were crossed with *unc-17 unc-5* hermaphrodites to produce a balanced semi-Dpy, non-Unc reference stock. Deficiencies obtained in this screen also were balanced over *nT1*.

Lethal phenotypes: The lethal phenotypes of the *ama-1* mutants were determined at 15°, 20° and 25° as follows. Initially, the progeny of hermaphrodites heterozygous for the *ama-1* lethal mutation (*e.g.*, *dpy-13 ama-1(m118m236)*/*nT1(IV)*; +/*nT1(V)*) were screened for Dpy segregants which, when present, were selected and placed on a separate plate. After 5–7 days these plates were examined to determine whether the worms were arrested as larvae or if they had become adults and, if so, whether they had laid any eggs.

A different procedure was used for the heterozygous strains that did not segregate Dpy progeny. Since strains carrying *nT1* produce arrested embryos and larvae, these strains were first crossed with N2 males to remove the translocation. Twenty hermaphrodites of genotype *dpy-13 ama-1(m118mx)*/+ +, where *mx* represents a lethal mutation, were placed on a plate, allowed to lay eggs for 2 hr and then removed. This plate was screened after 24 hr for unhatched eggs, the presence of which indicated that the mutant arrested development prior to hatching. To determine the stage of embryonic arrest, several embryos were transferred to an agar pad on a microscope slide and viewed with Nomarski optics (SULSTON and HORVITZ 1977). If no unhatched eggs were found, the plate was screened after another 24 hr for the presence of arrested larvae. When present, larvae were transferred to a separate plate and observed for 3–4 days. The arrested mutants were classified as early or mid-larval lethals based on their size and appearance. The characterization of the mutations defining other essential genes near *ama-1* was done in a similar manner.

Suppression tests: To determine whether any of the lethal *ama-1* alleles were suppressed by the amber nonsense suppressor, *sup-7 X* (WATERSTON 1981; BOLTEN *et al.* 1984), strains of genotype *unc-13(e450)I; dpy-13 ama-1(m118mx)IV/ + +; sup-7 X/+* were constructed. The *unc-13* mutation is dominantly suppressed by *sup-7* and was used to score suppressor activity. These strains were constructed by crossing *dpy-13 ama-1(m118mx)/+ +* males with *unc-13 I; sup-7 X* hermaphrodites, and selecting semi-Dpy, suppressed Uncs (*unc-13; dpy-13 ama-1(m118mx)/+ +; sup-7* or *sup-7/+*) in the F_2 generation. Since many hermaphrodites homozygous for *sup-7* proved to be sterile, the progeny of the *sup-7* heterozygotes were screened for the presence of adult Dpy, suppressed Unc hermaphrodites. The presence of this class of progeny would indicate that the lethal mutation was suppressed by a single copy of *sup-7*.

Determination of egg hatching and brood sizes: Genotypes of strains tested are given in RESULTS, Table 2. The number of fertilized eggs produced by DR432, DR680 and DR730 hermaphrodites was determined as follows. L4 hermaphrodites grown at either 20° or 25° were selected and placed onto Petri plates at their respective temperatures. These animals were transferred to new plates at 24-hr intervals for 3–4 days. The number of unfertilized eggs, fertilized eggs and larvae on each plate was counted 24 and 72 hr after the original parent had been placed on the plate. The total number of fertilized eggs laid was the sum of the fertilized eggs and larvae present at 24 hr, just after the parent had been removed from the plate. The fraction of unhatched eggs was calculated by subtracting the number of larvae present at 72 hr from the total number of fertilized eggs that were laid. The brood sizes of N2, CB184 and DR731 hermaphrodites were determined by placing L4 larvae on Petri plates and transferring them to new plates at 24-hr intervals until they ceased egg-laying. The number of progeny produced on each plate was counted after 3–4 days. The L4 larvae used in these experiments were grown at 20° and incubated at either 20° or 25° to lay eggs, or they were grown at 25° and also allowed to reproduce at this temperature.

Molting cycles: To obtain L1 larvae synchronized at hatching, fertilized eggs were collected by alkaline hypochlorite treatment of adult hermaphrodites (EMMONS *et al.* 1979) and suspended in M9 buffer (BRENNER 1974) for 22–24 hr at 20°. At this time, all of the eggs had hatched into the buffer, but larval development was arrested due to lack of food. Development resumed when the larvae were placed on plates with OP50. The molting cycles of several strains were determined at 25° by observing pharyngeal pumping (feeding). Cessation of pharyngeal pumping and decreased body movement (lethargus) accompanies each molt (BYERLY, CASSADA and RUSSELL 1976). The percentage of feeding worms was recorded every 30 or 60 min until just after the last molt. A sample of 100 worms was scored each time using a Wild M5 stereomicroscope with ×100 magnification.

Temperature-shift experiments: The L1 larvae used for the upshift experiments were synchronized at 20° as described above. Approximately 100–300 larvae/plate were then placed on plates with OP50 at 20°, and one plate was transferred from 20° to 25° every 12 hr. Adult fecundity after each temperature shift was determined by removing twenty L4 larvae from the original plate, placing them on a fresh plate at the appropriate temperature, and then transferring them to additional plates at 24-hr intervals until they had ceased egg-laying. The progeny (including arrested larvae) produced by these hermaphrodites were counted and expressed as a percentage of the total brood of control worms that had not been shifted to 25°. The procedure used for downshift experiments was the same as described above, except that larvae were synchronized by hatching into M9 buffer for 18 hr at 25°, and populations were shifted from 25° to 20°.

Mapping lethal and visible mutations: Lethal sites were mapped relative to *dpy-13* in crosses performed at 20°. All of the progeny were scored for recombinants as described by ROSE and BAILLIE (1979). Ten to 30 L4 hermaphrodites were placed on 60 mm Petri plates, one per plate, and transferred twice at 24-hr intervals. Since lethal (*let*) mutations were induced on a *dpy-13 ama-1(m118)* chromosome, recombinants were scored as Dpy self-progeny issuing from semi-Dpy (*dpy let /+ +*) parents. The F_1 phenotypes of all progeny were scored, and the recombination frequency was calculated using the formula, $p = 1 - \sqrt{1 - (3D/[D + W])}$, where p = recombination frequency, D = number of Dpy progeny and W = number of phenotypically Wild and semi-Dpy progeny. The recombination frequency between *dpy-13* and *unc-5* was determined by counting all of the progeny of 20 *dpy-13 + unc-5/+ ama-1(m118) +* hermaphrodites.

Lethal mutations were positioned to the right or left of *dpy-13* by the following protocol. The progeny of *unc-17 + + + unc-5/+ dpy-13 ama-1 let +* hermaphrodites were screened for Dpy recombinants. If a lethal mutation were to the right of *dpy-13*, the Dpy recombinants would segregate homozygous *dpy-13 unc-5* progeny. On the other hand, if the lethal were to the left of *dpy-13*, then *unc-17 dpy-13* progeny would be segregated. Dpy Unc segregants from each Dpy recombinant were selected, and their progeny were mated with males heterozygous for *unc-17* and with males heterozygous for *unc-5* for complementation testing.

Identification of lethal complementation groups: Semi-Dpy males carrying a lethal mutation (*dpy-13 ama-1 let-a /+ + +*) were crossed with hermaphrodites heterozygous for another lethal (*dpy-13 ama-1 let-b /+ + +*). The progeny were screened for males, to confirm that the parental males and hermaphrodites had crossed, and for the presence of Dpy cross-progeny. When present, several L4 Dpy hermaphrodites were put on a separate plate and examined for fertility. An absence of fertile, adult Dpy progeny indicated that the two mutations failed to complement. Most complementation tests were done in duplicate to decrease the chance of a false result due to recombination between a lethal mutation and the *dpy-13* marker.

Deficiency mapping: The lethal-bearing strains were tested for complementation with the deficiencies (*Df*) using the above procedure. In general, males heterozygous for the lethal mutation were mated with hermaphrodites carrying a deficiency, *dpy-13 mDf/+ +*. If no fertile, adult Dpy progeny were found, the *let* locus was considered to be included in the deficiency.

Isolation and mapping of duplications carrying *dpy-13*: Wild-type males that had been exposed to 1500 rad of γ-radiation were mated with *unc-17 dpy-13* hermaphrodites, and the F_1 generation was screened for the presence of rare Wild or Dpy hermaphrodites among the semi-Dpy cross-progeny. The Wild individuals, presumably, would carry a duplication (*Dp*) of the *dpy-13* region (*i.e.*, *dpy-13/+/+*), whereas the Dpys would carry a deficiency that included *dpy-13* but not *unc-17*. Individuals of genotype *unc-17 dpy-13 IV; mDp* were obtained among the progeny of the rare Wild hermaphrodites. These worms were either semi-Dpy or semi-Dpy Unc, depending on whether the *unc-17* gene was carried on the duplication.

