The Molecular Analyses of an Antimorphic Mutation of *Drosophila melanogaster*, *Scutoid*

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ABSTRACT

A dominant mutation of *Drosophila melanogaster*, *Scutoid* (*Sco*), acts as an antimorphic allele of the *no-ocelli* (*noc*) gene. In *Sco* the *noc* region has been transposed from 35B to 35D on chromosome arm 2L and the *noc* gene is now adjacent to *snail* (*sna*). Induced revertants of *Sco* are frequently mutant for *sna* or are aberrations broken very close to *sna*. A molecular analysis of the *Sco* chromosome has confirmed that *noc* is transposed and fused to the *sna* region. However, only part of the *noc* region is included within the transposition. The breakpoints of 19 chromosomally aberrant *Sco* revertants have been mapped at the molecular level. Fourteen of these breakpoints map to the *noc* region, spread over about 80 kb of DNA. The breakpoints of the remaining five are not within the DNA of the *noc* region and appear to map within sequences from the *sna* region. This has been shown directly for three of these, those associated with *T(2;3)Sco^{R+13}*, *In(2L)Sco^{R+24}* and *In(2L)Sco^{R+26}*. Thus mutation of either *noc* or *sna*, genes which are apparently unrelated in their wild-type functions, can revert the antimorphic phenotype of *Sco*.

MANY dominant mutations of *Drosophila melanogaster* result from a gain of genetic function. By genetic criteria, three different classes of such dominant mutations can be distinguished: hypermorphs, whose phenotypes result from an excess of otherwise wild-type gene products, antimorphs, whose phenotypes result from the action of the mutant gene products opposing those of their wild-type alleles, and neomorphs, whose phenotypes result from quite novel gene actions (MULLER 1932). All three classes of mutation can be reverted by deletion of the mutant allele. The phenotypes of hypermorphic alleles can be mimicked by the duplication of the wild-type allele, and are enhanced if duplications of the wild-type are added to the mutant genotype, an example is *Beadex* (GREEN 1953). Neomorphic and antimorphic alleles can be distinguished by the response of the phenotype to duplications of the wild-type allele: duplications have no effect on the phenotype of neomorphs, since their gene function is novel; by contrast duplications suppress the phenotype of antimorphs since the balance of gene products is then weighted toward the normal. Conversely, when heterozygous with deletions of the wild-type allele, the phenotype of antimorphs is more

severe than otherwise while that of neomorphs is unaffected (assuming viability). Examples of neomorphs are the dominant alleles of *Antennapedia* and *Bithorax* (e.g., *Cbx*), whose phenotypes apparently result from the expression of an essentially wild-type function in tissues which would not normally express these genes (WHITE and AKAM 1985; SCHNEUWLY, KLEMENZ and GEHRING 1987).

Very few dominant mutations of *D. melanogaster* have been rigorously shown to be antimorphs, though this is suspected to be the case for many of those that are unique (or very rare) mutational events. Previous genetic analysis has shown that *Scutoid* (*Sco*, 2–51.0), a unique mutation that maps close to *Adh* on chromosome arm 2L, is probably an antimorphic allele of the *no-ocelli* (*noc*) gene (ASHBURNER *et al.* 1983). The phenotype of *Sco* is enhanced by deletion, or mutation, of the *noc* gene and suppressed by *noc*⁺ duplications, indeed the phenotypic severity of *Sco* is apparently a function of the ratio of wild-type *noc* to mutant *noc* genes. The phenotype of *Sco* may be a consequence of a novel, modified, *noc* gene product that competes and interferes with the action of the *noc*⁺ gene product during development.

This interpretation of *Sco* was supported by the genetic structure of the *Sco* mutation. As summarized in Figure 1, *Sco* is a reciprocal interchange of two small genetic regions: the contiguous loci *noc*, *osp* and *Adh* exchanging places with *rd*, *l(2)35Cc*, *l(2)35Cd*, *l(2)35Ce*, *dgl* and *l(2)35Da* (the inclusion of *l(2)35c*, *l(2)35Ce* and *dgl* (= *double-glazed*) in this transposition will be documented elsewhere (M. ASHBURNER, P.

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FIGURE 1.—The genetic structure of the *Sco* chromosome, showing the reciprocal transpositions of the *noc-Adh* and *rd-l(2)35Da* regions. The genetic limits of the deletions associated with revertants of *Sco*, of the duplication associated with *Sco*^{R+23} and the genetic positions of the breakpoints associated with *Sco*^{R+9}, *Sco*^{R+11}, *Sco*^{R+13} and *Sco*^{R+17} are shown below the *Sco* map. The limits of the synthetic deletions used to map the genetic breakpoints of the translocation and inversions are shown above the wild-type map. The cloned region (see Figure 2) includes the *noc*, *osp* and *Adh* loci. Data from ASHBURNER *et al.* (1983) and ASHBURNER and HARRINGTON (1984).

THOMSON, Y. GRAU, M. EL MESSAL and P. SIMPSON, unpublished data). Two observations lead to the conclusion that the important part of this structure, for the phenotype of *Sco*, is the novel juxtaposition of *noc* and *sna*: first, a recombinant chromosome made by MARONI (1980) that lacks the distal transposition (*i.e.*, of *rd-l(2)35Da* to between *l(2)35Ba* and *l(2)35Bb*) remains Scutoid in phenotype; second, many induced revertants of *Sco* appear to be associated with genetic lesions at the transposed *noc* gene, at *sna* or are deleted for the *noc-sna* region (ASHBURNER, TSUBOTA and WOODRUFF 1982; ASHBURNER *et al.* 1983; ASHBURNER and HARRINGTON 1984).

The resolution of classical genetic analysis is insufficient to test this model. Since the entire *noc* to *Adh* region of chromosome arm 2L has been cloned (CHIA *et al.* 1985b), this has allowed us to characterize the structure of the *Sco* mutation and many of its revertants at the molecular level. The lesions associated with *Sco* and its revertants have revealed additional complexity in the organization of the *noc* gene (CHIA *et al.* 1985b).

These structural studies are a necessary preliminary to the functional analysis of *noc* and its antimorphic allele, *Sco*, and to an understanding of the molecular basis of antimorphy.

MATERIALS AND METHODS

Stocks: The *Sco* chromosome and its revertants have been described in previous papers (ASHBURNER, TSUBOTA and WOODRUFF 1982; ASHBURNER *et al.* 1983; ASHBURNER and HARRINGTON 1984).

TABLE 1

Correspondence between the previously used and new names of lethal loci in the *Adh* region

New	Old	New	Old
<i>l(2)35Ba</i>	<i>l(2)br22</i>	<i>l(2)35Ce</i>	<i>l(2)br43</i>
<i>l(2)35Bb</i>	<i>l(2)br3</i>	<i>l(2)35Da</i>	<i>l(2)br35</i>
<i>l(2)35Bc</i>	<i>l(2)br4</i>	<i>l(2)35Db = sna</i>	<i>l(2)br27</i>
<i>l(2)35Bd</i>	<i>l(2)br9</i>	<i>l(2)35Dc = lac</i>	<i>l(2)br36</i>
<i>l(2)35Be</i>	<i>l(2)br10</i>	<i>l(2)35Dd</i>	<i>l(2)br46</i>
<i>l(2)35Bf</i>	<i>l(2)br2</i>	<i>l(2)35De</i>	<i>l(2)br37</i>
<i>l(2)35Bg</i>	<i>l(2)br26</i>	<i>l(2)35Df</i>	<i>l(2)br44</i>
<i>l(2)35Bh = Su(H)</i>	<i>l(2)br7</i>	<i>l(2)35Dg</i>	<i>l(2)br45</i>
<i>l(2)35Ca = ck</i>	<i>l(2)br27</i>	<i>l(2)35Ea</i>	<i>l(2)br47</i>
<i>l(2)35Cb</i>	<i>l(2)br33</i>	<i>l(2)35Eb</i>	<i>l(2)br48</i>
<i>l(2)35Cc</i>	<i>l(2)br50</i>	<i>l(2)35Ec</i>	<i>l(2)br49</i>
<i>l(2)35Cd</i>	<i>l(2)br34</i>		

Nomenclature: We have used the new conventions of D. L. LINDSLEY and G. ZIMM (in preparation) for the naming of recessive lethal loci. The correspondence between these names and those previously used is given in Table 1.

