Aristapedioid: A Gain of Function, Homeotic Mutation in *Drosophila melanogaster*

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Manuscript received July 17, 1989
Accepted for publication September 25, 1989

ABSTRACT

The isolation of gain of function mutations has allowed the identification of a number of genes which are important in the normal development of the organism. We report here the isolation and characterization of *Aristapedioid*, a gain of function mutation which causes a partial transformation of arista towards tarsus and the loss or decrease in size of the dorso-central and scutellar bristles. *Aristapedioid* is the result of a *P* element mediated inversion which juxtaposes unrelated DNA adjacent to *Suppressor 2 of zeste*, causing a gain of function mutation in that gene.

A large number of genes that are essential for embryonic development in Drosophila have been identified via the systematic screening of zygotic recessive lethal (NÜSSLEIN-VOLHARD and WIESCHAUS 1980; JÜRGENS *et al.* 1984; NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984; WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984) and maternal effect mutations (NÜSSLEIN-VOLHARD 1979; SCHÜPBACH and WIESCHAUS 1986; PERRIMON, ENGSTROM and MAHOWALD 1984). Lethal mutations in many genes do not result in morphological defects suggestive of the gene having an essential regulatory function. Some of these mutations could, in fact, be in interesting regulatory genes where the essential embryonic function is largely met (rescued) by maternal gene expression (PERRIMON, ENGSTROM and MAHOWALD 1984). In the absence of a provocative lethal phenotype, these mutations would likely not be chosen for further study. Screens for lethal mutations also fail to identify functionally redundant genes. The *amalgam* gene, a member of the immunoglobulin superfamily located in the ANT-C, is apparently such a gene (SEEGER, HAFFLEY and KAUFMAN 1988). Thus, the standard loss of function mutant screens will likely fail to identify as such all "developmentally interesting" genes.

A number of developmentally important genes such as *Antennapedia* (*Antp*) (KAUFMAN, LEWIS and WAKIMOTO 1980; LEWIS *et al.* 1980a, b), *Ultrabithorax* (*Ubx*) (LEWIS 1978), and *Polycomb* (*Pc*) (LEWIS 1978) were initially isolated as dominant mutations. The dominant *Ubx* and *Pc* mutations are loss of function mutations and reflect the haplo-insufficiency of these genes. On the other hand, the dominant *Antp* mutations are gain of function neomorphic mutations that result from the inappropriate expression of the *Antp* gene (FRISCHER, HAGEN and GARBER 1986; SCHNEUWLY, KUROIWA and GEHRING 1987). The directed misexpression of regulatory genes such as *ftz* (STRUHL 1985), *Dfd* (KUZIORA and MCGINNIS 1988), *Antp* (SCHNEUWLY, KLEMENZ and GEHRING 1987) and *hairy* (ISH-HOROWICZ and PINCHIN 1987) act as conditional, gain of function mutations which result in developmental abnormalities. Thus, there is good reason to believe it should be possible to identify genes that play a regulatory role in normal development by screening for dominant, gain of function mutations that result in abnormal morphology. It would not be surprising if this approach could identify genes which may not be identified or recognized as developmentally interesting in standard screens for loss of function mutations.

There are examples of dominant, gain of function mutations resulting from either the insertion of a transposable element (SWAROOP, PACO-LARSON and GAREN 1985; LEVIS and RUBIN 1982) or chromosomal rearrangements such as inversions or translocations (FRISCHER, HAGEN and GARBER 1986; SCHNEUWLY, KUROIWA and GEHRING 1987). Both of these types of mutations are recovered when *P* elements are mobilized during hybrid dysgenesis (RUBIN, KIDWELL and BINGHAM 1982; ENGELS and PRESTON 1984). Thus, screening for flies carrying dominant mutations among the progeny of dysgenic parents is a reasonable strategy for identifying previously undiscovered genes of developmental importance. Additionally, the mutant gene is tagged by *P* element sequences and thus readily accessible to molecular analyses. We elected to screen for hybrid dysgenesis induced dominant mutations in a mutant screen which was initiated for other reasons. We report here the analysis of two allelic mutations isolated in this screen which we have

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TABLE 1

List of mutations

Mutation	Cytology	Comments	Reference[a]
Arp^{1}	In(2R)49B1-3;49E6-F1	Inversion, gain of function in *Su(z)2*	1
Arp$^{1.FBf1a}$	Df(2R)49B1-3;49E6-F1	*Arp*^{1} revertant, deletion between the terminal P elements of the *Arp*^{1} inversion	This paper
Arp$^{1.B2le}$	In(2R)49B1-3;49E6-F1	*Arp*^{1} revertant that deletes distally from *Arp*^{1} distal inversion junction	This paper
Arp$^{1.DG41}$	In(2R)49B1-3;49E6-F1	*Arp*^{1} revertant that deletes distally from the *Arp*^{1} distal inversion junction	This paper
Arp$^{1.C1}$	In(2R)49B1-3;49E6-F1	*Arp*^{1} revertant that deletes proximally from the *Arp*^{1} distal inversion junction	This paper
vg^{D}	Df(2R)49C1-2;49E2-6	Deletion including *vg* ending within 20 kb proximal to the distal *Arp* breakpoint	2
vg^{62}	Df(2R)B8-D1;49E1-F2	Deletion including *vg* ending within 12 kb proximal to the distal *Arp* breakpoint	3
vg^{C}	Df(2R)49B2-3;49E7-F1	Deletion which uncovers the proximal breakpoint of *Arp*	2
vg^{B}	Df(2R)49D3-4;50A2-3	Deletion which uncovers the distal breakpoint of *Arp*	2
Su(z)2^{1}	Normal	Dominant suppressor of the *zeste*^{1} interaction with *white*	4
Psc^{1}	Normal	Mutation in a *Pc* group gene and dominant suppressor of the *zeste*^{1}/*white* interaction	5–7
l387	Normal	Allele of *Brista* and dominant enhancer of the *Arp* antennae phenotype	8

[a] 1, ADLER (1984); 2, LINDSLEY and GRELL (1968); 3, LASKO and PARDUE (1988); 4, KALISCH and RASMUSON (1974); 5, NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING (1984); 6, WU (1984); 7, JÜRGENS (1985); 8, kindly provided by C.-T. WU.

named *Aristapedioid*^{1} (*Arp*^{1}) and *Arp*^{2}. Arp^{1} and *Arp*^{2} are homeotic, gain of function alleles of the *Suppressor 2 of zeste* (*Su(z)2*) gene, which was initially identified via a gain of function mutation (KALISCH and RASMUSSON 1974). Interestingly, the phenotype of loss of function alleles of *Su(z)2* is not provocative (ADLER, CHARLTON and BRUNK 1989) and would probably not result in *Su(z)2* being selected for further study.

MATERIALS AND METHODS

Drosophila stocks and culture: Marker mutations and balancer chromosomes are described in LINDSLEY and GRELL (1968). Flies were grown on a standard medium (ADLER and MACQUEEN 1984; CARPENTER 1950) at 25° unless stated otherwise. Dysgenic flies, and larvae used for salivary gland squashes were grown at 21°. Long-term stock maintenance was at 18°. Stocks are listed in Table 1.

