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A chromosomal walk in the region of polytene bands 7C–D of the *Drosophila* X chromosome

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Abstract. A chromosomal walk on the X chromosome of *Drosophila* in the region of polytene bands 7C1 to 7D5 is described. The region is of interest since three olfactory genes have been found to map here in addition to a haplo-inviable locus. Genomic clones spanning 160 kilobases have been isolated and their complete restriction map is presented. The clones have been aligned on the polytene chromosome bands by *in situ* hybridisation. In addition the end-points of a deficiency and duplication lying in this region have been mapped approximately, showing that an overlap exists between them.

Keywords. Chromosomal walking; *Drosophila*; olfactory genes; *singed*; haplo-inviable.

1. Introduction

Chromosomal walking, as a means of cloning genetically defined loci in *Drosophila melanogaster*, was first described by Bender *et al.* (1983). Subsequently a number of genes in *Drosophila* have been cloned by this method. Briefly, the method requires that the gene of interest should have been mapped to within a few bands on the polytene chromosomes, and a previous clone near the region of interest should be available. I have used this method to walk through a region of the X chromosome from polytene bands 7C1 to 7D2–3 (figure 1). This plus the neighbouring region upto band 7D5 is of interest since three olfactory genes have been found to map here (Ayyub *et al.* 1989).

The entry point for this walk was a plasmid clone from the *singed* locus. This locus has been mapped by both genetic and molecular techniques to band position 7D1 (Roiha *et al.* 1988). The purpose of the walk was to obtain an ordered restriction map of the complete region extending from bands 7C1 to 7D5. This had already been accomplished in part for a 70 kb region near band 7D5 (Digan *et al.* 1986; shown in figure 1). Here I describe the restriction map, with its orientation on the X chromosome, for the remaining region of 160 kb from band 7C1 to the point where it overlaps with the earlier published walk. In addition to obtaining a restriction map of the region it was also of interest to define which part of this walk was covered or uncovered by duplications or deficiencies of the region. Specifically I have looked at polytene chromosome squashes of the duplication $Dp(1;3)sn^{13a1}$ and the deficiency $Df(1)sn^{c128}$, after *in situ* hybridisation to various cloned DNA fragments from the walk. As a result it has been possible to define approximately the limits of this duplication and deficiency, and show that there exists between them a small region of overlap, which was previously not known.

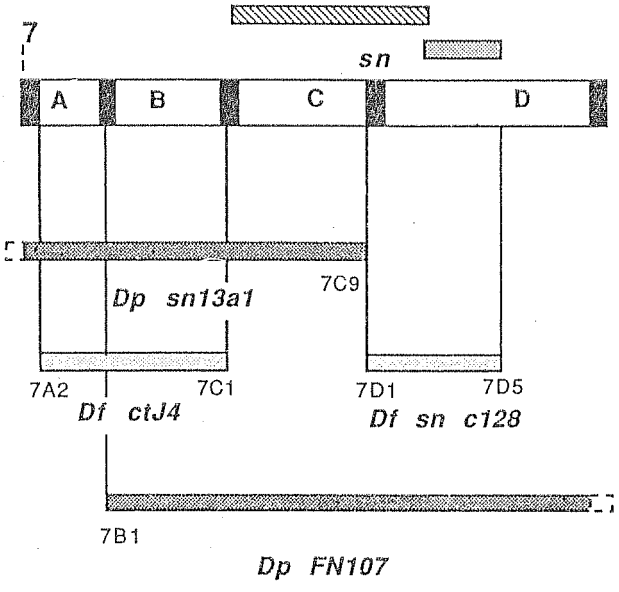

Figure 1. A schematic representation of the polytene band region 7C–7D of the X chromosome. The end points of the walk described here (▨) and of an earlier walk in the 7D5 region (⊞) are shown above the chromosome map. End points of various deficiencies and duplications mentioned in the text are indicated below the chromosome map. The drawing is not to scale.

