

# Genetic Analysis of Adult-Specific Surface Antigenic Differences Between Varieties of the Nematode *Caenorhabditis elegans*

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## ABSTRACT

We have studied developmental stage-specificity and genetic specification of surface antigens in the nematode *Caenorhabditis elegans*. Rabbit antisera directed against the adult *C. elegans* cuticle were used in conjunction with antiserum adsorption experiments to obtain antibody reagents with specificity for the adult surface. Adult-specific antibodies were used to identify several varietal strains of *C. elegans* that display antigen-negative phenotypes as adults. Genetic mapping results using the surface antigen phenotype as a marker indicated that a single gene (designated *srf-1*) or cluster of genes on linkage group II determines the adult surface antigen phenotype.

ALL roundworms of the phylum *Nematoda* undergo four postembryonic molts separating five developmental stages; at each molt, a new extracellular proteinaceous cuticle is synthesized and the old one shed. Cuticle formation is therefore a fundamental aspect of postembryonic nematode development.

Developmental changes in cuticle structure have been characterized in the free-living nematode *Caenorhabditis elegans*. Stage-specific aspects of *C. elegans* cuticle structure include surface morphology, layer organization, protein composition (COX, STAPRANS and EDGAR 1981), and collagen gene expression (POLITZ and EDGAR 1984; KRAMER, COX and HIRSH 1985; COX and HIRSH 1985). Together, these studies indicate that four distinct cuticle types are synthesized during wild-type development. Cuticle structure has been used as a phenotypic marker to describe expression of stage-specific developmental features at inappropriate developmental times in retarded and precocious mutants of *C. elegans* (AMBROS and HORVITZ 1984).

Genetic studies of *C. elegans* cuticle structure have emphasized the role of the cuticle as the nematode exoskeleton. Mutations that alter the body shape and cuticle morphology of *C. elegans* have been identified in over 50 genes (BRENNER 1974; HIGGINS and HIRSH 1977; COX *et al.* 1980); some of these genes have unusual genetic properties suggesting that they correspond to the structural genes encoding cuticle collagens (KUSCH and EDGAR 1986).

In addition to its role as exoskeleton, the cuticle also acts as the major contact point with the environment. Developmental changes in the structure of the cuticle surface may have adaptive significance, particularly for nematode parasites that encounter specific

host immune responses. For example, there is immunological and parasitological evidence that expression of adult-specific surface antigens in the vertebrate parasite *Trichinella spiralis* allows newborn progeny to escape detection by the immune response generated against the adult parents (reviewed in WAKELIN and DENHAM 1983).

Specification of the nematode surface has not heretofore been studied by a genetic approach in *C. elegans* or in other nematodes. In the work described here, we have extended characterization of the stage-specific features of the wild-type *C. elegans* cuticle to include a difference in surface antigenicity between adults and preceding larval stages, using rabbit antisera directed against the adult *C. elegans* cuticle.

Adult-specific antibody binding was used to search for surface antigenic differences between wild-type and other varietal strains of *C. elegans*. Several independently isolated varietal strains appeared to be antigenically deficient when tested against antibodies that bound specifically to the surface of wild-type adults. Discovery of these strain-specific differences allowed genetic analysis by linkage testing and mapping, using immunofluorescence of live animals as a phenotypic marker. Surprisingly, in linkage tests with markers on each of the five autosomal linkage groups, only markers on chromosome II showed significant linkage to the surface antigen marker, suggesting that a single gene or cluster of genes determined the adult surface antigen phenotype. The marker, designated *srf-1*, was further mapped on the right arm of chromosome II.

## MATERIALS AND METHODS

**Preparation of immunogens and antisera:** Preparation of rabbit antisera directed against *C. elegans* cuticle proteins

has been described (POLITZ, POLITZ and EDGAR 1986). Nematode growth conditions and preparation of cuticles and cuticle proteins were as described (COX, KUSCH and EDGAR 1981). Antiserum from a single bleeding of a rabbit immunized with adult solubilized cuticle proteins was used in all experiments except that of Figure 3. Antiserum from three other rabbits immunized with adult cuticle antigens behaved in a qualitatively similar way to the antiserum described here. An antiserum from a rabbit immunized with crude fourth larval stage (L4) cuticles was used in the experiment of Figure 3.

**Antiserum adsorption:** *C. elegans* strain N2 (wild type) was grown from dauer larvae on *Escherichia coli* OP50 lawns on NG plates (BRENNER 1974) containing 0.4% nutrient broth and 2.5% agar,  $5\text{--}10 \times 10^3$  animals/plate, for 41–44 hr (adults) or 17–18 hr (L4s) at 20°. Nematodes were harvested and washed three times with M9 buffer (BRENNER 1974). Adults were settled repeatedly to remove eggs and larvae. For adsorption with live worms, antiserum was added to adults or L4s in PBS (0.01 M Na-phosphate, pH 7.0, 0.15 M NaCl) in a final volume 10 (L4s) or 20 (adults) times the volume of antiserum. Ratios for adsorption were 40  $\mu$ l serum per 33,000 adults or 40  $\mu$ l serum per 50,000 L4s. After incubation with gentle shaking for 1 hr at room temperature, serum was separated from nematodes by filtration through a 12.0  $\mu$ m polycarbonate filter (13 mm diameter, Nucleopore) and stored at  $-20^\circ$ .

**Immunofluorescent staining of nematodes:** L4s or adults were grown and harvested as described above. Antibody staining of nematodes from plates showing bacterial contamination was avoided; nematodes from contaminated plates showed greatly reduced surface immunofluorescence. Aliquots containing 150 adults or 375 L4s in 80  $\mu$ l PBS were incubated with 30–60  $\mu$ l of a 1:10 dilution of anticuticle protein antiserum in PBS, or an equivalent volume of previously adsorbed serum. These amounts of antiserum were determined by separate radioimmunoassay titration (see below) to provide conditions of antibody excess. Samples were incubated for 1.5 hr at 21–23° with shaking (250 rpm) in flat-bottomed 2 ml glass vials. Nematodes were then washed three times in 3 ml conical centrifuge tubes by low speed centrifugation and resuspension in PBS. After washing, nematodes were incubated for one hour in fresh vials in 100–150  $\mu$ l PBS with 25  $\mu$ l of fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit globulins (Sigma F-6005). Samples were then washed six times as before. For photomicroscopy, nematodes were killed by gentle heating on a slide in PBS, before covering with a coverslip.

