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DNA map of mutations at the *scute* locus of *Drosophila melanogaster*

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The *achaete-scute* gene complex *(AS-C)* of *Drosophila melanogaster* is involved in the differentiation of innervated elements in the adult (chaetes) and in the embryo (central nervous system). Genetically, the *AS-C* is subdivided into four regions: *achaete, scute* α, *lethal of scute*, and *scute* β. Using a previously cloned fragment of *scute* DNA, we have now cloned 62 kb of wild-type DNA from the *scute* region. No repetitive sequences have been detected in this stretch of DNA. Of 16 *scute* mutants with chromosomal rearrangements studied (inversions, deletions, and translocations), nine, included genetically in *scute* β, have breakpoints in the cloned region. The remaining rearrangements, which genetically correspond to *scute* α, map outside and to the left of the cloned region. Of nine *scute* 'point mutants' studied, eight have large DNA alterations within the cloned region. These alterations include insertions (five) and deletions (three). The DNA alterations found in both 'point mutants' and rearrangements are interspersed and scattered over 40 kb. The relationship between the sites of the DNA alterations and the mutant phenotypes are discussed.

Key words: DNA cloning/chromosomal rearrangements/point mutations/complex loci/*scute* locus

Introduction

The *achaete-scute* gene complex *(AS-C)* of *Drosophila melanogaster* maps at the tip of the X chromosome (1 - 0.0) in the interval 1B1/2 - 1B4/5 of the salivary chromosomes (Figure 1). Chromosome rearrangements with breakpoints in this region have achaete and scute mutant phenotyes, i.e., the absence of microchaetes and macrochaetes from allele-specific positions in the cuticular pattern of the fly (see Lindsley and Grell, 1968). Genetic analysis of the *AS-C*, using chromosome rearrangements with different breakpoints, permits a subdivision of the complex into four regions: *achaete, scute* α, *lethal of scute*, and *scute* β (Muller, 1955; García-Bellido, 1979). Flies double-heterozygous for chromosome breakpoints separated by at least two thick salivary chromosomal bands do not show phenotypic complementation, neither do point mutations complement chromosomal rearrangements. Thus, the achaete-scute function can be impaired by chromosomal alterations far separated in the salivary chromosome DNA. Developmental analysis of *AS-C* mutants indicates that this system is involved: (1) in the differentiation of innervated elements in both the peripheral nervous system (sensory chaetes) and in the central nervous system; and (2) in the spatial and patterned distribution of chaetes in the adult (reviewed by García-Bellido, 1981).

The genetic and developmental peculiarities of the *AS-C* make it interesting for a molecular analysis. We have started cloning part of the DNA of the *AS-C* region entering the DNA of the *scute* region by using a small fragment of it (*sc* DNA) which has recently been cloned (J.Modolell, W.Bender, and M.Meselson, in preparation). This was accomplished by taking advantage of a DNA probe containing a transposon-like sequence known to be inserted in the *scute*[1] mutation. From this point we proceeded in both directions selecting clones from a library of wild-type *Drosophila* DNA. In the region analyzed so far (62 kb), we have found DNA alterations in almost all the point mutants tested and in the chromosome rearrangements genetically defining the *scute* β region of the complex.

Results

Cloning and restriction mapping of sc DNA

A cloned 2.9-kb fragment of *sc* DNA (J.Modolell, W.Bender, and M.Meselson, in preparation) was used to start 'walking' along the *AS-C* region. Initially, clones were selected from a cosmid library containing inserts of *D. melanogaster* strain Oregon-R DNA. One clone was recovered containing 14 kb of *AS-C* DNA and 21 kb of DNA hybridizing *in situ* elsewhere. A 6.4-kb *Bam*HI fragment of the *sc* DNA of this cosmid was then used to screen a λ Charon 4 library containing fragments of *D. melanogaster* Canton-S DNA flanked with *Eco*RI linkers (Maniatis *et al.*, 1978). Seventeen clones were recovered and the DNA of eight was digested with nuclease *Eco*RI and examined by agarose gel electrophoresis. Their inserts, congruent with each other, were aligned in an overlapping array. The phages with inserts extending the furthest to the right and to the left (λsc14 and λsc17) were analyzed with the nucleases *Eco*RI, *Sal*I, *Hind*III,

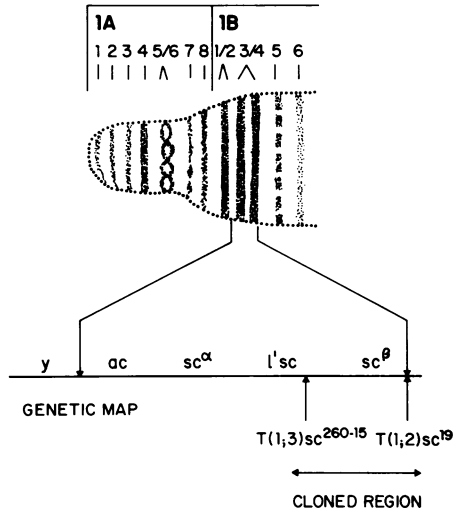

Fig. 1. Location of the cloned DNA with respect to the genetic map of *scute* and the cytological location of the *AS-C*. The leftmost and rightmost breakpoints of rearrangements that map within the cloned region are indicated. Genetic and cytological data have been taken from García-Bellido (1979).

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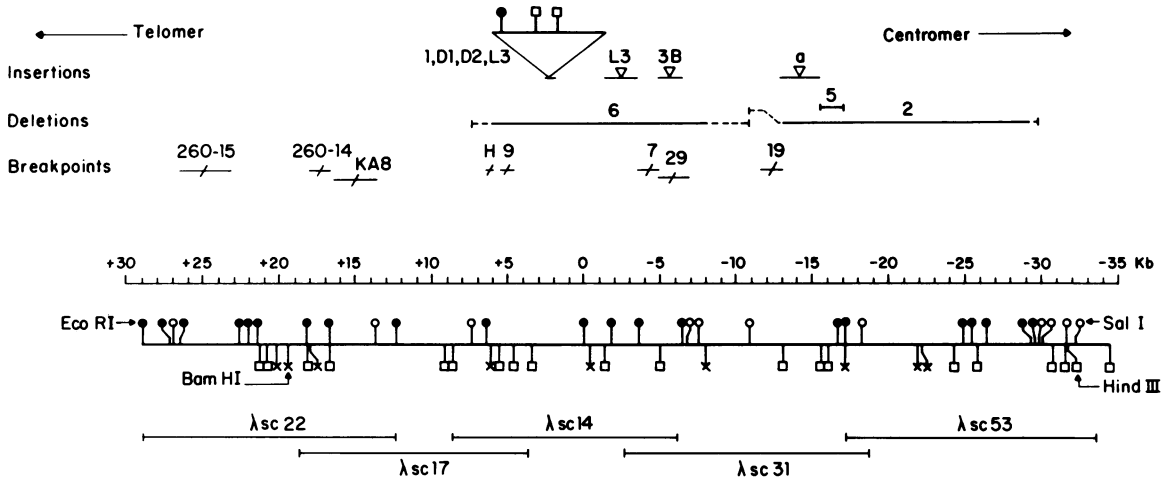

