The Drosophila *stubarista* Phenotype Is Associated With a Dosage Effect of the Putative Ribosome-Associated Protein D-p40 on *spineless*

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ABSTRACT

We describe the molecular characterization of the *Drosophila melanogaster* gene *stubarista* (*sta*) that encodes the highly conserved putative ribosome-associated protein D-p40. *sta* maps to cytological position 2A3-B2 on the *X* chromosome and encodes a protein (D-p40) of 270 amino acids. D-p40 shares 63% identity with the human p40 ribosomal protein. *P* element-mediated transformation of a 4.4-kb genomic fragment encompassing the 1-kb transcript corresponding to D-p40 was used to rescue both a lethal (sta^2) and a viable (sta^1) mutation at the *stubarista* (*sta*) locus. Developmental analysis of the sta^2 mutation implicates a requirement for D-p40 during oogenesis and imaginal development, which is consistent with the expression of *sta* throughout development. In addition, we have analyzed the basis of the sta^1 visible phenotype which consists of shortened antennae and bristles. sta^1 is a translocation of the 1E1-2 to 2B3-4 region of the *X* chromosome onto the third chromosome at 89B21-C4. We provide genetic evidence that $Dp(1;3)sta^1$ is mutant at the *spineless* (*ss*) locus and that it is associated with partial D-p40 activity. We demonstrate that sta^1 acts as a recessive enhancer of *ss*; reduction in the amount of D-p40 provided by the transposed *X* chromosomal region of sta^1 reveals a haplo-insufficient phenotype of the otherwise recessive *ss* mutations. This phenomenon is reminiscent of the enhancing effect observed with *Minute* mutations, one of which, rp49, has previously been shown to encode a ribosomal protein.

P40 is a ribosome-associated protein that is extremely well conserved among species as diverse as human and hydra. cDNAs encoding p40 were originally identified by various approaches which confused its identity. It was first isolated as a putative candidate for the gene encoding the 67-kD high affinity laminin receptor (RAO *et al.* 1989; WEWER *et al.* 1986). However, attempts to confirm a role for this molecule in laminin binding have not been successful, as no extracellular matrix binding activity has been found using the expressed product of the p40 gene (GROSSO, PARK and MECHAM 1991). In addition, p40 was identified as a positional marker in the mouse embryonic retina (RABACCHI, NEVE and DRAGER 1990), a transcript enriched in human colon carcinoma cells (YOW *et al.* 1988), and a cytoskeletal protein in hydra (KEPPEL and SCHALLER 1991). The p40 protein normally appears to be localized to the cytoplasm (AUTH and BRAWERMAN 1992; GROSSO, PARK and MECHAM 1991; KEPPEL and SCHALLER 1991; MCCAFFERY, NEVE and DRAGER 1990; RABACCHI, NEVE and DRAGER 1990). Immunocytochemistry (MCCAFFERY, NEVE and DRAGER 1990) as well as sucrose gradient analysis (AUTH and BRAWERMAN 1992) has shown that in the cytoplasm p40 is associated with polyribosomes. In dissociated ribosomes p40 apparently remains bound to 40S ribosomal small subunit (P. MCCAFFREY and U. DRAGER, personal communication). Further subcellular localization has revealed an association between p40 and the cytoskeleton (KEPPEL and SCHALLER 1991) consistent with studies showing an association between polyribosomes and the cytoskeleton (CERVERA, DREYFUSS and PENMAN 1981; HOWE and HERSHEY 1984).

In this study we report the cloning of the Drosophila homolog of the ribosomal associated protein p40. To analyze the function of this molecule during Drosophila development we have characterized the genetic properties of mutations in this gene. We demonstrate that Drosophila p40 (D-p40) protein corresponds to the *stubarista* gene and that one mutation in this gene elicits genetic properties reminiscent of the enhancing effect of *Minute* mutations.

MATERIALS AND METHODS

Stocks: *stubarista (sta) mutations:* Three mutations at the *sta* locus (1-0.3; 1E1-2B4) have been reported (LINDSLEY and ZIMM 1992); a viable X-ray-induced allele (sta^1) associated with visible phenotypes and a chromosome rearrangement, *Tp(1;3)1E1-2;2B3-4; 89B21-C4* (BELYAEVA *et al.* 1980); and two ethyl methanesulfonate-induced alleles, sta^2 (=$sta^{l(3)}$) and sta^3 (no longer available), that produce zygotic lethality. The stocks are kept as *FM3/*sta^1 and *FM6/y* sta^2. In addition, we built a sta^1 stock that contains a third chromosome balancer, *FM3/Df(1)*sta^1*/Y; Dp(1;3)*sta^1*/TM3, Sb* to facilitate the analysis of the sta^1 phenotype.

spineless (ss) mutations: In this study we used two viable

mutations at the *ss* locus (3-58.5; 89C1-2). The ss^1 mutation is associated with a reduction in size of all bristles, the "spineless" phenotype. The ss^a mutation is associated with a "spineless" phenotype as well as the "aristapedia" phenotype, that consists of a transformation of part of the antennae into leg structures (LINDSLEY and ZIMM 1992).

Tp(3;2)iab2^{P10} is Dp(3;2)29A-C;89C1-2;89E1-2 which is an insertion of the 89C1-2;89E1-2 region (*Dp(3;2)P10*), that carries ss^+, into 2L (LEWIS 1978).

Flies were raised on standard Drosophila media at 25°. Descriptions of balancers and mutations that are not described in the text can be found in LINDSLEY and ZIMM (1992).

Developmental analyses: Germline clones of sta^2 were generated as follows. Progeny from the cross between *FM6/y* sta^2 females with ovo^{D1} v^{24}/Y males were irradiated at the end of the first instar stage with a dose of 1000 rad (Torrex 120D X-ray machine; 100 kV, 5 mA, 3-mm aluminum filter). The incidence of X-ray-induced mitotic recombination in the ovo^{D1} background is around 5–8% (PERRIMON, ENGSTROM and MAHOWALD 1984) under these conditions. The *X*-linked dominant-female-sterile mutation ovo^{D1} (BUSSON *et al.* 1983) is maintained as an attached-*X* stock: *C(1)DX, y f/Y* females crossed to ovo^{D1} v^{24}/Y males.

To generate clones homozygous for sta^2 in the imaginal tissues we made use of the *FLP-FRT* system (GOLIC 1991). We used an *FRT*-insertion, $FRT^{9\text{-}2}$, located at 18E on the *X* chromosome (T. B. CHOU, E. NOLL and N. PERRIMON, unpublished) as well as the *FLP*-recombinase insertion FLP^{38} (CHOU and PERRIMON 1992). Progeny from the cross between *FM7/y* sta^2 f^{36a} $FRT^{9\text{-}2}$ females mated with $FRT^{9\text{-}2}$/*Y;F38/F38* males were heat shocked during early larval stages for 2 hr at 37°. As a control, we used the progeny from the cross between *y w* f^{36a} $FRT^{9\text{-}2}$ homozygous females crossed with $FRT^{9\text{-}2}$*/Y;F38/F38* males. Flies of the appropriate genotype (*y* sta^2 f^{36a} $FRT^{9\text{-}2}/FRT^{9\text{-}2}$*;F38/+*) and *y w* f^{36a} $FRT^{9\text{-}2}/FRT^{9\text{-}2}$*;F38/+*) were collected, cooked in 10% potassium hydroxide for 5 hr at 70°, dehydrated and mounted in Euparal. Clones of *y* f^{36a} bristles were scored in different regions of the fly cuticle. The markers used in this analysis, *y = yellow*, *w = white* and f^{36a} = *forked*^{36a}, are described in LINDSLEY and ZIMM (1992).