Isolation of *Arp*: The two *Arp* alleles were isolated in a large hybrid dysgenesis mutant hunt, whose primary purpose was to get a *P* element insertion mutation in the *frizzled* locus (VINSON, CONOVER and ADLER 1989). Several different genetic crosses were used. Most of the flies screened (including the original *Arp*^{1} and *Arp*^{2} carrying flies) were generated by the following crosses. *Oregon-R* virgin females (M) were crossed to Harwich males (P) (reviewed by ENGELS 1983). The dysgenic virgin female progeny from this cross were then crossed to *fz th st in Ubx/TM3* males. The progeny of these flies were screened for new *fz* mutations as well as potential dominant mutations. Over 200,000 flies were scored and more than 400 flies with an interesting morphological defect tested. One hundred sixty-four of these were females and hence good candidates for flies carrying new dominant mutations. Fifty-seven percent of these were sterile and an additional 28% failed to transmit the mutant phenotype. Additional mutant stocks were discarded due to poor penetrance and only 10 stocks carrying new dominant mutations were kept. Two of these were *Arp*^{1} and *Arp*^{2}.

Revertant screens: Hybrid dysgenesis induced revertants of the dominant *Arp*^{1} mutation were obtained in several independent experiments that used similar but slightly different genetic schemes. For example, in one experiment, *Arp*^{1}/*CyO* (P) males were crossed to *pr cn vg/CyO* (M) females. The dysgenic *Arp*^{1}/*CyO* male progeny of this cross were then crossed to *pr cn vg/CyO* (P) females and the resulting progeny scored for reversion of the dominant *Arp* phenotypes. In an alternative revertant screen, *Arp*^{1} *l387/CyO* (P) males were crossed to *pr cn vg/CyO* (M) females. *l387* is an allele of *Brista* (SUNKEL and WHITTLE 1987) also called *distal-less* (COHEN *et al.* 1989) isolated by C.-T. WU which, while not having a dominant visible phenotype by itself, causes a dominant enhancement of the antennae to tarsus transformation in flies carrying both *Arp*^{1} and *l387*. Dysgenic *Arp*^{1} *l387/CyO* flies were crossed to *pr cn vg/CyO* (P) flies and the progeny screened for reversion of the dominant phenotypes. Only male revertants were kept.

Stocks were established by crossing revertants to *pr cn vg/CyO* (P) flies and crossing the *Arp*^{rv}/*CyO* siblings from this cross. These stocks were crossed to T (2, 3) *CyO*, st *Ch red Tb/TM3* (*TbCh*) flies (kindly provided by W. GELBART via T. R. F. WRIGHT) and the resulting *Arp*^{rv}/*TbCh* progeny crossed to Oregon R. This enabled us to distinguish the *Arp*^{rv}/*OR* larvae which were used for the subsequent cytogenetic analysis from their *TbCh* carrying siblings, which are short and fat.

Cytogenetic analyses: Salivary gland polytene chromosomes were analyzed using standard techniques (LEFEVRE 1976), except that the wet chromosomes were examined under phase optics rather than being stained and examined under bright field. *In situ* hybridizations (ATHERTON and GALL 1972; PARDUE and GALL 1975) to salivary gland polytene chromosomes were done as described by BINGHAM, LEVIS and RUBIN (1981) using as a probe DNA labeled in a nick translation reaction in the presence of [^{3}H]-thymidine trisphosphate (TTP).

Genomic library construction: *Arp*^{1}/+ mutant females were outcrossed for several generations to M males to remove extraneous *P* elements. DNA was then isolated and inserted into the EMBL4 lambda vector essentially as described by FRISCHAUF *et al.* (1983). The library was plated on the P2 lysogen Q359, and the primary plating was screened (BENTON and DAVIS 1977) using as a probe nick translated *P* element DNA (pπ25.1) (SPRADLING and RUBIN 1982; RUBIN and SPRADLING 1982). Thirty-three *P* element-containing phage were isolated and DNA was obtained by a standard plate lysate procedure (MANIATIS, FRISCH and SAM-

BROOK 1982). The recombinant phage DNAs were then radioactively labeled with [^{3}H]TTP and used as probes for *in situ* hybridizations to the salivary gland chromosomes of Oregon R flies (which contain no *P* elements). Recombinants which hybridized to the endpoints of the *Arp* inversion were the phage of interest.

Cloning wild-type DNA: The genomic library used to isolate the wild type DNA was constructed by MANIATIS *et al.* (1979) in the lambda vector Ch4a using DNA isolated from Canton S flies. The libraries were plated on KH802 at a density of 10,000–20,000 plaques/100-mm plate and screened according to MANIATIS, FRISCH and SAMBROOK (1982).

Fly DNA isolation: Two hundred flies were etherized and placed into 1.5 ml of buffer A [0.2 M NaCl, 0.2 M sucrose, 10 mM EDTA, 30 mM Tris-HCl (pH 8.0), 0.15 mM spermine, 0.15 mM spermidine, 400 μg/ml ethidium bromide]. The flies were homogenized in a ground glass homogenizer and then 1.5 ml of lysis buffer [0.25 M EDTA, 0.5 M Tris-HCl (pH 9.2), 2.5% SDS] was added. Proteinase K (Sigma) was added to 100 μg/ml and allowed to digest 1 hr at 60°. The DNA was then extracted twice with phenol, twice with phenol/chloroform/isoamyl alcohol (24:24:1), and once with chloroform/isoamyl alcohol (24:1). This was followed by a 1-hr RNAse A digestion (20 μg/ml) at 37° and an ethanol precipitation. Whenever possible the DNA precipitate was spooled from the solution.

Lambda phage DNA isolation: The procedure was as described by GARBER, KUROIWA and GEHRING (1983) modified in the following manner. NaCl was added to 1.0 M and polyethylene glycol (PEG) to 10% to the lysed cultures and allowed to go into solution with gentle shaking. Cultures were placed on ice for at least 1 hr or at 4° overnight and then centrifuged 20 min in a GS3 rotor to pellet the phage and bacterial debris. The bacterial debris then separates into the interface during the subsequent chloroform extraction to remove the PEG.

Labeling probes: Labeled dCTP^{32} (3000 Ci/mmol) was obtained from New England Nuclear. Restriction fragments were labeled using the random primed oligo labeling technique (FEINBERG and VOLGELSTEIN 1983). Intact plasmid and bacteriophage DNAs were labeled by nick translation (RIGBY *et al.* 1977). Unincorporated nucleotides were removed by fractionation over Sephadex G50-80 (Sigma).

Genomic Southern analysis: All nonhomozygous viable fly stocks examined by genomic Southern analysis were balanced over an isogenic *CyO* balancer chromosome. Aliquots (10 μg) of DNA were digested overnight with at least three separate restriction enzymes, fractionated on a 0.9% agarose gel, blotted to nytran (Schleicher & Schuell) and hybridized with a ^{32}P-labeled DNA probe using the protocol suggested by Schleicher & Schuell. The blots were washed once in 0.1× SET [20× is 3 M NaCl, 0.4 M Tris-HCl (pH 7.8), 20 mM EDTA], 0.1% SDS for 15 min at room temperature then twice in the same buffer for 1 hr at 60°. Exposure times were from one to five days depending on the specific activity of the probe.