To determine whether a putative duplication included the wild-type allele of the *ama-1* gene, it was tested for

complementation with a lethal *ama-1* allele. Males of genotype *unc-17 dpy-13*/+ +; *mDp* (obtained by mating *unc-17 dpy-13*; *mDp* hermaphrodites with N2 males and selecting Wild male progeny) were mated with *dpy-13 ama-1(m118m236)/nT1(IV)*; +/*nT1(V)* hermaphrodites. The Wild hermaphrodite cross-progeny were selected, and those with the genotype *dpy-13 ama-1(m118m236)*/+ +; *mDp* were identified. Their progeny were counted to determine the ratio of Wild:semi-Dpy animals. A ratio of 7 Wild:2 semi-Dpy was expected if the *ama-1* gene were not carried on the duplication, whereas a ratio of 7 Wild:4 semi-Dpy was expected if the duplication included *ama-1*. The assumptions involved in calculating these expected ratios were that the *mDp* was lost at a low frequency and that the *mDp/mDp* homozygotes were rare. The semi-Dpy progeny obtained were selected and allowed to reproduce to confirm their genotype. When present, *dpy-13 ama-1(m118m236)IV*; *mDp* hermaphrodites were selected, and a stock established from a single animal. The *let-276* and *let-278* genes were tested for inclusion in *mDp2* in a similar manner.

The duplications, *mDp1* and *mDp2*, were tested for the presence of the wild-type alleles of several *unc* genes on chromosome *IV*. Males with the genotype *dpy-13 ama-1(m118m236)*/+ +; *mDp* were mated with *dpy-13 unc* hermaphrodites. Wild cross-progeny (*dpy-13 unc* /+ +; *mDp*) were cloned and their progeny were scored. The presence of semi-Dpy Unc progeny indicated that the *unc* gene was not carried on the duplication, whereas the absence of this progeny class indicated that the wild-type allele of the gene was present on the duplication.

A different protocol was used to determine if the wild-type allele of the *dpy-9* gene was included in *mDp1*. Males of genotype *dpy-13 ama-1(m118-m236)*/+ +; *mDp1* were mated with *dpy-9* hermaphrodites, and *dpy-9*/+; *mDp1* cross-progeny were identified. Their progeny were scored, and the ratio of Wild:Dpy was determined. A ratio of 11 Wild:1 Dpy would be obtained if the *dpy-9* gene were carried on this duplication, whereas a ratio of 9 Wild:3 Dpy would be obtained if *dpy-9* were not carried on the duplication.

The *mDp1* duplication was tested for linkage to *unc-13 I*, *unc-4 II*, *unc-32 III*, *unc-42 V* and the *X* chromosome. *X*-linkage was tested in a cross between males of genotype *unc-17 dpy-13*/+ +; *mDp1* and *dpy-13 ama-1(m118m236)/nT1(IV)*; +/*nT1(V)* hermaphrodites. The presence of Dpy hermaphrodite cross-progeny revealed that *mDp1* is not *X*-linked. To test linkage to the autosomal *unc* markers, strains of genotype *dpy-13*/+; *unc*/+; *mDp1* were obtained by mating *dpy-13*; *unc* hermaphrodites with *dpy-13 ama-1(m118m236)*/+ +; *mDp1* males, and selecting Wild hermaphrodite cross-progeny. The progeny of these animals were then scored. If the duplication were not linked to the *unc* marker, then a ratio of 21 Wild:12 semi-Dpy:11 semi-Dpy Unc + Unc:3 Dpy:1 Dpy Unc would be obtained. On the other hand, a ratio approaching 6:2:3:0:1 would indicate linkage.

RESULTS

Mapping the *ama-1* gene: We initially positioned *ama-1(m118)* approximately 0.03 map unit to the right of *dpy-13 IV* by three-factor recombination mapping. No amanitin-resistant recombinants were detected among 250 Dpy non-Unc progeny of *unc-17 dpy-13* +/+ + *ama-1(m118)* heterozygotes. However, Dpy recombinants carrying *ama-1(m118)* were found among the progeny of *dpy-13* + *unc-5*/+ *ama-1(m118)* + hermaphrodites, indicating that *ama-1* lies to the right of *dpy-13*. Since only two of the 150 Dpy non-Unc recombinants obtained were resistant to amanitin, the *ama-1* gene must be very close to *dpy-13*, approximately 1/75 of the distance between *dpy-13* and *unc-5*. A recombination frequency between *unc-5* and *dpy-13* of 0.022 (2.2 map units) was determined (Table 1), and this value was used to estimate a distance of 0.03 map unit from *dpy-13* to *ama-1*.

Isolation and characterization of *ama-1* lethal alleles: A *dpy-13 ama-1(m118)* chromosome constructed in the above mapping experiment was used in a screen designed to obtain lethal alleles of *ama-1* (Table 1). We first isolated lethal and sterile mutations closely linked to *dpy-13* on the *dpy-13 ama-1* chromosome, and then screened the mutant heterozygotes obtained for the loss of the dominant amanitin-resistant phenotype of *m118*. Strains carrying a mutation in an essential gene other than *ama-1* (*e.g.*, *dpy-13 ama-1 let-276*/+ + +) retained resistance to amanitin. However, hermaphrodites heterozygous for a mutation that altered or eliminated the *ama-1(m118)* gene product (*e.g.*, *dpy-13 ama-1(m118m236)*/+ +) were sensitive to amanitin. Twenty-nine mutations, most of which map within 2 map units of *dpy-13*, were isolated at a frequency of 1×10^{-2} after EMS mutagenesis of *dpy-13 ama-1* hermaphrodites, as described in MATERIALS AND METHODS. Six of these 29 strains were no longer able to grow and reproduce in the presence of amanitin when tested, and were presumed to carry a lethal or sterile mutation in *ama-1*. Complementation tests were performed to confirm that all six mutations were allelic. In addition, two of the *ama-1* lethal alleles were positioned relative to *dpy-13* by two-factor and three-factor recombination mapping (Table 1), placing these mutations at the *ama-1* locus.

One of the six EMS-induced lethal *ama-1* alleles is a deficiency, *mDf9*, based on complementation tests with other genes (see Table 4). The remaining five alleles apparently affect only the *ama-1* gene, and these were examined to determine their lethal phenotypes (Table 1). When incubated at 15° or 20°, animals homozygous for *ama-1(m118m252)* were arrested late in embryogenesis, after cell division was essentially complete (Figure 1). Although the actual stage of arrest varied, most of the embryos observed were blocked during morphogenesis, between the "lima bean" and "pretzel" stages (VON EHRENSTEIN and SCHIERENBERG 1980). By contrast, most eggs hatched at 25°, producing L1 larvae that moved well, but did not develop, and died in 2–3 days. Thus, the *m252* mutation conferred a more severe phenotype at lower temperatures. The phenotypes of the other four mutants also varied with temperature. Worms homozygous for *ama-1(m118m236)* developed into sterile

TABLE 1

Summary of genetic data and mutant phenotypes for *ama-1* and nearby essential genes and deficiencies

Gene (allele)	Lethal phenotype[a]	Position relative to *dpy-13*	2-Factor data		Map distance from *dpy-13*[b]
			Wild	Dpy	
let-273(m263)	Early larval	Left	2765	42	2.27 (1.77–3.02)
let-274(m256)	Early larval	Left	2740	30	1.66 (1.19–2.25)
let-275(m245)	Mid-larval	Left	2927	11	0.56 (0.32–0.94)
let-275(m257)	Mid-larval	Left			
let-278(m265)	Sterile adult	Left	7197	3	0.06 (0.02–0.16)
ama-1(m118m236)	Early larval (ts)	Right	8506	3	0.05 (0.014–0.14)
ama-1(m118m252)	Embryonic		7112	5	0.11 (0.041–0.22)
ama-1(m118m235)	Larval to sterile adult				
ama-1(m118m238)	Sterile adult (ts)				
ama-1(m118m251)	Sterile adult (ts)				
let-276(m240)	Early larval	Right	4489	19	0.63 (0.42–0.94)
let-276(m239)	Early larval	Right	4271	17	0.59 (0.38–0.90)
let-276(m241)	Early larval	Right			
let-276(m242)	Early larval	Right			
let-276(m246)	Early larval	Right			
let-276(m264)	Early larval	Right			
let-279(m261)	Sterile adult	Right	3704	17	0.69 (0.44–1.04)
let-288(m306)[c]	Sterile adult	Right			
let-280(m259)	Sterile adult	Right	3598	34	1.41 (1.08–1.95)
let-277(m262)	Mid-larval	Right	3409	35	1.55 (1.15–2.06)
let-281(m247)	Sterile adult	Right			
let-282(m258)	Early larval	Right			
let-282(m270)	Sublethal	Right			
let-284(m267)	Early larval	Right			
let-285(m248)	Sterile adult	Right			
let-286(m269)	Maternal-effect embryonic	Right			
let-287(m244)	Sterile adult	Right			
unc-5(e53)	Uncoordinated	Right[d]	4013	121[e]	2.20 (1.97–2.46)
unc-17(m324)		Left[d]	3379	69	3.02 (2.72–3.32)
mDf4[c]	Embryonic	Deletes?	11,038	0	<0.014
mDf5[c]	Embryonic	Deletes			
mDf6[c]	Embryonic	Deletes			
mDf7[c]	Embryonic	Right	1524	30	2.99 (2.15–4.09)
mDf8	Early larval	Right	2875	25	1.31 (0.91–1.84)
mDf9	Embryonic	Deletes			
mDf10[c]	Early larval	Deletes			