Clones: The lambda clones of the *noc* to *Adh* region used in this study were those described and characterized by CHIA *et al.* (1985b). All of those with *lob* or *lw* prefixes were from the LAUER-MANIATIS Canton-S genome library (MANIATIS *et al.* 1978); two subclones of *lob9.04*, the distal 7-kb *EcoRI* fragment and the proximal 8-kb *EcoRI* fragment were constructed in *pBR322* (*pob9.04D* and *pob9.04P*, respectively); the clones *plssRI-D* and *plssRI-P* are in *pUC9* and were subcloned from a *λEMBL4* library made from a wild-type stock from G. ISING (CHIA *et al.* 1985a). The extents of these phage are shown in Figure 2. A more detailed map of the region between coordinates -95 and -125 is given in Figure 3. The coordinates of this map assume an origin at the *EcoRI* site 5' to *Adh* and are negative in the direction of the telomere. Coordinates distal to -96.3 differ slightly from those used by CHIA *et al.* (1985b) since the genomic *EcoRI* fragment that hybridizes to *pob9.04P* is 11.5 kb in size, rather than 10.5 kb as previously thought. For ease of reference the coordinates of all clones used in this study are listed in Table 2.

Cytology: *In situ* hybridization of radioactive DNA probes to the polytene chromosomes was performed as described by GUBB *et al.* (1984).

Molecular techniques: The procedures used to extract and purify *Drosophila* DNA, for restriction enzyme digestion, electrophoresis and for the growth of bacteriophage and the purification of phage fragments are those described in CHIA *et al.* (1985b). Two different methods for Southern blotting were used: transfer of DNA to nitrocellulose filters (see CHIA *et al.* 1985b) and transfer to aminothiophenol (ATP) paper, as described by SEED (1982).

RESULTS

Experimental approach and rationale: The genetic model is that *Sco* is associated with a reciprocal transposition of the *noc-Adh* and *rd-l(2)35Da* regions, and that the antimorphic phenotype of *Sco* is a consequence of a chimeric *noc-sna* gene. This not only has obvious predictions with respect to the molecular structure of the *noc* gene in *Sco*, but also

FIGURE 2.—A molecular map of the wild-type *noc-Adh* region showing the limits of the deletions and breakpoints of aberrations used in this study, a summary of the breakpoints of *Sco* and its revertants, the molecular extents of the λ phage that comprise the chromosome walk and the limits of the *noc*, *osp* and *Adh* loci. The limits of the three components of the *noc* complex, *nocA*, *nocB* and *nocC* are taken from MCGILL (1985). Only *EcoRI* restriction enzyme sites are shown on this map; that marked with an asterisk is polymorphic. The 3.9-kb insertion in the λ ob4.01 region of the *Sco* chromosome is indicated by a triangle. DNA included within deletions is indicated by a solid line; uncertainties of deletion endpoints, and the limits of the breakpoints of other aberrations, are shown as dashed lines. Within this region the limits of the deletions associated with *Sco*^{R+7}, *Sco*^{R+10}, *Sco*^{R+14} and *Sco*^{R+18} are the same as that of *Sco*^{R+4}.

predicts that revertants of *Sco* will map to the *noc* or *sna* loci, or to both.

These predictions have been tested by three series of experiments. In the first we have confirmed, at a molecular level, that the *noc* region is indeed transposed in the *Sco* chromosome; in the second we have mapped the molecular limits of this transposition with respect to the *noc* gene itself; in the third we have mapped to the DNA of the *noc* gene the lesions associated with 14 different *Sco* revertants.

Molecular demonstration that *Sco* is a transposition: The obvious approach to determine whether or not the *noc* gene is transposed in *Sco* is by *in situ* hybridization to the *Sco* chromosome with *noc* region probes. The cytological appearance of the *Sco* chromosome (e.g. see Figure 3 of ASHBURNER and HARRINGTON 1984) makes this experiment difficult. For this reason the chromosomes of the *b el Sco* recombinant of MARONI (1980) were studied. This chromosome resulted from an exchange between *Sco* and a wild-type sequence homolog in the *l*(2)35Bb to *l*(2)35Cb interval. This recombinant is deleted for the genes that in *Sco* have been transposed distally (i.e., *rd-l*(2)35Da) and duplicated for those that have been transposed proximally (i.e., *noc*, *osp* and *Adh*). (The terms distal and proximal can be ambiguous when

used to describe regions that are inverted with respect to the wild type. In this paper these terms are used with respect to the wild-type gene order, unless explicitly stated to the contrary.) MARONI (1980) demonstrated that *Adh* is duplicated in *b el Sco*. That *noc* is also duplicated in this chromosome is inferred from the observation that the phenotype *b el Sco*/+ is less extreme than of *Sco*/+ (ASHBURNER, TSUBOTA and WOODRUFF 1982). Figure 4 shows the polytene chromosomes of *b el Sco*/Cy *Roi* hybridized with λ ob6, a recombinant phage that includes DNA covering the breakpoint of *In*(2LR)*noc7* (CHIA *et al.* 1985b). As predicted, there are two sites of hybridization on the *b el Sco* chromosome, at 35B and 35D, but only one on the Cy *Roi* homolog, at 35B. When the homologs are synapsed the number of grains at 35B is, as expected, higher than that at 35D. From genetic data it was proposed that the transposed *noc*, *osp* and *Adh* genes are inverted at their new location in *Sco* (ASHBURNER, TSUBOTA and WOODRUFF 1982). To confirm this, *in situ* hybridizations to the *In*(2LR)*Sco*^{R+1} chromosome were performed with λ ob5 and λ ob6 (Figure 5). The position of the *In*(2LR)*Sco*^{R+1} breakpoint had previously been mapped to coordinate -67 to -68, included within λ ob5 (CHIA *et al.* 1985b). If *noc* is inverted in *Sco* then the DNA included within λ ob6,

FIGURE 3.—A more detailed map of the region between coordinates -95 and -125 kb showing the various clones and subclones used in this study. The polymorphic *Bst*I site is marked with an asterisk. The *Eco*RI sites at the end of the phage that are marked with asterisks are synthetic.

FIGURE 4.—The polytene chromosomes of the *b el Sco* recombinant (heterozygous with *In(2L)Cy^Lt^R*, *Cy Roi*) hybridized *in situ* with *lob6*. In (a) the homologous chromosomes are synapsed, showing two sets of grains, at 35B and 35D. In (b) the homologs are asynapsed, showing only one site of hybridization on the *Cy Roi* homolog at 35B, but two on the *b el Sco* homolog. Note that in (a) the strength of hybridization to 35D is less than to 35B. The breakpoint of the *Cy* inversion is indicated (Cy). Hybridization to the *Sco*, or *Sco*-derivative homolog is indicated by the open arrow in this and all subsequent figures. The tip of chromosome arm 21 is indicated.

which, in the wild type, is distal to *lob5*, should be associated only with the right-hand break of *In(2LR)Sco^{R+1}*. In fact, this is so (Figure 5). Similar data were obtained with the paracentric inversion *In(2L)Sco^{R+17}* (see below).

Molecular limits of the *noc-Adh* transposition of *Sco*: Molecular analysis of the *Sco* chromosome has been complicated by the absence of a progenitor chromosome. However, over the relevant region of the DNA the *Eco*RI restriction map of *Sco* and the Cambridge Canton-S strain are very similar: the only

FIGURE 5.—The polytene chromosomes of *In(2LR)Sco^{R+1}/CyO* hybridized *in situ* with *lob5* (a) and *lob6* (b). The DNA included within *lob5* crosses the breakpoint of this inversion, showing two sites of hybridization (L and R). DNA included within *lob6* is entirely distal to the inversion, hybridizing only at the right-hand breakpoint. See Figure 6e for interpretation.