2. Materials and methods

2.1 *Construction and screening of genomic libraries*

A genomic DNA library from the *Drosophila* wild type strain Canton-S was constructed in the lambda vector EMBL3 (Frischauf *et al.* 1983). The procedure followed was essentially as described in Maniatis *et al.* (1982). Genomic DNA was partially digested with the restriction enzyme MboI and ligated to EMBL3 DNA, which had been simultaneously digested with the restriction enzymes BamHI and EcoRI. Restriction enzymes and other DNA modification enzymes were obtained from New England Biolabs, USA. The ligations were packaged *in vitro*, and plated out. Approximately 4×10^5 plaque forming units (pfu) were obtained and these were amplified further and stored as liquid lysates. Two other libraries used were a gift of Dr. Ruth Lehman. These were a Canton-S library in Charon 4A (Maniatis *et al.* 1978) and an Oregon-R library in EMBL4 (Frischauf *et al.* 1983).

The libraries were screened for specific sequences by plating out approximately 10^5 pfu on a 23×23 cm^2 plate. Hybond-N membranes obtained from Amersham International, UK, were used for doing the phage lifts from the plates. Hybridisation and detection of radioactive probes was according to published procedures (Maniatis *et al.* 1982). Radioactive probes were made using purified gel fragments which were labelled with [α-^{32}P] deoxyadenosine triphosphate by the random priming reaction (Feinberg and Vogelstein 1983).

2.2 *Preparation of lambda phage DNA*

Single well-isolated plaques obtained after a secondary or tertiary screen were used to make plate lysate stocks as described in Maniatis *et al.* (1982). Phage DNA was made from 400 ml liquid lysates, by precipitating the phage with 20% polyethylene glycol-6000 (PEG). The PEG pellet was purified further to yield pure phage DNA by following the glycerol gradient procedure in Maniatis *et al.* (1982).

2.3 *Drosophila stocks*

The wild type *Drosophila* strain used was Canton-Special (CS). Stocks carrying chromosomal rearrangements were obtained from *Drosophila* stock centres at Caltech, USA, and Umea, Sweden. Both the $Df(1)sn^{c128}$/Y; *DpFN107/bwD* stock and the $Df(1)ct^{j4}$; $Dp(1;3)sn^{13al}$ stock have been described (Cramer and Roy 1980; Lefevre and Johnson 1973). A schematic representation of these rearrangements is given in figure 1.

2.4 *In situ hybridisation to polytene chromosomes*

Polytene chromosome squashes and hybridisation of radiolabelled probes essentially followed standard procedures (Spierer *et al.* 1983). The probe was labelled by random priming as described by Feinberg and Vogelstein (1983). The label used was either adenosine 5'-α-[^{35}S]thiotriphosphate obtained from Amersham International (UK) or Biotinylated dUTP from Enzo Biolabs (USA). In case of the latter, detection of hybridisation was by immunological methods as described by Langer-Safer *et al.* (1982). All other chemicals used were of Analar grade or better.

3. Results and discussion

While a number of X-linked olfactory genes have been identified in *Drosophila melanogaster* (Rodrigues 1980; Helfand and Carlson 1989), so far none of these genes has been studied at a molecular level. In order to begin such a molecular analysis, a chromosomal walk, in a region where three such olfactory genes map, has been completed. A schematic representation of this region showing the area covered by the walk is given in figure 1. This walk was initiated from the *sn* locus at chromosomal band 7D1, using as a probe a previously existing plasmid clone pSn9. This clone and DNA from 40 kb of the flanking region have been described in Roiha *et al.* (1988). The first library used for screening was constructed in the lambda phage vector EMBL3 (Frischauf *et al.* 1983) using genomic DNA from the wild-type strain Canton-S. This library was screened with the insert from pSn9 (95–100 kb, figure 2), and four positive plaques isolated. Phage DNA from the positive plaques was digested with various restriction enzymes in single and double digests in order to obtain a restriction map (figure 2). Confirmation of the restriction map was obtained by Southern blotting (Southern 1975) using different restriction fragments as probes (data not shown). Two of the phages obtained from this first step were lambda CSp1 and lambda CSp2 (figure 2). As judged from their