Fig. 2. DNA map of the *scute* locus (β region) of *D. melanogaster*, with indication of the DNA modifications found in several *scute* mutants. The inserts of the λsc clones used to construct the composite restriction site map and the region covered by each insert are shown at the bottom of the figure. A preliminary restriction map of each clone was worked out by examining, after electrophoresis in 1% agarose gels, the fragments obtained in digests prepared with the four nucleases alone and in all the possible combinations, and taking into consideration the known fragments derived from the vector arms (Dewet *et al.*, 1980). The map was refined by preparing Southern blots from the gels and hybridizing them with selected fragments of the same clone in order to check fragment overlaps. Remaining ambiguities were solved by either hybridizing the blots of one clone with the DNA of another that partially overlapped with it or by performing analyses with additional restriction endonucleases. No attempts were made to resolve fragments smaller than ~0.3 kb, excepting in the map of λsc53. In this case, small fragments were fractionated in 5% polyacrylamide gels and blots were prepared as described by Smith and Summers (1980). The origin of coordinates of the composite restriction map has been arbitrarily set at the indicated *Eco*RI site. The nature and locations of the DNA modifications found in mutant alleles are shown above the coordinate axis. Only the superscript of the name of each mutant is indicated. a Corresponds to an insertion found in all chromosomes carrying the w^a marker examined in this work. Horizontal lines in the symbols for insertions and breakpoints of chromosomal rearrangements denote the extent of the uncertainty in the location of the DNA break. Uncertainty in the location of the ends of deletions has been marked with dashes. Restriction sites on the DNA of the *gypsy* insertion were kindly provided by W.Bender.

and *Bam*HI. These two overlapping clones allowed the construction of a restriction site map covering 25 kb of the *scute* region. To extend further the cloned region, new overlapping clones were selected from the same library, using as probes appropriate fragments of the previously selected clones. Figure 2 shows the composite restriction site map derived from five clones with overlapping inserts and spanning 62 kb.

The composite map faithfully reflects the genomic sequences in part of the *AS-C*. This was checked by comparing Southern blot analyses of the restriction fragments of genomic DNA homologous to each of the inserts with those expected from the map. Even though the Southern analyses were carried out with DNA of the strain Oregon-R and the composite map was constructed using DNA of the strain Canton-S, a complete match was found using the four restriction nucleases either singly or in combinations. Thus, all the restriction sites shown in the map are present in both Oregon-R and Canton-S DNA. As a rule, we have not attempted to detect small fragments (<0.3 kb) flanked by identical restriction sites.

The Southern blot analyses of genomic DNA also indicate that most, if not all, the cloned DNA is unique DNA. No genomic fragments, other than those predicted by the composite map, hybridized with the cloned DNA. Moreover, *in situ* hybridizations of the cloned inserts with salivary gland chromosomes showed consistent labeling only of the *AS-C* region.

Mapping of scute breakpoints

Many *scute* mutations are associated with chromosomal inversions and translocations that have breakpoints within the *AS-C*. We have mapped the breakpoints on the DNA map of *scute* by determining which of the λsc clones hybridizes *in situ*, with polytenic chromosomes of the mutant, to both ends of the rearrangement. This indicates that the cloned insert is homologous to DNA at both sides of the breakpoint and, therefore, that the breakpoint should be mapped within the region covered by the insert. Figure 3 shows that this was the case for λsc2 DNA (a clone with an insert extending between coordinates +12.3 and −2.7, Figure 2). It hybridized to both ends of the $In(1)sc^9$ (chromosomal regions 1B2/3; 18B8/9). Similar experiments allowed us to map the *scute* breakpoints of the following rearrangements within the indicated clones: $In(1)sc^{260\text{-}14}$, $T(1;3)sc^{260\text{-}15}$ and $T(1;3)sc^{KA8}$ within λsc22; $T(1;4)sc^{H}$ within λsc17; $In(1)sc^{7}$ within λsc14; $T(1;2)sc^{19}$ and $In(1)sc^{29}$ within λsc31. $In(1)sc^{4}$, $In(1)sc^{8}$, $In(1)sc^{L8}$, $In(1)sc^{S1}$, $In(1LR)sc^{V1}$ and $In(1)sc^{V2}$ map beyond the left end of the cloned region. The same experiments allowed us to orient unambiguously the DNA map with respect to the cytological map of the salivary chromosomes (Figure 2).

Mapping was refined by comparing Southern blot analyses of mutant and wild-type DNA in the region covering the breakpoint. The data, summarized below, distinguish between those mutations associated with breakpoints in either *scute* β, *achaete*, or *scute* α regions. Unless otherwise indicated, the origin of mutations and cytological data have been taken from Lindsley and Grell (1968).

a) Scute β region

In(1) sc^7. (X-ray-induced in w^a; *In(1)1B4-6; 5D3-6*). A 2.9-kb *Eco*RI fragment (coordinates −3.6, −6.5) is absent (Figure 4) and the overlapping 3.5-kb *Hind*III fragment is also missing (not shown). Consequently, the breakpoint maps between the *Eco*RI site at −3.6 and the *Hind*III site at −5.0 (Figure 2).

In(1)sc^9. (X-ray-induced; *In(1)1B2-3; 18B8-9*). Additional *in situ* hybridizations performed with the 6.4-kb *Bam*HI DNA fragment contained in clone λsc14 (coordinates +6.0, −0.4) indicate that this fragment contains the location of the breakpoint (not shown). Southern blot analyses of the

sc^9 DNA reveal that the 1.0-kb *Hind*III fragment (+5.6, +4.6) is missing (Figure 4). The breakpoint was mapped within this fragment (Figure 2).