Lethal phases were determined as previously described (PERRIMON, ENGSTROM and MAHOWALD 1989) and scanning electron microscopy of adult flies was performed as described by HODGKIN and BRYANT (1978).

cDNA and genomic library screening: A cDNA library prepared from 12–24 hr Drosophila embryonic mRNA and constructed in the vector pNB40 (BROWN and KAFATOS 1988) was screened at low stringency with a cDNA for the human 70-kD nerve growth factor (NGF) receptor (JOHNSON *et al.* 1986), labeled by random priming (FEINBERG and VOGELSTEIN 1983). Sequencing of one of the putative positives (cDNA *NB11*) revealed strong homology to human p40. *NB11* does not show significant homology to the NGF receptor indicating that this cDNA was isolated by accident. Genomic DNA corresponding to cDNA *NB11* was obtained by screening a Drosophila genomic library constructed in λ EMBL3 (BLACKMAN *et al.* 1987) using *NB11* as a probe as described (SAMBROOK, FRITSCH and MANIATIS 1989).

DNA sequencing: DNA sequencing was carried out using Sequenase (U.S. Biochemical Corp.) and a dideoxy chain termination protocol (DEL SAL, MANFIOLETTI and SCHNEIDER 1989) with the following modifications: double stranded plasmid DNA template was denatured at 70° for 15 min, and template and primer annealed at 37° for 15 min. The *NB11* cDNA was sequenced by primer walking. A restriction map of *sta* genomic DNA was generated and Southern analysis with a cDNA probe indicated which fragments contained the transcription unit. *sta* genomic DNA was subcloned for sequencing as follows: a *Bam*HI-*Sal*I fragment (from a *Bam*HI site, created by ligation of the partially MboI-cut phage insert, at the end of the phage 4 insert 5′ of the transcript, to a *Sal*I site 3 kb downstream) and an adjacent 1.4 kb *Sal*I-*Eco*RI fragment from phage 4 were each cloned into pBSK (Stratagene).

DNA sequence analysis was performed using the Wisconsin Genetics Computer Group (WGCG) sequence analysis package (DEVEREUX, HAEBRELI and SMITHIES 1984). Homology searches were performed using the BLAST Network Service (ALTSCHUL *et al.* 1990).

RNA analysis: Total RNA was isolated from staged embryos, larvae and pupae by the guanidinium/cesium chloride method (SAMBROOK, FRITSCH and MANIATIS 1989) and affinity-purified on oligo(dT) cellulose (Collaborative Research). Northern blot analysis was performed using standard methods (SAMBROOK, FRITSCH and MANIATIS 1989) with the genomic DNA used for transformation as random-primed probe.

***P* element transformation and rescue:** The 3-kb *Bam*HI-*Sal*I and 1.4-kb *Sal*I-*Eco*RI genomic fragments mentioned above were joined in a three part ligation with *Bam*HI/*Eco*RI digested and gel-purified pBSK. The resulting 4.4-kb insert was then cloned into the *Bam*HI-*Eco*RI sites of pCaSpeR2, a *P* element vector carrying the *white*$^+$ gene (THUMMEL, BOULET and LIPSHITZ 1988) kindly provided by C. THUMMEL. Following standard protocols (SPRADLING 1986) this construct was injected into *y w/y w; Δ2-3, Sb/TM6* (ROBERTSON *et al.* 1988) embryos prior to cellular blastoderm. Five independent transformants were identified by rescue of white eye color to near wild type, four of which were not *X*-linked. The four autosomal lines were used to demonstrate rescue of mutations at the *sta* locus. Similar results were obtained with all the autosomal transformant lines tested which are referred to collectively as *Tr-sta*$^+$.

***In situ* hybridization to polytene chromosomes:** *In situ* hybridization to polytene chromosomes was carried out as described by GALL and PARDUE (1971). *NB11* cDNA probe for *in situ* hybridizations was nick translated with biotinylated dUTP (LANGER-SAFER, LEVINE and WARD 1982), and detected using a Detek-1-HRP kit from ENZO Diagnostic Inc.

***In situ* hybridization to embryos:** *In situ* hybridization to whole mount embryos was carried out as described by TAUTZ and PFEIFLE (1989) using the Genius kit (Boehringer Mannheim). Single stranded sense and anti-sense *sta* cDNA probes were hybridized to embryos 0–20 hr old. As a positive control, whole mount *in situ* hybridization was performed using a *bnb* cDNA probe (EBERL *et al.* 1992).

RESULTS

Cloning of the *stubarista* (*sta*) gene: A cDNA (*NB11*) encoding a Drosophila *p40* homolog was cloned from a low stringency screen of a 12–24-hr embryonic cDNA library, using a human 70-kD nerve growth factor receptor cDNA probe (see MATERIALS AND METHODS). *NB11* cDNA was mapped by *in situ* hybridization to polytene chromosomes to cytological position 2A3-B2 (data not shown). Twenty-eight kilobases of genomic DNA encompassing the transcription unit were isolated in the form of two genomic clones (phages 4 and 2 shown in Figure 1) using the *NB11* cDNA as a probe. A comparison of the restric-

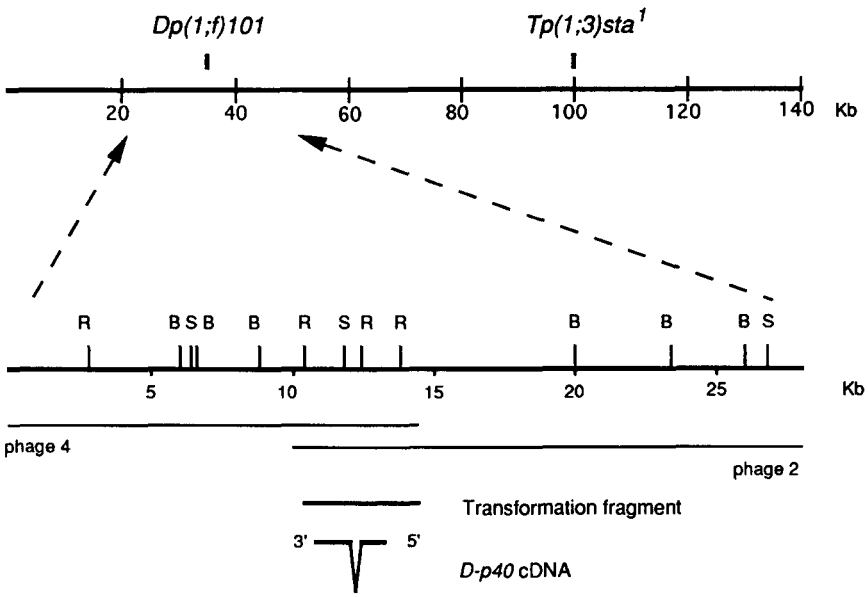

FIGURE 1.—Correlation between our genomic DNA and a portion of the 2B chromosomal walk performed by CHAO and GUILD (1986). The restriction map of two phage clones spanning a total of 28 kb of genomic DNA around the p40 transcription unit was found to correspond to coordinates 23–50 of CHAO and GUILD's chromosomal walk. Positions of the *Dp(1;f)101* and *Tp(1;3)sta*^{1} breakpoints, identified by CHAO and GUILD, are indicated. R, *Eco*RI; B, *Bam*HI; and S, *Sal*I.

tion map of this genomic DNA with that of a chromosomal walk through the 2B5 ecdysone-inducible "early" puff region by CHAO and GUILD (1986) indicated that our genomic DNA extended from coordinates 23–50 of their walk, spanning the *Dp(1;f)101* breakpoint (Figure 1). Genomic DNA corresponding to the *D-p40* transcription unit was sequenced and the intron-exon structure of the gene determined by comparison of genomic and cDNA sequence, revealing a single intron of 192 bp (Figure 2).