RESULTS

Two independently derived alleles of *Arp* (*Arp^1* and *Arp^2*) were isolated in the hybrid dysgenesis mutant screen. Both alleles are homozygous lethal and fail to complement each others recessive lethality. As will be described later, the phenotype, genetics and cytology of *Arp^1* and *Arp^2* are indistinguishable.

Dominant phenotypes of *Arp*: Two dominant phenotypes are associated with both *Arp* alleles. One is a partial transformation of arista toward tarsus. As can be seen from the panels in Figure 1, the expressivity of the mutation is variable (all the flies represented could have come from the same bottle). The penetrance, however, is essentially 100%. The transformed arista does not appear to be a simple mosaic of aristal and tarsal cells as some individual structures appear to be intermediate in phenotype. This suggests that the *Arp* mutation does not cause a simple segmental transformation from antenna to leg, but rather is modulating in some fashion the expression of antenna and leg specific genes such that an intermediate cellular phenotype can result. The second dominant phenotype associated with *Arp* is the loss or decrease in size of the dorsocentral and scutellar bristles (Figure 2). As is seen in the arista transformation phenotype, this phenotype also shows variable expressivity and complete penetrance. Finding a continuum of bristle phenotypes (complete loss to decreased size of varying extent) again suggests that the *Arp* mutation does not result in a simple loss of cell determination or complete transformation to an alternative developmental fate.

Genetic mapping of the *Arp* mutation: Initial genetic mapping experiments indicated that both *Arp* alleles were located around map position 65 on the second chromosome. More precise mapping was done by crossing *cn vg/Arp^1* females to *cn vg* males and examining the F_1 progeny for crossovers in the 9.5 cM interval between *cn* (57.5) and *vg* (67). One-hundred-seventy progeny were recovered that carried crossovers in the *cn-vg* interval. Eighty-two were *cn Arp^1* while 88 were *vg*. Similar results were obtained in analogous crosses involving *Arp^2* (75 *cn Arp^2* and 106 *vg* progeny). Thus, *Arp* maps to position 67, very close to *vg*. We were unable to separate *vg* and *Arp*, which is consistent with the following cytological examination of the *Arp* chromosomes.

The *Arp* mutation is associated with a small cytologically visible inversion: Cytological examination of the salivary gland polytene chromosomes of *Arp/+* larvae reveals a small region of mispairing which includes the location of the *vg* locus (49C-D) and extends from cytological position 49B1-3 to 49E6-F1 (Figure 3B). The mispaired region appears to be the same length on both homologs, suggesting it could be due to a small inversion. Genetic data consistent with this hypothesis has also been obtained. The *vg^D* deletion (diagrammed in Figure 5), which extends from 49C-D to 49E6-F1, is viable in trans with the *Arp^1* chromosome. The viability of *vg^D/Arp^1* flies is consistent with the mispaired region being a small inversion as all of the genes uncovered by the *vg^D* deletion would still be present and presumably functional inside the inversion. It seems unlikely that this DNA could be

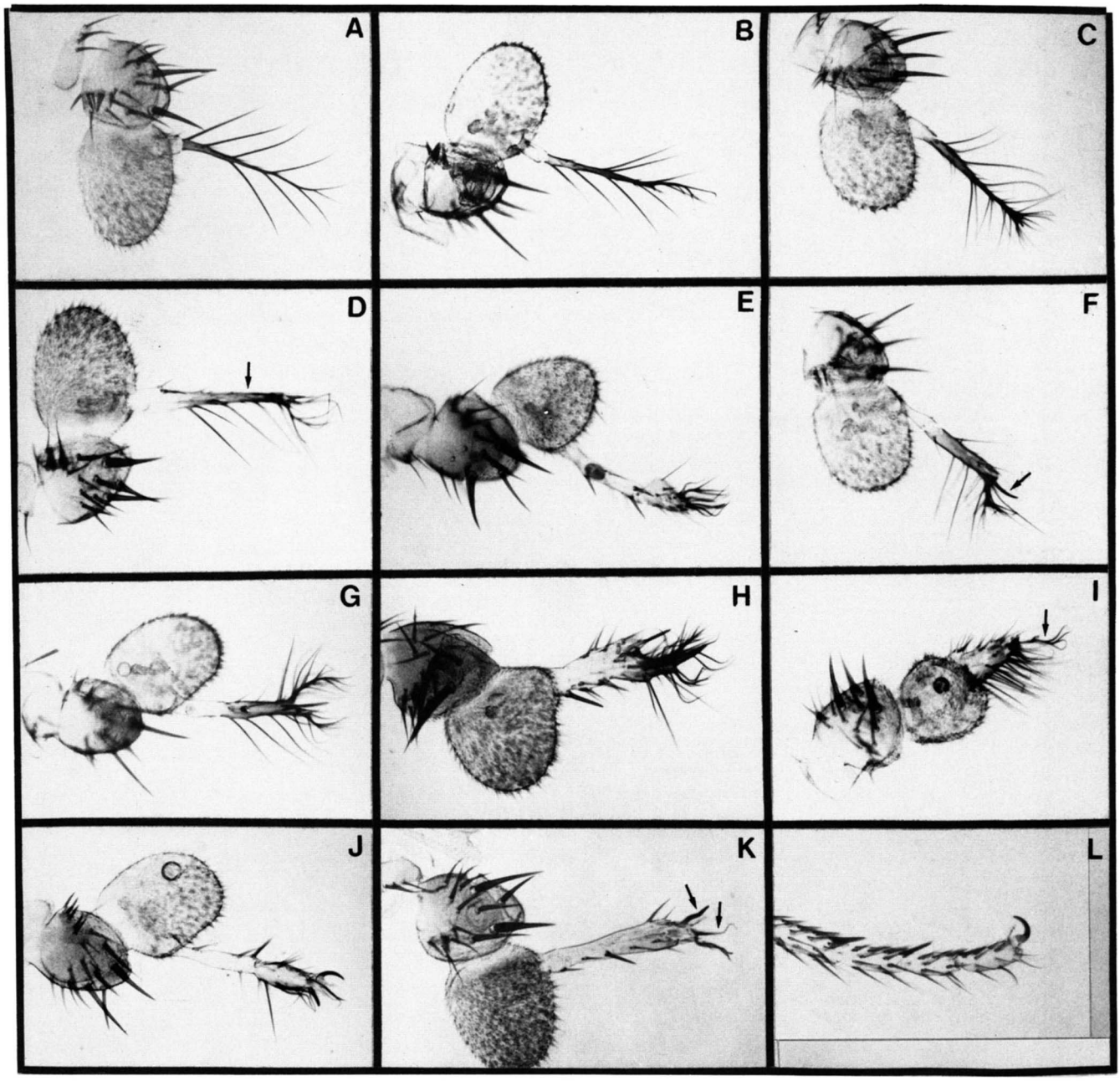

FIGURE 1.—Antennae transformation phenotype of the *Arp*^{1} mutation. A, Wild-type antennae; L, wild-type tarsus; B–K, *Arp*^{1} antennae showing the variable expressivity of the phenotype. The arrows indicate some of the structures which have qualities intermediate between arista and tarsus, suggesting that the mutation does not cause a simple transformation from arista to tarsus.

present and functioning at an alternative chromosomal location as our mapping experiments indicate there is a functional *vg*$^{+}$ gene very tightly linked to the *Arp* mutation.