T(1;2)sc^{19}. (X-ray-induced; *T(1;2)1B1-2; 1B4-7; 25-26*). In the *Bam*HI plus *Sal*I digest of the DNA of this mutant either a 6.4-kb *Bam*HI/*Sal*I or a 6.3-kb *Sal*I/*Bam*HI fragment (coordinates −0.4, −6.8 and −10.8, −17.1) is missing and two new fragments are present (5.5 and 3.1 kb, Figure 4). The 6.3-kb *Sal*I/*Bam*HI fragment is modified, since the fragments 2.3-kb *Sal*I/*Hind*III (−10.8, −13.1) and 5.1-kb *Bam*HI/*Hind*III (−8.0, −13.1), which overlap with it, are enlarged ~0.3 kb (not shown). Therefore the sc^{19} breakpoint is situated between the *Sal*I (−10.8) and *Hind*III (−13.1) sites. Moreover, of the two new fragments that are generated in the *Bam*HI plus *Sal*I digest by the presence of the translocation (Figure 4), only the 5.5-kb fragment can span the *Bam*HI site at −17.1 and the *Hind*III site at −13.1. Hence, the breakpoint lies between a point 5.5 kb to the left of the *Bam*HI site and the *Hind*III site at −13.1 (Figure 2). The duplication element of *T(1;2)sc*^{19} can be mapped to position 25A of the salivary chromosomes using the probe λsc14.

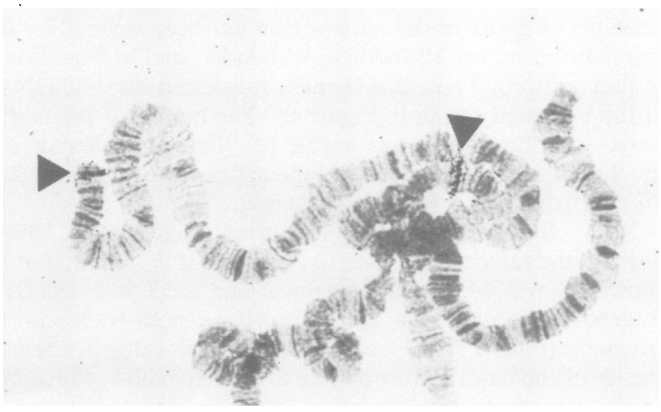

Fig. 3. Hybridization *in situ* of ^{125}I-labeled λsc2 DNA to chromosomes bearing the *In(1)sc*^{9}.

In(1)sc^{29}. (*In(1)1B; 13A2-5*). The pattern of the *Hind*III plus *Bam*HI digest of sc^{29} DNA hybridized with the λsc31 probe is complex (Figure 4). Two noncontiguous fragments disappear [3.0-kb *Hind*III/*Bam*HI (−5.0, −8.0) and 2.6-kb *Hind*III (−13.1, −15.7)] and two new fragments (1.9 and 0.85 kb) hybridize with the probe. The disappearance of the 2.6-kb *Hind*III fragment and the appearance of the 1.9- and 0.85-kb fragments were also found in the *Hind*III genomic blots of the mutants *In(1)sc*^{7}, *In(1)sc*^{9}, sc^6 and *In(1)ac*^{3} but the absence of the 3.0-kb *Hind*III/*Bam*HI fragment is specific to the sc^{29} DNA. Since the overlapping 0.7-kb *Sal*I and 3.3-kb *Sal*I fragments are not modified (not shown), the breakpoint lies between the *Hind*III site at −5.0 and the *Sal*I site at −6.8 (Figure 2).

The sc^7, sc^9, sc^6, ac^3, and sc^{29} X-chromosomes carry a *white apricot* (w^a) allele. Moreover, we have found the changes described above in the *Hind*III genomic blot of a gt^1, w^a strain (Figure 4). Thus, the modification seems peculiar to the X-chromosome carrying the w^a marker and probably has no bearing on the scute phenotype. The modification is not simply the appearance of a new *Hind*III site within the 2.6-kb *Hind*III fragment, since the overlapping *Sal*I and *Eco*RI fragments are also modified (not shown). We tentatively conclude that it is an insertion. Possibly, this insert is a relic of the w^a-chromosome on which the mutations were induced (see *Df(1)sc*$^{10-1}$).

In(1)sc$^{260-14}$. (X-ray-induced; *In(1)1B2-3; 11D3-8*). A 1.45-kb *Eco*RI fragment (coordinates +16.7, +18.1) is missing (Figure 4); the overlapping 1.4-kb *Hind*III fragment is also lost (not shown). The breakpoint should be within these fragments (Figure 2).

T(1;3)sc$^{260-15}$. (X-ray-induced; *T(1;3)1B4-5; 71CD*). A 5.5-kb *Sal*I/*Hind*III fragment (coordinates +21.4, +26.9) is absent (Figure 4). Moreover, the overlapping 3.7-kb *Eco*RI fragment is enlarged (not shown). Hence, the breakpoint is within this *Eco*RI fragment (Figure 2).

T(1;4)sc^H. (X-ray-induced; *T(1;4)1B4-C3; 101-102*). A 3.0-kb *Hind*III fragment (coordinates +5.6, +8.6) is missing (Figure 4). Of the three overlapping fragments, 6.5-kb