TABLE 2

Molecular coordinates of the λ and plasmid clones used in this analysis

Clone	Distal	Proximal
$\lambda w4.04$	+26.5	+41.1
$\lambda w3.13$	+14.0	+29.0
$\lambda w2.01$	+5.5	+18.0
$\lambda gAC2$	-3.7	+9.9
$\lambda gAC3$	-13.1	+3.3
$\lambda ob1.12$	-24.6	-11.2
$\lambda ob2.01$	-31.6	-20.5
$\lambda ob3.04$	-46.0	-31.2
$\lambda ob4.01$	-57.4	-43.8
$\lambda ob5$	-70.5	-56.2
$\lambda ob6$	-79.0	-67.3
$\lambda ob7.06$	-89.8	-75.1
$\lambda ob8.10$	-103.3	-87.8
$\lambda ob9.04$	-114.8	-99.8
<i>pob9.04P</i>	-107.8	-99.8
<i>pob9.04D</i>	-114.8	-107.8
<i>pIssRI-P</i>	-107.8	-96.3
<i>pIssRI-D</i>	-119.8	-107.8
0.7-kb <i>Sal</i> I- <i>Eco</i> RI	-108.5	-107.8
0.7-kb <i>Hind</i> III- <i>Sal</i> I	-109.2	-108.5
2.7-kb <i>Sal</i> I- <i>Hind</i> III	-111.9	-109.2
$\lambda ob10.02$	-124.0	-111.8

significant difference seen, when *Eco*RI-digested DNA from *Sco* is probed with DNA from the walk, is with the proximal 8-kb *Eco*RI fragment of *lob9.04*. Then, instead of the expected 11.5-kb genomic *Eco*RI fragment, two fragments are seen, one of which co-

FIGURE 6.—The polytene chromosomes of *In(2LR)Sco^{R+1}/CyO* *in situ* hybridized with *lob10.02* (a), *pob9.04P* (b), the 2.7-kb *SalI-HindIII* subclone (c) and the 0.7-kb *SalI-EcoRI* fragment (d). With *lob10.02* there is only one site of hybridization at 35B (and not at the right break of the inversion (open arrow)) with *p9.04P* there are two sites, one on 2L (open arrow L) distal to the inversion breakpoint (bp) and one at the right end of the inversion (open arrow R). With the 2.7-kb *SalI-HindIII* clone the hybridization pattern is similar to that with *lob10.02*, with one site of hybridization at 35B (open arrow) but with the more proximal 0.7-kb *SalI-EcoRI* clone there are two sites. One of these, the major site (open arrow L), is at the left end of the inversion, the other, the minor site (open arrow R), at the right end, showing that this inversion is broken within the DNA included within this clone. (e) is an interpretation of these data and that shown in Figure 5. The junctions of *In(2LR)Sco^{R+1}* are shown enlarged with the cloned DNA indicated by heavy lines (with coordinates). The sites of hybridization of the probes are indicated by a "+" sign. A "-" sign indicates no hybridization to a site. The *noc-Adh* transposition of *Sco* is indicated as *Tp1* and the *rd-l(2)35Da* transposition as *Tp2*.

migrates with the 11.5-kb fragment of Canton-S and the other which is 5.5 kb in size. (Differences between the restriction maps of the Cambridge Canton-S strain and the Canton-S strain used for the LAURMANIATIS genome library, due to polymorphic sites, are indicated in the legend to Figure 2.)

One group of *Sco* revertants (group 3a of ASHBURNER *et al.* 1983) are genetically deleted for *noc*, *osp* and *Adh* (and for genes proximal to the transposition in *Sco*, e.g., *sna* and *lac*) but not for those genes (*rd-l(2)35Da*) transposed distally in *Sco*. At the molecular level these revertants (*i.e.*, *T(2;3)Sco^{R+7}*, *Sco^{R+10}*, *Sco^{R+14}* and *Sco^{R+18}*) lack all of the DNA between *λw4.04* and *λob8.10* from the *noc-Adh* region and, presumably, sequences from the *sna* region too (data not shown). When DNA from these genotypes is probed with *pob9.04P* they show the 5.5-kb *EcoRI* fragment of *Sco*, but not the 11.5-kb *EcoRI* fragment (data not shown, but see Figure 8, below). This means that the 5.5-kb fragment must represent the fusion of distal *noc* sequences to the transposed *rd-l(2)35Da* region, since the genetic data show that any sequences contained within the *noc-Adh* transposition must be deleted from these revertants.

The distal limit of the *noc-Adh* transposition (which, of course, is proximal on the *Sco* chromosome since this region is then inverted) was mapped with *In(2LR)Sco^{R+1}*. As mentioned above, the 2L breakpoint of this inversion is within *lob5*. In the inverted chromosome those sequences between this break and the distal limit of the *noc-Adh* transposition are juxtaposed to 44C3-5 on 2R. With *lob10.02* as an *in situ* hybridization probe to this inversion all of the grains are at 35B, distal to the inversion breakpoint. All of the DNA included in this phage must, therefore, be distal to the distal limit of the *noc-Adh* transposition (Figure 6a). With *pob9.04P* as a probe three sites of hybridization are seen: one on the *CyO* homolog, and two on the inversion, one on 2L at 35B and one at the right-hand 35D/44C junction (Figure 6b). Two subclones from *pIssRI-D*, the 2.7-kb *SalI-HindIII* subclone and the 0.7-kb *SalI-EcoRI* subclone, were then used *in situ* with *In(2LR)Sco^{R+1}*. The results (Figure 6c and d) show that the more distal of these subclones (*SalI-HindIII*) hybridizes only to 2L at 35B, distal to the inversion break. The *SalI-EcoRI* subclone, however, hybridizes both to this site and to the 35D/44C breakpoint on 2R. This shows that the *noc-Adh* transposition must include sequences from this fragment. Given the relative strengths of the *in situ* hybridization signals the limit of this transposition must be close to the *EcoRI* site at -107.8. This result leads to the conclusion that the original *noc-Adh* transposition is not simple, since the junctions between *noc* and the *rd-l(2)35Da* transposition distally and between *noc* and *sna* proximally do

FIGURE 7.—*SalI*, *EcoRI* and *BstI* digested DNA of *Sco* and *b el Sco* probed with *pob9.04P* (a) and the 0.7-kb *HindIII-SalI* (b) and *SalI-EcoRI* (c) fragments. DNA from *b el rd^s pr cn* and Canton-S are included as controls. Note that the *BstI* site at -118.1 is polymorphic.

not correspond. *Sco* is associated with an approximately 5-kb duplication of *noc* sequences. The interpretation of the *in situ* analysis of *In(2LR)Soc^{R+1}* is shown in Figure 6e.

These conclusions were confirmed, and made more precise, by a Southern blot hybridization analysis of the *Sco* chromosome. The three probes used in the *in situ* analysis (*i.e.*, *pob9.04P* and the *SalI-EcoRI* and *HindIII-SalI* subclones of *pIssRI-D*) were used to probe DNA from *Sco/Df(2L)fn3* and *b el Sco/Df(2L)fn3*. *Df(2L)fn3* removes all the sequences included in this walk. DNA from the Canton-S and *b el rd^s pr cn* strains were used as controls. The latter chromosome is that which recombined with *Sco* to give *b el Sco*. Since the *b el Sco* chromosome has lost the *rd-l(2)35Da* transposition by exchange, it should only show fusion fragments from the *noc/sna* junction. The *Sco* chromosome should show both these and the *noc/rd* fusion fragment.