Sequencing of the 990-bp *NB11* cDNA reveals an open reading frame of 270 amino acids that shows excellent Drosophila codon usage (data not shown). The predicted protein shows a striking degree of homology to p40 from other organisms; we therefore refer to the fly protein as D-p40. p40 has been isolated from many different species including humans, mouse, bovine and hydra (GROSSO, PARK and MECHAM 1991; KEPPEL and SCHALLER 1991; MAKRIDES *et al.* 1988; YOW *et al.* 1988). In Figure 3A the degree of identity with p40 proteins from two evolutionarily distant species, human (63%) and hydra (58%), is shown. Consensus phosphorylation sites for protein kinase C (WOODGETT, GOULD and HUNTER 1986) and cdc2 kinase (PETER *et al.* 1990) which are conserved in all known p40 sequences are also found in D-p40. D-p40 also reveals a lower (27%) but still significant homology to a prokaryotic small subunit ribosomal protein, S2 from *Escherichia coli* (AN *et al.* 1981; Figure 3B).

Expression of *NB11* during development: Northern analysis indicates that *NB11* cDNA detects a single 1-kb transcript expressed maternally and through all developmental stages (data not shown). To determine whether *NB11* is spatially regulated during embryogenesis, whole mount *in situ* hybridization of embryos with both sense and anti-sense *NB11* cDNA was performed, showing that *NB11* is ubiquitously expressed during embryogenesis (data not shown).

D-p40 transformants rescue a lethal mutation at the *stubarista* (*sta*) locus: A *P* element transposon was constructed that contains genomic DNA extending 2 kb upstream and 1 kb downstream of the *NB11* open reading frame (Figure 1). Following injections, five transformant lines were recovered (see MATERIALS AND METHODS). We tested whether the autosomal transformants were able to rescue the lethality associated with zygotic lethal mutations in the 2A-B interval (Figure 4). We found that the zygotic lethality associated with the *sta*^{2} mutation was fully rescued by the transformants (Table 1A). We therefore refer to these transformants as *Tr-sta*$^+$. *sta*^{2} hemizygous animals derived from heterozygous mothers die during the first to second instar stage with no obvious cuticle defects (Table 1A, line 1). In the presence of an autosomal *Tr-sta*$^+$ transformant, they are viable and fertile with no obvious visible defects (Table 1A, lines 3 and 4).

The *stubarista* phenotype: A viable allele at the *sta* locus (*sta*^{1}), associated with a transposition of the 1E1-2 to 2B3-4 region of the *X* chromosome to the third chromosome at 89B21-C4 (AIZENZON and BELYAEVA 1982; BELYAEVA *et al.* 1982; LINDSLEY and ZIMM 1992), has been previously identified. *sta*^{1} is associated with a set of phenotypes that includes a shortened blunt third joint of the antennae, aristae with thickened and irregular branches (Figure 5C), all bristles short and sparse (Figure 5D), and female sterility (Table 1B, lines 8 and 11).

To demonstrate that the *sta*^{1} phenotypes are associated with D-p40, we tested the ability of the transformant lines to rescue the *sta*^{1} phenotype. Interestingly, all aspects of the *sta*^{1} phenotype, except the female sterility, are rescued by the transformant lines

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   1  acgtggagccccttctttgaggttatgtccactaaccatatgcctgtctcgttcacagGT   60

  61  CCGTAACTCTTTCCACGTTAACATGTCGGGAGGCTTAGATATTCTGTCCCTCAAGGAGGA  120
                          M  S  G  G  L  D  I  L  S  L  K  E  D

 121  CGAGATCACCAAGATGTTGGTGGCCACCACCCATCTGGGTTCCGAGAACGTCAACTTCCA  180
       E  I  T  K  M  L  V  A  T  T  H  L  G  S  E  N  V  N  F  Q

 181  GATGGAGCAGTACGTGTACAAGCGCCGCGCTGATGGCGTCAACATCCTCAACCTGGGCAA  240
       M  E  Q  Y  V  Y  K  R  R  A  D  G  V  N  I  L  N  L  G  K

 241  GACCTGGGAGAAGCTGCAGCTGGCGGCCCGCGCCATCGTGGCCATCGATAACCCCTCGGA  300
       T  W  E  K  L  Q  L  A  A  R  A  I  V  A  I  D  N  P  S  D
                      ▼EcoRI
 301  Cgtgagtacaagtgcagaattccatggcataccagtgcggcgtggctagcgtgatgagtt  360

 361  tattactaatctttcgggtatgaaattcaatgatttcaacttcactgctgaccagccacg  420

 421  cctcaacgcctcggagtgtttgcccccagtctctgatagatacaccatattctaatacac  480

 481  acttattttgcagATCTTCGTCATCTCGTCGCGTCCGATCGGCCAGCGCGCGGTGCTGAA  540
                   I  F  V  I  S  S  R  P  I  G  Q  R  A  V  L  K

 541  GTTCGCCAAGTACACCGACACCACTCCGATCGCCGGCCGCTTCACGCCCGGTGCCTTCAC  600
       F  A  K  Y  T  D  T  T  P  I  A  G  R  F  T  P  G  A  F  T

 601  CAACCAGATCCAGCCCGCTTTCCGCGAGCCGCGTCTGCTCGTGGTGACCGACCCCAACAC  660
       N  Q  I  Q  P  A  F  R  E  P  R  L  L  V  V  T  D  P  N  T

 661  CGATCACCAGCCCATCATGGAGGCCAGCTACGTGAACATCCCCGTGATTGCCTTCACGAA  720
       D  H  Q  P  I  M  E  A  S  Y  V  N  I  P  V  I  A  F  T  N

 721  CACCGACTCGCCTCTGCGCTACATCGACATTGCCATTCCGTGCAACAACAAGTCGGCCCA  780
       T  D  S  P  L  R  Y  I  D  I  A  I  P  C  N  N  K  S  A  H

 781  CTCTATCGGTCTGATGTGGTGGCTGTTGGCCCGCGAGGTGCTCCGCCTGCGTGGCACCAT  840
       S  I  G  L  M  W  W  L  L  A  R  E  V  L  R  L  R  G  T  I

 841  CTCTCGCAGCGTCGAGTGGCCCGTAGTCGTCGATCTGTTCTTCTACCGCGATCCCGAGGA  900
       S  R  S  V  E  W  P  V  V  V  D  L  F  F  Y  R  D  P  E  E

