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Overlapping Deficiencies Refine the Map Position of the Sex-Linked Actin Gene of *Drosophila melanogaster*

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Abstract. A ^{3}H-labelled actin-specific probe was hybridized to *Drosophila melanogaster* X chromosomes heterozygous for deficiencies in the 5C region. The results suggest that the sex-linked actin gene resides in the overlap region of *Df(1)C149* and *Df(1)N73* at 5C3-4.

Introduction

How the morphology of a cell is molded as it occupies its final position in a developing tissue is a major question in understanding cell and tissue differentiation. There is now no doubt that cytoskeletal molecules such as tubulins and actins play important roles in this developmental phenomenon. In addition to maintaining cell structure and cytoskeleton, actin is involved in the contractile apparatus of muscle, cell motility, and cytokinesis. A family of closely related actin proteins appears to be involved in these processes and seems to function in a tissue specific manner (Huxley, 1969; Pollard and Weihing, 1974; Korn, 1978). Since the actins differ in their amino acid sequences, they must be coded for by separate genes (Vandekerckhove and Weber, 1978a, b). Actin genes from a number of different species have been cloned and characterized, and in a majority of the cases, it appears that the actins are coded by a multigene family. Depending on the species, the number of members in the family varies from only a few to about twenty.

In *Drosophila*, six actin genes have been found and localized to six disperse loci on the polytene chromosomes by in situ hybridization: 5C on the X chromosome, 42A and 57A on 2R, 79B on 3L, and 87E and 88F on 3R (Fyrberg et al., 1980, 1981). These genes are being characterized by molecular techniques and it would be worthwhile to combine genetic approaches with this accumulating biochemical information. To do so, it is important to know linkage relationships of the actin genes, and whether they are associated with any known morphological lesions. Map assignment of the actin genes by in situ hybridization was based on invariability of

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high grain density at the six regions since the probe used in the study gave higher backgrounds than probes for a number of other genes (Fyrberg et al., 1980). Furthermore, it is clear from Bridges and Bridges (1939) and Bridges' (1941) cytological maps of the salivary gland chromosomes that the number of bands in these regions varies from 3 in 79B to 19 in 42A. A more precise localization of actin DNA sequences to the polytene chromosomes can be achieved by using overlapping deficiencies or other chromosomal aberrations where the breakpoints are known. We chose the 5C region for analysis since overlapping deficiencies in this region are available, and a number of mutant genes that affect wing morphogenesis such as *curlex, crossveinless* and *deformed antennae* are associated with bands spanning the region 5A8-5D6 (Lindsley and Grell, 1968). 5C is the locus for λDmA2 which codes for a cytoplasmic actin (Fyrberg et al., 1980).

Materials and Methods

Three sex-linked deficiencies isolated by Dr. George Lefevre were obtained from the Drosophila Stock Center, California Institute of Technology. The *Df(1)HF366* chromosome lacks bands 3E8 through 5A7 and is deficient for markers *cho, ec, bi, peb, rb, rg*, but complements *bo* and *cx* mutants (for a description of symbols, see Lindsley and Grell, 1968). This deficiency does not extend into the 5C region but was selected to visualize hybridization at a 5C locus near a heterozygous deficient region. *Df(1)C149* extends from 5A10 to 5C4±; it is deficient for the *cv* locus but not *rux*. The limits of the third deficiency used, *Df(1)N73,* are defined by breakpoints at 5C3 and 5D5-6; it is deficient for *rux* but not *cv* and *vs*. The deficiency (*Df*) chromosomes are maintained against an *FM6* or *FM7* balancer chromosome carrying *yellow* and *Bar* markers. Females heterozygous for each of the *Df* chromosomes were crossed to *yellow, chocolate* males. Larvae from the crosses were raised on cream of wheat medium seeded with live yeast for 72 h at 27° C. They were then transferred to an 18° C incubator for 12–18 h when the female larvae with black mouthparts were selected for chromosome squashes.

The actin coding sequence was isolated from a lambda-*Drosophila* DNA recombinant, λDmA2 shown in Figure 1. The Sal I fragments indicated in the Figure were subcloned into pBR322, and the 0.8 kb fragment, representing the internal actin coding region only, was used to construct a radioactive probe. The plasmid containing the 0.8 kb fragment was cut with Sal I restriction endonuclease, separated on 0.7% agarose gel by electrophoresis and electroeluted from the gel (Sodja et al., 1982). The electroeluted fragment was labelled with tritium by nick translation (Maniatis et al., 1975). ^{3}H-dCTP (107 Ci/m mol) was the radioactive nucleotide used. Specific activities of approximately 5×10^5 cpm/μg were obtained.