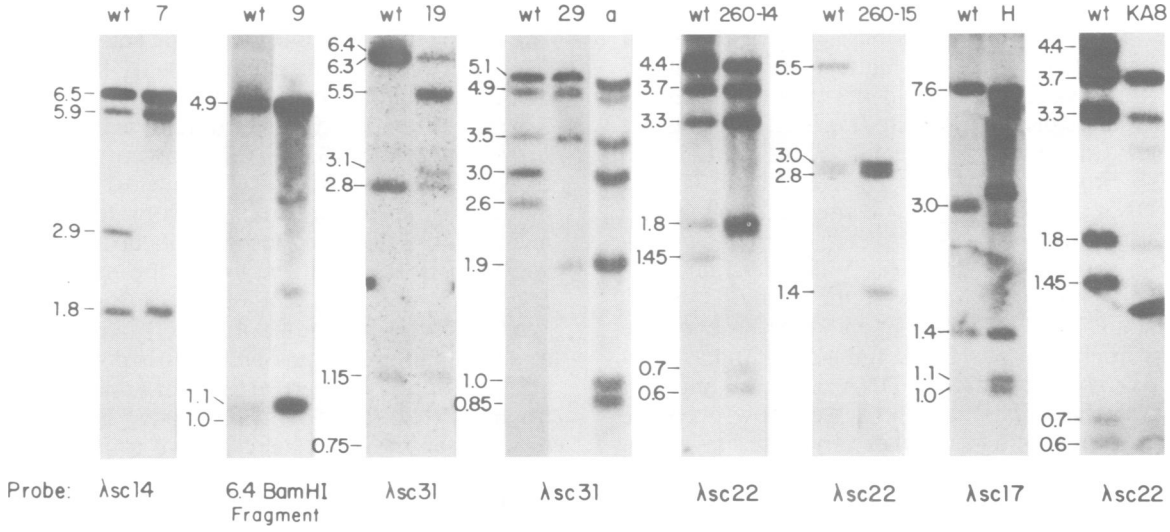

Fig. 4. Identification of the restriction fragments modified by the presence of a breakpoint in *scute* mutants displaying chromosomal rearrangements. DNA from wild-type Oregon-R embryos (wt) and mutant adult flies were digested with restriction nucleases and analyzed in a Southern blot. The nucleases used in each analysis are described in the text. Lanes corresponding to mutant DNA are identified by the superscript of the mutation. **Lane a** contains DNA from a gt^1 w^a stock. Sizes of selected fragments are indicated in kilobases. Bands corresponding to 0.75 kb (**lanes 5** and **6** from the left), 0.7 and 0.6 kb (**lanes 10** and **17**) and 1.1 and 1.0 kb fragments (**lane 14**) were clearly visible in the original autoradiographs but were lost in the photographic process.

*Eco*RI, 5.9-kb *Eco*RI, and 6.4-kb *Bam*HI, only the first is affected (not shown). Thus, the breakpoint lies between the *Bam*HI site at +6.0 and the *Eco*RI site at +6.4 (Figure 2).

T(1;3)sc^{KA8}. (X-ray-induced (G.Lefevre, unpublished); *T(1;3)1B4-5; 98±)*. A 4.4-kb *Eco*RI fragment (coordinates +12.3, +16.7) is absent (Figure 4). Moreover, the overlapping 3.0-kb *Hind*III/*Sal*I and 4.3-kb *Bam*HI/*Sal*I fragments are also missing (not shown). The breakpoint lies between the *Eco*RI site at +16.7 and the *Sal*I site at +13.7 (Figure 2).

T(1;2)sc^{S2}. (Unreported origin, *T(1;2)1B4-7; 60C-E*). Hybridizations *in situ* using as probes the DNA of either λsc22 or a clone overlapping with λsc22 and extending 9 kb to the left label both breakpoints of the translocation (not shown). However, genomic blots of *sc*^{S2} DNA with *Eco*RI, *Hind*III, *Sal*I, and *Bam*HI show no alterations in the region covered by these clones. Alterations have been detected in the region homologous to λsc14. Cloning of the altered regions will help to understand the nature of the modifications.

b) Achaete and scute α regions

In(1)ac^{3}. (X-ray-induced; *(In(1)1B2-3; 1B14-C1)*. The Southern blot analyses only detected the modification associated with the presence of the *w*^{a} marker (see above). The breakpoints are outside the cloned region.

Df(1)sc$^{10\text{-}1}$. (X-ray-induced in a *In(1)ac*^{3} chromosome; associated with a deletion for a chromosomal band in 1B1-4). Southern blots indicate that the deletion is outside the cloned region (not shown), probably to the left of it (García-Bellido, 1979). As with the parent inversion, the same blot analyses detect the insertion associated with the chromosomes bearing the *w*^{a} marker (see above). However, the *sc*$^{10\text{-}1}$ chromosome no longer carries the *w*^{a} allele, which was possibly removed some time ago by recombination.

DNA alterations in scute 'point mutations'

We have searched for DNA alterations in *scute* 'point mutations' by comparing the *Eco*RI and *Hind*III restriction patterns of wild-type and mutant genomic DNA, as determined by autoradiographs of Southern blots hybridized with the ^{32}P-labeled DNA of each of the five λsc clones shown in Figure 2.

sc^{1}, *sc*^{D2}. (Spontaneous mutations). The patterns of restriction fragments for these two mutants are identical and differ from wild-type DNA only in the region covered by λsc14: the 6.5-kb *Eco*RI and 4.9-kb *Hind*III fragments are absent and new fragments are present (8.8-kb *Eco*RI, 5.6-kb *Eco*RI, 6.2-kb *Hind*III, and 4.2-kb *Hind*III, Figure 5). In a clone of *sc*^{1} DNA the element *gypsy* is inserted precisely into the missing fragments (somewhere between coordinates +1.9 and +2.4, Figure 2) (J.Modolell, W.Bender, and M. Meselson, in preparation). Moreover, the sizes of the new fragments (Figure 5) are in agreement with the restriction site map of *gypsy* inserted into *sc* DNA in the orientation shown in Figure 2. We conclude that the DNA of the *sc*^{1} and *sc*^{D2} alleles (and also that of *sc*^{D1} and *sc*^{L3}, see below) have the *gypsy* element inserted into identical or very close sites and in the same orientation.

sc^{D1}. (Found by Dobzhansky in an X-ray experiment). The DNA shows fragments characteristic of the *gypsy* element inserted into the same position as in *sc*^{1} and *sc*^{D2} (5.6-kb *Eco*RI, 6.2-kb *Hind*III, and 4.2-kb *Hind*III, Figure 5). However, the expected 8.8-kb *Eco*RI fragment is absent and a new 3.2-kb *Hind*III fragment is seen (Figure 5). The

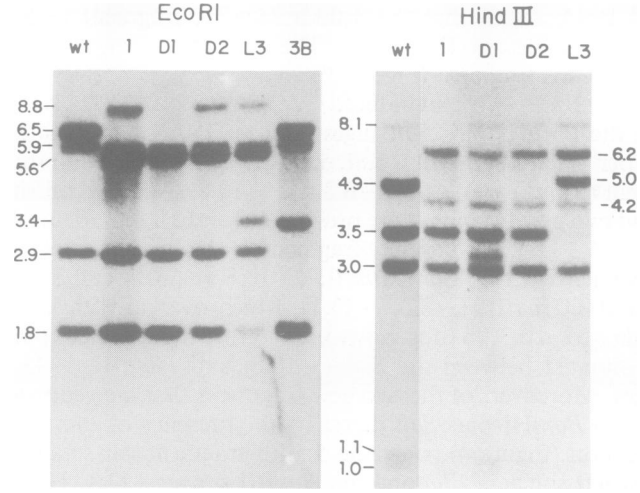

Fig. 5. Identification of the restriction fragments modified by the presence of insertions in the DNA of *scute* 'point mutants'. DNA from wild-type Oregon-R embryos (wt) and mutant adult flies were analyzed in Southern blots using the indicated restriction nucleases and the DNA of the λsc14 clone as probe. The nomenclature used is as in Figure 4.

presence of *gypsy* in the *scute* region has been verified by *in situ* hybridizations (J.Modolell, W.Bender, and M.Meselson, in preparation). Thus, this element is inserted into the DNA at the indicated position (Figure 2). The nature of the additional modification(s) responsible for the disappearance of the 8.8-kb *Eco*RI fragment and the appearance of the 3.2-kb *Hind*III fragment is being investigated.