[a] A lethal phenotype is the stage of developmental arrest exhibited by homozygous mutant animals segregating from a heterozygous parent. Embryonic, mutant arrests prior to hatching; early larval, mutant arrests during the L1 or early L2 stage; mid larval, mutant arrest during the late L2 or L3 stage; sterile adult, mutant completes larval development but is sterile as an adult.

[b] The 90% confidence intervals are shown in parentheses.

[c] Induced by γ-radiation; all other mutations were EMS-induced.

[d] EDGLEY and RIDDLE (1987).

[e] Includes both Dpy and Unc recombinants.

adults at 15° and 20°, but stopped developing during the L1 stage at 25°. Animals homozygous for *ama-1(m118m235)* arrested larval development earlier at 15° or at 25° than they did at 20°. At both 15° and 25°, all *m118m235* homozygotes were mid-larval lethals, whereas the phenotype at 20° was somewhat variable: some animals arrested development as larvae, and others became adults that occasionally produced arrested embryos. The two remaining *ama-1* alleles exhibited *ts* adult sterile phenotypes. Mutant hermaphrodites were fertile at 15° and 20°, but they were essentially sterile at 25° (Table 2).

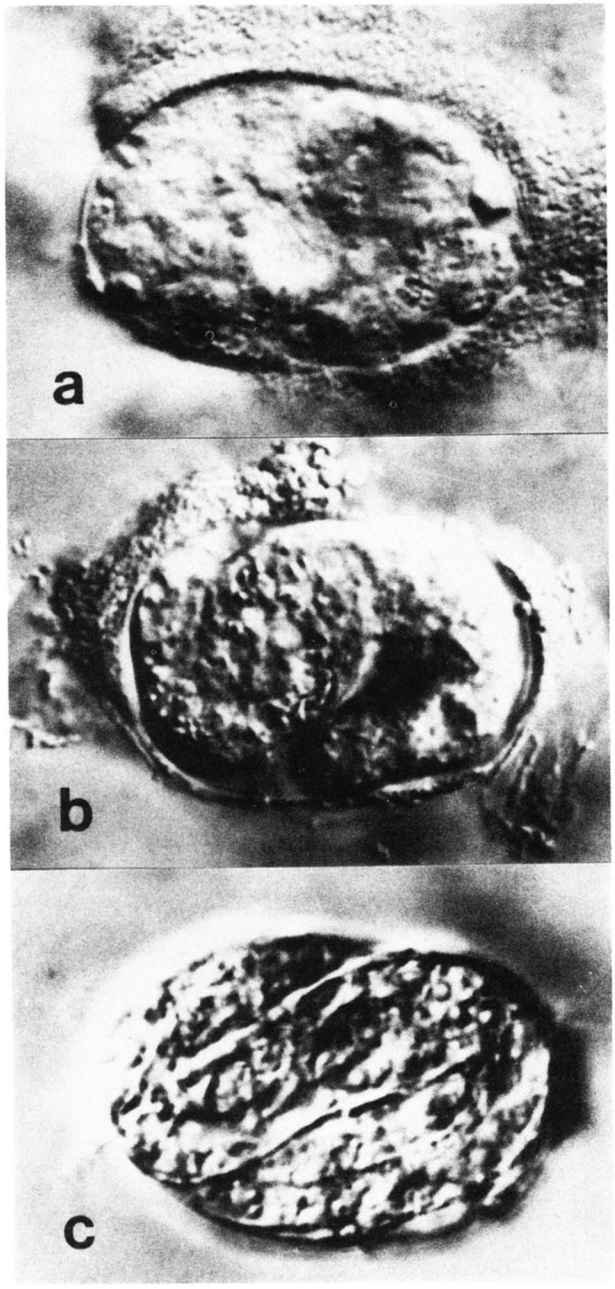

FIGURE 1.—Nomarski micrographs of three *dpy-13 ama-1(m118m252)* embryos showing their terminal phenotypes at 20°: (a) the "lima bean stage," (b) the "tadpole stage," (c) the "late pretzel stage." The arrested embryos were viewed through a Zeiss Universal microscope and photographed with an Olympus 35 mm camera. The photographs were taken approximately 24 hr after the eggs were laid (magnification, ×1200).

We used the amber nonsense suppressor, *sup-7 X* (WATERSTON 1981; BOLTEN *et al.* 1984), to determine whether *m235*, *m236* or *m252* are suppressible amber mutations. None of these mutations were suppressed, but it is possible that an amber allele might not be suppressed if the amino acid, tryptophan, inserted by the *sup-7* tRNA does not restore RNA polymerase activity.

Temperature-sensitive *ama-1* mutants: Two independent *ts* alleles, *m118m238*ts and *m118m251*ts, exhibit similar *ts* sterile phenotypes (Table 2), and are viable as homozygotes at 20°. A *m118m238*ts strain was chosen for detailed comparison with the *dpy-13 ama-1(m118)* parent. The adult sterile phenotype of the strain carrying *m118m238*ts is the result of two effects. First, the effect of the *m238*ts mutation was to reduce the number of fertilized eggs produced at both temperatures. In comparison with the parent strain, there was almost a 60% reduction at 20° (Table 2, column 3), and almost an 85% reduction at 25° (column 4). Thus the number of fertilized eggs laid at 25° compared to 20° also was reduced. Second, there was a decrease in the fraction of fertilized eggs that hatched at the restrictive temperature (columns 8 and 9). This second effect also was observed with the *dpy-13 ama-1(m118)* control strain, and thus is not attributed to the *m238*ts mutation.

The effect of growth temperature on brood size that is characteristic of wild-type *C. elegans* (BYERLY, CASSADA and RUSSELL 1976) was seen for all the strains listed in Table 2, as shown by comparison of columns 5 and 7. Furthermore, the reduction in brood size resulting from incubation at higher growth temperature was substantially less pronounced in all strains grown at 20° until the L4 stage, then shifted to 25° for egg laying (Table 2, column 6). This indicates that the effects of higher temperature are partially executed prior to gametogenesis in both wild-type and mutant strains. The *dpy-13* and *ama-1(m118)* broods were smaller than those of N2 under all conditions tested, and only about half the size of N2 broods at 25°. The *dpy-13 ama-1(m118)* double mutant had a markedly reduced brood size at both temperatures relative to either single mutant, as well as increased temperature-sensitivity with regard to the fraction of eggs hatched. As stated above, this latter character is similar to the embryonic-arrest component of the *ama-1(m118m238*ts) phenotype, and suggests that it is not the result of the *m238* mutation.

The *m238*ts mutation accentuated the effect of temperature on egg production (Table 2, columns 3 and 4). Egg production in the *dpy-13 ama-1(m118)* strain was reduced approximately 50% at 25° compared to 20°, whereas an 85% reduction was observed in the strain carrying *m238*ts. This sterile phenotype of *m118m238*ts was not rescued by wild-type sperm (*i.e.*, by mating wild-type males with the mutant hermaphrodites grown at 25°), indicating that functional oocytes were not being produced. Finally, not all of the *m118m238*ts larvae that hatched grew to the adult stage. At 25°, 30% were arrested during larval development, whereas 12% were arrested when grown at 20° (data not shown).