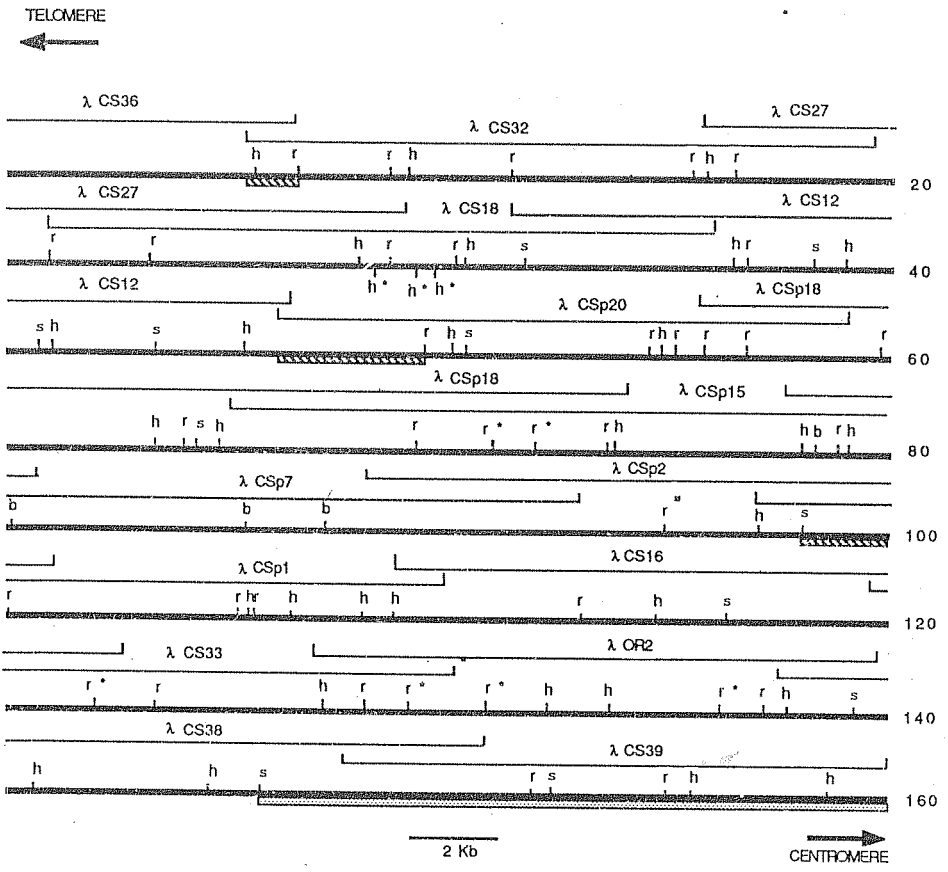

Figure 2. A restriction map of the chromosomal walk. Restriction enzymes have been indicated as follows: h—HindIII; r—EcoRI; s—SalI; b—BamHI (mapped for the area covered by phage CSp7 only). Restriction sites marked with an asterisk are tentative. Numbers along the side indicate kilobase pairs. Lambda phage clones used for obtaining the restriction map are drawn above the map and have been labelled. Restriction fragments used as probes for *in situ* hybridisation have been indicated as underlined hatched areas (▨). The region of overlap with the walk of Digan *et al.* (1986) is also marked (⊞).

restriction maps these two phages extend in opposite directions with an overlap of 4 kb between them. A restriction fragment from the end of lambda CSp2 that did not overlap with lambda CSp1, was used as a probe for the next step. In this way, a series of overlapping phages, viz. lambda CSp7, CSp15, CSp18, CSp20 were obtained. Together these phages cover a distance of 54 kb. A complete restriction map of the area covered by these and subsequent phage clones is given in figure 2.

At this stage the direction and position of the walk, with respect to bands on the polytene X chromosome, was determined. This was done by *in situ* hybridisation of the end fragment of lambda CSp20 to polytene chromosomes. As shown in figure 3a, hybridisation was to a position approximately halfway between bands 7C1 and 7D1. The remainder of the walk in this direction, upto band 7C1 was carried out using a Canton-S genomic library made in the lambda vector Charon 4A. Phages