Surface immunofluorescence was photographed under the 6.3 Neofluar objective of a Zeiss Universal microscope, using epifluorescence optics, a narrow band FITC filter set, and Kodak TriX Pan film. Exposures were timed for 5.0 min. Negatives were printed to the same background density for direct comparison.

**Radioimmunoassay:** L4s or adults were grown, harvested, and incubated with anticuticle serum as described above for immunofluorescent staining. After the first incubation and washing, nematodes in PBS were incubated for 1 hr with 14 or 16  $\mu$ l of  $^{125}$ I-labeled *Staphylococcus aureus* protein A. Protein A was labeled by the chloramine T method (CAMPBELL *et al.* 1970); specific activities were typically about  $2 \times 10^7$  cpm/ $\mu$ g. After washing six additional times with PBS, total radioactivity bound to each nematode pellet was measured in a gamma counter.

Initial radioimmunoassay experiments tested saturability of binding of antibodies to nematodes. Titration with nematodes, antiserum or protein A produced a saturable binding curve in each case. Binding to 300 adult nematodes was

saturated by 10  $\mu$ l of antiserum and 14  $\mu$ l of  $^{125}$ I-protein A. Subsequent radioimmunoassay experiments were performed under conditions of antibody limitation and protein A saturation to minimize variability caused by pipetting nematode suspensions. Results from duplicate samples were reproducible within *ca.* 5%.

To compare binding of unadsorbed and adsorbed antiserum to nematodes, equivalent volumes of diluted serum were added to each sample, and final incubation volumes were made equal by addition of PBS. In order to make total surface areas per sample equivalent, 2.5 times as many L4s as adults per sample were used. Relative surface areas of L4s and adults were calculated from data in Table I of COX, STAPRANS and EDGAR (1981). Relative lengths of adults of different *C. elegans* strains were measured using a dissecting microscope ocular micrometer after growth under standard conditions and were similar for N2, PA-1, DH424, and *lin-4(e912)* adults. For experiments comparing antibody binding of *lin-4(e912)* animals to wild-type, *lin-4* populations were synchronized by treatment of gravid adults with sodium hypochlorite (EMMONS, KLASS and HIRSH 1979). This treatment dissolves *C. elegans* adults and larval stages but leaves eggs intact and viable. After hatching overnight in M9 buffer, newly hatched L1s were plated onto NGM plates and grown to sexual maturity for 92 hr at 20°. Wild-type (N2) animals to be compared to *lin-4* were synchronized similarly.

**Strains:** Wild-type (strain N2) (BRENNER 1974), deficiency-containing, and mutant strains of *C. elegans* var. Bristol were obtained from the *Caenorhabditis* Genetics Center, University of Missouri, Columbia, Missouri 65211. We obtained *lin-4(e912)* and *lin-29(n546) rol-1(e91)/mnC1 dpy-10(e128) unc-52(e444)* from V. AMBROS. Standard *C. elegans* genetic nomenclature are used throughout (HORVITZ *et al.* 1979). Mutations used were previously described by BRENNER (1974), unless otherwise noted, and are listed by linkage group, as follows:

LG I: *dpy-5(e61)*.

LG II: *lin-4(e912)* [isolated by P. BABU after  $^{32}$ P mutagenesis, cited in HORVITZ and SULSTON (1980); mapping described by HODGKIN (1974)], *lin-29(n546)* (AMBROS and HORVITZ 1984), *dpy-10(e128)*, *unc-4(e120)*, *rol-1(e91)*, and *unc-52(e444)*. Deficiency-containing strains were genotypically *unc-4(e120) Df/mnC1 dpy-10(e128) unc-52(e444)* and contained *mnDf83*, *mnDf89*, or *mnDf77*. *mnC1* is a dominant crossover suppressor for the *dpy-10* to *unc-52* interval that maps very close to these markers (HERMAN 1978). Identification and mapping of deficiencies was described by STIGURDSON, SPANIER and HERMAN (1984).

LG III: *dpy-18(e364)*.

LG IV: *dpy-13(e184)*.

LG V: *dpy-11(e224)*.

Varietal strains obtained from the *Caenorhabditis* Genetics Center were interfertile with Bristol (strain N2) males and were originally collected from the wild as follows (M. EDGLEY, personal communication): AB1 and AB3, A. BIRD, Adelaide, Australia; PA-1 and PA-2, E. HEDGECOCK, Pasadena, California, 1973; GA-1, GA-2, and GA-5, C. JOHNSON, Altadena, California, 1973–74; CL2a, Claremont, California, 1972; DH424, El Prieto Canyon, California. Bergerac BO is descended from the Bergerac stock first described by NIGON (1949).

Visible mutant phenotypes were as follows: *Unc*, uncoordinated movement; *Dpy*, short fat body; *Rol*, cuticle helically twisted so that animals roll over as they move. *lin-29(n546)* mutant adults have a protruding vulva and are defective in egg laying.

**Genetic methods:** *C. elegans* strains were grown and ge-

netic crosses were performed at 20° as described previously (BRENNER 1974).

The surface antigen phenotype of N2/PA-1, N2/DH424 or PA-1/DH424 F<sub>1</sub> hybrids was assessed as follows. N2 or PA-1 males were mated with PA-1, N2, or DH424 hermaphrodites in hybrid combinations. At least 100 L4 or young adult F<sub>1</sub> progeny males were picked onto a fresh plate and allowed to grow for 41 hr at 20°. F<sub>1</sub> males were stained with L4-adsorbed, adult-specific antiserum as described above. The sample was scored in 100 µl PBS for the number of fluorescent *vs.* nonfluorescent males at ×25 magnification under the FITC optics of a Wild M5 stereomicroscope equipped with an epifluorescence attachment (Leitz, Rockleigh, New Jersey). Male control samples were stained and scored in a similar fashion.

Linkage analysis of the adult surface antigen marker was performed in crosses between strains carrying a visible linkage marker in the Bristol (antigen-positive) genetic background and antigen-negative strains. N2 males were mated with Bristol homozygous mutant hermaphrodites. Heterozygous cross-progeny males were then mated with antigen-negative hermaphrodites (strain PA-1, AT1, or AT2). Immature F<sub>1</sub> hermaphrodites were picked onto separate plates. F<sub>1</sub> hybrid cross progeny were identified as those segregating the Bristol marker in the F<sub>2</sub> by self-fertilization, and phenotypically nonmarker F<sub>2</sub> segregants of F<sub>1</sub> outcross parents were picked onto separate plates. Those that segregated no marker progeny by self-fertilization were assumed to be homozygous for the PA-1 (nonmutant) allele of the visible marker; these clones were saved for analysis of the surface antigen marker. Use of homozygous nonmarker clones allowed testing of surface antigen phenotype after only one generation of self-fertilization, because of larger brood sizes of the nonmarker hermaphrodites, and did not require additional assumptions of noninteraction of visible marker phenotypes with the surface antigen phenotype.