Although the majority of *m118m238*ts larvae survive, they exhibit slowed development at both restric-

TABLE 2

Effect of temperature on the fecundity of *ama-1* mutant hermaphrodites

Strain	Genotype	Fertilized eggs per hermaphrodite		Hatched progeny per hermaphrodite[a]			Fraction of eggs hatched	
		20°	25°	20°	25°(20°)[b]	25°	20°	25°
1	2	3	4	5	6	7	8	9
N2	Wild type			350 ± 8	281 ± 13	243 ± 21		
CB184	*dpy-13(e184)*			268 ± 11	252 ± 8	139 ± 16		
DR432	*ama-1(m118)*	297	132	293 ± 11	187 ± 21	126 ± 13	0.99	0.96
DR680	*dpy-13(e184)ama-1(m118)*	153	83	139 ± 23	80 ± 7	51 ± 5	0.91	0.61
DR730	*dpy-13(e184)ama-1(m118m238*ts)	66	13	57 ± 2		7 ± 0.5	0.86	0.54
DR731	*dpy-13(e184)ama-1(m118m251*ts)			93 ± 3		9 ± 2.5		

[a] Hatched progeny include those that arrested as larvae, as well as those that grew to the adult. Average broods in samples of 10–60 hermaphrodites are given with standard errors.

[b] Animals grown at 20° and transferred to 25° as L4 larvae.

TABLE 3

Developmental profiles of *ama-1* mutants

Strain	Genotype	Time (hr) after hatching at 25°				
		L1-L2 molt	L2-L3 molt	L3-L4 molt	L4-adult molt	Onset of egg laying
CB184	*dpy-13(e184)*	13.5	22.0	31.0	41.0	49
DR680	*dpy-13(e184)ama-1(m118)*	15.5	25.0	35.0	46.5	53
DR730	*dpy-13(e184)ama-1(m118m238*ts)	19.0	30.0	41.0	57.0	68

tive and permissive temperatures. There was an increase in the duration of each larval stage in *dpy-13 ama-1(m118m238*ts) animals compared to the *dpy-13 ama-1(m118)* parent strain. The greatest increase in developmental time (27 hr *vs.* 18 hr, a 50% increase) occurred between the L3-L4 molt and the onset of egg-laying. Egg-laying for the *dpy-13 ama-1(m118)* strain at 25° commenced 53 hr after hatching, whereas the *dpy-13 ama-1(m118m238*ts) strain commenced egg-laying 15 hr later, for an overall increase of 30%. The data in Table 3 also indicate that the *ama-1(m118)* amanitin-resistant allele has only a slight effect on developmental rate. The *dpy-13 ama-1(m118)* mutant commenced egg-laying only 4 hr later than the *dpy-13 ama-1*(+) strain.

Temperature-sensitive period: Temperature-shift experiments were performed to determine the critical period for the function affected by the *m238*ts mutation (Figure 2). For downshift experiments, synchronous populations of L1 larvae were allowed to grow for various lengths of time at the restrictive temperature (25°), and then were shifted to permissive temperature (20°) to complete development. The effect of these temperature shifts on hermaphrodite fecundity was then determined by progeny counts, and expressed as a percentage of the total brood size of *dpy-13 ama-1(m118m238)* control hermaphrodites that were raised at 20° and not shifted. Reciprocal experiments, in which mutant larvae growing at 20° were shifted to 25°, also were done. Temperature upshifts at any time during larval development resulted in the mutant phenotype. Upshifts after the L4-adult molt resulted in a gradual increase in fecundity up to the

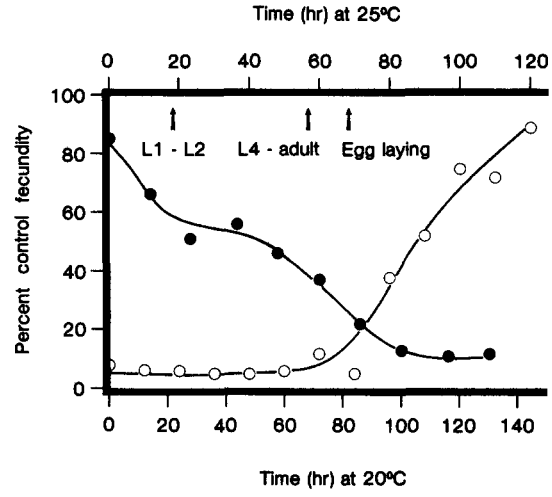

FIGURE 2.—Temperature-shift studies of the *dpy-13 ama-1(m118m238)* strain. To construct the upshift (○) and downshift (●) curves synchronous populations of L1 larvae were incubated at 20° or 25° and shifted to the other temperature at defined intervals. The larvae were allowed to continue development and their brood sizes were determined and expressed as a percentage of the brood size of control (*dpy-13 ama-1(m118m238)*) hermaphrodites that were raised at 20° and not shifted. The time scales are adjusted so that hatching and the initiation of egg-laying are aligned. The times of the L1-L2 and L4-adult molts at 25° (taken from Table 3) are indicated by *arrows*.

level of the control hermaphrodites. The reverse occurred with the downshifts, but exposure to 25° even for a short period of time during larval development affected adult fecundity. For example, worms shifted down at the L1-L2 molt produced only about 55–60% as many offspring as the control animals.

Each shift curve is characterized by its slope as well as its transition midpoint (the point at which 50% of control fecundity was observed). According to a standard interpretation of such shift studies (SUZUKI 1970), the downshift transition marks the beginning of the temperature-sensitive period (tsp) and the upshift transition marks the end. The slope indicates how well the endpoints of the tsp are defined. The data in Figure 2 define a major tsp that begins shortly before the L4-adult molt and is centered around the initiation of egg-laying, and a minor tsp beginning during the L1 stage. It is important to note that these data represent the sum of the two major effects of incubation at the higher temperature: the reduction in the number of fertilized eggs produced, and the increase in embryonic arrest. Only the former *ts* phenotype is attributed to the *m238*ts mutation (see Table 2). Thus, it is not possible at this time to say whether both the early and late effects of temperature seen in these experiments are due to the *m238*ts allele. It should be noted, however, that the 40–45% reduction in brood size seen after downshifts at early times (Figure 2) roughly corresponds in amount to the reduction in the fraction of eggs hatched at 25° compared to 20° in both parent and *m118m238*ts mutant strains (Table 2, columns 8 and 9).

Essential genes around *ama-1*: Analysis of the EMS-induced mutants that retained resistance to amanitin (Table 1) identified 14 new essential genes in a region of approximately 4.5 map units around *dpy-13*. One additional essential gene, *let-288*, was defined by a γ-ray-induced mutation, as described below. A lethal allele of the *unc-17* gene, *unc-17(m324)*, also was identified. The *unc-17* gene was previously defined by mutations conferring an uncoordinated, coiler phenotype when homozygous. All of these Unc mutants are resistant to acetylcholinesterase inhibitors (BRENNER 1974), and several are deficient in choline acetyltransferase activity (RAND and RUSSELL 1984). The *m324* allele was identified by its lack of complementation when attempting to construct a strain in which the lethal mutation was balanced by *unc-17(e113)*. The other 22 mutations were positioned relative to *dpy-13* by three-factor crosses, and mutations mapping on the same side of *dpy-13* were complemented with each other. Four of the new genes map to the left of *dpy-13*, and ten map to the right. Three of the genes are represented by more than one mutation (there are two mutant alleles of *let-275* and *let-282*, and six alleles of *let-276*). Representative alleles of eight of these genes were positioned relative to *dpy-13* by two-factor recombination mapping (Table 1) to construct a genetic map of the region (Figure 3). The complementation analysis of EMS-induced mutations also identified two small deficiencies, *mDf8* and *mDf9*, which include several essential