 901  GGCCGAGAAGGAGGAGGCCGCCGCCAAGGAGCTGTTGCCGCCACCCAAGATTGAGGAGGC  960
       A  E  K  E  E  A  A  A  K  E  L  L  P  P  P  K  I  E  E  A
       ▼SalI
 961  CGTCGACCACCCGGTCGAGGAGACCACCAACTGGGCCGATGAGGTTGCCGCCGAGACCGT  1020
       V  D  H  P  V  E  E  T  T  N  W  A  D  E  V  A  A  E  T  V

1021  TGGCGGAGTGGAGGACTGGAACGAGGACACCGTCAAGACCTCCTGGGGTAGCGACGGCCA  1080
       G  G  V  E  D  W  N  E  D  T  V  K  T  S  W  G  S  D  G  Q

1081  GTTCTAAGGAATCGTCCGGGCCACAGATGGCACTACATCAGCACAGCGTTTTGTCAGGAG  1140
       F  *

1141  CCTTTTCAACGGCATAAATAAACAGCTGTTTATATCTAAAcacctcgttttttttttctg  1200

1201  catagcgtagcccaatatcacgtgcttccaccttattatgcacgatatatttactagtat  1260

1261  cgaagcgatcaa  1272
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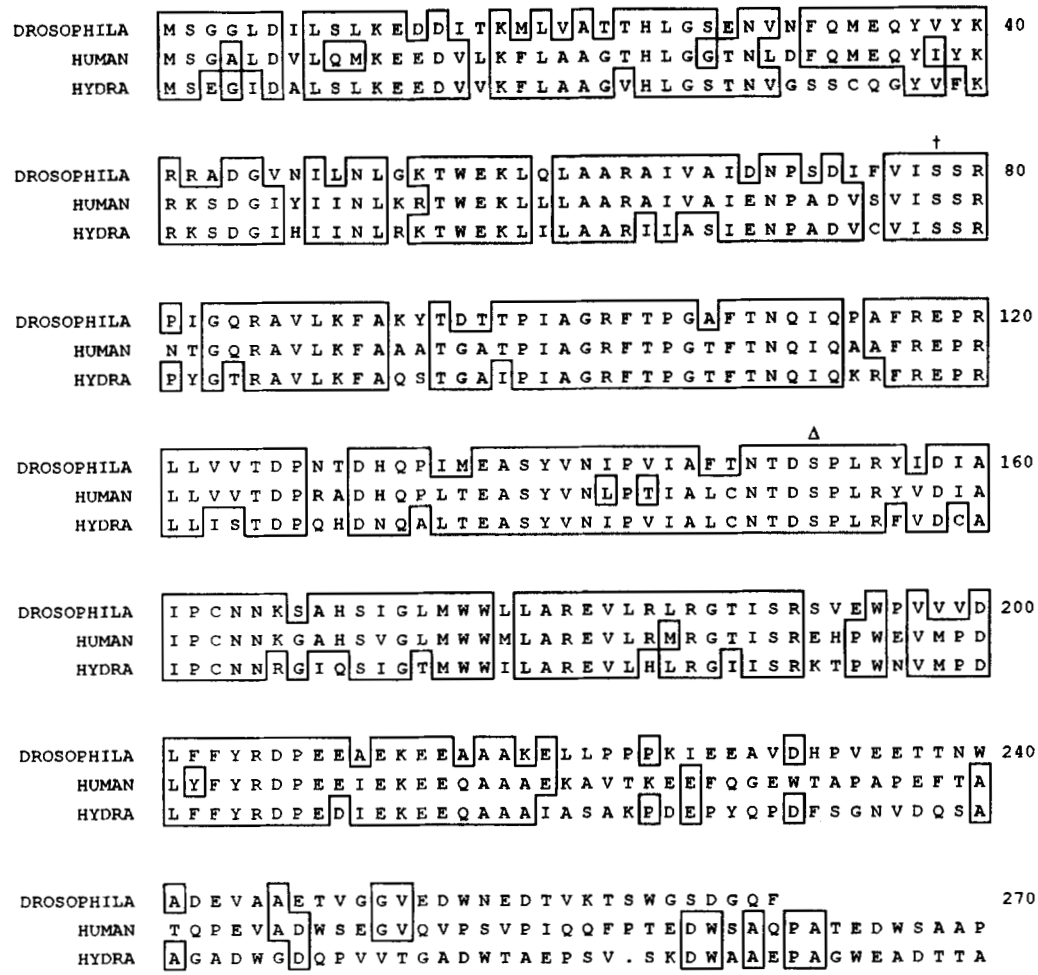
FIGURE 2.—Structure of the *sta* gene. DNA sequence represented in both the genomic DNA and cDNA is in uppercase, sequence represented only in the genomic DNA is in lowercase. Genomic sequence is available in GenBank under accession number M90422. The translation initiation site is in good agreement with the consensus described by CAVENER (1987). Indicated splice sites show good agreement with established consensus (BROWN *et al.* 1989).

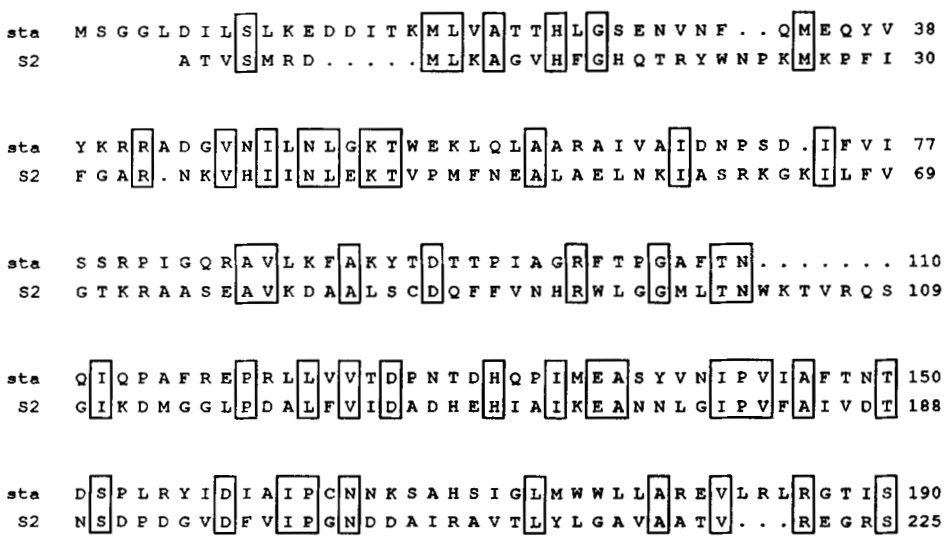
(Table 1B, lines 9 and 12). These results indicate that the antennal and bristle phenotypes are associated with D-p40. Our failure to rescue the female sterility of sta^1 females may reflect insufficient expression of the transgene or that a *D-p40* ovarian enhancer was not included in the transgene. Alternatively, it is possible that another mutation(s) located on the sta^1 chromosome causes female sterility.