Progeny from each homozygous nonmarker clone were harvested and tested separately for binding of adult-specific antibodies by immunofluorescence. Antibody screening was similar to that described above. For samples containing more than 150 adults, a fraction containing 150 adults was incubated to insure that samples were screened under conditions of antibody saturation. Each sample was scored as either antigen-positive or antigen-negative. Tables of the binomial distribution (MOSTELLER, ROURKE and THOMAS 1970) were used to decide whether linkage data deviated significantly from the result expected for independent assortment (BRENNER 1974). Symmetric 95% confidence intervals were used.

In practice, negative clones were easily distinguishable from positive ones. Negative clones were defined as those similar to the negative parental strain (>95% negative adults) and all positive clones had >70% positive adults. In several cases, true-breeding negative segregant lines were established by selecting individuals from a negative clone and retesting their progeny with antibodies, confirming the negative phenotype of the original clone in each case. Strain AT1 was derived from a non-Unc antigen-negative F<sub>2</sub> segregant obtained from a cross between PA-1 males and *unc-4(e120)II* hermaphrodites. Strain AT2 was obtained similarly by crossing AT1 to *dpy-11(e224)V* and selecting a non-Dpy antigen-negative segregant. AT1 and AT2 were used in some linkage testing and genetic mapping experiments in place of PA-1.

Three-factor crosses were performed similarly to two-factor crosses described above, except that LGII double mutant markers in the Bristol genetic background were used instead of single mutant markers. Bristol heterozygous males carrying the markers were mated with hermaphro-

ditis from an antigen-negative strain. Phenotypically recombinant segregants were picked and allowed to eliminate the nonrecombinant chromosome by self-fertilization. Homozygous recombinant clones from all three-factor crosses were screened with adult-specific antibodies as described above.

Details of three-factor crosses were as follows. In a three-factor cross between *dpy-10 unc-4/+* + males and AT2, Dpy non-Unc or Unc non-Dpy recombinant segregants were picked. An Unc antigen-negative clone was saved and an individual hermaphrodite was picked to establish AT3, an antigen-negative strain marked with *unc-4*. AT4, an antigen-negative strain marked with *unc-4* but derived from strain DH424, was obtained in a similar fashion. In a three-factor cross with *unc-4* and *rol-1* mutant alleles in *trans*, Bristol males of genotype *rol-1/+* were mated with strain AT3 hermaphrodites. Homozygous Rol Unc or wild-phenotype recombinant segregants were picked. In a three-factor cross between *lin-29 rol-1/mnC1 dpy-10 unc-52* hermaphrodites and PA-1 males, only Rol recombinant segregants could be identified and picked, because *lin-29* is a recessive suppressor of *rol-1* (V. AMBROS, personal communication).

Complementation between the surface antigen marker and chromosome II deficiencies (SIGURDSON, SPANIER and HERMAN 1984) was measured in heterozygotes. For complementation testing, antigen-negative PA-1 males were mated with antigen-negative AT3 hermaphrodites, or antigen-negative DH424 males were mated with antigen-negative AT4 hermaphrodites. Cross-progeny males were assumed to be homozygous for *srf-1(yj1)* or *srf-1(yj2)*, the PA-1 or DH424 allele of the putative gene determining the surface antigen phenotype. These males were mated with *unc-4 Df/mnC1 dpy-10 unc-52* hermaphrodites. F<sub>1</sub> outcross progeny containing the deficiency were recognizable by their Unc phenotype. Immature Unc progeny were picked and allowed to grow to adulthood on a separate plate. These were then screened with adult-specific antibodies to assess the surface antigen phenotype.

## RESULTS

**Characterization of wild-type adult-specific surface antigen phenotype:** Antibody binding to the surface of live wild-type (*C. elegans* var. Bristol, strain N2) nematodes was detected initially by indirect immunofluorescence observed microscopically (Figure 1). Antibodies contained in a rabbit antiserum to adult cuticle proteins reacted very uniformly with the surface of live adults (Figure 1A). The antiserum also cross-reacted uniformly with the surface of live fourth stage larvae (L4s) (Figure 1B) and all other larval stages (data not shown). Preimmune serum showed little reaction with either the L4 or adult surface (Figure 1, E and F), although gut fluorescence was sometimes observed (*e.g.*, Figure 1E), probably due to ingestion of the FITC second antibody.

Although crude serum cross-reacted with L4s, it could be made adult-specific by adsorption. The anti-adult cuticle protein serum used in the experiment of Figure 1, A and B, was cross-adsorbed with large numbers of live L4s to remove L4-reactive antibodies. After L4 cross-adsorption, antibody binding of residual serum to the L4 surface was reduced to back-

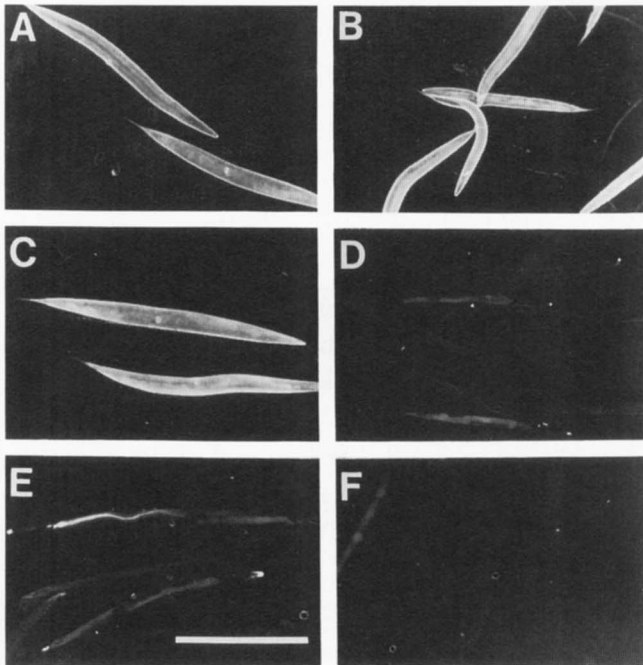


FIGURE 1.—Indirect immunofluorescence staining of *C. elegans* wild-type (strain N2) adults and L4s. Nematodes were stained with rabbit anticuticle antibodies and FITC goat anti-rabbit second antibody as described in MATERIALS AND METHODS. (A) Adults and (B) L4s stained with whole anti-adult serum. (C) Adults and (D) L4s stained with anti-adult serum that was previously cross-adsorbed with L4s. (E) Adults and (F) L4s stained with preimmune serum from the same rabbit as in panels A–D. The bar in (E) indicates 500  $\mu\text{m}$ .

ground levels (Figure 1D) compared to the level of binding seen with an equivalent amount of unadsorbed serum (Figure 1B). Binding to other larval stages was also eliminated by L4 cross-adsorption (not shown). In contrast to the result with L4s, L4-adsorbed serum retained substantial capacity to bind to the adult surface (Figure 1C), with immunofluorescence of adults qualitatively similar to that seen with unadsorbed serum (Figure 1A). The effect of adsorption required the presence of L4s in the adsorption reaction; incubation and filtration of antiserum in their absence had no effect on subsequent binding (S. POLITZ, unpublished data).