The *Arp* mutation proved to be unstable, reverting at a spontaneous frequency of 0.02%. When hybrid dysgenesis was induced by crossing *Arp/CyO* (P) males to *pr cn vg/CyO* (M) females, the frequency of reversion of the dominant phenotype increased as much as 100-fold to 2%, indicating the likely involvement of *P* elements in the mutation. *In situ* hybridizations to *Arp/+* chromosomes using the P element probe pπ25.1 (SPRADLING and RUBIN 1982; RUBIN and SPRADLING 1982) gives hybridization signals at each end of the mispaired region on the *Arp* chromosome (Figure 3C) indicating the presence of *P* elements at those locations. Thus, it seems likely that the *P* elements mediated the original mutational event and are facilitating subsequent reversions of the *Arp* phenotype to wild type.

Cytological examination of hybrid dysgenesis induced revertants of the dominant phenotypes indicates that one class of revertants has a normal, wild-type cytology, consistent with the putative inversion reinverting to give a wild-type orientation. Complementation analysis supports this hypothesis. *vg*^{C}, which

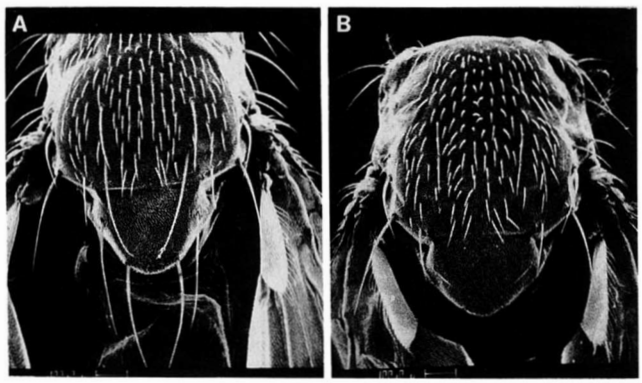

FIGURE 2.—Bristle loss phenotype of the Arp^1 mutation. A, Wild-type dorsal thorax, B, Arp^1 dorsal thorax. Again the expressivity is variable as we see a continuum of sizes in the bristle phenotype. The Arp^1 fly pictured here is one of the stronger phenotypes we see. The loss of polarity of the bristles in the Arp^1 fly is an artifact caused by preparing the specimen for scanning electron microscopy.

uncovers the proximal end of the mispaired region (see Figure 5), is lethal over the *Arp* chromosome. vg^B, which uncovers the distal end of the mispaired region (Figure 5) is viable over the *Arp* chromosome. The *Arp* revertant (Arp^{rv}) class which appears to be cytologically wildtype is viable over both vg^B and vg^C, indicating that the lesion in the *Arp* mutation uncovered by vg^C has been repaired. These data argue that the mispairing is due to a *P* element mediated inversion, and a reinversion to wild type orientation restores function to a gene within the vg^C deletion which had been broken by the inversion. *In situ* hybridization experiments to the polytene salivary gland chromosomes of this class of Arp^{rv} chromosomes using *P* element probes indicates that the *P* element may or may not remain at the site of the proximal breakpoint (data not shown). Thus, we hypothesize that the *P* element at the proximal breakpoint is not in an exon as the presence of *P* element sequences at this location does not result in a mutation.

Molecular cloning of the endpoints of the *Arp* inversion: The endpoints of the *Arp* inversion were cloned utilizing the transposon tagging technique (BINGHAM, LEVIS and RUBIN 1981; SEARLES *et al.* 1982). A genomic library of *Arp* DNA was constructed and 33 recombinant phage were isolated which hybridized with a *P* element probe (SPRADLING and RUBIN 1982; RUBIN and SPRADLING 1982). Three of these phage contained inserts which hybridized to one or both of the *Arp* inversion breakpoints on Oregon R salivary gland chromosomes. Figure 4A shows a preparation obtained using as a probe DNA from the phage (BB26) that is most symmetrically situated about an inversion junction such that it hybridizes relatively equally to both 49B1-3 and 49E6-F1.

These three recombinant phage were restriction mapped and DNA fragments flanking but not including the *P* elements were isolated and used to probe

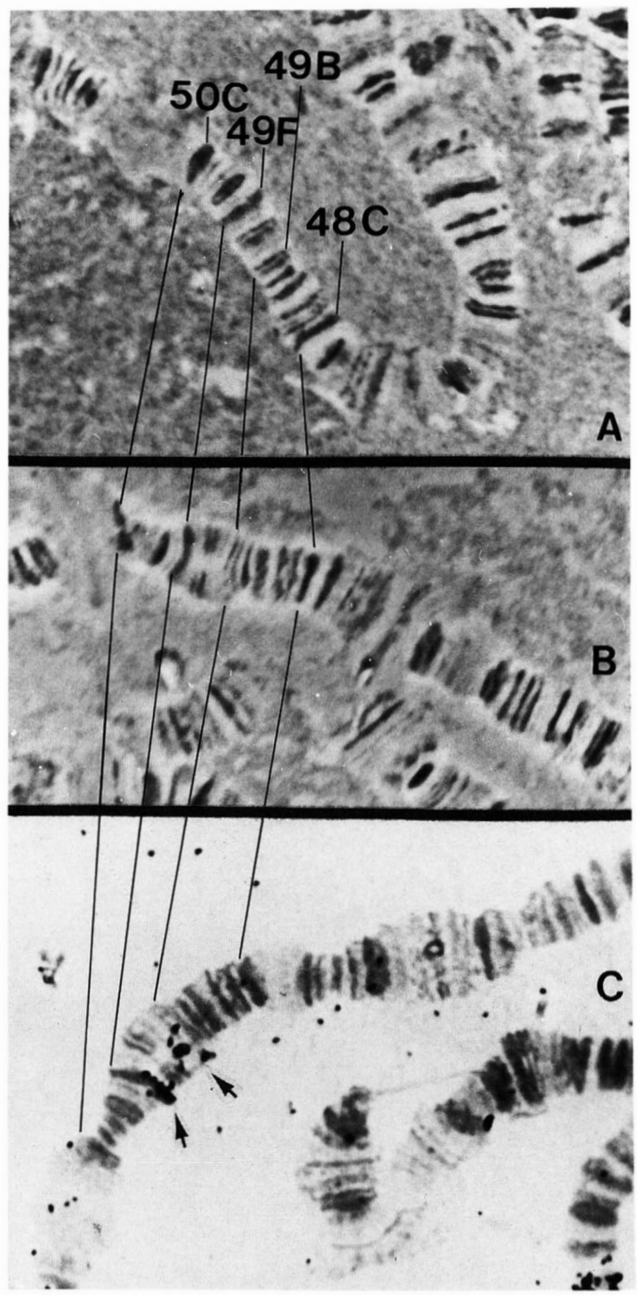

FIGURE 3.—Cytology of the Arp^1 mutation. A, Salivary gland polytene chromosome preparation of a wild type larvae. B, Chromosome preparation from an $Arp^1/Oregon\ R$ larvae. Note the mispairing of the homologs from 49B to 49F. C is an *in situ* hybridization to chromosomes from $Arp^1/Oregon\text{-}R$ larvae using a *P* element probe pπ25.1 (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1982). The arrows indicate the sites of hybridization at the ends of the inversion on the Arp^1 chromosome.