Sc^{L3}. (Found by Levy; unreported origin). The DNA displays the same altered pattern as *sc*^{1} and *sc*^{D2}, except that it shows a new 3.4-kb *Eco*RI fragment and the 3.5-kb *Hind*III fragment (coordinates −1.5, −5.0) is replaced by a 5.0-kb fragment (Figure 5). Moreover, in the *Eco*RI digest, the intensity of the band corresponding to the two wild-type 1.8-kb *Eco*RI fragments suggests it is a single fragment (Figure 5), implying that one of the two 1.8-kb *Eco*RI fragments has been replaced by the 3.4-kb *Eco*RI fragment. Thus, in both digests one fragment is replaced by another 1.5−1.6 kb larger. Since the modified fragments overlap from coordinate −1.5 to −3.6, it is likely that, in addition to *gypsy*, *sc*^{L3} DNA has a second insertion of ~1.5 kb within this overlapping region (Figure 2).

sc^{3B}. (Found by Bridges; unreported origin). The 2.9-kb *Eco*RI fragment (−3.6, −6.5) is absent in *sc*^{3B} DNA and is replaced by a 3.4-kb *Eco*RI fragment (Figure 5), suggesting that a small insertion (~0.5 kb) is present within this fragment. Confirmatory evidence obtained with *Hind*III/*Sal*I and *Hind*III/*Bam*HI digest locates the insertion between sites *Hind*III at −5.0 and *Eco*RI at −6.5 (Figure 2).

sc^{6}. (X-ray-induced). All *Eco*RI fragments between sites at +12.3 and −16.6, and all *Hind*III fragments between sites at +8.6 and −15.7 are absent. In addition, very few new fragments hybridized with the cloned DNA covering these regions; the *sc*^{6} DNA therefore has a deletion. Confirming this, no DNA homologous to the cloned 6.4-kb *Bam*HI fragment (coordinates +6.0, −0.4) was detected in *sc*^{6} DNA by genomic blot analysis and *in situ* hybridization. To define more precisely the ends of the deletion, *Sal*I/*Hind*III digests were analyzed (Figure 6). These results indicate that the left end of the deletion lies at a point within 0.95 kb to the right of the *Sal*I site at +7.4 and the right end lies within the 3.3-kb

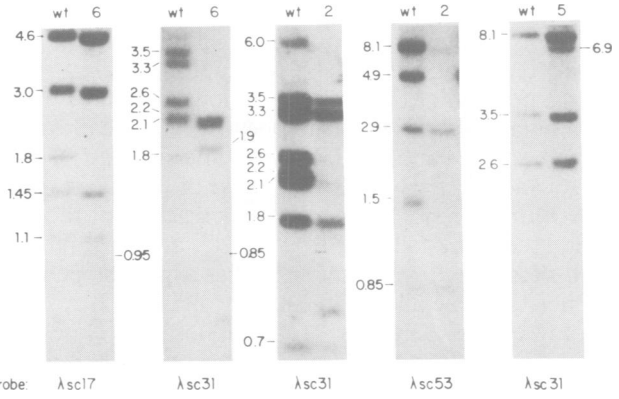

Fig. 6. Identification of the restriction fragments containing the left and right termini of deletions associated with mutations sc^6, sc^2, and sc^5. DNA from wild-type Oregon-R embryos (wt) and mutant adult flies were digested with nucleases *Sal*I plus *Hind*III **(lanes 1–6**, starting from the left) or *Hind*III **(lanes 7–10)** and were analyzed in Southern blots. The labeled probes used in each analysis are indicated. The nomenclature used is as in Figure 4.

*Sal*I fragment (−7.5, −10.8). Moreover, in a *Bam*HI/*Hind*III digest, the 5.1-kb *Bam*HI/*Hind*III fragment (−8.0, −13.1) was absent, so the right end is mapped between the *Bam*HI (−8.0) and *Sal*I (−10.8) sites (Figure 2). It is not clear whether foreign DNA is inserted into the deletion.

sc^2. (X-ray-induced). The DNA of this mutant has a deletion in the region covered by λsc31 and λsc53. The left end was mapped at a point between sites *Sal*I (−10.8) and *Hind*III (−13.1) (Figure 6, lanes 5 and 6). The right end is located within the 4.9-kb *Hind*III fragment (−25.8, −30.7) (Figure 6, lanes 7 and 8) and additional evidence obtained with *Sal*I and *Eco*RI/*Hind*III digests locates it between the *Eco*RI and *Sal*I sites at −29.6 and −30.0, respectively. As with the deletion of sc^6, we do not know whether or not that of sc^2 is an insertional deletion.

sc^5. (X-ray-induced). The preliminary analysis indicated that the wild-type 10.1-kb *Eco*RI fragment (coordinates −6.5, −16.6) was replaced by a slightly shorter fragment (not shown) and that either a new *Hind*III fragment of ~6.9 kb was present, or one of the two wild-type 8.1-kb *Hind*III fragments (coordinates −5.0, −13.1 and −16.1, −24.2) was shortened in the mutant by ~1.2 kb (Figure 6). Moreover, in a *Sal*I/*Bam*HI digest, one of two 6.3–6.4 kb fragments with homology to λsc31 (−0.4, −6.8 and −10.8, −17.1) was also replaced by a shorter fragment (5.1 kb, not shown). These results are explained assuming that a deletion of ~1.2 kb has removed most of the DNA between the sites *Hind*III at −15.7 and *Bam*HI at −17.1, including the restriction sites *Hind*III (−16.1) and *Eco*RI (−16.6) (Figure 2). Other analyses with *Hind*III/*Bam*HI and *Bam*HI/*Sal*I digests confirm this.