The apparent stage-specific antibody binding observed by immunofluorescence was confirmed by indirect radioimmunoassay. Binding of equivalent amounts of unadsorbed or L4-adsorbed antiserum to L4s or adults was measured. Results are shown in Figure 2. Unadsorbed serum reacted with both L4s and adults (Figure 2, *clear bars*), in agreement with the immunofluorescence results. After L4 cross-adsorption, an equivalent amount of residual serum did not react with live L4s (Figure 2, *left panel, shaded bar*) detectably above preimmune background (level of preimmune binding is indicated in legends to Figures 2–4 and 6). In contrast, reaction of the adsorbed serum with adults was reduced quantitatively (Figure

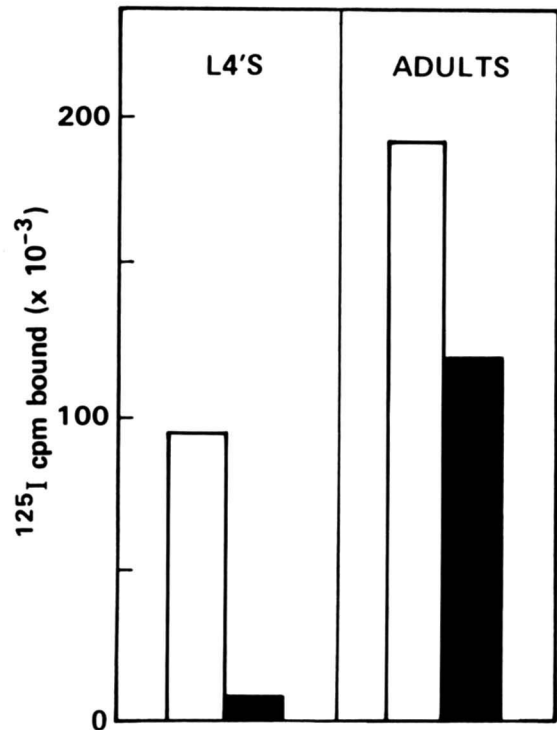


FIGURE 2.—Radioimmunoassay of anti-adult antibody binding to L4s and adults. Serum was raised by immunization with an adult SDS-2-mercaptoethanol-soluble cuticle protein immunogen. Each nematode sample was incubated with the equivalent of 5  $\mu\text{l}$  of anti-cuticle serum and then with 16  $\mu\text{l}$  of  $^{125}\text{I}$ -protein A. The height of each bar represents the total  $^{125}\text{I}$  radioactivity bound by the nematode pellet in a sample. *Clear bars* represent binding of whole antiserum; *black bars* represent binding of L4 cross-adsorbed antiserum. *Left panel*: binding to L4s; 750 synchronous L4s per sample. *Right panel*: binding to adults; 300 synchronous adults per sample. Level of preimmune serum binding to 300 N2 adults (not shown) was 7891 cpm.

2, *right panel, shaded bar*), but was still significantly higher than the preimmune background.

A rabbit antiserum directed against an L4 cuticle fragment immunogen was used to search for a reciprocal pattern of L4-specific antibody binding. Anti-L4 serum was subjected to cross-adsorption with adults and then tested for L4-specific antibody binding. These results are shown in Figure 3. Unadsorbed anti-L4 serum cross-reacts with the adult surface (Figure 3, *right panel, clear bar*). In contrast to the anti-adult serum results (Figures 1 and 2), however, after cross-adsorption with saturating numbers of live adults, residual antiserum reacted with neither the adult (Figure 3, *right panel, stippled bar*) nor the L4 (Figure 3, *left panel, stippled bar*) surface.

**Adult surface antigen phenotype of a heterochronic mutant:** A mutation on linkage group II, *lin-4(e912)*, causes expression of a larval cuticle type in sexually mature adults (AMBROS and HORVITZ 1984; CHALFIE, HORVITZ and SULSTON 1981). Both cuticle surface morphology and cross-sectional layering pattern are of larval appearance in sexually mature *lin-4* animals, and the electrophoretic pattern of cuticle

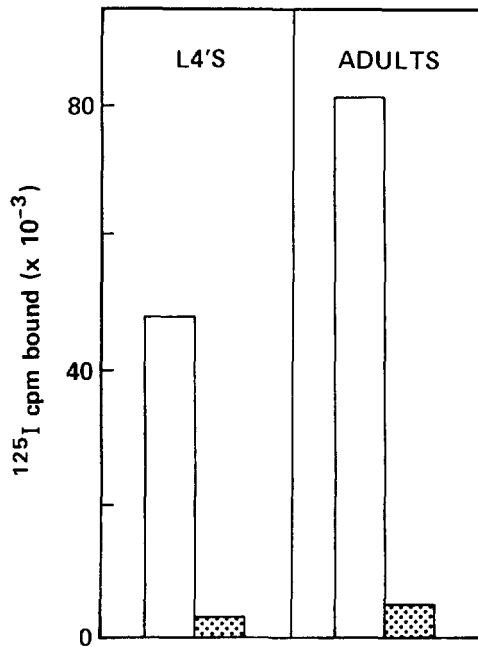


FIGURE 3.—Radioimmunoassay of anti-L4 antibody binding to L4s and adults. Results were obtained with rabbit antisera raised by immunization with an L4 cuticle fragment immunogen. Each nematode sample was incubated with the equivalent of 5  $\mu$ l of anticuticle antiserum and 14  $\mu$ l of  $^{125}$ I-protein A. The height of each bar indicates the total amount of radioactivity bound by the nematodes in a sample. *Clear bars* indicate whole serum and *stippled bars* indicate anti-L4 serum cross-adsorbed with adults. *Left panel*: binding to L4s; 1250 synchronous L4s per sample. *Right panel*: binding to adults; 500 synchronous adults per sample. Level of preimmune binding to similar samples was 3037 cpm for adults and 2608 cpm for L4s (not shown).

proteins extracted from mature adults is characteristic of the larval cuticle (EDGAR *et al.* 1982). Recognition of cuticle type was tested by measuring binding of unadsorbed and L4-adsorbed antibody to *lin-4* and wild-type animals by radioimmunoassay. Unadsorbed anti-adult serum cross-reacted with sexually mature live *lin-4* animals (Figure 4, *clear bar*), as would be expected for wild-type L4s. However, L4-adsorbed antiserum reacted at a reduced level with wild-type adults (Figure 4, *shaded bar*), but showed almost no reaction with sexually mature *lin-4* animals (Figure 4, *shaded bar*).