salivary gland chromosomes of *Arp*/+ larvae in order to orient the cloned DNA on the chromosome. Probe 1 (Figure 5) hybridizes distally (49E6-F1) on both chromosomes (Figure 4B) indicating that it is outside the inversion and that it represents the most distal DNA of the recombinant phage. This result is confirmed by probe II (Figure 5), which hybridizes to both ends of the mispaired region (Figure 4C) indi-

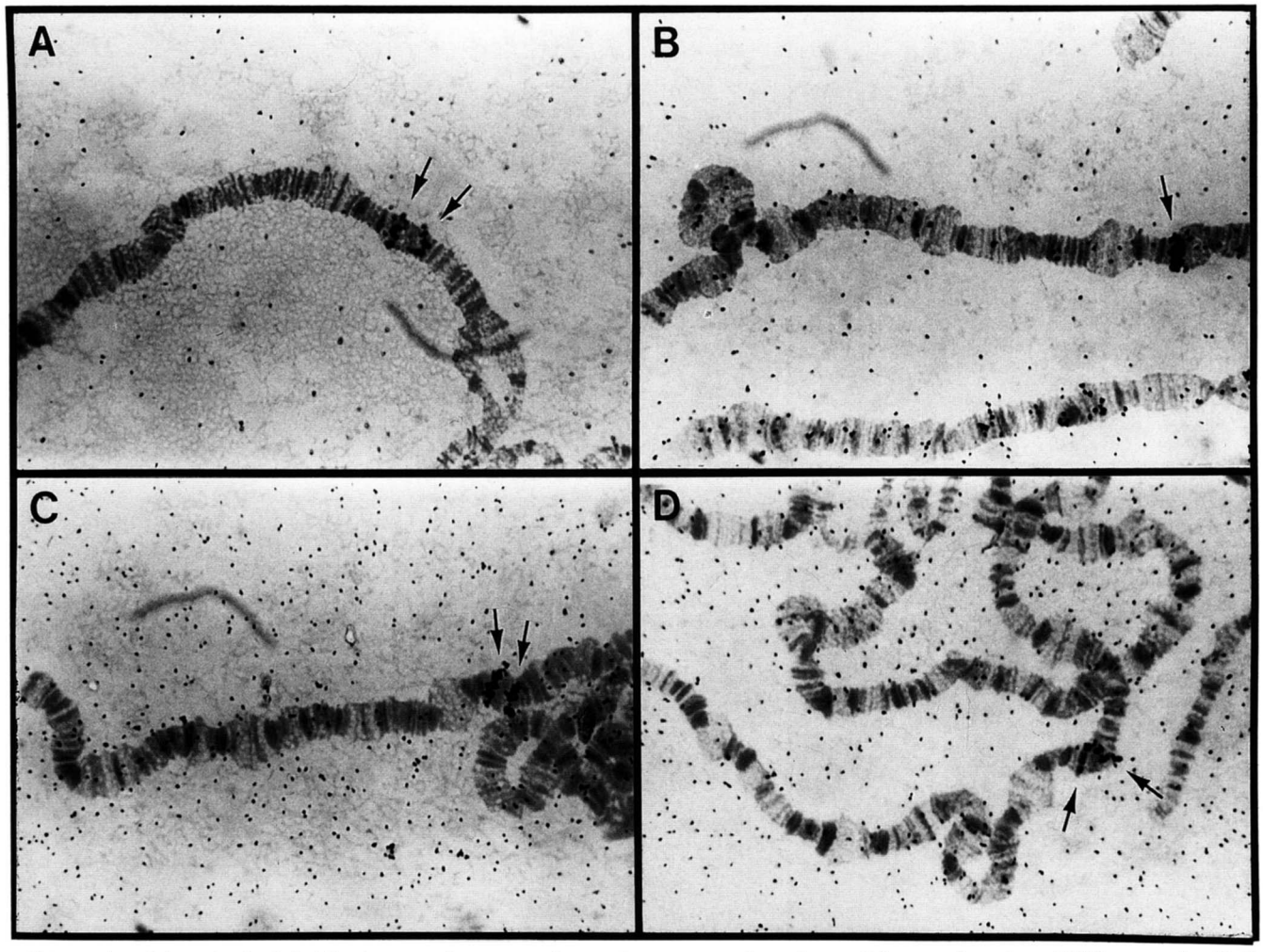

FIGURE 4.—A is an *in situ* hybridization experiment to polytene chromosomes from *Oregon-R* larvae using phage BB26 as a probe. B–D are all *in situ* hybridizations to Arp^1/*Oregon-R* chromosomes. The probe used in B is number 1 (Figure 5); C is number II (Figure 5) and D is number III (Figure 5). Arrows indicate the sites of hybridization.

cating that this DNA is contained within the inversion. Phage BB23 was oriented with respect to the chromosome by probe III, which hybridizes to both ends of the mispaired region (Figure 4D). This indicates that probe III is within the inversion and thus distal to the proximal *P* element.

Insert DNA from the recombinant phage was used to screen a wild-type Drosophila Canton S genomic library (MANIATIS *et al.* 1979). A number of recombinant phage were isolated and restriction mapped. The data are presented in Figure 5. Comparing the restriction maps of DNA from the Arp^1 and wild-type chromosomes indicates that the Arp^1 mutation is due to a simple inversion with *P* element sequences inserted at both ends. There do not appear to be (at least at the level of restriction mapping) any other DNA alterations such as deletions or other inversions. A genomic Southern analysis comparing the Arp^1 and Arp^2 alleles indicates that the breakpoints for both mutations are identical at this level of analysis. The only detectable difference is in the amount of *P* element sequences present at the proximal junction (the *P* element in Arp^2 is 0.8 kb smaller than in Arp^1). The two alleles arose from different parents and as such are independent. Thus, we hypothesize that there were *P* elements resident at these positions on some chromosomes in the parental Harwich stock which were mobilized in this screen or, alternatively, these positions are hot spots for *P* element insertion. *In situ* hybridization of *P* element sequences to salivary gland chromosomes derived from Harwich larvae failed to detect any chromosomes with *P* element sequences at either 49B1-3 or 49E6-F1 (data not shown). We did detect substantial polymorphisms in the location of *P* elements in the ten Harwich chromosomes examined, thus it is possible that *P* elements were located at 49B and/or 49EF in a fraction of the chromosome population.