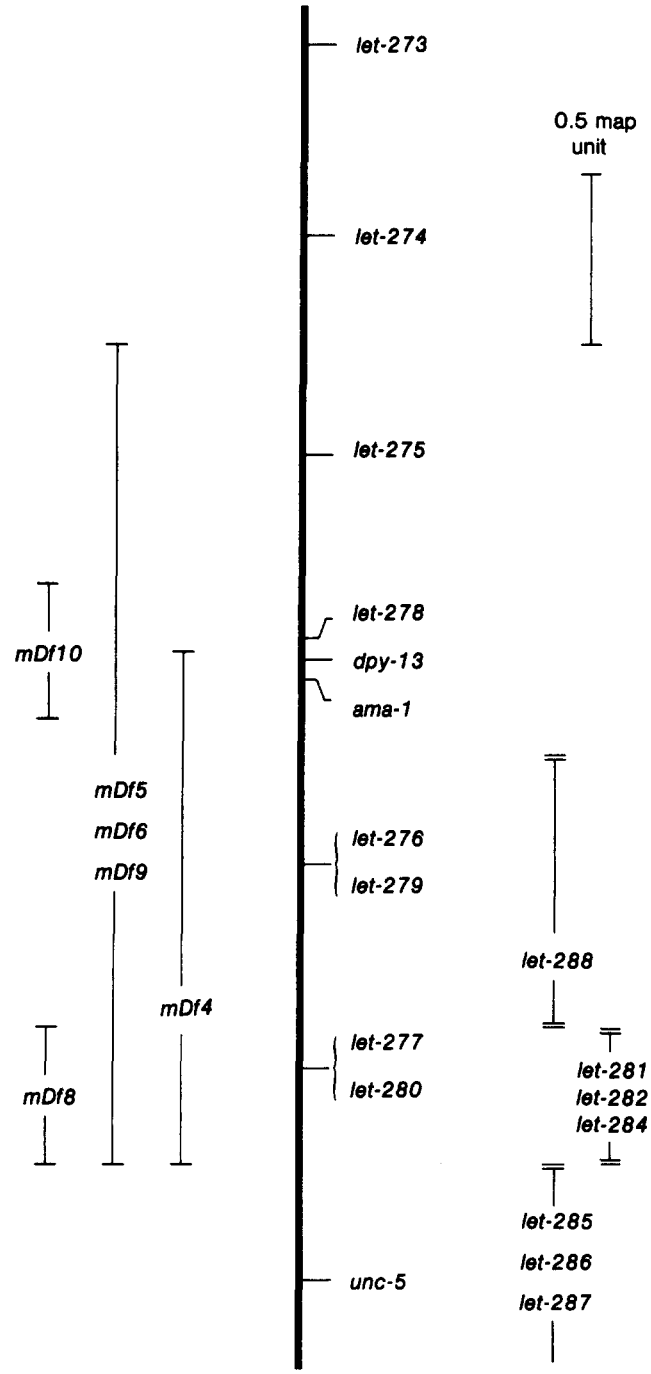

FIGURE 3.—Genetic map of essential genes, nonessential genes and deficiencies in the region surrounding *ama-1*. The genes placed on the main line have been positioned relative to *dpy-13* by two-factor recombination mapping, whereas those placed to the right of the line have not. The positions of the six deficiencies are shown to the left of the main line (*mDf7* is not shown). On this vertical map, the left arm of LG *IV* is up and the right arm is down.

TABLE 4

Results of complementation tests between mutations and deficiencies in the *ama-1* region[a]

Gene (allele)	*mDf4*	*mDf5*	*mDf6*	*mDf8*	*mDf9*	*mDf10*
let-273(m263)	+	+	+		+	+
let-274(m256)	+	+	+		+	+
let-275(m245)[b]	+	−	−		−	+
let-278(m265)	+	−	−		−	−
ama-1(m118m235)[c]	−	−	−	+	−	−
let-276(m240)[d]	−	−	−	+	−	+
let-279(m261)	−	−	−	+	−	+
let-288(m306)	−	−	−	+	−	+
let-280(m259)	−	−	−	−	−	
let-277(m262)	−	−	−	−	−	
let-281(m247)	−	−	−	−	−	
let-282(m258)	−	−	−	−	−	
let-284(m267)	−	−	−	−	−	
let-285(m248)	+	+	+	+	+	
let-286(m269)	+	+	+	+	+	
let-287(m244)	+	+	+	+	+	

[a] Complementation is indicated by + and noncomplementation by −.

[b] Both alleles of *let-275* were tested.

[c] The *ama-1(m118m236)* allele was used for some of the crosses.

[d] All six alleles of *let-276* were tested with *mDf4*, *mDf8* and *mDf10*.

genes (Table 4). Whereas *mDf9* includes *ama-1*, *mDf8* does not.

Deficiencies around *ama-1*: A set of 45 γ-ray-induced lethal and sterile mutations linked to *dpy-13 ama-1(m118)* was isolated after screening the progeny of 3229 F_1 hermaphrodites (see MATERIALS AND METHODS). Three of these mutations affected the *ama-1* gene as determined by loss of the amanitin-resistant phenotype of *m118*, and were shown to be deficiencies (*mDf4*, *mDf5* and *mDf6* in Table 4) by complementation tests with EMS-induced mutations. The deficiency, *mDf4*, fails to complement eight essential genes in addition to *ama-1* (Table 4). Based on two-factor positioning of the *let* genes included within *mDf4*, this deficiency is approximately 1.5 to 2.0 map units in length. Its left endpoint is between *let-278* and *ama-1*, an interval of only 0.1 map unit. Since *mDf4* was isolated on a chromosome carrying *e184*, it could not be determined genetically whether the *dpy-13* gene is included in this deficiency, but we have not been able to separate the deficiency endpoint from *dpy-13* by two-factor recombination mapping (Table 1). Another γ-ray-induced deficiency, *mDf10*, was obtained in the screen for duplications and deficiencies (see below). This latter deficiency is small, and includes only three identified genes: *let-278*, *dpy-13* and *ama-1*. It complements *let-273*, *let-274* and *let-275* on the left, and *let-276*, *let-279*, *let-288* and *mDf8* on the right (Table 4).

To identify additional deficiencies with breakpoints near *ama-1*, we crossed 42 γ-ray-induced lethals with males heterozygous for *mDf4*. Several of the heterozygous mutant strains grew very slowly and had greatly reduced brood sizes. Ten of these strains were too "sick" to mate efficiently and no complementation results were obtained. Twenty-six complemented *mDf4*, and were not characterized further. One of the remaining mutations failed to complement *mDf4*, and was shown to define at least one new essential gene, *let-288*, by virtue of its complementation with the other essential genes included in *mDf4* (Figure 3).

The results obtained with the remaining five mutations in the set of 42 were more complicated. Since these mutations are all embryonic or early larval lethals, we expected no adult Dpy progeny in the complementation tests if the mutation were uncovered by *mDf4*, and fertile Dpy progeny if the mutation were outside the deficiency. In all five cases, sterile Dpy progeny were obtained in the complementation tests. Tests that produced sterile adults could reflect a small overlap of *mDf4* with a second deficiency, such that only genes affecting fertility were deleted. Alternatively, sterility in animals heterozygous for *mDf4* and another nonoverlapping deficiency could result from haplo-insufficiency. In fact, one of the mutations, *mDf7*, is a nonoverlapping deficiency, so sterility in this case apparently did result from haplo-insufficiency. The *mDf7* deficiency failed to complement mutant alleles of *unc-22*, *unc-43* and *daf-14* and complemented *unc-5*, *daf-15*, *let-52*, *unc-31*, *unc-30* and *dpy-4*. These results place the left endpoint of *mDf7* between *daf-15* and *daf-14*, approximately 5 map units from *dpy-13*, and the right endpoint between *unc-22* and *let-52*. The left endpoint of *mDf7* was located approximately 3 map units from *dpy-13* by two-factor recombination mapping (Table 1). This distance is shorter than expected, since *daf-15* is about 5 map units from *dpy-13* (EDGLEY and RIDDLE 1987), suggesting that recombination may be suppressed by *mDf7*, or that there may be a lethal site between *dpy-13* and *mDf7*.

Developmental arrest in lethal mutants: The lethal phenotypes of the mutations that define the 15 essential genes around *ama-1* were determined, and are listed in Table 1. The phenotypes observed range from early larval lethal to maternal-effect lethal. Seven of the 15 genes are represented only by mutations causing sterility in adult hermaphrodites. The terminal phenotypes of the deficiency homozygotes also were determined (Table 1). The four largest deficiencies are embryonic lethals, whereas animals homozygous for either of the two smaller deficiencies (*mDf8* and *mDf10*) arrest development as L1 larvae.