**Surface antigenic differences between *C. elegans* varietal strains:** Several varietal strains of *C. elegans* were tested for surface antigenic differences from wild type by indirect immunofluorescent staining with L4-adsorbed antiserum and FITC-labeled second antibody under conditions of primary and secondary antibody excess. The number of fluorescent and non-fluorescent adults was scored at  $\times 50$  under an epifluorescent stereomicroscope; no larvae in any of the strains were observed to fluoresce. In the positive control, 95% of wild-type (strain N2) adults showed fluorescence. Adults of strains PA-2, GA-1, GA-2, AB1, AB3, CL2a, and GA-5 showed fluorescence

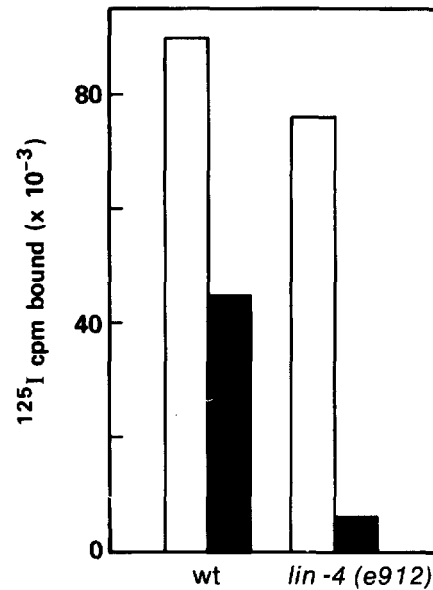


FIGURE 4.—Binding of anti-adult antibodies to *lin-4(e912)* adults. Each sample containing 300 nematodes was incubated with the equivalent of 6  $\mu$ l of total anti-adult antiserum and 14  $\mu$ l of  $^{125}$ I-protein A. The height of each bar indicates the total radioactivity bound by the nematodes in a sample. *Clear bars* indicate whole antiserum; *black bars* indicate antiserum cross-adsorbed with N2 L4s. *Left*: binding to synchronous N2 adults. *Right*: binding to synchronous *e912* adults. Level of binding of preimmune serum to similar samples was 1501 cpm for N2 adults and 2868 cpm for *lin-4* adults.

similar to wild type. In contrast, adults of three varietal strains (PA-1, DH424, and Bergerac BO) were essentially nonfluorescent. In these negative strains, immunofluorescent adults were occasionally observed (0–5% of all adults). Conversely, 0–5% of N2 adults were negative by immunofluorescence. In either case, progeny testing excluded a genetic explanation; 95–100% of progeny of cloned variant animals showed the nonvariant phenotype. The apparent variation was therefore treated as partial penetrance of a genetically determined trait.

Surface antigen phenotypes of strains PA-1, DH424, and N2 were examined in more detail. At higher magnification, the head, tail, and vulva of PA-1 adults labelled with adult-specific antibody showed fluorescence (Figure 5A). Localization of fluorescence to small areas surrounding body openings was strikingly different from the N2 pattern, which was typified by uniform surface fluorescence over the whole body (Figure 1C and 5B). The adult-specific labeling pattern of strain DH424 was similar to that observed for PA-1 (data not shown).

Reduction in amount of adult-specific antibody binding to strains PA-1 and DH424 was confirmed by radioimmunoassay (Figure 6). Synchronous populations of N2 L4s or PA-1, DH424, or N2 adults were tested under conditions of nematode excess. Unadsorbed serum contained antibodies that cross-reacted with adults of all three strains (Figure 6, *left panel*).

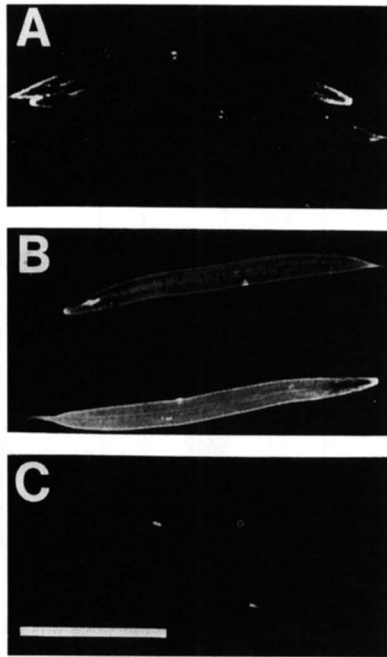


FIGURE 5.—Indirect immunofluorescence staining of N2 and PA-1 adults with L4-adsorbed antibodies. Each sample containing 150 synchronous adults or 375 L4s was incubated with 6  $\mu$ l of L4 cross-adsorbed anti-adult cuticle rabbit antiserum and FITC second antibody. (A) PA-1 adults, (B) N2 adults, and (C) N2 L4s. The bar in (C) indicates 500  $\mu$ m.