With *EcoRI* digested DNA probed with *pob9.04P* all four genotypes show 11.5-kb fragments and only *Sco/Df(2L)fn3* shows the 5.5-kb fragment, which must, therefore, come from the fusion of distal *noc* sequences to the transposed *rd-l(2)35Da* region. The restriction map of the wild-type predicts two fragments, of 5.2 and 5.5 kb, were *SalI* digested DNA to be probed with the same clone. These do not resolve in our gels. Both *Sco* and *b el Sco* have an extra fragment, of approximately 12 kb and *Sco*, only, has an extra 1.9-kb fragment (Figure 7). We interpret the 12-kb fragment as being from the *noc/sna* fusion and the 1.9-kb as being from the fusion unique to *Sco*, *i.e.*, the *noc/rd* fusion. Finally, *BstI* digested DNA was probed. The 7.8-kb fragment predicted from the wild-type map is observed in all four genotypes. Both *Sco* and *b el Sco* also show a 5.4-kb fragment and *Sco*,

only, a 4.3-kb fragment. In addition, there is a large fragment whose size varies between the stocks. This is almost certainly due to polymorphism for the *BstI* site at -118.1 , a site which is present in the Ising wild type and *Sco* but absent from Canton-S, *b el rd^s pr cn* and *b el Sco*.

This same filter was reprobed with the two small subclones from *pIssRI-D*: with the 0.7-kb *SalI-EcoRI* probe all four genotypes show the 12-kb fragment in *EcoRI*-digested DNA. *Sco* and *b el Sco* both show a weakly hybridizing band of 3.8 kb, this is the *noc-sna* fusion fragment. The weak homology of the probe to this fragment suggests that the breakpoint in *noc* is close to the *EcoRI* site at -107.8 . In *SalI*-digested DNA all genotypes show the expected 5.5-kb fragment. *Sco* and *b el Sco* show the 12-kb *noc/sna* fusion fragment. The 1.9-kb fragment seen when *SalI*-digested *Sco* DNA was probed with *pob9.04P* was not seen with the *SalI-EcoRI* probe. With *BstI*-digested DNA the large fragment seen with the *pob9.04P* probe in all genotypes is clear. Both *Sco* and *b el Sco* also have the 5.4-kb fragment seen formerly (from the *noc/sna* fusion) but *Sco* lacks the 4.3-kb fragment observed with *p9.04P* probe. The important conclusion is that, with this probe, the DNAs of *Sco* and *b el Sco* are the same, hence the *noc/rd* fusion must map proximal both to the *BstI* site at -105.7 and to the *SalI* site at -103.3 .

With the 0.7-kb *HindIII-SalI* probe the 12-kb fragment observed previously in *EcoRI* digests of both *Sco* and *b el Sco* is seen. The 3.8-kb fragment, seen in *Sco* and *b el Sco* with the *SalI-EcoRI* probe does not hybridize. After *SalI* digestion all genotypes show only the predicted 3.4-kb fragment and after *BstI* digestion all show only the large fragments seen previously with the other two probes. The *noc/sna*

FIGURE 8.—(a) DNA from the *Sco* revertants that are deleted for the transposed *noc-Adh* region digested with *Bst*I (left-hand six tracks) and *Sal*I (right-hand six tracks) and then hybridized with *pob9.04P*. DNA from *Sco* and Canton-S included as controls. All of the revertants lack the 7.8-kb *Bst*I fragment of Canton-S and *Sco*, and the 5.5-kb *Bst*I and 12-kb *Sal*I fragments of *Sco*. Note the faintly hybridizing 7.8-kb *Bst*I fragment in *Sco*^{R+4}, this is a fusion fragment and is discussed in the text. (b) *Eco*RI digested DNA of *Sco*^{R+4}, *Sco*^{R+25} and *Sco*^{R+26} probed with *pob9.04P*. DNA from *Sco* and Canton-S included as controls. This shows the deletion of the 11.5-kb *Eco*RI fragment from *Sco*^{R+4} and *Sco*^{R+25} and the 3.2-kb *Eco*RI fusion fragment of *Sco*^{R+4}.

fusion fragment is not detected by this probe, in any of the three digests, confirming that this break is proximal to the *Sal*I site at -108.5 .

These data show that the break in *noc* at the *noc/sna* fusion must be within the 0.7-kb *Sal*I-*Eco*RI region from coordinates -107.8 to -108.5 . The strength of the hybridization signals, both on the chromosome *in situ* and on the Southern blots, when this region is used as a probe, suggest this break is very near the -107.8 *Eco*RI site. The break in *noc* at the *noc/rd* fusion must be proximal to the *Sal*I site at -103.3 . When Canton-S DNA is probed with *p9.04P*, after *Sal*I digestion, two approximately 5.5-kb fragments are seen. In *Sco* one of these is replaced by a 1.9-kb fusion fragment. This fusion fragment is not seen with the *Sal*I-*Eco*RI probe, therefore the breakpoint must be in the more proximal of the two wild-type 5.5-kb *Sal*I fragments, *i.e.*, between coordinates -103.3 and -97.8 . The *Eco*RI fusion fragment from the *noc/rd* junction is 5.5-kb long. So the break must be within 5.5 kb of the *Eco*RI site of -107.8 . These data refine the limits of this break to between -103.3 and -102.3 , that is to say some 5-kb proximal to the *noc/sna* fusion.

These conclusions were confirmed by an analysis of deleted revertants of *Sco*. DNA from *Df*(2L)*Sco*^{R+4}, *Df*(2L)*Sco*^{R+10}, *Df*(2L)*Sco*^{R+14} and *T*(2;3)*Sco*^{R+7} (also a *noc-Adh* deletion), all heterozygous with *Df*(2L)*fn3*, was digested with either *Sal*I or with *Bst*I and probed with *pob9.04P* (Figure 8).

With *Sal*I all have the expected 5.5-kb fragment predicted from the restriction map of *pIssRI-D* and the 1.9-kb fragment expected from the *noc/rd* fusion, which is unaffected in these revertants. The 12-kb fusion fragment, expected from the *noc/sna* fusion, is deleted in these revertants. With *Bst*I all have the 12-kb fragment predicted from the wild-type restriction map and the 4.3-kb fragment of the *noc/rd* fusion. Only *Sco* retains the 5.4-kb *noc/sna* fusion. Both Canton-S and *Sco* have a 7.8-kb *Bst*I fragment predicted from the wild-type map. This is deleted in all of the revertants. *Df*(2L)*Sco*^{R+4} does show a faintly hybridizing 7.8-kb fragment; this is a fusion fragment, and will be discussed below. These data are entirely consistent with the interpretation of the molecular structure of *Sco* and the fact that, genetically, these revertants are deleted for the *noc-Adh* transposition. *Sco*^{R+7}, *Sco*^{R+10} and *Sco*^{R+14}, but not *Sco*^{R+4}, are also deleted for *sna* and other genes proximal to the insertion site of this transposition. A summary of the molecular interpretation of the structure of the *Sco* mutation is shown in Figure 9.

Molecular analysis of *Sco* revertants: Twenty-one different induced revertants of *Sco*, previously characterized both genetically (ASHBURNER *et al.* 1983) and cytologically (ASHBURNER and HARRINGTON 1984), have been analyzed by both *in situ* hybridization and Southern blotting. *Eco*RI digested DNA from all these revertants had been probed with clones from the entire region from $\lambda ob1.12$ to $\lambda ob10.02$. By genetic criteria the revertants fell into several groups (ASHBURNER *et al.* 1983). Group 1 consisted of two revertants, *Sco*^{R+1} and *Sco*^{R+27}; both included a lethal or semilethal distal to *noc*. This was named *l*(2)*br29*. Subsequently, it has become clear that this lethal locus does not exist and that the recessive lethality of *Sco*^{R+1} is associated with a gain of function mutation that shows negative complementation for viability with some *noc* mutations (ASHBURNER and HARRINGTON 1984; CHIA *et al.* 1985b). The breakpoint of the pericentric inversion associated with *Sco*^{R+1} has been mapped to between -67 and -68 (CHIA *et al.* 1985b). It is also, now, clear that the recessive semilethality of *Sco*^{R+27}, uncovered by deletions of the *el-noc* region, is due to this revertant carrying a leaky allele of *l*(2)*35Ba* (M. ASHBURNER, unpublished data). Cytologically, *Sco*^{R+27} is indistinguishable from *Sco*. Similarly, hybridization of *Sco*^{R+27}/*Df*(2L)*A178* DNA with probes from the entire $\lambda ob1.12$ to $\lambda ob10.02$ region, showed no molecular differences between it and *Sco* (data not shown). These probes probably include the entire *l*(2)*35Ba* locus since we now know that $\lambda ob10.02$ spans this gene (T. DAVIS, unpublished data).