The distal inversion junction is responsible for the gain of function mutation: We examined more than 100 hybrid dysgenesis induced revertants of the dominant antennae phenotype and in all cases the

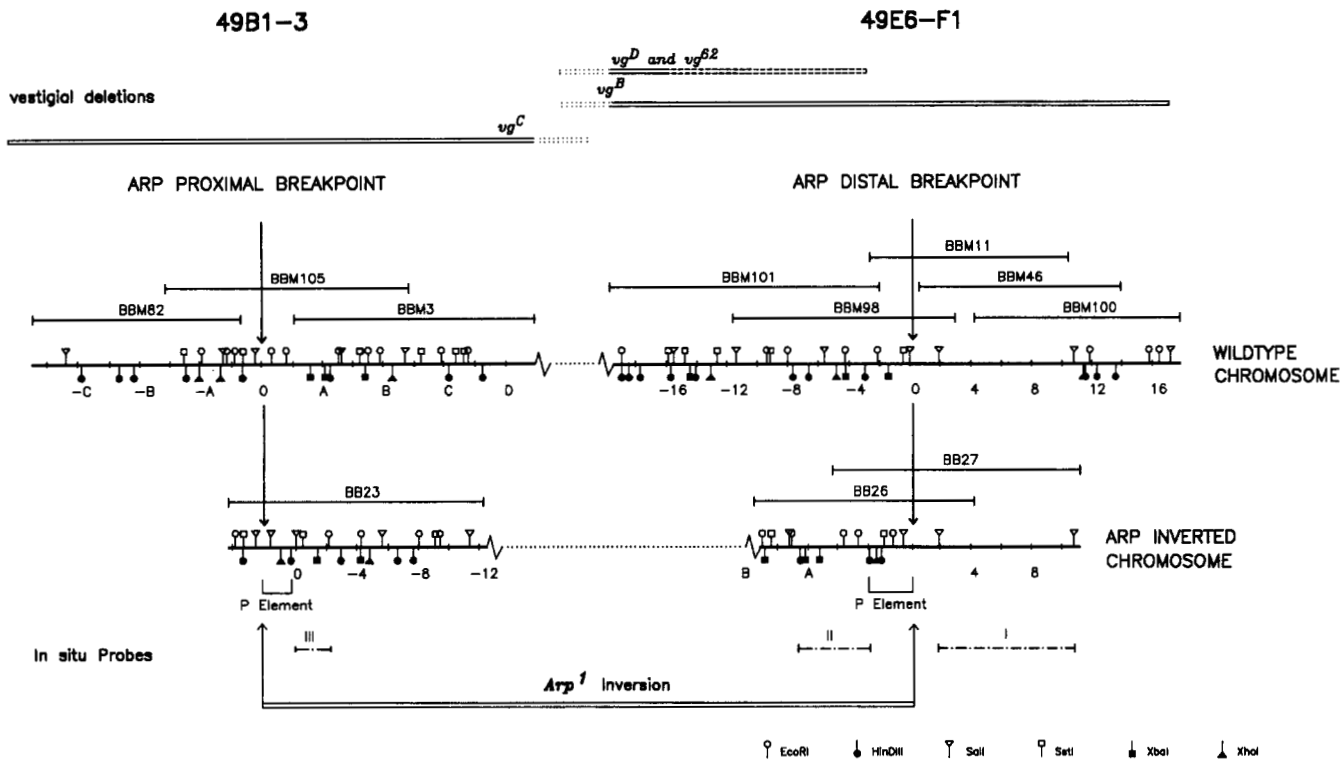

FIGURE 5.—Restriction map of cloned DNA. Cytologically visible deletions and the Arp^1 inversion are indicated by the double lines. Cloned DNA is represented by a single bold line and the phage which were restriction mapped are indicated above the map. Probes used to orient the cloned DNA onto the chromosome are indicated near the bottom of the figure. Probe I is the large *Sal*I fragment isolated from phage BB27, probe II is the 4.3 kb *Hin*dIII fragment from phage BB26 and probe III is the 2.3-kb *Eco*RI/*Sal*I fragment from phage BB23. At the level of restriction mapping, there do not seem to be any other DNA alterations such as inversions or deletions on the Arp^1 chromosome.

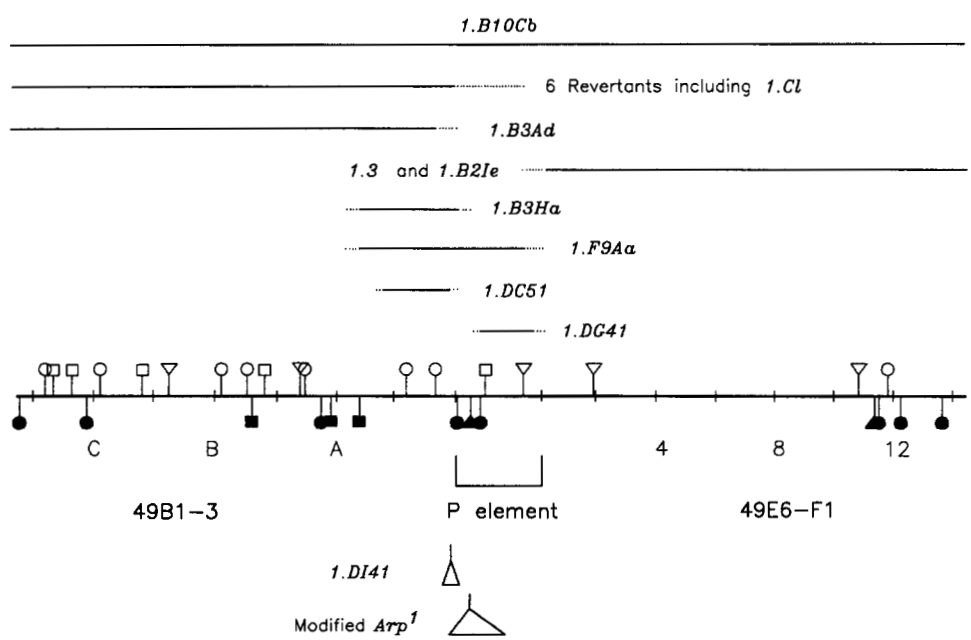

FIGURE 6.—Localization of the DNA alterations in revertants of the gain of function *Arp* phenotype which retain the *Arp* inversion. Deletions are represented by lines. Deletions which extend beyond the map are represented by lines which end at the edge of the cloned DNA. Two small insertions are indicated at the bottom. $Arp^{1.DI41}$ reverts the dominant phenotype while the insertion within the *P* element in the modified Arp^1 chromosome has no noticeable effect on the phenotype.

bristle loss phenotype reverted as well. Because the experiments were done by screening for reversion of the antennae phenotype, we cannot say the converse is true, *i.e.*, that a reversion of the antennae phenotype always accompanies a reversion of the bristle loss phenotype, although we suspect that to be the case as limited attempts to isolate partial revertants of this sort have not been successful. Thus, it seems probable that the two phenotypes are due to the same lesion. The *Arp* inversion resulted in the formation of novel

combinations of DNA sequences at both junctions of the inversion. Either or both of these junctions could be responsible for either or both of the dominant phenotypes. We have determined that the distal inversion junction is responsible for both dominant phenotypes via a genomic Southern analysis of hybrid dysgenesis induced revertants.

Revertants of the dominant *Arp* phenotype fall into the four cytological classes listed below. The relative frequencies of these classes varied from one experiment to another.

Class I: The *Arp* inversion is still present.

Class II: The *Arp* inversion has reinverted and the Arp^{rv} chromosome appears to be cytologically wild type.

Class III: DNA between the *P* elements has been deleted.

Class IV: Complex rearrangements with other regions of the genome. In most cases the rearrangements appear to involve the distal *P* element.