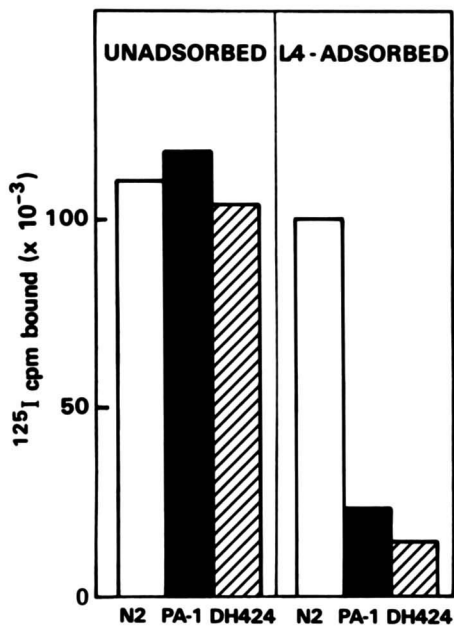


FIGURE 6.—Radioimmunoassay of adult-specific antibody binding to PA-1 and DH424 adults. Each sample containing 300 synchronous adults or 750 synchronous L4s was incubated with the equivalent of 5  $\mu$ l of total anti-adult cuticle antiserum and 16  $\mu$ l of  $^{125}$ I-protein A. The height of each bar represents the total amount of  $^{125}$ I radioactivity bound by the nematodes in a sample. *Left panel*: whole anti-adult cuticle serum. *Right panel*: L4 cross-adsorbed anti-adult serum. *Clear bars*: binding to N2 adults. *Shaded bars*: binding to PA-1 adults. *Cross-hatched bars*: binding to DH424 adults. Level of preimmune serum binding to 300 N2 adults was 14,042 cpm (not shown).

TABLE 1

Adult surface antigen phenotype of PA-1/N2 and PA-1/DH424 hybrids

| Genotype                                  | No. of adult males showing phenotype |                  |
|---|--------------------------------------|------------------|
|   | Antigen-positive                     | Antigen-negative |
| Bristol (N2)                              | 139                                  | 0                |
| PA-1                                      | 4                                    | 92               |
| PA-1/N2 (PA-1 $\delta$ $\times$ N2)       | 114                                  | 3                |
| N2/PA-1 (N2 $\delta$ $\times$ PA-1)       | 130                                  | 0                |
| DH424                                     | 0                                    | 134              |
| N2/DH424 (N2 $\delta$ $\times$ DH424)     | 141                                  | 32               |
| PA-1/DH424 (PA-1 $\delta$ $\times$ DH424) | 42                                   | 187              |

Phenotype was tested by adult-specific antibody staining as described in MATERIALS AND METHODS.

In contrast, L4-adsorbed serum reacted well (although at a quantitatively reduced level) only with N2 adults (Figure 6, *right panel*, *clear bar*). Antibody binding to PA-1 (Figure 6, *right panel*, *shaded bar*) adults was reduced to a level only about twice the pre-immune background, while binding to DH424 (Figure 6, *right panel*, *cross-hatched bar*) adults was reduced to background level.

**Genetic analysis of surface antigenic differences between varietal strains:** Table 1 shows that 97–100% of N2/PA-1 males were antigen-positive, similar to the control sample in which 100% of N2 males were positive; and 96% of negative control PA-1 males were antigen-negative. Thus the antigen-negative phenotype is recessive to the antigen-positive phenotype in PA-1/N2 heterozygotes. The antigen-negative phenotype of DH424 is also recessive in N2/DH424 heterozygotes (Table 1). PA-1/N2 males were almost all antigen-positive regardless of which parent (male or hermaphrodite) was antigen-negative, indicating that the adult surface antigen trait is not inherited in a sex-linked fashion. Only 18% of PA1/DH424 heterozygotes were antigen-positive (Table 1), indicating that the genotypes of PA-1 and DH424 fail to complement each other for this phenotype.

Table 2 summarizes linkage data obtained using markers on each of the five autosomes. Only markers on chromosome II showed significant linkage to the PA-1 surface antigen marker; with *dpy-10*, *unc-4*, or *rol-1*, about 80% of the homozygous non-marker clones screened were antigen-negative. Markers on the other linkage groups showed no significant linkage. The PA-1 and DH424 surface antigen markers on LGII are hereafter designated *srf-1(yj1)* and *srf-1(yj2)*, respectively, for convenience, although there is presently no evidence other than complementation to indicate that a single gene, rather than a cluster of linked genes, determines the adult surface antigen phenotype. Positions of chromosome II linkage markers are shown in Figure 7.

Three-factor crosses using LGII markers indicated



TABLE 2  
Linkage analysis

| Linkage group | Marker/antigen-negative strain | No. of homozygous nonmarker segregant clones |                  |
|---------------|--------------------------------|--|------------------|
|               |                                | Antigen-positive                             | Antigen-negative |
| I             | <i>dpy-5</i> /AT1 <sup>a</sup> | 6  | 5                |
| II            | <i>dpy-10</i> /PA-1            | 2  | 8                |
|               | <i>dpy-10</i> /AT1             | 4  | 17               |
|               | <i>unc-4</i> /AT2 <sup>b</sup> | 3  | 8                |
|               | <i>rol-1</i> /AT1              | 2  | 9                |
|               | <i>unc-52</i> /AT1             | 4  | 5                |
| III           | <i>dpy-18</i> /PA-1            | 7  | 4                |
|               | <i>dpy-18</i> /AT1             | 5  | 7                |
| IV            | <i>dpy-13</i> /AT1             | 10   | 2                |
| V             | <i>dpy-11</i> /PA-1            | 10   | 2                |
|               | <i>dpy-11</i> /AT1             | 8  | 4                |

<sup>a</sup> AT1, an antigen-negative segregant of a cross between *unc-4(e120)* and PA-1.

<sup>b</sup> AT2, an antigen-negative segregant of a cross between *dpy-11(e224)* and AT1.

that *srf-1* was either to the right of *rol-1* or very close to it on the left (see Table 3 and Figure 7). Without further mapping information, *srf-1* could not be located more precisely; *i.e.*, no position between *rol-1* and the right end of linkage group II could be excluded. However, linkage data indicated that *unc-52*, the most distal marker mapped on the right arm of chromosome II (Figure 7), is only weakly linked to *srf-1* (Table 2), suggesting that *srf-1* is closer to *rol-1* than to *unc-52*.

To distinguish between a *srf-1* map position to the left versus to the right of *rol-1*, the ability of *srf-1* to complement deficiencies in the region was tested. *mnDf83* is a deficiency of *bli-1*, *sqt-1*, and *lin-29* but complements *rol-1* (SIGURDSON, SPANIER and HERMAN 1984). Its right endpoint therefore maps between *lin-29* and *rol-1* (Figure 7). Complementation between *mnDf83* and *srf-1* was assessed in heterozygotes of genotype *unc-4 srf-1/unc-4 mnDf83*. With *srf-1(yj1)*, 85% of adults were antigen-negative, and with *srf-1(yj2)*, 98% of adults were antigen-negative (Table 4), indicating that both PA-1 and DH424 alleles of *srf-1* fail to complement *mnDf83*. A similar result (97% negative adults, Table 4) was obtained with *srf-1(yj1)* and *mnDf89*, whose right endpoint also maps between *lin-29* and *rol-1* (Figure 7). These results exclude a position for *srf-1* to the right of *rol-1*. Complementation was also assessed between *srf-1(yj1)* and *mnDf77*, a smaller deficiency that is within *mnDf83* but does not extend into the region between *lin-29* and *rol-1*. 93% of adults of genotype *unc-4 srf-1/unc-4 mnDf77* were antigen-positive (Table 4), and therefore *mnDf77* complements *srf-1*. This experiment excludes a map position for *srf-1* within *mnDf77*.