The group 2 revertants, *Sco*^{R+8}, *Sco*^{R+9}, *Sco*^{R+11}, *Sco*^{R+12} and *Sco*^{R+17} are all lethal when *trans*-heterozygous *inter se* or with *Sco* and are not obviously

FIGURE 9.—A molecular interpretation of the *Sco* chromosome showing the 5-kb duplication of *Sco* and the fusion fragments observed in *Sco* DNA and *SalI*, *EcoRI* or *BamHI* digestion and probing with *pob9.04P* (Figure 7). The DNA duplicated in *Sco* is indicated by the heavier line, the broken line indicates DNA that has not been cloned. The coordinates are with respect to the wild-type map.

FIGURE 10.—(a) *EcoRI* digested DNA of *Sco* revertants probed with $\lambda ob6$, showing the deletion of these sequences from *Sco*^{R+25}, the replacement of the 10.8-kb fragment of *Sco* by two faintly hybridizing fragments of similar size in *Sco*^{R+9} and the replacement of the 5.5-kb *EcoRI* fragment of *Sco* in *Sco*^{R+12} by two fusion fragments (5.3 and 2.0 kb). (b) *EcoRI* digested DNA of *Sco* revertants probed with $\lambda ob8.10$. Note particularly the absence of the 4.6-kb restriction fragment from *Sco*^{R+8}. The size of the 3.6-kb restriction fragment is affected by a polymorphism. (c) *EcoRI* digested DNA of *Sco*^{R+11} and *Sco*^{R+27} probed with $\lambda ob2.01$ showing the 5.5-kb fusion fragment in *Sco*^{R+11} and the corresponding decrease in hybridization to the 3.8-kb doublet. (d) *EcoRI* digested DNA of *Sco* revertants probed with $\lambda ob3.04$ showing the 2.5-kb fusion fragment of *Sco*^{R+17} and the replacement of the 6.0- and 1.4-kb fragments of *Sco*^{R+25} by a 8.5-kb fusion fragment. In all of these blots DNA from Canton-S and *Sco* was included as controls.

deleted for any of the loci involved in the *Sco* mutation. All are chromosomal aberrations (ASHBURNER and HARRINGTON 1984). They all have molecular lesions within the DNA covered by the walk, but these extend over a very long interval, from coordinates -24.6 to -92.6.

The only difference between *In(2L)Sco*^{R+8} and *Sco* is observed when its DNA is probed with $\lambda ob7.06$ or $\lambda ob8.10$ (Figure 10b). The 4.6-kb *EcoRI* fragment hybridized by these probes is missing from this revertant and is replaced by a 3.9-kb fusion fragment. Since the genomic 4.6-kb *EcoRI* fragment is in the region common to these adjacent clones the breakpoint of *In(2L)Sco*^{R+8} is between coordinates -88 and -92.6.

The molecular lesion of *Sco*^{R+9}, a pericentric inversion broken also in the centric heterochromatin

of 2R, is in the DNA covered by $\lambda ob6$ (Figure 10a). A 10.8-kb *EcoRI* fragment, seen in the wild type, *Sco* and all nondeleted revertants, is missing from *Sco*^{R+9} DNA and is replaced by two slightly larger fusion fragments. Only 3.5 kb of the wild-type genomic 10.8-kb fragment is actually contained within $\lambda ob6$, therefore the breakpoint of *In(2LR)Sco*^{R+9} is between -75.8 and -79.2. This was confirmed by *in situ* hybridization to its polytene chromosomes with $\lambda ob6$ (Figure 11a).

The only molecular abnormality of *In(2L)Sco*^{R+11} is seen when *EcoRI*-digested DNA is probed with $\lambda ob2.01$. In the wild type and *Sco*, this probe hybridizes to a 3.8-kb doublet and to 3.2-kb and 2.1-kb fragments. In *Sco*^{R+11} an extra 5.5-kb fragment is seen and the intensity of hybridization to the 3.8-kb band suggests that one of the members of the doublet

FIGURE 11.—(a) The polytene chromosomes of *In(2LR)Sco^{R+9}/CyO* hybridized *in situ* with λ ob6 showing a hybridization signal at both ends of the inversion (open arrows, L and R). (b) Polytene chromosomes of *In(2L)Sco^{R+11}/CyO* hybridized *in situ* with λ ob2.01 showing hybridization to both ends of this inversion (open arrows, L and R). The breakpoint of *In(2LR)O* is indicated by O. (c and d) Polytene chromosomes of *In(2L)Sco^{R+17}* hybridized *in situ* with λ ob3.04 (c) showing hybridization to both ends of the inversion, with the left end having the stronger signal and λ ob4.01 (d) showing this DNA to be proximal to the inversion breakpoint.

is missing (Figure 10c). One of the two normal 3.8-kb fragments hybridizes to both λ ob2.01 and to λ ob1.12, the phage immediately proximal to it. *Sco^{R+11}* DNA is normal when probed with λ ob1.12 (data not shown); therefore the breakpoint of the inversion associated with this revertant is in the 3.8-kb genomic *Eco*RI fragment from λ ob2.01 (−24.6 to −28.4). This result was confirmed by the *in situ* hybridization of *Sco^{R+11}* with λ ob2.01—this phage spans the inversion breakpoint (Figure 11b). The approximately equal intensities of the signals at either end of this inversion suggest that this breakpoint is roughly in the middle of this phage.

Sco^{R+12} is a complex transposition of the *Sco* region to 34A8–34B1.2. The relevant breakpoint maps to the region of overlap between λ ob5 and λ ob6, since when *Sco^{R+12}* DNA, digested with *Eco*RI, is probed with these clones the 5.5-kb *Eco*RI fragment seen in *Sco* and all its nondeleted revertants is missing and is replaced by a 5.3-kb fusion fragment (Figure 10a). The breakpoint is between coordinates −68.3 and −73.8.

In(2L)Sco^{R+17} DNA is abnormal when probed with

λ ob4.01 and λ ob3.04. In Canton-S λ ob4.014 hybridizes to two fragments, of 9 kb and 4.6 kb. In *Sco*, and all of its revertants not actually deleted for this DNA, the 4.6-kb fragment is replaced by one of 8.5 kb, suggesting an insertion relative to Canton-S. *Sco^{R+17}* has an additional 2.1-kb fragment. This fragment also hybridizes when λ ob3.04 is used as a probe, suggesting that the breakpoint of this inversion is in the region where these phage overlap, *i.e.*, between coordinates −43.8 and −46.0 (Figure 10d). This was confirmed by *in situ* hybridization (Figure 11, c and d).

The group 3 revertants of *Sco* are all mutant for *sna*. Two different subgroups were recognized: group 3a, deleted for the *noc-Adh* region and (except for *Sco^{R+4}*) for loci proximal to the insertion site, and group 3b, not deleted for *osp-Adh*, but mutant for *sna* and, in many cases deleted for *sna* and more proximal loci (Figure 1).

The breakpoints of the group 3a revertants have already been mentioned: when *Eco*RI digested DNA is probed with *pob9.04P* then, in comparison to *Sco*, a 11.5-kb *Eco*RI fragment is missing and is replaced

by a 5.5-kb fusion fragment. The deletions of all group 3a revertants include the entire *noc-Adh* transposition. *Sco*^{R+4} is slightly different from the others of this group, genetically it is deleted for *noc*, *osp*, *Adh* and ten proximal loci, up to and including *l(2)35Cb* (Figure 1). It too lacks the 11.5-kb *Eco*RI fragment and has a 5.5-kb *Eco*RI fusion fragment when probed with *pob9.04P*. This hybridization also shows an extra 3.2-kb *Eco*RI fusion fragment and a 7.8-kb *Bst*I fusion fragment (Figure 8), indicating that the proximal limit of this deficiency is within the *noc-Adh* transposition.