The DNA sequences necessary for the gain of function *Arp* phenotype were identified by genomic Southern analyses (SOUTHERN 1975) of classes I and IV. When these analyses were initiated, there was some evidence from the cytogenetic analysis of class IV revertants that the distal junction of the *Arp* inversion was necessary for the dominant phenotype. This observation was confirmed by the genomic Southern analysis. Thirty-one class IV revertants were examined. All 31 revertants showed alterations in the DNA at the distal junction while only two showed alterations at the proximal junction (data not shown). Additionally, all 14 of the class I revertants examined showed DNA alterations at the distal junction while only three showed alterations at the proximal junction. Taken together, this is strong evidence that it is the DNA at the distal junction of the *Arp* inversion that is responsible for the dominant, gain of function phenotype.

Sequences from both 49B1-3 and 49E6-F1 are required for the *Arp* phenotype: More precise genomic Southern mapping was done to localize the DNA alterations associated with revertants which retain the *Arp* inversion (class I). This class was chosen because the DNA changes should be relatively small and thus able to be mapped on a molecular scale. Deletions both proximal to the *P* element as well as distal to the *P* element revert the dominant phenotypes (Figure 6). Thus, the dominant phenotypes of the *Arp* mutation are due to the juxtaposition of these two normally non-contiguous pieces of DNA, and both pieces are necessary for the gain of function phenotypes. It is probable that sequences from 49B located close to the *P* element are important for the *Arp* phenotype since a small 0.4 kb insertion just proximal to the *P* element ($Arp^{1.DI41}$) causes a phenotypic reversion. Additionally, a 2.5-kb deletion ($Arp^{1.DC51}$) of 49B DNA results in reversion of the *Arp* phenotype. DNA sequences located just distal to the *P* element (49EF DNA) are also important for the gain of function phenotypes. A phenotypic reversion is caused by a small deletion ($Arp^{1.DG41}$) which, based on the genomic Southern analysis, appears to end near the distal edge of the *P* element. The failure of this revertant to complement vg^{B} (Table 2) indicates that a gene from 49EF was inactivated by the reversion event. Thus, the deletion presumably extends a small distance beyond the *P* element. Larger deletions of 49EF DNA such as $Arp^{1.B2Ie}$ also revert the gain of function phenotypes.

TABLE 2

Complementation matrix between Arp^{1}-revertants and mutations affecting DNA at the distal *Arp* breakpoint (49E6-F1)

	vg^{B}	vg^{62}	Psc^{1}	$Su(z)2^{1}$
Arp^{1}	+	+	+	+
$Arp^{1.B2Ie}$	−	+	+	−
$Arp^{1.DG41}$	−	+	+	−
$Arp^{1.CI}$	+	+	+	+
$Arp^{1.FBf1a}$	−	−	−	+

A − indicates lethal; + indicates viable.

The role of the *P* element remains unclear from this analysis. The Arp^{1} stock sustained a 2-kb insertion internal to the *P* element during the course of this investigation without affecting the dominant phenotype. VOELKER *et al.* (1984) have shown for a *P* element induced lethal mutation in the gene for a subunit of RNA polymerase II that a substantial fraction (up to 65%) of all mobilizations of the resident *P* element resulted in precise excision of that *P* element. While this percentage would likely be affected by the specific *P* element and its surrounding sequences, we would still expect some mobilizations of the *P* element at the *Arp* distal junction to result in precise excision of that element. As we did not isolate any revertants in which the *P* element has been precisely deleted, we suggest that such an event does not revert the dominant phenotypes. Thus, it is probable that the *P* element is only important for mediating the inversion event and does not play an active role in the resulting gain of function mutation.

The distal breakpoint of the *Arp* inversion is located between and close to *Posterior sex combs* and *Suppressor 2 of zeste*: The *Arp* inversion does not induce a loss of function mutation at the distal breakpoint, nor does it interact with any of the known mutations in the 49E,F region. Therefore, it is not possible in a simple way to place the distal breakpoint of the inversion onto a genetic map. However, certain revertants of the dominant phenotypes are associated

with loss of function recessive lethal mutations in the region. These revertants have enabled us to localize the distal *Arp* breakpoint to the *Posterior sex combs* (*Psc*)/*Suppressor 2 of zeste* (*Su(z)2*) region. The results of several crosses between *Arp* revertants and *Psc*/*Su(z)2* region mutations are shown in Table 2. Cytologically normal, molecularly defined deletions which extend distally from the *Arp* distal breakpoint (e.g., $Arp^{1.B2le}$ and $Arp^{1.DG41}$) fail to complement $Su(z)2^1$, indicating that $Su(z)2^1$ maps distal to the *Arp* breakpoint. Since $Arp^{1.DG41}$ is associated with a very small deletion, it is likely that sequences essential for *Su(z)2* function are located quite close to the *Arp* breakpoint (within 500 bp). Revertants which remove sequences proximal to the 49EF breakpoint such as $Arp^{1.FBf1a}$, which deletes the DNA between the terminal *P* elements of the *Arp* inversion (class III), fail to complement Psc^1 but do complement $Su(z)2^1$. Thus, Psc^1 maps proximal to the *Arp* distal breakpoint. Since Psc^1 is not uncovered by the vg^C deletion, it must map between the distal endpoints of vg^C and $Arp^{1.FBf1a}$ (49E1-6 and 49E6-F1). Thus, at least at the cytogenetic level, *Psc* maps close to the distal *Arp* breakpoint. Molecular analyses (our manuscript in preparation) have confirmed these conclusions and show that the genes flanking the distal breakpoint of *Arp* are indeed *Psc* and *Su(z)2* and that both are contained within the region of DNA we have cloned.

DISCUSSION

The *Arp* mutations are associated with a *P* element mediated inversion that juxtaposes DNA sequences from 49B1-3 and 49E6-F1. The finding that deletion of distal junction DNA reverts the dominant phenotypes of *Arp* is evidence that the phenotypes are due to a gain of function mutation (DENELL 1973; STRUHL 1981b). Deletions in the region that span either inversion breakpoint (vg^C and vg^B) but fail to produce similar phenotypes also supports this hypothesis. Revertant analysis indicates that the novel sequence created at the distal junction is responsible for both gain of function phenotypes. A number of other gain of function mutations have been mapped to the 49EF region. These include two cytologically visible *vestigial* deletions (vg^D and vg^{62}) which share an endpoint at 49E6-F1 and are associated with dominant cheatae loss phenotypes (CRIPPS and SPARROW 1989). Other gain of function mutations that map to the 49EF region are two alleles of *Suppressor 2 of zeste* (*Su(z)2*) (KALISCH and RASMUSON 1974; WU 1984) and *Posterior sex combs* (Psc^1) (NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984), a Polycomb group gene (JÜRGENS 1985). Psc^1 has a number of gain of function phenotypes including suppression of the $zeste^1$ eye color phenotype (WU *et al.* 1989). In addition to a similar *zeste* suppression phenotype, $Su(z)2^1$ and Psc^1 fail to complement each others recessive lethality (WU 1984; JÜRGENS 1985; WU *et al.* 1989) raising the possibility that these are mutations in the same gene. Our data argue that *Su(z)2* and *Psc* are separate genes and that *Psc* maps proximal and *Su(z)2* distal to the *Arp* distal breakpoint, which separates the two genes without causing a loss of function mutation in either. Thus, the failure of the $Su(z)2^1$ and Psc^1 mutations to complement each others recessive lethality is presumably due to their gain of function nature. Further support for this hypothesis has been obtained where homozygous lethal revertants of $Su(z)2^1$ complement Psc^1 and vice versa (WU 1984; C.-T. WU and M. HOWE, in preparation).