By the criterion of deficiency mapping, *srf-1* could

be located within *mnDf83* to the left or the right of *mnDf77*. However, the relevant three-factor cross excludes the possibility that *srf-1* is to the left of *lin-29*. Therefore, we conclude that *srf-1* maps between *lin-29* and *rol-1*, but very close to *rol-1*. Positions of deficiencies and the map position of *srf-1* are diagrammed in Figure 7.

## DISCUSSION

There are striking correlations between stage-specificity of surface antigen expression described here for *C. elegans* and the results of experiments on surface antigenicity of the parasitic nematode *Trichinella spiralis*. Evidence for adult-specific and larval-specific surface antigens in *Trichinella* was first obtained by OLIVER-GONZALEZ (1941), who performed antiserum adsorption experiments similar in principle to those described here. Stage-specificity of *Trichinella* surface antigens has been demonstrated more recently by modern immunochemical methods (PHILIPP, PARKHOUSE and OGILVIE 1980; PHILIPP *et al.* 1981). Inability of larval stages to express adult-specific surface antigens might have adaptive significance for *Trichinella*, which reproduces in the same host individual in which it develops. Newborn larvae appear after host immune responses to larval and adult stages of the parental generation have been stimulated, so that restriction of immunodominant antigens to the adult stage may allow migration of newborn larvae to the muscles without interference from the host immune system (WAKELIN and DENHAM 1983). The enteral and parenteral stages of *Trichinella* are treated as different animals by the host; immunity produced against one stage has no adverse effect on the other (JAMES, MOLONEY and DENHAM 1977; JAMES and DENHAM 1975).

We have used rabbit anticuticle antibodies to detect two distinct classes of surface antigens in *C. elegans*. The results suggest that all antigens on the larval surface are also found on the adult surface, but that a distinct class of adult-specific surface antigens also exists. The similarity of these results to those obtained with *Trichinella* indicates that information about the genes specifying surface antigens in *C. elegans* might also be relevant to understanding parasite surface antigen expression.

Expression of the adult-specific surface antigen class depends on the presence of the adult-specific cuticle type, and not simply sexual maturity of the animal. Adult-specific antibodies did not recognize sexually mature *lin-4(e912)* adults that express a larval cuticle as an adult. Thus, expression of the adult-specific antigen class appears to be controlled coordinately with other cuticle structural features that are missing in *lin-4(e912)*, such as the vulva, adult-specific lateral

FIGURE 7.—Partial genetic map of *C. elegans* linkage group II. Map shows *srf-1*, relevant Bristol genetic loci and deficiencies. Map is to scale except for right terminus. Positions of all loci except *srf-1* and *lin-29* are from SWANSON, EDGLEY and RIDDLE (1984). Position of *lin-29* is from AMBROS and HORVITZ (1984). Positions of *mnDf83*, *mnDf89* and *mnDf77* are from SIGURDSON, SPANIER and HERMAN 1984. Mapping of *srf-1* is described in the text. The bar length for *srf-1* position represents the 95% confidence interval for the distance from *rol-1* inferred from a three-factor cross with flanking markers *lin-29* and *rol-1*.

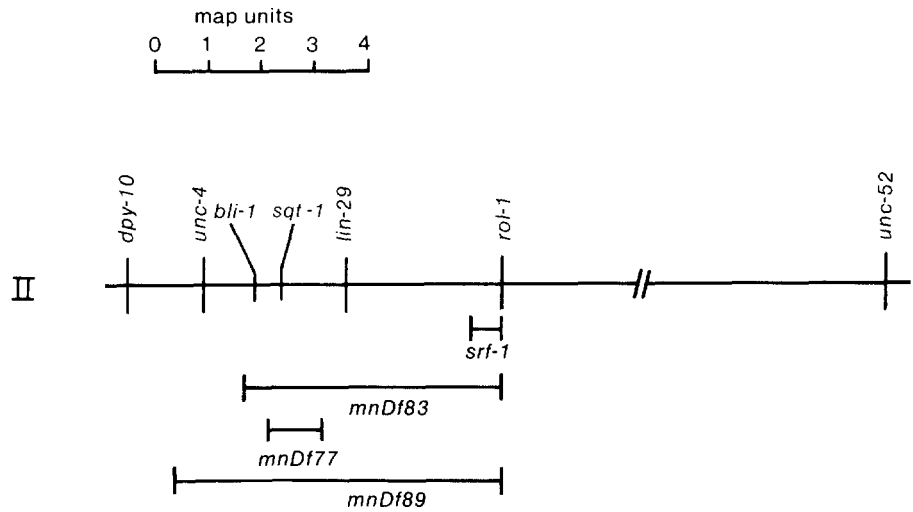


TABLE 3  
Three-factor crosses

| Parental genotype <sup>a</sup> | Recombinant phenotype | No. of recombinants showing phenotype |                  |
|--------------------------------|-----------------------|---------------------------------------|------------------|
|                                |                       | Antigen-positive                      | Antigen-negative |
| <i>dpy-10 unc-4 +</i> (N2)     | Dpy                   | 0                                     | 7                |
| <i>+ + srf-1</i>               | Unc                   | 11                                    | 2                |
| <i>unc-4 srf-1 +</i>           | Rol Unc               | 12                                    | 0                |
| <i>+ + rol-1</i> (N2)          | wt                    | 0                                     | 13               |
| <i>lin-29 + rol-1</i> (N2)     | Rol <sup>b</sup>      | 14                                    | 0                |
| <i>+ srf-1 +</i>               |                       |                                       |                  |

<sup>a</sup> All nonmutant alleles, whether from PA-1 or N2, are indicated by + for simplicity. *srf-1* indicates *yj1*, the allele introduced from PA-1. (N2) denotes Bristol chromosomes. Genes are shown in inferred order.

<sup>b</sup> Reciprocal *lin-29* (non-Rol) recombinants could not be identified because *lin-29* is a recessive suppressor of *rol-1*.

alae, and the ultrastructural pattern of cuticle layers characteristic of the adult cuticle (AMBROS and HORVITZ 1984).