The group 3b revertants share a common lethality with EMS induced lethal alleles of *sna*. Three, *Sco*^{R+16}, *Sco*^{R+23} and *Sco*^{R+25}, are mutant for more proximal loci in addition to *sna* (Figure 1). However, only one of these, *Sco*^{R+25}, has been shown to possess a molecular lesion within the limits of the cloned DNA: all of the others (*i.e.*, *Sco*^{R+16}, *Sco*^{R+19}, *Sco*^{R+23} and *Sco*^{R+26}) are indistinguishable from *Sco* within this region. *Sco*^{R+25} is a small deletion whose proximal end is within *lob3.04*. When *Sco*^{R+25} *Eco*RI digested DNA is probed with this phage three expected fragments of 9 kb, 6 kb and 1.3 kb are absent but a novel 8.5-kb fusion fragment is seen (Figure 10d). This suggests that the proximal limit of this deletion is between coordinates -35.5 and -41.5. *Sco*^{R+25} lacks all homology to all more distal phage until *lob9.04*. When the 0.7-kb *Sal*I-*Eco*RI fragment is used to probe *Sco*^{R+25} DNA the expected 3.8-kb *Eco*RI *noc/sna* fusion fragment is missing, as in the group 3a revertants (data not shown).

The failure to find any molecular evidence of breakpoints associated with *Sco*^{R+16}, *Sco*^{R+19}, *Sco*^{R+23} and *Sco*^{R+26} is notable, since these group 3b revertants are all, genetically, mutant for *sna*. Two, *Sco*^{R+16} and *Sco*^{R+23}, are deletions that include loci proximal to *sna*. Although it is possible that, with digestions using only a single restriction enzyme, some molecular lesions may have been inadvertently missed this is unlikely to have been the case for four independent mutations.

Sco^{R+26} is cytologically aberrant, a paracentric inversion. This allowed a direct test of whether or not the inversion break was within the cloned region, by *in situ* hybridization. When the chromosomes of *In(2L)Sco*^{R+26} were probed with *pob9.04P* only two very closely adjacent sets of grains were seen (Figure 12). Were the breakpoint of this inversion to be within the transposed *noc* gene, then this probe would have hybridized to two sets of grains, well separated, one of each end of the inversion. The conclusion must be, therefore, that this inversion is broken proximal to the *noc-Adh* transposition, presumably in *sna* sequences since this reversion is a semilethal allele of *sna*, but is not mutant for any more proximal loci.

FIGURE 12.—Polytene chromosomes of *In(2L)Sco*^{R+26} hybridized *in situ* with *pob9.04P* showing hybridization of this probe to the left, but not to the right, ends of the inversion. The signal to the left end of the breakpoint is very stretched.

FIGURE 13.—(a) *Eco*RI digested DNA from *Sco* revertants probed with *pob9.04P* showing the 6-kb fusion fragment of *Sco*^{R+21}. (b) *Eco*RI digested DNA of *Sco* revertants probed with the 0.7-kb *Sal*I-*Eco*RI subclone showing the replacement of the 3.8-kb *Eco*RI fragment of *Sco* by a 7-kb fusion fragment in *Sco*^{R+24}. DNA from Canton-S and *Sco* included on these blots as controls.

In addition to these three groups of revertants there is a miscellaneous fourth group. This includes *Sco*^{R+2}, *Sco*^{R+13}, *Sco*^{R+21} and *Sco*^{R+24}. Molecular lesions associated with *Sco*^{R+21} and *Sco*^{R+24}, both paracentric inversions, have been found on the chromosomal walk. When DNA from *Sco*^{R+21} is probed with *pob9.04P* three fragments are seen, the expected 11.5-kb and 5.5-kb fragments and a novel one of 6.5 kb (Figure 13a). This 6.5-kb fragment is not seen when this DNA is probed with *lob8.10*, so the breakpoint of this inversion must be in that region of *lob9.04* that is not overlapped by *lob8.10*, *i.e.*, between coordinates -103.3 and -107.8. The 0.7-kb *Sal*I-*Eco*RI subclone, corresponding to DNA between -107.8 and -108.5 was used to probe *Eco*RI cut DNA from *Sco*^{R+24} (Figure 13b). This hybridizes to

a 12-kb fragment, but the expected 3.8-kb fusion fragment (seen with *Sco* DNA and that of all other revertants except group 3a and *Sco*^{R+25}) is missing and is replaced by a faint 7-kb fusion fragment. Since the degree of homology between the 3.8-kb genomic *Eco*RI fragment and this probe is small (see above) any aberration within the region of homology would be expected to have a dramatic effect on signal strength. The 7-kb fusion fragment from *Sco*^{R+24} and the 3.8-kb fragment of *So* hybridize to about the same intensity (given that these gels are not well controlled for the amount of DNA loaded per lane); this suggests that the *Sco*^{R+24} break is not within that part of the 3.8-kb *Eco*RI fusion fragment to which the 0.7-kb *Sal*I-*Eco*RI clone is homologous. This leads to the conclusion that the breakpoint of *In*(2L)*Sco*^{R+24} is within *sna* sequences juxtaposed to *noc* in *Sco*. Indeed, this breakpoint must be between this junction and the nearest *Eco*RI site (that which marks the proximal end of the 3.8-kb *Eco*RI fusion fragment of *Sco*) in the *sna* region.

Neither the breakpoints of *In*(2L)*Sco*^{R+2} nor *T*(2;3)*Sco*^{R+13} have been identified at the molecular level. This fact suggests that they may be proximal to the *noc-Adh* transposition. The pattern of restriction enzyme fragments seen for both over the entire region from *lob1.12* to *lob10.02* is similar to that of *Sco*. The polytene chromosomes of *T*(2;3)*Sco*^{R+13} have been probed with *pob9.04P* (Figure 14, a and b). Due to the complexity of this aberration, a *T*(2;3) plus an *In*(3LR), the results are not easy to interpret. The observations are that on the translocated elements hybridization is seen at two sites, one on the 2^D3^P element at the 35B | 71B junction, as expected, but also one at the 71B | 81 junction of the pericentric inversion. In very stretched polytene chromosomes this site appears to be double. No hybridization is seen on the 3^D2^P element, consistent with the interpretation that the translocation break point on chromosome 2 occurred proximal to the *noc-Adh* transposition. Cytologically *T*(2;3)*Sco*^{R+13} was interpreted to be 21-[*Sco*] | 71B · 81 | 71B-61 (2^D3^P) and 60 · 35D | 81-100 (3^D2^P) (ASHBURNER and HARRINGTON 1984). A few extra bands at the 71B | 81 junction were never satisfactorily interpreted (*e.g.*, see Figure 6c of ASHBURNER and HARRINGTON 1983). *lob9.04* hybridizes to both ends of the third chromosome inversion. Since *pob9.04P* is partly duplicated in *Sco* this means that the breakpoint of the inversion within 2L material must have been between the two copies of these sequences. A model for the origin of this aberration is shown in Figure 14c. The duplicated region 35 is adjacent to centric heterochromatin, which may account for the relatively lower grain density, seen in the *in situ* hybridizations, compared to that over the 35B | 71B junction.

FIGURE 14.—(a and b) Polytene chromosomes of *T*(2;3) *Sco*^{R+13}/*CyO* hybridized *in situ* with *pob9.04P*. Hybridization to the 2^D3^P element of the translocation is marked by open arrows. The inversion is indicated as *In* and the open arrows indicate hybridization to the right-hand end of this inversion. The *noc-Adh* transposition of *Sco* is indicated as *Tpl* and the *rd-l(2)35Da* transposition as *Tp2*. In (a) the inverted homolog is very stretched and, proximally, lies alongside proximal 3L. In these nuclei this hybridization occurs at two sites, these were not seen in all nuclei. (c) The interpretation of the pattern of *in situ* hybridization to *T*(2;3)*Sco*^{R+13}. The top part of (c) shows the presumed consequence of the simple translocation between chromosomes 2 and 3, and the bottom part the 2^D3^P element after the occurrence of the third chromosome inversion. The breakpoint of this inversion within 2L material is arbitrarily shown as being between the two transpositions of the *Sco* chromosome. In fact it could be anywhere distal to the cloned DNA included within the *noc-Adh* transposition (see text). The sites expected to hybridize to *pob9.04P* are indicated by arrows. The chromosome regions are not to scale.