Chromosomal duplications: To complete our analysis of this chromosomal region, we isolated two duplications that carry the *ama-1* gene (Figure 4). These gamma-ray-induced duplications were obtained at a frequency of about 1.3×10^{-3} in a screen designed to identify both duplications and deficiencies (see MA-

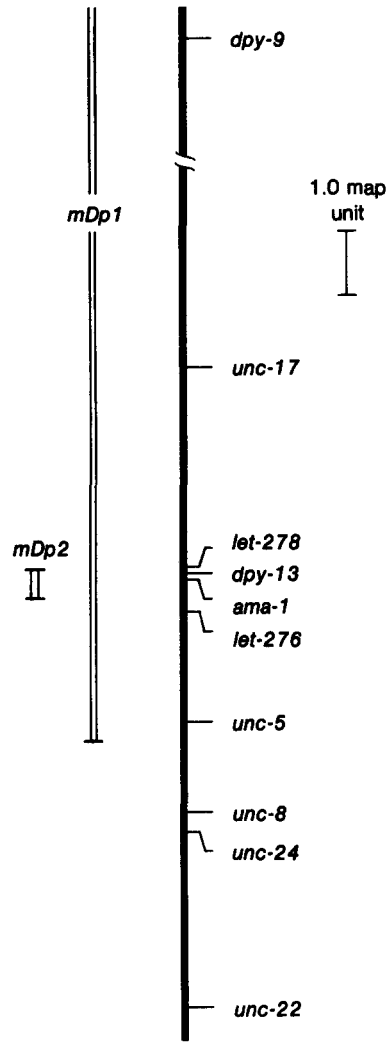

FIGURE 4.—Partial genetic map of LG *IV* showing the known extents of *mDp1* and *mDp2*. The *mDp1* duplication was tested with mutations in all of the genes shown on the map except *let-276* and *let-278*, whereas *mDp2* was tested with mutations in *unc-17*, *let-278*, *dpy-13*, *ama-1*, *let-276*, *unc-5* and *unc-22*. Genes on the left arm of LG *IV* are up, and those located to the right are down.

TERIALS AND METHODS). The largest duplication, *mDp1*, carries wild-type alleles of *dpy-9*, *unc-17*, *dpy-13*, *ama-1* and *unc-5*, as determined by complementation testing. This suggests that the entire left arm of chromosome *IV* may be duplicated. The *unc-8*, *unc-24* and *unc-22* genes are not included in *mDp1*, thereby placing the duplication breakpoint in the 1.5 map units interval between *unc-5* and *unc-8*.

Duplication *mDp1* recombines with the normal fourth chromosome. Both Dpy and semi-Dpy Unc recombinant self-progeny issued from an *unc-17 dpy-13*; *mDp1* strain. The latter recombinants carried the duplication and were of genotype *unc-17 dpy-13*; *mDp1*[*unc-17*], whereas the former did not carry the duplication and were of genotype + *dpy-13*/*unc-17 dpy-13*. These recombination events involving the duplication were approximately 20-fold less frequent than those between normal homologues (Table 5). The recombination frequency of 0.031 obtained between homologous chromosomes was reduced to 0.0016 in a strain with the genotype *unc-17 dpy-13*; *mDp1*.

The ratio of 2 semi-Dpy:1 Dpy Unc obtained in Table 5 for the *unc-17 dpy-13*; *mDp1* strain is consistent with two hypotheses. Either *mDp1* is a free duplication that is lost at a low frequency, or it is linked to another chromosome and has a lethal phenotype when homozygous. The data suggest that *mDp1* may be a free duplication. First, the *unc-17 dpy-13*; *mDp1* strain did not produce arrested embryos or larvae, indicating that the duplication homozygotes were not lethal. Second, we obtained hermaphrodites from the above strain that appeared to carry two copies of *mDp1*, and they segregated progeny with only one copy of the duplication at a frequency of about 1%. The segregants with only one copy of *mDp1* were semi-Dpy, and they segregated Dpy Unc progeny. Such loss is expected if *mDp1* is a free duplication. The *mDp1*/*mDp1* homozygotes represented only about 5% of the progeny of *unc-17 dpy-13*; *mDp1* hermaphrodites. The homozygotes were recognized by their relatively transparent, slender appearance, their slow development, and the fact that they did not segregate Dpy Unc progeny. The underrepresentation of *mDp1*/*mDp1* animals may result from their slow growth, as well as loss of the duplication from the germ line.

Finally, we determined that *mDp1* is not linked to the *X* chromosome, nor to *unc-13 I*, *unc-4 II*, *unc-32 III*, *unc-25 III*, *unc-45 III*, *daf-7 III* or *unc-42 V*. To test for linkage to these autosomal markers, hermaphrodites of genotype *dpy-13*/+; *unc*/+; *mDp1* were constructed, and their progeny were scored as described in MATERIALS AND METHODS. When these initial results were inconclusive, a second experiment also was performed in which the progeny of *dpy-13*/*dpy-13*; *unc*/+; *mDp1* hermaphrodites were scored. In this case, a ratio of 6 semi-Dpy:3 Dpy:2 semi-Dpy Unc:1 Dpy Unc would be obtained if *mDp1* and the *unc* marker were not linked. By these criteria, none of the above chromosomal markers were found to be linked to the duplication (data not shown).

The genetic evidence that *mDp1* is a free duplication is not conclusive. Translocated duplications can be lost from homozygotes (HERMAN, MADL and KARI 1979), and a duplication located on the end of LG I, LG II or LG V might not have shown linkage to the markers tested.

In contrast to *mDp1*, the *mDp2* duplication appears to carry only a small region of chromosome IV. Of the seven genes tested, only *dpy-13* and *ama-1* are included in *mDp2*, whereas *unc-17*, *let-278*, *let-276*, *unc-5* and *unc-22* are not. It seems likely that *mDp2* is also a free duplication; it is apparently lost at a higher frequency than *mDp1*. A ratio of 1.3 semi-Dpy Unc:1

TABLE 5

Recombination frequency between normal LG *IV* chromosomes and *mDp1*

Parental genotype	Number of progeny				Recombination frequency[a]
	Wild or semi-Dpy	Dpy Unc	Dpy	Unc or semi-Dpy Unc	
unc-17(e113)dpy-13(e184) / + +	3710	1158	70	85	0.0313[b] (0.0284–0.0342)
unc-17(e113)dpy-13(e184) ; *mDp1* / *unc-17(e113)dpy-13(e184)*	2233	1199	3	2	0.00165[c] (0.00086–0.0046)

[a] The 90% confidence intervals are shown in parentheses.

[b] Recombination frequency (p) was calculated using the formula $p = 1 - \sqrt{1-2R}$ where R = (Dpy + Unc)/(Wild + Dpy + Unc + Dpy Unc).

[c] Recombination frequency (p) was calculated using the formula $p = 0.75\ (1 - \sqrt{1-4R})$, where R = (Dpy + semi-Dpy Unc)/(semi-Dpy + Dpy + semi-Dpy Unc + Dpy Unc).

Dpy Unc was obtained with the *dpy-13 unc-5*; *mDp2* strain. Apparently, about 45% of the self-progeny are nullo-*Dp*, whereas a strictly mendelian expectation would be 25%. This discrepancy could be accounted for by premeiotic loss or meiotic loss (or both) in one or the other or both germ lines. The higher frequency of loss makes this duplication harder to manipulate than *mDp1*.

DISCUSSION

An important part of the genetic analysis of RNA polymerase II in *C. elegans* is the isolation of mutations in genes encoding subunits of this enzyme. Mutations that affect RNA polymerase II function are useful tools because they identify regions in particular polypeptides that are important for enzyme structure and function. The EMS-induced alleles described here, identified as second-site mutations in the *ama-1(m118)* gene by scoring for the loss of the dominant amanitin-resistant phenotype of *m118*, were isolated at a frequency of 1.5×10^{-3}. Hence, this method provides an efficient means to detect the desired mutations.

We tentatively conclude that the L1 lethal phenotype exhibited at 25° by *m118m236* and *m118m252* is the null phenotype, because mutants homozygous for the small deficiency, *mDf10*, also arrest development at this stage. This suggests that there is sufficient maternal RNA polymerase II to complete embryonic development, and that *de novo* RNA polymerase II synthesis is not required until the first larval stage. It will be necessary to characterize additional alleles of *ama-1* to verify this conclusion. The *m118m252* allele results in larval arrest at 25°, but embryonic arrest at 15° or 20°. This allele may produce an altered protein that interferes with maternal RNA polymerase II function during embryogenesis. The *m252* mutation has been positioned within the fine-structure map of *ama-1* (A. M. E. BULLERJAHN and D. L. RIDDLE, unpublished data) suggesting that it is not a multiple mutant or a deficiency. Consistent with this, the two-factor map distance between *dpy-13* and *ama-1(m118m252)* indicates that recombination is not suppressed in this interval. Furthermore, an *mDp1* strain of genotype *m252/m252/+* is much slower developing at 20° than *m252/+* or *m252/+/+* strains, indicating that a predominance of the *m252* product antagonizes function of the wild-type product (T. M. ROGALSKI, A. M. E. BULLERJAHN and D. L. RIDDLE, unpublished data). Interference with the function of maternally derived enzyme could account for the embryonic lethal phenotype at 20°. Such interference may be reduced at 25° if the abnormal function is thermolabile.