$sc^{3\text{-}1}$. (Spontaneous derivative of sc^3, which was X-ray-induced). No alteration was detected in the DNA of this mutant along the region studied.

Discussion

DNA anatomy of wild-type and mutant alleles

The results described here allow us to construct a restriction map of part of the *achaete-scute* gene complex of *D. melanogaster*. We have localized on this map the breakpoints of eight *sc* mutant alleles with chromosomal rearrangements and have characterized other DNA alterations (insertions and deletions) associated with several 'point mutations'. The fact that breakpoints, insertions and deletions are scattered over a region of >40 kb, and that breakpoints of other *sc* mutations lie beyond the left end of this region, confirm previous inferences, based on genetic and cytological evidence, that the *AS-C* extends over a large stretch of DNA.

All the cloned DNA (62 kb) seems to be unique DNA. Repetitive sequences have not been detected by either hybridizations *in situ* to polytenic chromosomes or Southern blot analyses of genomic DNA. However, we are investigating the possibility that small regions of homology between the cloned *sc* DNA and other genomic regions might have escaped detection. At the resolution of our restriction analysis, the cloned DNA of the *scute* region is conserved in the strains Canton-S, Oregon-R and in the non-altered regions of the mutants studied. In contrast, genomic blots of DNA of *D. simulans* and *D. mauritiana* show differences with *D. melanogaster* (unpublished results).

It is surprising that DNA alterations in many points along 62 kb of DNA cause, in *D. melanogaster*, the same scute phenotypic effects. This situation is not unique to the *AS-C* since it also occurs at the *bithorax* complex (discussed by Marx, 1981). Thus, in the genome of *Drosophila*, there are long stretches of DNA, which can be altered at widely separated sites but show similar mutant phenotypes.

The DNA alterations associated with the 'point mutants' deserve special consideration. The 'point mutations' sc^1, sc^{D1}, sc^{D2}, and sc^{L3} are, at the DNA level, very similar. All have the insertion of the *gypsy* transposon-like element in the same orientation and at the same (or very similar) position. In addition, sc^{L3} has a second, smaller (1.5 kb) insertion 5 kb to the left of *gypsy*, and sc^{D1} has another still undefined modification. The similarities in the perturbations of the DNA correlate with the fact that the four alleles have similar, albeit not identical, phenotypes and that sc^1, sc^{D1}, and sc^{D2} are suppressible by the suppressor of *Hairy wing* (Lindsley and Grell, 1968). The suppressibility of sc^{L3} has not been investigated. Evidently, the correlation between mutant phenotypes, presence of *gypsy* and suppressibility suggests that this insertion is the primary cause of the mutant phenotype. However, the possibility remains that there may be additional smaller modifications of the DNA that are undetectable at the present level of analysis and are responsible for the mutant phenotype. Obviously, the same uncertainty applies to the interpretation of phenotypes in mutations with large DNA alterations such as chromosomal rearrangements or deletions.

That consideration is specially pertinent in the case of the small (~0.5 kb) insertion found in sc^{3B}. This mutant originally resembled sc^1 (Lindsley and Grell, 1968), but in the stocks now available the mutation only affects microchaetes, typical of the *achaete* locus (García-Bellido, 1979). The sc^{3B} insertion is close to the breakpoints of sc^7 and sc^{29}, both showing clear scute phenotypes. Thus, it might be expected that if the insertion was responsible for the phenotype, sc^{3B} flies would be scute rather than achaete. In this connection it is of interest that in revertants of *bithorax* alleles that had the *gypsy* insertion, the terminal 0.5-kb repeat of *gypsy* is still found at the insertion site, but the flies show no mutant phenotype (W.Bender, personal communication). Therefore, it is tempting to suggest that the sc^{3B} insertion may be the remainder of a larger insertion that, when present, caused the original scute phenotype. The achaete phenotype could result

from the small fragment left behind or from a spontaneous secondary mutation. Secondary mutations are a rather common phenomenon in the *AS-C* (see Lindsley and Grell, 1968).

We have analyzed most of the *scute* point mutants available. The DNA of nine of them has been examined and eight show DNA alterations that modify the restriction map by at least several hundred pairs. Thus, at the *scute* locus, recoverable mutations with small DNA modifications, such as base substitutions or deletions and insertions of a few base pairs, seem to be rare or undetectable. The same occurs at the *bithorax* locus (discussed by Marx, 1981). However, at the *rosy* locus, which is probably smaller and less complicated and codes for the enzyme xanthine dehydrogenase, a large number of electrophoresis variants have been described (Chovnick *et al.*, 1977) and the majority of *rosy* null mutations, induced with ethyl methanesulfonate or X-ray irradiation, show no large DNA lesions (W.Bender and A.Chovnick, personal communication). It is conceivable that in complex loci, most small modifications are phenotypically undetectable unless they affect regions such as *cis*-regulatory or critical coding sequences. In contrast, large insertions or deletions may cause structural changes in the DNA, which impair transcription even if they do not alter directly the coding or regulatory sequences.

Comparison between the DNA map and the mutant phenotypes

By genetic manipulation of the *AS-C*, using chromosome rearrangements with achaete and scute phenotypes, internal duplications and deletions of the complex can be generated. The phenotypes of these duplications and deletions reveal four regions within the complex: *achaete, scute* α, *lethal of scute*, and *scute* β (García-Bellido, 1979). Whereas the rearrangements which define the *scute* α region have a second breakpoint in the heterochromatin (excepting $T(1;3)sc^{J4}$ and $Df(1)sc^{10\text{-}1}$), those which define the *scute* β region are euchromatic. All the *scute* mutations we studied that are associated with euchromatic rearrangements, except $Df(1)sc^{10\text{-}1}$, have breakpoints within the cloned region. In contrast, those associated with heterochromatic rearrangements have their breakpoints outside and to the left of this region. Thus, the distinction between *scute* α and *scute* β, proposed on genetic grounds has a correlate in the DNA map (Figures 1 and 2). Moreover, since the list of euchromatic rearrangements examined includes most of the available ones, it is likely that most of the β region is comprised within the cloned *sc* DNA. Its limits are at present undefined.