The *Su(z)2* gene is intact on the *Arp* chromosome: The *Arp* inversion does not remove any cis acting sequences that are essential for normal regulation of the *Su(z)2* gene as the *Arp* mutation is viable over a deletion for the entire region (*e.g.*, vg^B). Hence, the *Arp* chromosome can supply all essential *Su(z)2* functions. This is not due to 49B1-3 sequences providing a substitute function as revertants which delete proximally from the distal *P* element ($Arp^{1.Cl}$ is an example) are almost always viable in trans with the vg^B deletion. On the other hand, revertants which delete even small amounts of DNA distal to the *P* element ($Arp^{1.DG41}$) are lethal over vg^B and $Su(z)2^1$. Taken together, these data suggest that *Su(z)2* is intact on the *Arp* chromosome and that sequences essential for *Su(z)2* function are located very close to the distal breakpoint of the *Arp* inversion.

The *Arp*, vg^{62}, and vg^D phenotypes likely result from the misexpression of wild-type *Su(z)2* protein: The similarity of the *Arp*, vg^{62}, and to a lesser extent, vg^D bristle loss phenotypes suggests that these phenotypes have a similar molecular basis and are due to gain of function mutations in the same gene. Support for this hypothesis comes from our finding that the distal end points of both the vg^{62} and vg^D deletions map between 2 and 20 kb proximal to the distal *Arp* breakpoint (BRUNK 1989). Northern and cDNA analyses (BRUNK 1989) indicate that there is a single transcription unit in the cloned DNA located distal to the breakpoint that corresponds to the *Su(z)2* gene. Thus, all three of these gain of function mutations juxtapose foreign DNA sequences next to the *Su(z)2* gene. Evidence that the simple loss of *Su(z)2* regulatory sequences does not cause a gain of function comes from the existence of chromosomes that carry deletions of DNA sequences proximal to the *Arp* breakpoint that do not result in a loss or gain of function in *Su(z)2*. *Arp* revertants which delete the DNA between the terminal *P* elements (class III) and class I revertants which delete proximally from the distal *P* element (*e.g.*, $Arp^{1.Cl}$) are two examples of such chromosomes.

It is easy to imagine an inversion or deletion resulting in a gain of function mutation by causing the inappropriate expression of a gene via juxtaposition of foreign regulatory sequences to a promoter. It is also easy to imagine such DNA rearrangements resulting in a gain of function mutation by causing a novel protein to be produced, either by fusing DNA sequences that normally encode separate proteins so that a fusion mRNA and fusion protein are produced or by producing a truncated mRNA and protein. In the case of *Arp*, our revertant analysis indicates that sequences from both 49B and 49EF are required for the gain of function mutation, thus the truncation hypothesis seems unlikely although a gain of function resulting from the formation of a fusion protein jointly encoded by DNA sequences from 49B and 49EF remains a possibility. However, this also seems unlikely given the similar phenotypes of the *Arp*, vg^{D} and vg^{62} mutations, each of which juxtapose different sequences to the *Su(z)2* gene. Thus, our working hypothesis is that misexpression of the wild type *Su(z)2* protein is responsible for the gain of function phenotypes of these three mutations. A prediction of this hypothesis is that misexpression of the *Su(z)2* protein directed by an engineered *Su(z)2* gene reintroduced into the Drosophila germ line should produce similar phenotypes. Experiments to test this hypothesis are in progress.

There are a number of ways that misexpression of the *Su(z)2* gene could be imagined to cause the *Arp* gain of function phenotypes. It is important to note at this juncture that *Su(z)2*, although not a typical *Polycomb* (*Pc*) group gene, appears to be related to the *Pc* group of genes which have been shown to be essential for the maintenance of homeotic selector gene expression patterns (STRUHL 1981a; DUNCAN 1982; INGHAM 1984; DURA, BROCK and SANTAMARIA 1985). The derived *Su(z)2* protein shows substantial sequence similarity to the product of the neighboring *Psc* gene (manuscript in preparation), a *Pc* group gene (JÜRGENS 1985), suggesting that *Su(z)2* could have a molecular function similar to this *Pc* group gene. Several *Pc* group genes including *Psc* can mutate to give a suppressor of *zeste* phenotype (WU 1984; WU *et al.* 1989). Additionally, mutations in *Su(z)2* have been found to interact with mutations in a number of *Pc* group genes (ADLER, CHARLTON and BRUNK 1989). Finally, some *zeste* mutations have been found to alter expression of the BX-C (BABU and BHAT 1980; GELBART and WU 1982) and the *zeste* protein has been shown to activate transcription from the *Ubx* promoter in vitro (BIGGIN *et al.* 1988). Considering its relationships with the *zeste* gene and *Pc* group genes, it would not be surprising if inappropriate expression of the *Su(z)2* protein resulted, either directly or indirectly via interaction with one or more *Pc* group genes, in aberrant expression of the homeotic selector genes. Thus some cells in the arista of *Arp* mutants could have a tarsal pattern of selector gene expression and produce typical tarsal structures. Other cells could have a novel pattern of expression of homeotic selector genes and produce novel cuticular structures with both aristal and tarsal qualities. Alternatively, misexpression of the *Su(z)2* protein could perturb development downstream of the homeotic selector genes. This hypothesis is particularly attractive in explaining the loss/reduction in size of the dorsocentral and scutellar bristles. For example, mis-expression of the *Su(z)2* protein could repress genes such as *scute*, that are essential for macrocheatae development (reviewed by GHYSEN and DAMBLY-CHAUDIÈRE 1988).

Designing a better screen for gain of function mutations: A major limitation in screening the progeny of dysgenic parents for provocative gain of function mutations is their relative rarity (~1/20,000 in our experiments). *P* elements do not contain strong promoter elements and as such cause mutations primarily by insertional inactivation or chromosomal rearrangements, as is the case with *Arp*. It should be possible to increase the likelihood of causing dominant *P* element induced mutations by placing a strong promoter/enhancer near the edge of a *P* element which could then be transformed into the genome and subsequently jumped around by inducing hybrid dysgenesis. In this manner, one could increase the frequency of dominant mutations as genes which come under the control of the heterologous promoter/enhancer within the *P* element would, in most cases, be expressed inappropriately. Whether one isolates gain of function mutations serendipitously in a screen for other reasons (as we did) or attempts to specifically generate gain of function mutations in the manner just described, these mutations can be valuable as resources to identify and clone genes of potential interest that might not otherwise be easily identified.

We thank CHARLES VINSON and TING WU for helpful discussions and JEANNETTE CHARLTON for technical assistance with stocks and photography. This work was supported by National Science Foundation grants DCB 8502683 and DCB 8812076. BPB was supported by a National Institutes of Health predoctoral training grant in developmental biology (HDO-7192). P.N.A. was supported during the initial phase of this work by a National Institutes of Health Career Development Award (KHDO-0361).

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Communicating editor: W. M. GELBART