***sta*^{1} is mutant at the *spineless* locus:** Dan Lindsley (personal communication) suggested to us that *Dp(1;3)sta*^{1}, which is inserted at 89B21-C4 on the third chromosome, might be mutant at the *spineless* (*ss*) locus. To test this possibility, a set of crosses between *Dp(1;3)sta*^{1} and two different *ss* mutations, ss^a and ss^1 (STRUHL 1982; LINDSLEY and ZIMM 1992), were performed (Table 1C). ss^a homozygous mutant animals show a transformation of the arista and distal portion of the third antennal segment into distal mesothoracic leg segment (the "aristapedia" phenotype, Figure 6A) as well as a reduction in bristle size (the "spineless" phenotype, Figure 6B). ss^1/ss^1 as well as ss^1/ss^a animals do not show the aristapedia phenotype but exhibit the "spineless" phenotype (Table 1C, lines 14 and 17). *Dp(1;3)sta*$^1/ss^a$ show both the aristapedia transformation (Figure 6C) and a strong spineless phenotype (Figure 6D) which are more severe than in ss^a/ss^a homozygous animals, an observation consistent with *Dp(1;3)sta*^{1} being a null allele of *ss* (Table 1C, lines 18 and 19). In addition, *Dp(1;3)sta*$^1/ss^1$ animals have a stronger spineless phenotype than ss^1 homozygous animals but no antennal transformation (data not shown).

In conclusion, these complementation analyses demonstrate that *Dp(1;3)sta*^{1} is mutant at *ss*. *Dp(1;3)sta*^{1} behaves as a null mutation at the *ss* locus since similar complementation results and phenotypes have



A



B

FIGURE 3.—Sequence comparison of the p40 proteins. The comparison of D-p40 with human and hydra p40 protein is shown in A. Conserved protein kinase C phosphorylation site (in the consensus S/T-*X*-K/R; WOODGETT, GOULD and HUNTER 1986) and cdc2 phosphorylation site (in the consensus S/T-P-*X*-R/K; PETER *et al.* 1990) are indicated by the symbols † and Δ, respectively. The C-terminal 55 amino acids of the human and C-terminal 53 amino acids of the hydra proteins are not shown. The sequence comparison of D-p40 protein and *E. coli* ribosomal protein S2 is shown in B. An S2 "insert domain" relative to p40 (residues 110–148 of the S2 protein) is not shown; neither are the C-terminal 15 amino acids of S2 and C-terminal 80 amino acids of D-p40.

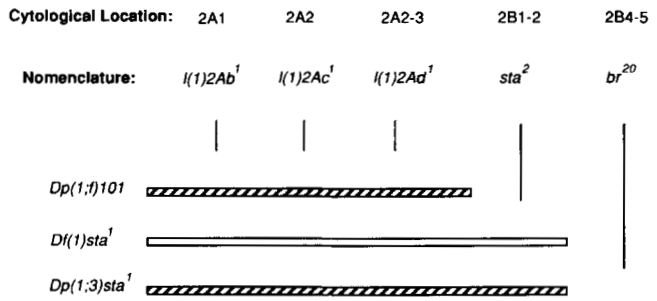


FIGURE 4.—Genetics of the region 2A-B. *Dp(1;f)101* refers to *Dp(1;f)2A2-B1;19F5-20A*. *Df(1)sta*^{1} refers to *Df(1)1E1-2;2B3-4*. *Dp(1;3)sta*^{1} refers to *Dp(1;3)1E1-2;2B3-4; 89B21-C4*. *Df(1)sta*^{1} and *Dp(1;3)sta*^{1} are aneuploid segregants of *Tp(1;3)sta*^{1}. Hatched lines indicate the extent of duplicated DNA; the open line indicates DNA missing due to a deficiency. The nomenclature of the mutations tested for rescue using the *Tr-sta*$^+$ is according to LINDSLEY and ZIMM (1992).

been observed using a deficiency of the *ss* locus in trans with both *ss*^{1} and *ss*^{a} (I. DUNCAN, personal communication).

Partial D-p40 activity is associated with *Dp(1;3)sta*^{1}: *sta*^{1} is a transposition of a portion of the *X* chromosome onto the third chromosome and the proximal break is 60 kb upstream of the *D-p40* gene (CHAO and GUILD 1986; Figure 1). Genetic crosses designed to test the level of *sta*$^+$ gene activity provided by *Dp(1;3)sta*^{1} indicate that the duplicated segment contains only partial *sta*$^+$ activity. *sta*^{2}*/Df(1)sta*^{1}*; Dp(1;3)sta*^{1}*/+* have a weak stubarista phenotype in which the third joint of their antennae is slightly shorter with missing sensilla and the aristae slightly fused (Table 1D, line 22; Figure 7B). Since all sequences necessary for D-p40 gene activity are present in a 4.4-kb fragment (Table 1A, lines 3 and 4), this result strongly suggests that *Dp(1;3)sta*^{1} is associated with reduced D-p40 activity.

The stubarista phenotype is due to a dosage effect of D-p40 on *ss*: We tested the hypothesis that the stubarista phenotype is caused by a recessive enhancing effect of *sta*^{1} on *ss*. In this case, one would expect that increasing the dosage of the *ss*$^+$ gene might, at least partially, suppress the *sta*^{1} phenotype. To test this hypothesis, we used *Dp(3;2)P10* which is a duplication of the region that contains *ss*$^+$ onto the second chromosome (see MATERIALS AND METHODS). Consistent with this prediction, we found that *Df(1)sta/Y; Dp(3;2)P10/+; Dp(1;3)sta*^{1}*/+* flies have weak stubarista antennal and bristle reduction phenotypes (Table 1E, line 26; Figure 7, E and F).

That mutations in D-p40 behave as recessive enhancers of *ss* is further supported by the following observations: *+/Df(1)sta*^{1}*; Dp(1;3)sta*^{1}*/ss*^{a} flies have a more severe antennal phenotype than +/+; *Dp(1;3)sta*^{1}*/ss*^{a} flies (Table 1E, line 24; Figure 7A) suggesting that a reduction in the amount of D-p40 enhances the aristapedia phenotype associated with the *ss*^{a} mutation; similarly, the antennal and bristle phenotypes of *Df(1)sta*^{1}*/Y; Dp(1;3)sta*^{1}*/ss*^{a} animals are worse than the phenotype of *Dp(1;3)sta*^{1}*/ss*^{a} (Table 1E, line 25; Figure 7, C and D).

***D-p40* activity is required for cell viability both in the germline and imaginal tissues:** To determine whether D-p40 is required during oogenesis, we analyzed the phenotype associated with homozygous germline clones of *sta*^{2} (see MATERIALS AND METHODS). 300 females of genotype *sta*^{2}*/ovo*^{D1} were examined for the presence of germline clones. No females possessing developed ovaries were recovered, indicating that *sta*$^+$ activity is required for germ cell viability. Since *sta*^{2}*/sta*^{2} homozygous females that carry a copy of the *Tr-sta*$^+$ transformant are viable and fertile (Table 1), this result cannot be attributed to the presence of a second site mutation on the *sta*^{2} chromosome.

The zygotic requirement during imaginal cell development of *sta*$^+$ activity was analyzed by the production of clones of homozygous *sta*^{2} cells during zygotic development (MATERIALS AND METHODS). As shown in Table 2, only a few *y sta*^{2} *f*^{36a} clones were recovered indicating that D-p40 is required for imaginal cell viability. These clones were of smaller size than those found in the control sample (data not shown) and most likely survived due to perdurance of D-p40.