The adult-specific surface antigens expressed by wild-type *C. elegans* adults are missing or expressed at a very low level in adults of several *C. elegans* varietal strains. These strains are only antigen-negative with respect to the adult-specific antibodies; an unadsorbed antiserum that cross-reacts with the L4 surface also cross-reacts with adults of strains PA-1 and DH424 (Figure 6). Thus the adult-specific antigens comprise a more restricted class of antigens than the L4-adult shared class by genetic as well as developmental criteria.

Adults of the antigen-negative strains PA-1 and DH424 have apparently wild-type adult lateral alae,

TABLE 4  
Complementation between *srf-1* and deficiencies

| F <sub>1</sub> genotype              | Phenotypes of F <sub>1</sub> adults |                                      |
|--------------------------------------|-------------------------------------|--------------------------------------|
|                                      | Total no. adults scored             | Percent antigen-positive (± range/2) |
| <i>unc-4 srf-1(yj1)/unc-4 mnDf83</i> | 135                                 | 15 ± 2                               |
| <i>unc-4 srf-1(yj1)/unc-4 mnDf89</i> | 147                                 | 3 ± 1                                |
| <i>unc-4 srf-1(yj1)/unc-4 mnDf77</i> | 149                                 | 93 ± 5                               |
| <i>unc-4 srf-1(yj2)/unc-4 mnDf83</i> | 128                                 | 2 ± 2                                |

F<sub>1</sub> heterozygotes of the genotypes shown were constructed and tested for antibody binding as described in MATERIALS AND METHODS. *yj1* and *yj2* refer to the *srf-1* alleles carried by strains PA-1 and DH424, respectively. Results with *mnDf83* and *mnDf89* are the sum of two samples; results with *mnDf77* are the sum of three samples. The range was calculated as the difference in percentages between the highest and lowest samples.

and cuticle collagens extracted from adults of strain DH424 show the SDS-polyacrylamide gel electrophoresis pattern characteristic of wild-type adults (S. POLITZ and D. L. HERMAN, unpublished data). These preliminary results suggest that the lesion in these strains is specific to surface antigen expression, unlike mutations such as *lin-4(e912)* that cause extensive cuticle alterations and changes in underlying cell lineage patterns (AMBROS and HORVITZ 1984).

Linkage of the *srf-1* gene to chromosome II markers was demonstrated in crosses between antigen-negative strains and visibly marked Bristol strains. F<sub>2</sub> segregant clones derived from PA-1/N2 F<sub>1</sub> hybrids gave an approximate 3:1 distribution of antigen-positive to antigen-negative populations (Table 2). Thus, although the antigen-negative strains used were not congenic with Bristol (N2), no evidence was found for polygenic determination of the adult surface antigen phenotype. Moreover, the failure of *yj1* and *yj2* to



complement each other (Table 1) suggests that the same gene function is altered in both PA-1 and DH424. Because *mnDf83* failed to complement both *yj1* and *yj2* (Table 4), the location of these mutations is also consistent with the idea that they are alleles. However, at present we cannot eliminate the possibility that *srf-1* is a cluster of interacting genes.

Mapping of *srf-1* to a well-defined locus on chromosome II is surprising considering the polyclonal nature of the antiserum used. Such an antiserum might have contained antibodies specific for a variety of genetically determined surface antigens, even after adsorption. The antisera used here have been demonstrated by immunoblotting methods to bind a number of different collagen species isolated from the *C. elegans* cuticle (POLITZ, POLITZ and EDGAR 1986). The *in situ* binding experiments described here may have been specific for the antigens specified by *srf-1* because only a restricted class of antigenic determinants is accessible on the cuticle surface. Chemical description of the adult surface antigens may ultimately require development of monoclonal antibody probes or molecular genetic analysis of the *srf-1* locus.

The molecular nature of the *srf-1* antigen may have consequences for complete understanding of genetic control of surface antigenicity. For example, if the *srf-1* antigenic determinant is a post-translational modification (e.g., a sugar group on a surface glycoprotein) rather than a primary gene product, *srf-1* could be a gene encoding a modification enzyme. An explanation for the greatly reduced immunofluorescence of animals grown on bacterial contaminants (MATERIALS AND METHODS) might be that the contaminants secrete hydrolytic enzymes capable of removing the post-translational modification. If this model were correct, one might expect that other *C. elegans* genes in addition to the one encoding the modifying enzyme could mutate to give a *srf-1* phenotype.

At present, the simplest interpretation of the data is that PA-1 and DH424 each contain a mutant allele of a single gene that determines the surface antigen phenotype. Do these negative strains carry null alleles of *srf-1*, or do they carry alleles that code for structurally distinct antigen types that are not recognized by anti-Bristol antisera? Evidence for the latter possibility might be obtained by raising antibodies to immunogens derived from the negative strains. It is possible that multiple alleles of *srf-1* determine different surface serotypes, like those characterized for alloantigens such as the human blood group antigens (RACE and SANGER 1975).

A third possibility is that the antigen-negative strains regulate expression of *srf-1* incorrectly. The fact that occasional antigen-positive adults are found in antigen-negative strains by immunofluorescence suggests that some individuals in these populations are

capable of expressing an N2-like adult antigen. Correct adult-specific expression of *srf-1* might be a phenomenon analogous to enzyme induction in bacteria, with *srf-1* inactive in larval stages, active in wild-type adults, and only rarely active in PA-1 adults because of defective induction. In this regard, it is interesting that wild-type *srf-1* expression appears more penetrant in older adult males (>95% positive, e.g., Table 1) than in young, recently molted adult males (ca. 80% positive) (S. POLITZ, unpublished data).

Expression of stage-specific cuticle features at inappropriate developmental times has been characterized in heterochronic mutants that may carry mutations in major regulatory genes specifying the character of developmental stage transitions (AMBROS and HORVITZ 1984). Thus far, heterochronic phenotypes have been identified by defects that result from cell lineage alterations, such as egg-laying defects. The adult-specific surface antigen phenotype responds to at least one gene of this type in mutants altered in the gene *lin-4*. The effects of heterochronic mutations on the expression of a specific gene, *srf-1*, can now be studied in tests of epistasis between precocious heterochronic mutants and *srf-1(yj1)*. In turn, it may be possible to select heterochronic mutants directly as individuals that bind adult-specific antibody at inappropriate developmental times. Such an investigation would allow the number of genes that can be mutated to cause heterochronic phenotypes to be estimated.

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