The other four *ama-1* mutants obtained in this study appear to be hypomorphs with some residual RNA polymerase II function. All four mutants are able to complete larval development at 20°, and they all exhibit a more severe phenotype at 25° than at 20°. The two mutants that are fertile at 20° become sterile at 25°, whereas the two mutants that are sterile at 20° cannot complete larval development at the higher temperature. The *m118m236* allele is likely to encode the most thermolabile product. This mutant product has sufficient function at 20° for mutant hermaphrodites to complete larval development, though they are sterile. However, at 25° the mutants arrest development during the first larval stage. Therefore, it appears that *m118m236* gene activity may be eliminated at the higher temperature. The degree of temperature sensitivity of the other three mutant strains is less pronounced, and may be due partially to the general stress of growth at 25°. The sterile phenotypes of these latter mutations are more severe at the higher temperature, but they do not become L1 lethals as do the *m118m236* mutants. In wild-type *C. elegans*, brood size was reduced by 30% at 25° in comparison with 20°, whereas the *m118m238*ts and *m118m251*ts broods were reduced by almost 90% (see Table 2).

A common defect of the *ama-1* hypomorphs is their inability to produce functional oocytes, as deduced

from the observation that fertility was not increased by mating with wild-type males. Depending on the incubation temperature, these mutants either produce very few fertilized eggs (*m118m235* at 20°; *m118m238* and *m118m251* at 25°) or none at all (*m118m236* at 20°). We have not yet determined whether this phenotype is due to a specific defect in oogenesis or to an earlier defect in gonadal development. The effect of these mutations on oocyte production may be due to the inability of the altered enzymes to meet an increased need for mRNA synthesis during this period. Alternatively, these mutations may affect the interaction of RNA polymerase II with one or more specific transcription factor(s). Enzymes isolated from strains carrying the *ama-1* alleles described here are being characterized biochemically (G. TULLIS and M. GOLOMB, unpublished results).

Temperature-shift experiments performed with the *dpy-13 ama-1*(*m118m238*ts) strain define a temperature-sensitive period (tsp) late in development, as expected for a mutation affecting fecundity, and oogenesis in particular. The major tsp begins during the late L4 stage, and ends approximately 18 hr after the initiation of egg-laying. The developmental events that occur during the tsp include differentiation of the somatic gonad and the production of occytes (HIRSH, OPPENHEIM and KLASS 1976; KIMBLE and HIRSH 1979). Sperm production occurs during the L4 stage, prior to the tsp. The tsp corresponds well with the stage (L4 to egg-laying) in which development is most slowed at 25° (see Table 3).

The temperature-shift experiments also suggest that events occurring as early as the L1 stage of mutant development affect fecundity in adult hermaphrodites, since temperature downshifts at these early times failed to prevent reduced fecundity in comparison with animals that had developed at 20° exclusively. Therefore, the higher temperature may be affecting the earlier development of the somatic gonad and/or the germ-line in this mutant strain. The interpretation of the temperature-shift data is complicated by the presence of two different temperature effects, only one of which is attributed to the *m118m238*ts allele (see Table 2). This *ama-1* mutation affects the number of fertilized eggs produced, but the *ts* embryonic lethal phenotype also is present in the parent strain. This latter phenotype is not due to *ama-1*(*m118*), but is either an effect of the combined *dpy-13* and *ama-1*(*m118*) mutations, or an unrelated mutation acquired during the construction of the *dpy-13 ama-1*(*m118*) chromosome. It will be necessary to compare our data with temperature-shift data on the parent strain to accurately define the tsp of *m118m238*ts.

The *ama-1* gene in *C. elegans* is analogous to the *RpII215* gene in *D. melanogaster*. Both genes encode subunits of RNA polymerase II and can be mutated to produce an amanitin-resistant enzyme. Lethal alleles of *RpII215* have been isolated and characterized genetically and/or biochemically. The biochemical analysis has identified both null and hypomorphic mutations (GREENLEAF *et al.* 1980). Although the developmental effects of most of these alleles have not been described, one *ts* allele has been examined in detail and is believed to be defective in RNA polymerase II assembly at the restrictive temperature (MORTIN and KAUFMAN 1984). Another allele, *Ubl*, exhibits a dominant phenotype similar to that of *Ultrabithorax* mutants (MORTIN and LEFEVRE 1981; MORTIN and KAUFMAN 1982). This mutation may produce an abnormal gene product that interferes with the function of the wild-type RNA polymerase II. Based on the terminal phenotype of flies hemizygous for a deficiency that deletes the *RpII215* gene, MORTIN and KAUFMAN (1982, 1984) have determined that *de novo* synthesis of RNA polymerase II is not required to complete embryonic development in *D. melanogaster*.

We have isolated seven new deficiencies as well as the first free duplications of chromosome *IV*. Five of the deficiencies obtained include the *ama-1* gene, and the smallest of these is *mDf10*. The region of overlap between this deficiency and *mDf4* defines an interval of, at most, 0.6 map unit. At present, *ama-1* is the only gene that we have identified in this region of overlap. These two deletion mutations should be useful in the molecular analysis of *ama-1* and the adjacent genes. In fact, the *mDf4* deficiency has been used to identify a recombinant DNA clone carrying *ama-1* (D. BIRD and D. L. RIDDLE, unpublished results). Both of the duplications obtained carry *ama-1*, and one of these, *mDp1*, has been used in the construction of heteroallelic *ama-1* strains to position lethal alleles relative to each other in the fine-structure map (A. M. E. BULLERJAHN and D. L. RIDDLE, unpublished data). Using *mDf10* and *mDp1*, we plan to construct strains carrying one, two, three and four copies of *ama-1* to determine if expression of this gene is regulated. Although the deficiencies and duplications were isolated primarily to study the *ama-1* gene, they are suitable genetic tools for mapping other genetically defined genes and cloned DNA fragments as well. In addition, the duplications can be used for cytological mapping (ALBERTSON 1984) and mosaic analysis (HERMAN 1984).

A number of duplications of the *C. elegans* genome have been described (HERMAN, MADL and KARI 1979; HODGKIN 1980; HERMAN, KARI and HARTMAN 1982; ROSE, BAILLIE and CURRAN 1984). Several of these are free duplications similar to *mDp1* and *mDp2*, whereas others are translocated to different chromosomes. The *mDp1* duplication is the second duplication described that recombines with its normal hom-

ologue. The free duplication, *sDp1*, which includes the entire right arm of chromosome *I* also recombines with the normal homologue (ROSE, BAILLIE and CURRAN 1984). Both *sDp1* and *mDp1* are large, covering approximately 25–30 map units. The *mDp2* duplication, which includes less than 0.6 map unit, is one of the smallest to be reported. At present, only *dpy-13* and *ama-1* appear to be included in *mDp2*. However, it is possible that this duplication may carry noncontiguous segments of chromosome *IV*, or even regions of another chromosome.

The genetic map of *C. elegans* shows clustering of genes on all of the autosomes (EDGLEY and RIDDLE 1987), and *dpy-13* is located at the left end of a gene cluster. Recent evidence (GREENWALD *et al.* 1987) suggests that the observed clustering is due to differential recombination frequencies in various regions of the *C. elegans* genome, rather than to an uneven distribution of genes on the physical map. An asymmetric distribution of essential genes was observed in this study, because 12 of the 16 genes listed in Table 1 lie to the right of *dpy-13*. Nine of these rightward genes lie in the region defined by *mDf4*. Not all of the essential genes in this 1.5 map units interval have been identified, since only three of the nine genes are represented by more than one allele. Over half of the mutations positioned in *mDf4* were found to be alleles of either *ama-1* or *let-276*. This high EMS-induced mutation frequency could mean that *ama-1* and *let-276* are both large genes. The *ama-1* gene is large, approximately 8.5 kb in length, and encodes a 200-kD polypeptide (D. BIRD and D. L. RIDDLE, unpublished results). Although it is still too early to obtain a good estimate of the number of essential genes in the interval defined by *mDf4*, the region is similar in size to that defined by *sDf2*, also on chromosome *IV* (ROGALSKI and BAILLIE 1985). A total of 32 essential genes has been estimated to lie within *sDf2*.

We have isolated mutations in 15 essential genes in addition to *ama-1* and determined their lethal phenotypes. Although there are three genes with more than one mutant allele, in only one case do the multiple alleles exhibit different terminal phenotypes (see Table 1). The *let-282(m270)* allele conveys a sublethal phenotype, whereas the *m258* allele conveys an early larval lethal phenotype. Seven of the 15 genes apparently produce products that are essential for larval development, based on the fact that each of these genes is represented by at least one allele with an early or mid-larval arrest phenotype. The eight remaining essential genes are defined by mutations that affect either reproduction in adult hermaphrodites or embryonic development in the progeny of homozygous mutants. Since only one allele has been obtained for each of these genes, we do not know whether the phenotypes observed reflect complete, or only partial loss of gene activity.