A closer comparison between the phenotypic map of the *AS-C* (García-Bellido, 1979) and the molecular map (Figure 2) reveals, however, an important difference: the linear order of breakpoints in *scute* β from left to right, according to decreasing scute phenotype, does not correspond with the linear order of breakpoints in the DNA. This is apparent comparing the position on the molecular map of each breakpoint with the strength of the associated scute phenotype, as indicated by the number of positions in the seriation map (García-Bellido, 1979) whose macrochaetes are affected (figures in parenthesis). Starting from the left side we find: $sc^{260\text{-}15}$ (16), $sc^{260\text{-}14}$ (11), sc^{KA8} (14), sc^{H} (10−11), sc^{9} (11−13), sc^{7} (10−15), sc^{29} (9), sc^{19} (2). Furthermore, the few breakpoints studied do not seem to be clustered within this *scute* region. This poses the question as to whether any breakpoint in the region will have a scute phenotype. The analysis of new *scute* mutations associated with breakpoints may help to establish a correlation, if any, between the site of the breakpoint and the strength of the phenotype.

Similarly, it is not yet possible to establish a correlation between the position of the DNA alterations associated with the 'point mutants' and their scute phenotypes. The phenotypes of sc^{1} (8), sc^{D2} (6) and sc^{L3} (5), which interrupt the DNA between sc^{7} and sc^{9}, are weaker than those of the two rearrangements. Moreover, sc^{6} (5), which has a deletion of the region covering the breakpoints of sc^{H}, sc^{9}, sc^{7}, and sc^{29} and the insertion sites of sc^{1}, sc^{D2}, and sc^{L3}, has a phenotype weaker than most of these alleles. In contrast, the sc^{19} (2) rearrangement and the sc^{2} (2) and sc^{5} (2) deletions map close to one another (Figure 2) and have similar phenotypes. It is possibly significant that those 'point mutations' associated with insertions have these alterations clustered around the 0 position in the DNA map; whereas DNA breakpoints in mutations associated with rearrangements are scattered throughout the DNA region studied. Hopefully, the study of the RNA transcripts coded in this region, in both wild-type and mutant flies, will help explain the relation between DNA alteration and mutant phenotype.

Materials and methods

Drosophila stocks

The stocks were from the collections of A.García-Bellido and Bowling Green *Drosophila* Stock Center. The sc^{1} stock was a gift from W.Bender. See Lindsley and Grell (1968) for the description of mutants and balancer chromosomes.

DNA from adult flies was obtained from the following stocks:
sc^{1} *cv* ct^{6} *v;* $su(Hw)^{70a}$ e^{s}*/TM2*
$sc^{D1}y$
$sc^{D2}y$
sc^{2} *pn/CDX*
$sc^{3\text{-}1}$ *w/CDX*
sc^{3B}
sc^{5}
sc^{6} w^{a}
$Df(1)sc^{10\text{-}1}/FM6/B^{S}Y$
sc^{L3} f^{36}*/CDX*
$In(1)ac^{3}$ w^{a}
gt^{1} w^{a}
When the X chromosome was balanced over *CDX* or *FM6*, only males or B^{+} flies were used, respectively.

Salivary glands for hybridizations *in situ* were obtained from the following stocks:
$In(1)sc^{4}$, *y*
$In(1)sc^{8}$
$In(1)sc^{L8}$, *car m* w^{a}*/*$In(1)dl^{49}$, *y w* lz^{s}
$In(1)sc^{S1L}$ sc^{8R}, *y*
$In(1)sc^{V1}$, *v/*$In(1)dl^{49}$ + $In(1)sc^{8}$, y^{31} *v f B*
$In(1)sc^{V2}$
Only male y^{+} larvae were used with the $In(1)sc^{V1}$ stock. Adult males $In(1)sc^{8}$ were crossed to *CDX* females and only male larvae from the progeny were used.

Both DNA from adult flies and salivary glands from larvae were prepared from the following stocks:
$In(1)sc^{7}$, w^{a}
$In(1)sc^{9}$, w^{a} *Bx*
$In(1)sc^{29}$, w^{a}*/CDX*
$In(1)sc^{260\text{-}14}$
$Df(1)sc^{19}$, f^{36}; $Dp(1;2)sc^{19}$, *b pr c/*$In(2)Cy$, *pr*
$T(1;2)sc^{S2}/In(2L + 2R)Cy$
$T(1;2)sc^{S2}$, *pwn/*$In(2LR)Gla$
$T(1;3)sc^{260\text{-}15}/FM7c$
$T(1;4)sc^{H}$
$T(1;3)sc^{KA8}/CDX$
With the $In(1)sc^{29}$ and $T(1;3)sc^{KA8}$ only male individuals were used. With the $T(1;3)sc^{260\text{-}15}$, chromosomes were prepared from the male larvae and the presence of the translocation was verified after squashing. To prepare DNA from this stock, non *FM7c* males were selected. With the $T(1;2)sc^{S2}$, crosses were performed with wild-type flies and the presence of the translocation was verified in salivary gland nuclei.

Library screening

The library prepared by J.Lauer and T.Maniatis (Maniatis *et al.*, 1978) containing *D. melanogaster* Canton-S DNA inserted in λ Charon 4 was used. 10^4 phages were grown at 37°C in each of 10 plates (9 cm diameter) containing L medium (Lennox, 1955) and 1% agar. Phage particles from the resulting plaques were absorbed on nitrocellulose filters (Millipore HAWP or HATF) and their DNA denatured and fixed on the filters as described by Benton and Davis (1977). Filters were prehybridized in 50 ml of 6 x SSC (1 x SSC = 0.15 M NaCl, 0.015 M sodium citrate), 1 x Denhardt reagent [0.2 g/l each of bovine serum albumin, polyvinyl pyrrolidone, and Ficoll 400 (Pharmacia)], and 0.1 mg/ml of denatured salmon sperm DNA for 2–5 h at 65°C. Hybridization was carried out in the same solution (20 ml), but containing, in addition, 10% dextran sulfate (average mol. wt. 500 000) and 10^6-10^7 c.p.m. of denatured, ^{32}P nick-translated probe ($10^7-2 \times 10^8$ c.p.m./μg). Incubation at 65°C overnight was followed by washing of the filters once in 6 x SSC, 1 x Denhardt reagent at 65°C for 0.5 h and in 2 x SSC, 0.1% SDS and 0.1 x SSC, 0.1% SDS as described by Wahl *et al.* (1979). Exposures were performed at −70°C for 1 h to overnight using a Dupont Lighting Plus intensifying screen. Regions of the plates giving positive signals were cored, the phages were extracted into 1 ml of phage suspension medium (SM, 85 mM NaCl, 1 mM $MgSO_4$, 20 mM Tris-HCl pH 7.4, 0.1% gelatin, saturated with $CHCl_3$), the solutions were titered and a second screening was carried out at low plaque density to identify individual positive plaques.