DISCUSSION

Structure of the *Sco* chromosome: A genetic analysis of the *Sco* mutation (ASHBURNER, TSUBOTA and WOODRUFF 1982; ASHBURNER *et al.* 1983) led to the model that this mutation was associated with a reciprocal transposition of the *noc-Adh* and *rd-l(2)35Da* regions. A recombinant between *Sco* and a structurally normal chromosome separated these transpositions: this recombinant [*b el Sco*, MARONI (1980)] retains only the transposition of the *noc-Adh* region. This recombinant is phenotypically Scutoid, hence this phenotype must be associated with this transposition rather than that of the *rd-l(2)35Da* region. Genetic analysis of the revertants of *Sco* led to the suggestion that the important part of the *Sco* chromosome, as far as the Scutoid phenotype was concerned, was the novel juxtaposition of *noc* and *sna* (ASHBURNER, TSUBOTA and WOODRUFF 1982).

The evidence that the gene adjacent to *noc* in *Sco* is *sna* was, originally, simply that *sna* was the closest identified locus to the transposition. Subsequently, this conclusion has been strengthened by the fact that an extensive screen for mutations in the *sna* region, carried out for quite a different purpose (M. ASHBURNER, P. THOMSON, Y. GRAU, M. EL MESSAL and P. SIMPSON, unpublished data), has failed to identify any other vital or visible locus closer to the transposition than *sna*. Moreover, the molecular cloning of *sna* by BOULEY, DENNEFELD and ALBERGA (1987) has, independently, allowed the discovery that this gene is within about 16 kb of the transposition (A. ALBERGA, personal communication).

The molecular analysis of the *Sco* chromosome confirms the general features of the model for the structure of the *Sco* chromosome. Sequences from *noc* are indeed transposed from 35B to 35D, are inverted in their new position and are adjacent to sequences from the *sna* region. In particular, two of the four novel junctions in *Sco* have been characterised, the most distal junction, between *noc* and *rd*, and the most proximal junction, between *noc* and *sna*. The remaining junctions, those between the *rd* and *l(2)35Bb* regions and between the *l(2)35Cb* and *Adh* regions have not been studied, since DNA covering them has not been cloned. The proximal limit of the *noc-Adh* transposition, which in *Sco* is fused to *l(2)35Cb*, is at least 40 kb proximal to *Adh*.

The distal breakpoint of the *noc-Adh* transposition is not simple, since the same 5-kb DNA sequence is found both at its original site and in the transposition (Figure 9). *Sco* was an X-ray-induced mutation (KRIVSHENKO 1959) and we presume that this duplication reflects post-replicative repair of a damaged chromosome occurring after fertilization.

Structure of the revertant chromosomes: A model

was proposed whereby a fusion led to the synthesis of a novel *noc* gene product (*noc**) which interfered with the function of the wild-type gene product (*noc*⁺), to cause the *Sco* phenotype (ASHBURNER, TSUBOTA and WOODRUFF 1982). Mutational inactivation of this fusion gene would then revert the *Sco* phenotype. Since *Sco* is a gain-of-function mutation, the positions of breakpoints that revert its phenotype must define those sequences whose integrity is required for this phenotype. Twenty-one different induced reversions of *Sco* have been analyzed molecularly. Of these 19 are associated with gross aberrations of the *Sco* chromosome (that is to say, all except *Sco*^{R+19} and *Sco*^{R+27}). We have mapped, to the DNA covered by *lob1.12* to *lob10.02* the breakpoints of 14 of these aberrations. The surprising result is that these are spread over a very long region of the DNA, about 80 kb from coordinates -24.6 to -28.4 to -103.3 to -107.8 (Figure 2). Indeed they are spread over a longer region of DNA than we had previously thought to be associated with the *noc* locus (CHIA *et al.* 1985b). This point will be discussed below. We have failed to discover the molecular lesions associated with the aberration breakpoints of six *Sco* revertants (*i.e.*, *Sco*^{R+2}, *Sco*^{R+13}, *Sco*^{R+16}, *Sco*^{R+23}, *Sco*^{R+24} and *Sco*^{R+26}) within the cloned region. The supposition is, therefore, that these aberrations revert *Sco* as a consequence of a breakpoint outside the *noc-Adh* transposition. Genetically three of these breakpoints are associated with mutation of *sna* (*i.e.*, *Sco*^{R+16}, *Sco*^{R+23} and *Sco*^{R+26}). *Sco*^{R+19} and *Sco*^{R+27} are cytologically similar to *Sco* and the failure to find any molecular lesion associated with these may be because they are "point" mutations.

The breakpoint of *In(2L)Sco*^{R+24} is close enough to the fusion of *noc* and *sna* region sequences (within 3.5 kb) to have been mapped to the DNA. Since *Sco*^{R+24} is *sna*⁺ (ASHBURNER *et al.* 1983) this breakpoint must revert *Sco* by separating the fused *noc* and *sna* sequences, rather than mutating either.

T(2;3)Sco^{R+13} is another *sna*⁺ revertant that may also separate *noc* and *sna*. The translocation breakpoint has not been found within the cloned region and is, by *in situ* analysis, proximal to the *noc-Adh* transposition. Furthermore, genetic data (ASHBURNER *et al.* 1983; ASHBURNER and HARRINGTON 1984) show that this breakpoint is distal to *sna*, since a deletion synthesized with the 3^P2^P element of this translocation is *sna*⁺.

Sco^{R+23} is associated with an insertional translocation from chromosome 2 to the base of the X chromosome. *Dp(2;1)Sco*^{R+23} carries the entire *noc-Adh* transposition of *Sco*. This duplication does not cover the lethality of *sna* (ASHBURNER *et al.* 1983). *Dp(2;1)Sco*^{R+23}/+; *sna/sna*⁻ hemizygotes are semilethal and surviving adults are usually hemithorax or

lack halteres (GRAU, CARTERET and SIMPSON 1984). These data suggest that this duplication carries a mutant, but not amorphic, allele of *sna*.

The revertant breakpoint of *In(2L)Sco^{R+26}* is associated with a leaky lethal allele of *sna*. Again, this breakpoint cannot be found on the cloned DNA from the *noc* region and by *in situ* analysis is proximal to the transposition.

The final breakpoints unaccounted for are those of *Sco^{R+2}* and *Sco^{R+16}*. We have no information on the position of the breakpoint of *In(2L)Sco^{R+2}*, except that it has not been found on the cloned DNA and that this revertant is *sna*⁺. Cytologically *Sco^{R+16}* is not grossly aberrant, but is, by genetic criteria, associated with a deletion of *sna* and at least nine complementation groups more proximal (M. ASHBURNER, P. THOMSON, Y. GRAU, M. EL MESSAL and P. SIMPSON, unpublished data).

In addition to these chromosomally rearranged revertants of *Sco* two that are cytologically similar to *Sco* are mutant for *sna*, *Sco^{R+15}* and *Sco^{R+19}* (ASHBURNER *et al.* 1983). DNA from *Sco^{R+15}* has not been studied but that of *Sco^{R+19}* has, and no lesion has been found in the region covered by *lob1.12* to *lob10.02*.

The evidence is, therefore, that reversion of *Sco* can result from at least three distinct classes of mutational lesion: mutation or deletion of sequences associated with *noc*, mutation or deletion of *sna* and breakpoints that separate these juxtaposed regions in the *Sco* chromosome. The critical point to be emphasized is that breakpoints associated with the *Sco* revertants map to either side of the junction between the *noc-Adh* transposition and *sna*, demonstrating that sequences from both sides of this junction are required for the dominant gain-of-function phenotype of *Sco*.