We have isolated the first lethal allele of *unc-17*. Mutations in *cha-1* and *unc-17* exhibit a complex complementation pattern, and are thought to define two different domains of choline acetyltransferase (RAND and RUSSELL 1984). All of the nonlethal mutations in this complex gene have the samer coiler, Unc phenotype and confer resistance to acetylcholinesterase inhibitors. The new recessive-lethal allele, *m234*, fails to complement *unc-17(e113)* and all *cha-1* alleles tested (J. RAND, personal communication). It provides genetic evidence that biosynthesis of acetylcholine is essential for development.

The lethal *ama-1* alleles isolated in this study have been characterized by determining the effects that they have on development. Some of these mutations may provide altered enzymes suitable for biochemical study. To continue the genetic analysis we are isolating additional lethal alleles of *ama-1* (T. M. ROGALSKI, A. M. E. BULLERJAHN and D. L. RIDDLE, unpublished data). Some of these lethal alleles may be cloned and sequenced to identify regions critical for enzyme function. Potentially, an important use of lethal *ama-1* alleles will be to identify genes encoding interacting proteins, such as other RNA polymerase II subunits or transcription factors, by means of suppressor mutations selected as revertants of *ama-1* lethals.

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LITERATURE CITED

ALBERTSON, D. G., 1984 Localization of the ribosomal genes in *Caenorhabditis elegans* chromosomes by *in situ* hybridization using biotin-labeled probes. EMBO J. **3:** 1227–1234.

BOLTEN, S. L., P. POWELL-ABEL, D. A. FISCHOFF and R. H. WATERSTON, 1984 The *sup-7(st5)X* gene of *Caenorhabditis elegans* encodes a transfer RNA-Trp-UAG amber suppressor. Proc. Natl. Acad. Sci. USA **81:** 6784–6788.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

BYERLY, L., R. C. CASSADA and R. L. RUSSELL, 1976 The life cycle of the nematode *Caenorhabditis elegans*. I. Wild-type growth and reproduction. Dev. Biol. **51:** 23–33.

COULTER, D. E., and A. L. GREENLEAF, 1982 Properties of mutationally altered RNA polymerase II of *Drosophila*. J. Biol. Chem. **257:** 1945–1952.

EDGLEY, M. L., AND D. L. RIDDLE, 1987 *Caenorhabditis elegans*. In: *Genetic Maps 1987*, Vol. 4, Edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

EMMONS, S. W., M. R. KLASS and D. HIRSH, 1979 Analysis of the constancy of DNA sequences during development and evolution of the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **79:** 1333–1337.

FERGUSON, E. L., and H. R. HORVITZ, 1985 Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. Genetics **110:** 17–72.

GREENLEAF, A. L., 1983 Amanitin-resistant RNA polymerase II mutations are in the enzyme's largest subunit. J. Biol. Chem. **258:** 13403–13406.

GREENLEAF, A. L., L. M. BORSETT, P. F. JIAMACHELLO and D. E. COULTER, 1979 Amanitin-resistant *D. melanogaster* with an altered RNA polymerase II. Cell **18:** 613–622.

GREENLEAF, A. L., J. R. WEEKS, R. A. VOELKER, S. OHNISHI and B. DICKSON, 1980 Genetic and biochemical characterization of mutants at an RNA polymerase II locus in *D. melanogaster*. Cell **21:** 785–792.

GREENWALD, I., A. COULSON, J. SULSTON and J. PRIESS, 1987 Correlation of the physical and genetic maps in the *lin-12* region of *Caenorhabditis elegans*. Nucleic Acids Res. **15:** 2295–2307.

HERMAN, R. K., 1984 Analysis of genetic mosaics of the nematode *Caenorhabditis elegans*. Genetics **108:** 165–180.

HERMAN, R. K., C. K. KARI and P. S. HARTMAN, 1982 Dominant X-chromosome nondisjunction mutants of *Caenorhabditis elegans*. Genetics **102:** 379–400.

HERMAN, R. K., J. E. MADL and C. K. KARI, 1979 Duplications in *Caenorhabditis elegans*. Genetics **92:** 419–435.

HIRSH, D., D. OPPENHEIM and M. R. KLASS, 1976 Development of the reproductive system of *Caenorhabditis elegans*. Dev. Biol. **49:** 200–219.

HODGKIN, J., 1980 More sex-determination mutants of *Caenorhabditis elegans*. Genetics **96:** 649–664.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. K. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

INGLES, C. J., J. BIGGS, J. K.-C. WONG, J. R. WEEKS and A. L. GREENLEAF, 1983 Identification of a structural gene for a RNA polymerase II polypeptide in *Drosophila melanogaster* and mammalian species. Proc. Natl. Acad. Sci. USA **80:** 3396–3400.

KIMBLE, J., and D. HIRSH, 1979 Post-embryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev. Biol. **70:** 397–417.

LEWIS, M. K., and R. R. BURGESS, 1982 Eukaryotic RNA polymerases. pp. 109–153. In: *The Enzymes*, Vol. XV, Edited by P. BOYER. Academic Press, New York.

MORTIN, M. A., and T. C. KAUFMAN, 1982 Developmental genetics of a temperature sensitive RNA polymerase II mutation in *Drosophila melanogaster*. Mol. Gen. Genet. **187:** 120–125.

MORTIN, M. A., and T. C. KAUFMAN, 1984 Developmental effects of a temperature-sensitive RNA polymerase II mutation in *Drosophila melanogaster*. Dev. Biol. **103:** 343–354.

MORTIN, M. A., and G. LEFEVRE, JR., 1981 An RNA polymerase II mutation in *Drosophila melanogaster* that mimics *Ultrabithorax*. Chromosoma **82:** 237–247.

RAND, J. B., and R. L. RUSSELL, 1984 Choline acetyltransferase-deficient mutants of the nematode *Caenorhabditis elegans*. Genetics **106:** 227–248.

ROGALSKI, T. M., and D. L. BAILLIE, 1985 Genetic organization of the *unc-22 IV* gene and the adjacent region in *Caenorhabditis elegans*. Mol. Gen. Genet. **201:** 409–414.

ROSE, A. M., and D. L. BAILLIE, 1979 The effect of temperature and parental age on recombination and nondisjunction in *Caenorhabditis elegans*. Genetics **92:** 409–418.

ROSE, A. M., D. L. BAILLIE and J. CURRAN, 1984 Meiotic pairing behavior of two free duplications of linkage group I in *Caenorhabditis elegans*. Mol. Gen. Genet. **195:** 52–56.

ROSENBLUTH, R. E., C. CUDDEFORD and D. L. BAILLIE, 1983 Mutagenesis in *Caenorhabditis elegans*. I. A rapid eukaryotic mutagen test system using the reciprocal translocation *eT1(III;V)*. Mutat. Res. **110:** 39–48.

SANFORD, T., M. GOLOMB and D. L. RIDDLE, 1983 RNA polymerase II from wild type and amanitin-resistant strains of *Caenorhabditis elegans*. J. Biol. Chem. **258:** 12804–12809.

SANFORD, P., J. P. PRENGER and M. GOLOMB, 1985 Purification and immunological analysis of RNA polymerase II from *Caenorhabditis elegans*. J. Biol. Chem. **260:** 8064–8069.

SEARLES, L. L., R. S. JOKERST, P. M. BINGHAM, R. A. VOELKER and A. L. GREENLEAF, 1982 Molecular cloning of sequences from a *Drosophila* RNA polymerase II locus by *P*-element transposon tagging. Cell **31:** 585–592.

SENTENAC, A., 1985 Eukaryotic RNA polymerases. CRC Crit. Rev. Biochem. **18:** 31–91.

SULSTON, J. E., and S. BRENNER, 1974 The DNA of *Caenorhabditis elegans*. Genetics **77:** 95–104.

SULSTON, J. E., and H. R. HORVITZ, 1977 Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. Dev. Biol. **56:** 110–156.

SUZUKI, D. T., 1970 Temperature-sensitive mutations in *Drosophila melanogaster*. Science **170:** 695–706.

VON EHRENSTEIN, G., and E. SCHIERENBURG, 1980 Cell lineages and development of *Caenorhabditis elegans* and other nematodes. pp. 1–72. In: *Nematodes as Biological Models*, Vol. 1, Edited by B. M. ZUCKERMAN. Academic Press, New York.

WATERSTON, R. H., 1981 A second informational suppressor, *sup-7 X*, in *Caenorhabditis elegans*. Genetics **97:** 307–325.

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