It should be noted that the *sta*^{2} mutation we used in this analysis probably retains partial *sta*$^+$ activity since *sta*^{2}*/Y; Dp(1;3)sta*^{1}*/+* as well as *sta*^{2}*/sta*^{2}*; Dp(1;3)sta*^{1}*/+* animals, unlike *sta*^{2}*/Df(1)sta*^{1}*; Dp(1;3)sta*^{1}*/+* (Figure 7B), have a wild-type phenotype (Table 1D, lines 20, 21 and 22). Regardless, our results demonstrate that *sta*$^+$ is required for both germline and imaginal disc development.

DISCUSSION

The D-p40 protein: The *sta* gene described in this paper encodes a molecule that is highly conserved between such evolutionarily distant organisms as human (YOW *et al.* 1988) and hydra (KEPPEL and SCHALLER 1991). Figure 3A compares the Drosophila, human and hydra p40 sequences in a way that may be informative regarding essential functional regions of the molecule. D-p40 shows 63% identity to the human and 58% identity to the hydra proteins (human and mouse p40 are by contrast 99% identical). The sequences diverge toward the C terminus. In addition, the predicted D-p40 protein is more than 50 amino acids shorter than other known p40 molecules.

Potentially relevant to the role of p40 in translation is the homology between p40 proteins and the prokaryotic ribosomal protein, S2, from *E. coli* (AN *et al.* 1981; DAVIS, TZAGOLOFF and ELLIS 1992; Figure 3B). D-p40 and S2 proteins are 27% identical, and both S2 and p40 are associated with the small ribosomal subunit (STERN *et al.* 1989). S2 is found on the surface

TABLE 1

Characterization of *stubarista*

Genotype	Phenotype
A. sta^2 is rescued by $Tr\text{-}sta^+$	
1 sta^2/Y	Larval lethal
2 $sta^2/+$	Wild type
3 sta^2/Y; $Tr\text{-}sta^+/+$	Wild type
4 sta^2/sta^2; $Tr\text{-}sta^+/+$	Wild type
B. $Tr\text{-}sta^+$ rescues $Tp(1;3)sta^1$	
5 $Df(1)sta^1/+$	Wild type
6 $+/Y$; $Dp(1;3)sta^1/+$	Wild type
7 $+/+$; $Dp(1;3)sta^1/+$	Wild type
8 $Df(1)sta^1/Y$; $Dp(1;3)sta^1/+$	Stubarista (Figure 5, C and D)
9 $Df(1)sta^1/Y$; $Dp(1;3)sta^1/Tr\text{-}sta^+$	Wild type
10 $Df(1)sta^1/+$; $Dp(1;3)sta^1/+$	Wild type
11 $Df(1)sta^1/Df(1)sta^1$; $Dp(1;3)sta^1/+$	Stubarista, sterile (Figure 5, C and D)
12 $Df(1)sta^1/Df(1)sta^1$; $Dp(1;3)sta^1/Tr\text{-}sta^+$	Wild-type, sterile
C. $Dp(1;3)sta^1$ is mutant at *ss*	
13 $ss^1/+$	Wild type
14 ss^1/ss^1	Spineless
15 $ss^a/+$	Wild type
16 ss^a/ss^a	Spineless-aristapedia (Figure 6, A and B)
17 ss^1/ss^a	Spineless
18 $ss^1/Dp(1;3)sta^1$	Spineless
19 $ss^a/Dp(1;3)sta^1$	Spineless-aristapedia (Figure 6, A and B)
D. Partial D-p40 activity associated with $Dp(1;3)sta^1$	
20 sta^2/Y; $Dp(1;3)sta^1/+$	Wild type
21 sta^2/sta^2; $Dp(1;3)sta^1/+$	Wild type
22 $sta^2/Df(1)sta^1$; $Dp(1;3)sta^1/+$	Weak stubarista (Figure 7B)
23 $sta^1/Df(1)sta^1$; $Dp(1;3)sta^1/Tr\text{-}sta^+$	Wild type
E. Dosage interaction between sta^1 and *ss*	
24 $Df(1)sta^1/+$; $Dp(1;3)sta^1/ss^a$	Strong spineless-aristapedia (Figure 7A)
25 $Df(1)sta^1/Y$; $Dp(1;3)sta^1/ss^a$	Strong spineless-aristapedia (Figure 7, C and D)
26 $Df(1)sta^1/Y$; $Dp(3;2)P10/+$; $Dp(1;3)sta^1/+$	Weak stubarista (Figure 7, E and F)

sta^1 is *Tp(1;3)1E1-2;2B3-4; 89B21-C4* which can also be written as $Df(1)sta^1$; $Dp(1;3)sta^1$. Only the genotypes for the *X*, *Y*, second and third chromosomes are indicated. Genotypes were constructed using standard genetic crosses. Numbers in the left column indicate the line numbers referred to in the text. The effect of two copies of $Dp(1;3)sta^1$ could not be tested since homozygosity of the $Dp(1;3)sta^1$ chromosome is lethal to the organism. Thirty percent of $sta^2/Df(1)sta^1$; $Dp(1;3)sta^1/+$ flies have incised wings, a phenotype also reported for sta^2 in combination with a variegating duplication of the region, $Dp(1;2)dor^{var7}$ (DEMAKOVA and BELYAEVA 1988). All the autosomal transformant lines tested gave similar results and are referred to collectively as $Tr\text{-}sta^+$. See text and figure legends for phenotypic descriptions.

of the small subunit where it helps stabilize conformation (MARION and MARION 1988), and is involved in tRNA binding (SHIMIZU and CRAVEN 1976; THOMAS *et al.* 1975). These observations may offer clues in trying to elucidate the function of p40.

Phosphorylation of p40 may provide one mechanism of regulating its activity, based on consensus phosphorylation sites for protein kinase C (WOODGETT, GOULD and HUNTER 1986) and cdc2 kinase (PETER *et al.* 1990) conserved in all known p40 sequences. The possibility of regulation by cdc2 kinase has previously been noted in the context of changes in p40 cellular localization with the cell cycle: in mouse fibroblasts p40 appears to be cytoskeleton-associated in non-dividing cells but more diffusely distributed throughout the cytoplasm in dividing cells (KEPPEL and SCHALLER 1991). Cytoskeletal association has been reported for a number of other translation factors including initiation factors (HOWE and HERSHEY 1984) and at least one elongation factor (YANG *et al.* 1990). The latter case, namely elongation factor 1a, sets an interesting precedent by having both actin-binding and tRNA-binding activities, with which it helps provide the ribosome with aminoacyl tRNAs.

p40 function may be regulated on at least two other levels. YENOFSKY *et al.* (1983) suggested a translational repression mechanism by showing that a considerable fraction of p40 mRNA can be found in untranslated ribonucleoprotein (RNP) particles as opposed to polysomes, and that this fraction and thus the extent of p40 mRNA utilization can change dramatically with cell differentiation. Second, MCCAFFERY, NEVE and DRAGER (1990), looking at p40 expressed in the mouse retina, showed a difference in the sensitivity to trypsin of p40 from the dorsal *vs.* ventral retina, suggesting a conformational difference in the protein potentially significant for its function. These observations are reminiscent of the many levels at which the activity of other ribosomal proteins is regulated, in the context of development and of coordinated