In fact, there is evidence that a fourth class of mutation can also revert *Sco*. This comes from a consideration of the unusual revertant *Sco^{R+27}*. This revertant is cytologically indistinguishable from *Sco*. It is unique in two respects. First, it is the only revertant of *Sco* that does not act as a dominant enhancer of *Sco*. All revertants other than *Sco^{R+27}* are either completely lethal when heterozygous with *Sco* (*i.e.*, group 2) or have an extreme Scutoid phenotype when heterozygous with *Sco* (ASHBURNER *et al.* 1983, Table 5). Although *Sco/Sco^{R+27}* are semi-lethal the survivors are *Sco*⁺. Second, it is the only revertant that acts as a recessive allele of *l(2)35Ba*. (*Sco^{R+1}* also fails to complement *l(2)35Ba* alleles, but this is due to a gain-of-function, see ASHBURNER and HARRINGTON (1984)). All four known EMS alleles of *l(2)35Ba* show a weak, but clear, failure of complementation with mutant *noc* alleles and all enhance the phenotype in *Sco/l(2)35Ba* heterozygotes (ASH-

BURNER, TSUBOTA and AARON 1982). These data are explained if *l(2)35Ba*⁺ acts as a *cis*-activator of *noc*. Then, *noc* function would be reduced in *l(2)35Ba*⁻, altering the balance of *noc*⁺ and *noc*^{*} products in *l(2)35Ba*⁻/*Sco* heterozygotes in favor of *noc*^{*}, enhancing the Scutoid phenotype. Moreover a *l(2)35Ba*⁻ mutation on *Sco* itself would reduce the amount of *noc*^{*} product, reverting the Scutoid phenotype. Such may be the case for *Sco^{R+27}*.

Relationship between *Sco* and the *noc* gene: The region of DNA associated with *noc* has been defined only by the mapping of breakpoints associated with mutant *noc* alleles. Four chromosomally aberrant alleles of *noc* have been mapped to the DNA; these span from coordinates -61.5 to -66 (*In(2L)noc2*) to -109 to -113 (*In(2LR)noc4*) (CHIA *et al.* 1985b). Proximally, deletions of DNA up to -56.2 to -57.4 (*i.e.*, the distal end of *Df(2L)fn52*) are *noc*⁺, while deletions extending distal to -61.5 (*e.g.*, *Df(2L)A379*) are *noc*⁻. Distally, a deletion extending to -121.9 to -125 (*Df(2L)b84a2*, T. DAVIS, unpublished data) is *noc*⁺ but a deletion extending as far proximal as -103.3 to -98.5 (*Df(2L)TE146 (Z)GW12*) is *noc*⁻. The proximal end of *noc*, therefore, lies between -56.2 and -61.5 and the distal end between -109 and -125.

With respect both to the distribution of those *Sco* revertant breakpoints that have been mapped to the cloned DNA, and to the distal breakpoint of the *noc-Adh* transposition, two facts are immediately apparent. One is that some revertant breakpoints are not only proximal to the *In(2L)noc2* breakpoint, but are in sequences which do not result in a *noc* phenotype when deleted. Thus the breakpoints of both *In(2L)Sco^{R+11}* (between -24.6 and -28.4) and *In(2L)Sco^{R+17}* (between -43.8 and 46.0) are within sequences deleted by *Df(2L)fn52* (CHIA *et al.* 1985b); heterozygotes between *Df(2L)fn52* and a deletion of the entire cloned DNA (*e.g.*, *Df(2L)fn2*) are phenotypically *noc*⁺. The second fact is that the *noc-Adh* transposition does not include all of the DNA defined, by mutation, as being required for *noc* function; its distal breakpoint is just proximal to the insertion site of *TE146*, a strong *noc*⁻ allele and well proximal of the breakpoint of *In(2LR)noc4*, also a strong *noc*⁻ allele.

The *noc* locus: The genetic complexity of *noc* is evident from several independent observations: (1) alleles of both the elbow (*el*) and *l(2)35Ba* loci fail to complement mutant *noc* alleles or *noc*⁻ deletions (ASHBURNER, TSUBOTA and WOODRUFF 1982); (2) the *Sco* revertant *In(2LR)Sco^{R+1}* shows negative complementation for viability with some *noc* alleles and some *noc*⁻ deletions, so distinguishing two classes of *noc* mutation. For example, *In(2LR)Sco^{R+1}/TE146* heterozygotes are inviable, despite the fact that *TE146*

is homozygous viable (ASHBURNER *et al.* 1983). The breakpoint of the *Sco*^{R+1} inversion is between coordinates -67.1 and -68.3 (CHIA *et al.* 1985b). Mutations or deletions of *noc* that affect sequences proximal to -80 are viable when heterozygous with *Sco*^{R+1}, whereas mutations or deletions of *noc* that affect sequences distal to -80 are not (CHIA *et al.* 1985b); (3) homozygous deletion of the entire *noc* region is lethal. This lethality is not the result of deleting any single vital gene, since the distal and proximal *noc* regions can be independently homozygously deleted without lethality. Lethality results only when both are deleted. For example, heterozygotes between *Df(2L)A446* and *l(2)35Ba⁻ noc⁻* deletions are viable unless such deletions extend proximal to -37.5: *Df(2L)b84a4/Df(2L)A446* are viable, but *Df(2L)A446/Df(2L)TE146(Z)GW8* are lethal. The proximal end of *Df(2L)b84a4* is at about -37.5, that of *Df(2L)TE146(Z)GW8* at about -11.5; (4) the molecular region defined as *noc* (see above and CHIA *et al.* 1985b) cannot be a single gene since at least one breakpoint within it, that of *T(2;3)Mpe* at -103 to -106.5, is not mutant for *noc* when heterozygous with deletions of the entire region; and (5) within the *noc* region map two distinct clusters of tRNA_{gly} genes, at -91.2 to -93.1 (3 genes) and -79.2 to -82.4 (2 genes), whose functional relationship to *noc* is obscure (S. MCGILL and B. MENG, unpublished results).

These, and other data (MCGILL 1985), suggest that the *noc* region can be functionally divided into at least three units, *nocA*, *nocB* and *nocC* (Figure 2). One very clear-cut distinction between these units is the strength of the *noc* phenotypes seen when these regions are individually mutated or deleted. Deletion or mutation of *nocA* gives a very strong mutant phenotype, that of *nocB* a weak phenotype while deletion of *nocC* is phenotypically *noc*⁺. However, deletion of *nocC* does enhance *Sco* and two revertants of *Sco* are broken within *nocC*. Further evidence for this subdivision of *noc* will be published elsewhere. Suffice it to state now that the *Sco* transposition includes only *nocB* and *nocC*; *nocA* remains at its normal chromosomal location. A breakpoint between *nocA* and *nocB* (*i.e.*, that of *T(2;3)Mpe*) is *noc*⁺. The two *Sco* breakpoints, those at -107.8 to -108.5 and -103.3 to -102.3, appear to be in the region between *nocA* and *nocB*. It is, therefore, unlikely that these transposition breakpoints themselves have anything to do with the *Sco* phenotype but, rather, that this phenotype is a consequence of the fusion of *nocB* and *nocC* with sequences in the *sna* region.

The important consequence of this fusion had originally been considered to be the formation of a chimeric *noc-sna* gene coding for a product that antagonized the effect of that of a *noc*⁺ gene. Molecular mapping of the breakpoints that revert *Sco* makes

this hypothesis unlikely. For example, the breakpoint of *Sco*^{R+24} is within *sna* region sequences, it interrupts the putative fusion gene, yet is *sna*⁺. This does not, of course, invalidate the general hypothesis but suggests that the fusion may act as an antimorphic *noc* allele as a consequence of a fused transcriptional domain, rather than a fused translational domain. There are several mechanisms for such an effect, but any discussion of these will remain speculative until the normal transcriptional organization of both *noc* and *sna* is understood.

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