Phage DNA preparations

Phage were prepared by a method suggested to us by W.Bender (Harvard Medical School). Approximately 3 x 10^6 p.f.u. were grown in each of 2–4 fresh 15 cm plates containing NZ-amine medium (10 g/l NZ-amine, 5 g/l yeast extract, 5 g/l NaCl and 1% agar) for 6–8 h at 37°C until lysis of most of the cell colonies was visible. 12 ml of SM saturated with chloroform were added and the plates were gently rocked in the cold room overnight. The overlaying fluid was removed, clarified by low speed centrifugation and the phage sedimented by centrifugation for 1 h at 90 000 *g*. The pellets were broken up with the help of a Pasteur pipette into 3 ml SM and, after 4 h of gentle shaking at 4°C, were layered on top of a discontinuous CsCl gradient formed with 2 ml of 1.5 g/ml and 2 ml of 1.45 g/ml CsCl and were centrifuged for 1 h at 80 000 *g*. The phage band, visible in the interphase between the two layers of CsCl, was aspirated with a drawn-out Pasteur pipette and was diluted with an equal volume of H_2O. To disrupt the phage, 2 vol formamide were added to the diluted suspension and the DNA was precipitated with 2 vol ethanol. The DNA was recovered by centrifugation, dissolved in 0.2–0.4 ml TNE (10 mM Tris-HCl pH 7.7, 5 mM NaCl, 1 mM EDTA) and, sometimes, twice extracted with phenol-chloroform. Yields ranged from 5 to 50 μg DNA per plate.

Drosophila DNA preparations

D. melanogaster (Oregon-R) embryo DNA was prepared as described by Holmgren *et al.* (1979). DNA of *scute* mutants was extracted from ~100 adult etherized flies by disrupting them in a glass homogenizer containing 2 ml of 80 mM EDTA, 100 mM Tris-HCl pH 8.0, 160 mM sucrose, 0.5% SDS and 25 μl of diethylpyrocarbonate. The homogenizer was rinsed with 1 ml of the homogenizing solution and the pooled extracts were incubated at 65°C for 30 min. 0.45 ml of 8 M potassium acetate were added, the mixture was kept on ice for 10 min, centrifuged for 10 min at 13 000 *g*, the supernatant was separated, and the DNA was precipitated by addition of 1 vol ethanol. After 5 min at room temperature, the DNA was pelleted by centrifugation, washed with 70% ethanol, briefly desiccated under vacuum and dissolved in 0.25 ml TNE. The yields ranged from 20 to 35 μg DNA per 100 flies.

Southern blot analyses

1–2 μg of either cloned or genomic DNA was digested by restriction endonucleases following the instructions of the manufacturer. The digested DNA was fractionated in 1% agarose gels in TAE (40 mM Tris-base, 5 mM sodium acetate, 1 mM EDTA, adjusted to pH 8.8 with acetic acid) and 0.5 μg/ml ethidium bromide. Blotting the DNA fragments on the nitrocellulose filters was carried out as described by Southern (1975). Hybridizations were performed at 65°C as described under 'library screening' or at 42°C in the presence of 50% formamide, 3 x SSC, 1 x Denhardt reagent, 50 mM HEPES pH 7.4, 50 μg/ml denatured salmon sperm DNA, and ^{32}P nick-translated probe. In the second case the filters were washed once with 0.5 l of 3 x SSC, 1 x Denhardt reagent at room temperature for 30–60 min followed by several changes of 0.1 x SSC, 0.1% SDS at 30–45°C.

Hybridization in situ

Glands were dissected under 45% acetic acid and mounted on subbed slides (Bingham *et al.*, 1981). The chromosomes were heat treated for 30–60 min at 65°C, denatured for 80 s in 0.07 M NaOH, immediately washed twice in 70% ethanol and twice more in 95% ethanol (5–10 min in each wash) and air dried. The hybridization mixture contained 50% formamide, 0.42 M NaCl, 10 mM Pipes buffer pH 6.5, 8% sodium dextran sulfate, 200 μg/ml denatured salmon sperm DNA and 7–15 μg/ml of ^{3}H- or ^{125}I nick-translated probe (Rigby *et al.*, 1977). Hybridization at 37°C overnight, was followed by washing of the slides with 2 x SSC for several hours at room temperature, dehydration in ethanol, and autoradiography for 2–15 days.

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References

Benton,W.D., and Davis,R.W. (1977) *Science*, **196**, 180-182.
Bingham,P.M., Levis,R., and Rubin,G.M. (1981) *Cell*, **25**, 693-704.
Chovnick,A., Gelbart,W., and McCarron,M. (1977) *Cell*, **11**, 1-10.
Dewet,J.R., Daniels,D.L., Schroeder,J.L., Williams,B.G., Deniston-Thompson,K., Moore,D.D., and Blattner,F.R. (1980) *J. Virol.*, **33**, 401-410.
García-Bellido,A. (1979) *Genetics*, **91**, 491-520.
García-Bellido,A. (1981) in Lloyd,C.D., and Rees,D.A. (eds.), *Cellular Controls in Differentiation*, Academic Press, NY, pp. 281-301.
Holmgren,R., Livak,K., Morimoto,R., Freund,R., and Meselson,M. (1979) *Cell*, **18**, 1359-1370.
Lennox,E.S. (1955) *Virology*, **1**, 190-206.
Lindsley,D.L., and Grell,E.H. (1968) *Genetic Variations of Drosophila melanogaster*, 627, published by Carnegie Institution, Washington.
Maniatis,T., Hardison,R.C., Lacy,E., Lauer,J., O'Connell,C., Quon,D., Sim,G.K., and Efstratiadis,A. (1978) *Cell*, **15**, 687-701.
Marx,J.L. (1981) *Science (Wash.)*, **213**, 1485-1488.
Muller,H.J. (1955) *Brookhaven Symp.*, **8**, 126-147.
Rigby,P.W.J., Dieckmann,M., Rhodes,C., and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237-251.
Smith,G.E., and Summers,M.D. (1980) *Anal. Biochem.*, **109**, 123-129.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.
Wahl,G., Stern,M., and Stark,G.R. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3683-3687.