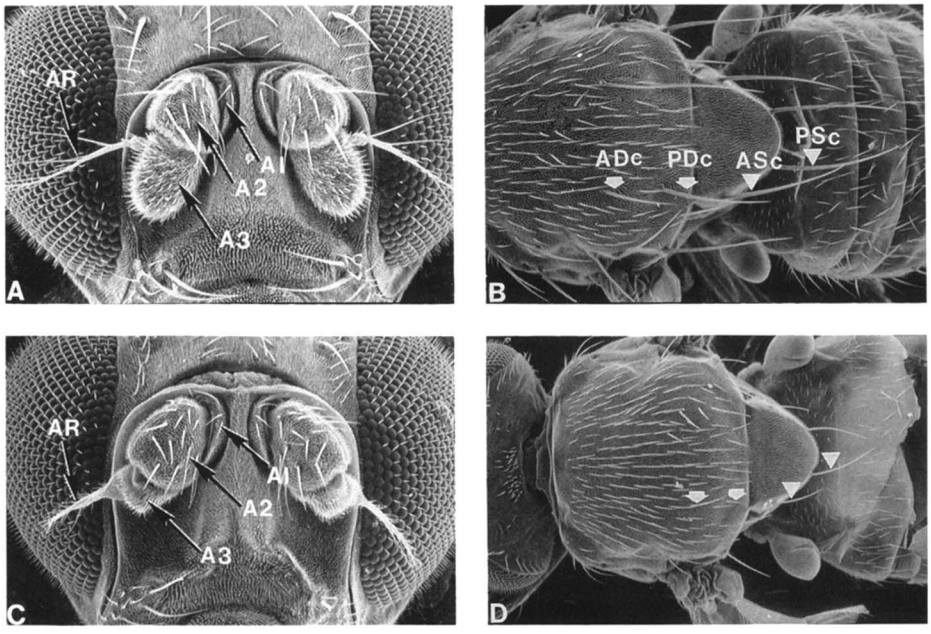


FIGURE 5.—The stubarista phenotype. Scanning electron micrographs (SEM) of the antennal and bristle phenotypes of wild-type (*OreR*) and *sta*^{1}(*Df(1)sta*^{1}/*Y*; *Dp(1;3)sta*^{1}/+ or *Df(1)sta*^{1}/*Df(1)sta*^{1}; *Dp(1;3)sta*^{1}/+) animals. Wild-type morphology of the antennae (A) and of the scutellar and thoracic bristles (B). In a *sta*^{1} animal the third joints of the antennae are extremely short, the bases of the aristae are thickened (C) and the thoracic and scutellar bristles are reduced in size (D). *Nomenclature:* AR (aristae); A1, A2 and A3 refer to the three antennal segments; ASc (anterior scutellars), PSc (posterior scutellars), ADc (anterior dorsocentrals) and PDc (posterior dorsocentrals) bristles. Nomenclature is from HODGKIN and BRYANT (1978).

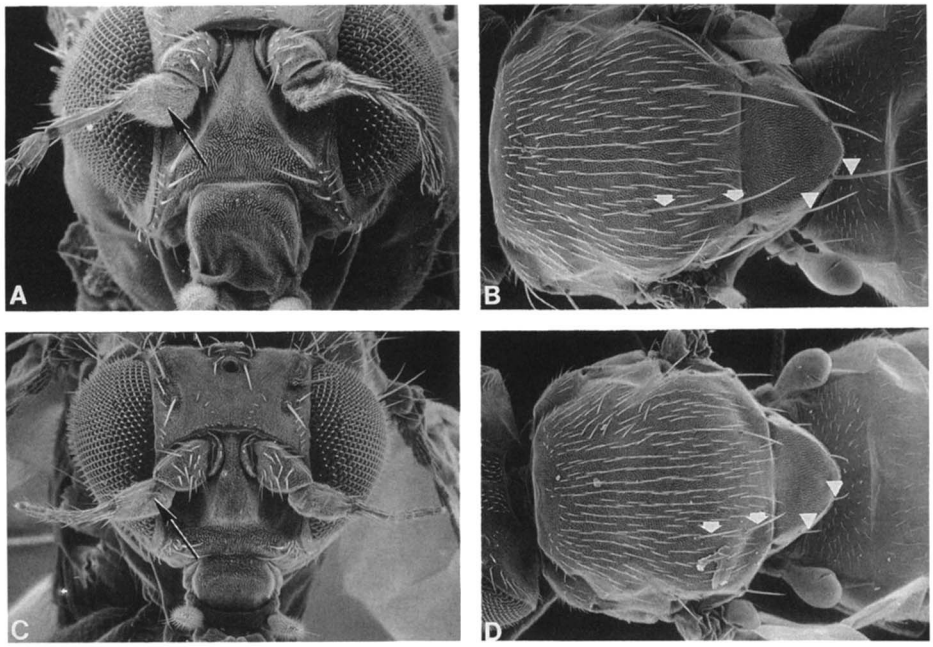


FIGURE 6.—*Dp(1;3)sta*^{1} is mutant at the *spineless* locus. The antennal transformation to leg structures (indicated by an arrow in A, the "aristapedia" phenotype) and the reduction in bristle sizes (B, the "spineless" phenotype, compare with the wild-type phenotype in Figure 5B) of an *ss*^{a} homozygous animal are shown. *ss*^{1}/*ss*^{1} and *ss*^{1}/*ss*^{a} have a normal antennal morphology while *ss*^{a}/*ss*^{a}, *ss*^{1}/*ss*^{1} and *ss*^{1}/*ss*^{a} animals have a similar reduction in bristle size (data not shown). *Dp(1;3)sta*^{1}/*ss*^{a} animals show both the aristapedia (C) and spineless (D) phenotypes. Note that the spineless phenotype shown in D is more severe than in B. *Dp(1;3)sta*^{1}/*ss*^{1} animals only exhibit the severe spineless phenotype as seen in D (data not shown).

expression of ribosomal genes (PATEL and JACOBS-LORENA 1992; WARNER *et al.* 1985).

The stubarista gene: Using *P* element rescue we have been able to demonstrate that mutations at the *stubarista* (*sta*) locus correspond to D-p40. Our developmental analysis of *sta* mutations indicates that D-p40 is required during oogenesis and imaginal development as demonstrated by the germline and somatic mosaic analysis of *sta*^{2} and the bristle, wing and antennal adult phenotypes of *sta*^{1}. This ubiquitous requirement of *D-p40* is consistent with a basic cellular function such as that of a ribosomal protein.