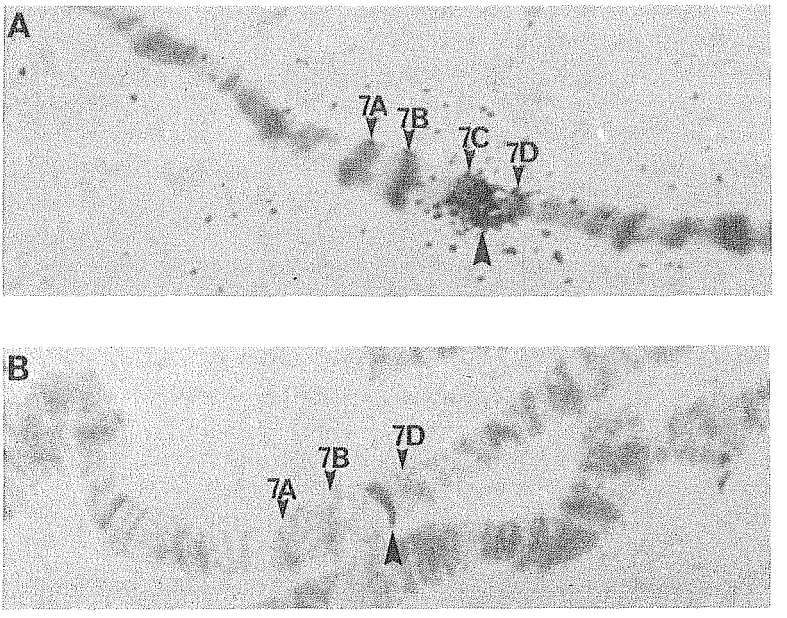

Figure 3. *In situ* hybridisation of the end fragments of (**A**) phage CSp20, and (**B**) phage CS32, to the X chromosome of Canton-S females. Hybridisation is indicated by the large arrowheads. The smaller arrow heads indicate bands of the polytene X chromosome. The probe was labelled with 5'-α-[^{35}S] dATP in (A) and with biotinylated dUTP in (B) as described in § 2.

obtained from this library, viz. lambda CS12, CS18, CS27, CS32 and CS36, together comprise 46 kb. Genomic clones extending beyond phage CS36 have not been isolated since the end-fragment of lambda CS32 was found to hybridise within band 7C1 (figure 3b). However, this hybridisation does not necessarily mean that band 7C1 has been entirely covered by the phage clones CS32 and CS36. In order to be certain of this one would need to obtain clones that hybridise between 7B and 7C1. This was not considered essential since the olfactory genes of interest are known to map closer to band 7D1. The region from 7C1 to 7D1 comprising 9 bands was thus covered by walking a distance of just 100 kb. This small amount of DNA between the two major bands 7C1 and 7D1 very likely reflects the fact that the polytene bands in this region are extremely faint. A correlation between the thickness of a band and its DNA content has been made earlier (Spierer *et al.* 1983).

The walk from 7D1 to the point of overlap with the previously described walk of Digan *et al.* (1986) is 60 kb long. The first clone in this direction lambda CS16 was isolated from the Canton-S genomic library in the vector Charon 4A. This clone appears to be under-represented in most libraries since approximately 100,000 phages from the Charon 4A library and a similar number from an Oregon-R library in the vector EMBL3, were screened, before a single positive phage was obtained. The walk was continued in both of the above mentioned libraries but only phages with a minimal overlap are shown in figure 2. The last phage in the walk, lambda CS39, was found on comparison to have a long region of overlap with the first phage in the walk described by Digan *et al.* (1986). In addition it contains a region

that is polymorphic between the Canton-S and Oregon-R strains due to insertion of different repetitive elements. A similar region has also been described by Digan *et al.* (1986). Thus it was clear that all of phage CS39 and 4·5 kb of phage CS38 overlap with the walk of Digan *et al.* (1986). Phages extending beyond lambda CS39 have therefore not been isolated.

My final interest in the walk described here is to define within it the limits of various olfactory genes that have been shown to map in this region (Ayyub *et al.* 1989). The mapping of these genes was done with the help of essentially two chromosomal rearrangements which are the following: (1) $Df(1)sn^{c128}$ which extends from bands 7D1 to 7D5 and (2) $Dp(1;3)sn^{13al}$ which extends from 6C11 to 7C9. These have been depicted schematically in figure 1. It was therefore of interest to place the ends of these rearrangements within the clone of this walk. One end of $Df(1)sn^{c128}$ at 7D5 has been mapped by Digan *et al.* (1986). In order to begin mapping the other end of this deficiency a 2 kb EcoRI-SalI fragment (98–100 kb, figure 2) known to hybridise to band 7D1 (Roiha *et al.* 1988) was used as a probe on polytene chromosomes from male larvae of the following strain:

$Df(1)sn^{c128}/Y;\ Dp(1;2)FN107/bwD.$

In this case no hybridisation was observed to the X chromosome indicating that this fragment is uncovered by $Df(1)sn^{c128}$ (data not shown). The breakpoint for this deficiency must therefore lie further in the telomeric direction. The same fragment was then used as a probe for hybridisation to male larvae of the following strain: $Df(1)ctJ4/Y;\ Dp(1;3)sn^{13al}$. In this case hybridisation was observed to both the X chromosome and the third chromosome carrying the duplication (figure 4). Hybridisation of this fragment to the X chromosome is expected since $Df(1)ctJ4$

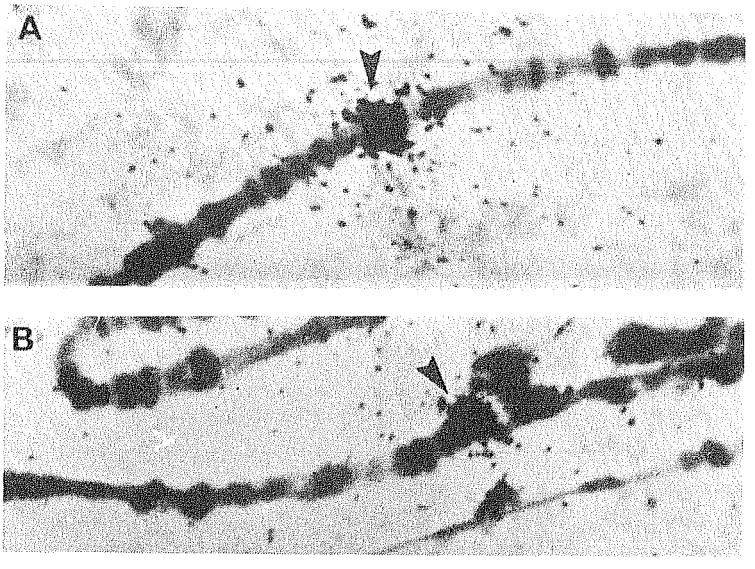

Figure 4. *In situ* hybridisation of the 2 kb EcoRI-SalI fragment of psn9 to polytene chromosomes from males of the strain $Df(1)ct^{14}/Y;\ Dp(1;3)sn^{13al}/Ki$. (**A**) X chromosome; (**B**) third chromosome. Arrowheads indicate position of hybridisation. The probe was labelled with 5'-α-[^{35}S]dATP.

uncovers the region from 7A2 to 7C1. However hybridisation to the duplication was not expected since this fragment is uncovered by $Df(1)sn^{c128}$ which uncovers from 7D1, while $Dp(1;3)sn^{13a1}$ covers till 7C9. From these data it is clear that a small region of overlap must exist between $Df(1)sn^{c128}$ and $Dp(1;3)sn^{13a1}$. The precise size of this overlap requires mapping of the breakpoints of both $Df(1)sn^{c128}$ and $Dp(1;3)sn^{13a1}$ in the 7C9–7D1 region. This is currently in progress.

A somewhat unexpected finding of this walk was the complete absence of any repetitive sequences in the 100 kb region from 7C1 to 7D1. As a general rule such sequences are encountered more frequently in the genome. For example, in the 70 kb walk described by Digan *et al.* (1986) there are two regions of repetitive DNA within 10 kb of each other. A possible explanation for the absence of repetitive sequences from the 7C1–7D1 interval may be the presence of a haplo-inviable region in this interval (Lefevre and Johnson 1973). The extent of this haplo-inviable region has not been determined. However it seems likely that insertion of repetitive elements in such a region of the chromosome would prove lethal, hence selecting for their absence. In order to prove such a hypothesis the extent of the haplo-inviable region would need to be established. I hope to do this by generating smaller deficiencies in this region and subsequently mapping their breakpoints within the clones of this walk. This should shed some light on the nature of haplo-inviable loci in *Drosophila* hitherto not understood.

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