Ribosomal proteins have been well characterized in Drosophila, both biochemically (CHOOI 1980; CHOOI *et al.* 1980) and molecularly. Among the Drosophila ribosomal protein genes to have been cloned are those for S6 (WATSON *et al.* 1992; STEWART and DENELL 1993), S14 (BROWN *et al.* 1989), S17 (MAKI *et al.* 1989), S18 (BURNS *et al.* 1984), S26 (ITOH *et al.* 1989b), S31 (ITOH *et al.* 1989a), L1 (RAFTI *et al.* 1988), L12 (BURNS *et al.* 1984), rpA1 (QIAN *et al.* 1987), rp7/8 (BURNS *et al.* 1984), rp21 (KAY, ZHANG and JACOBS-LORENA 1988) and rp49 (O'CONNELL and ROSHBASH 1984). In only one case has a ribosomal protein gene been assigned to a known genetic locus; namely, rp49 to the *Minute(3)99D* locus (KONGSUWAN *et al.* 1985). This locus is 1 of some 50 *Minute* loci scattered throughout the Drosophila genome which, as heterozygotes, share a characteristic phenotype of short thin bristles and delayed development. Homo-

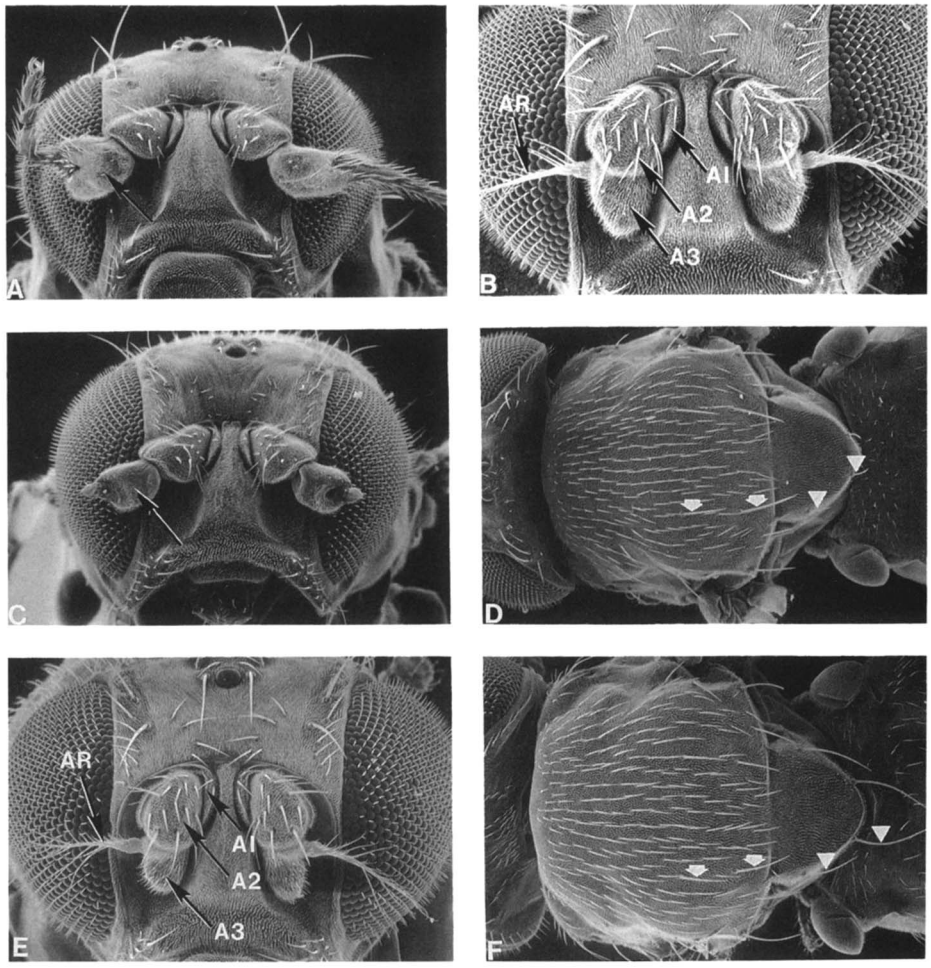


FIGURE 7.—Dosage interactions between *stubarista* and *spineless*. The antennal phenotype of a $+/Df(1)sta^1$; $Dp(1;3)sta^1/ss^a$ animal is shown in A. The antennal transformations are more severe in these animals than in +/+; $Dp(1;3)sta^1/ss^a$ (compare with Figure 6C). In a sta^2/sta^1 ($sta^2/Df(1)sta^1$; $Dp(1;3)sta^1/+$) animal (B), the third joints of the antennae are slightly shorter than in wild-type animals shown in Figure 5A and the bases of the aristae are also slightly fused. In these animals some of the long bristles are missing rendering the appearance of the antennal 3 segment rather smooth. The antennal and bristle phenotypes of a $Df(1)sta^1/Y$;$Dp(1;3)sta^1/ss^a$ animal are shown in C and D, respectively. Note the extreme effect on both antennal and bristle morphology when compared with the phenotype of $Dp(1;3)sta^1/ss^a$ animals shown in Figure 6, C and D. The antennae (E) and bristles (F) of a *Df(1)sta1/Y;Dp(3;2)P10/+*; $Dp(1;3)sta^1/+$ animal are partially rescued by the introduction of *Dp(3;2)P10* that carries an extra copy of the ss^+ gene (compare E and F to the phenotype of a *Df(1)sta1/Y;* +/+; $Dp(1;3)sta^1/+$ animal shown in Figure 5, C and D).

TABLE 2

Recovery of sta^2 clones in imaginal disc derivatives

Genotype	Labial	Thorax	Leg	Tergite	Sternite
Control ($N = 25$)	21	79	82	122	36
sta^2 ($N = 55$)	0	2	3	5	2

N = number of adults examined. Control genotype = ywf^{36a} FRT^{9-2}/FRT^{9-2};*F38/+*; sta^2 genotype = $y\ sta^2\ f^{36a}\ FRT^{9-2}/FRT^{9-2}$; *F38/+*.

zygotes are late embryonic to early larval lethals [see WRIGHT (1970) for review; LINDSLEY and ZIMM (1992)]. Many other ribosomal protein genes map near known *Minute* loci (KAY and JACOBS-LORENA 1987), though in a number of cases this correlation was shown not to be significant (DORER, ANANE-FIREMPONG and CHRISTENSEN 1991; KAY, ZHANG and JACOBS-LORENA 1988). The *Minute* phenotype is also shared by mutants at the *bobbed* and *mini* loci, which are partial deletions of the rRNA genes (KAY and JACOBS-LORENA 1987). The current model is that reduction in the amount of a ribosomal protein reduces the overall rate of protein synthesis which results in at least two developmental effects, a slow mitotic rate of $M/+$ cells relative to +/+ cells and a reduction in the rate of bristle elaboration.

Minute mutations are known to act as dominant enhancers of a variety of mutations and exhibit dominant lethal effects with specific mutations (LINDSLEY and ZIMM 1992). Similar to this phenomenon, we propose that the stubarista phenotype is due to a recessive enhancing effect of sta^+ on *ss*. In the context of $Tp(1;3)sta^1$, the reduced amount of D-p40 provided by the duplication $Dp(1;3)sta^1$ reveals an haplo-insufficient phenotype for *ss*. This model is supported by the observations that: (1) $Dp(1;3)sta^1$ is associated with reduced D-p40 activity most likely as a result of a position effect and (2) that the visible stubarista phenotype can be suppressed by either introduction of an extra copy of D-p40 (from a transformant line) or a duplication of the ss^+ gene. It should be kept in mind that since no transformants of the ss^+ gene are available, we cannot as yet disprove the possibility that an unidentified gene present on *Dp(3;2)P10* is responsible for the suppressive interaction.

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Note added in proof: Y. KIM and B. BAKER have reported the sequence of an apparently non-full length D-p40 cDNA under GenBank accession number M77133.

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