The preparation of the salivary gland chromosomes and subsequent hybridization with the ^{3}H-actin probe was essentially according to the standard procedures (Gall and Pardue,

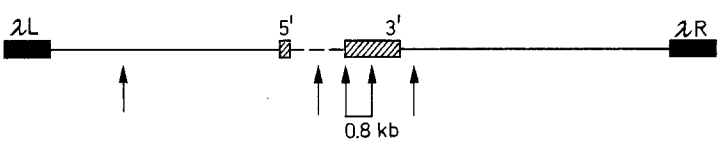

Fig. 1. A partial map of λDmA2 with the Sal I restriction endonuclease sites indicated by arrows. The Dm DNA insert is between the λL and λR blocks, the actin coding sequence is indicated with barred blocks, and the intervening sequence with a dashed line. Transcriptional orientation of the actin gene is also noted. The Sal I fragments were subcloned into pBR322 and the 0.8 kb fragment prepared for nick translation and labelled with ^{3}H-dCTP as described in Materials and Methods

1971; Bonner and Pardue, 1970). We used a nick translated DNA probe to facilitate its handling and reduce the background. Hybridization was under siliconized coverslips using 10 µl of 50% formamide (3 × recrystallized), 0.5 M NaCl, 0.01 M PIPES (pH 7.2) containing 130 µg/ml each of yeast tRNA and calf thymus DNA. Approximately 300–500 cpm were used per slide. The hybridization followed standard procedure, except that in the post-hybridization washes, the RNase step was omitted since the probe was nick translated DNA. Slides were developed and stained with Giemsa after 24 days of autoradiography.

Results and Discussion

DfHF366 is a relatively large deficiency distal to the 5C region. We selected this chromosome which is not deficient for 5C to serve as a control. Since both homologues of the HF366 heterozygous chromosome carry the 5C region, hybridization of the actin probe to these chromosomes should show silver grains over both of the paired homologues. Figure 2a shows the Df^+/*DfHF366* loop and the position of silver grains as expected to the right of the proximal breakpoint. We examined at least 20 such chromosomes where the silver grains were clearly distributed across both synapsed chromosomes. *HF366* is a large deficiency so squashes of this region of the X chromosome generally spread flat and the 5C region with its overlying silver grains was displayed clearly. However, the Df^+/*DfHF366* larvae were not as healthy as larvae from the crosses with the two smaller deficiencies and their salivary gland chromosomes were small.

Examples of in situ hybridization in chromosomes heterozygous for *Df(1)C149* are given in Figure 2b–d. Since the synapsed Df^+/*Df* region is relatively small, the chromosomes were often twisted or bent in this region making analysis difficult. Ideal spreads where the Df^+ region with the extra bands and coincident silver grains were found, however, such as in Figure 2b, c. The preparation in Figure 2d shows the pattern commonly seen where the Df^+ region protrudes as a wedge of extra bands with silver grains localized on it. This pattern is quite distinct from that found in Figure 2a. However, if the *Df* in the squashed preparation comes to rest directly below the focal plane of the Df^+ region, then the Df^+ region appears lozenge-shaped and the silver grains in this case are localized across the width of the chromosome. Thus, our interpretation of the hybridization site required evaluation of the position of the two synapsed homologues and the silver grains. In the Df^+/*DfC149* preparations we found 7 chromosomes where asynapsis extended from the telomere to the proximal region, or from the telomere to the middle section of the X chromosome. In all cases, only one of the homologues had silver grains in the 5C region. The absence of silver grains in the other homologue is consistent with the observations presented above, but we were reluctant to score these cases since the two arms were spatially separated in all cases.

Figure 2e shows an asynaptic region involving *Df(1)N73* where the asynapsis occurs over a short distance and the banding patterns of the two homologues can be clearly discerned. Silver grains are limited to the Df^+ region of the asynapsed homologue where the extra bands are clearly visible. In addition to this preparation we found 2 asynapsed Df^+/*DfN73*

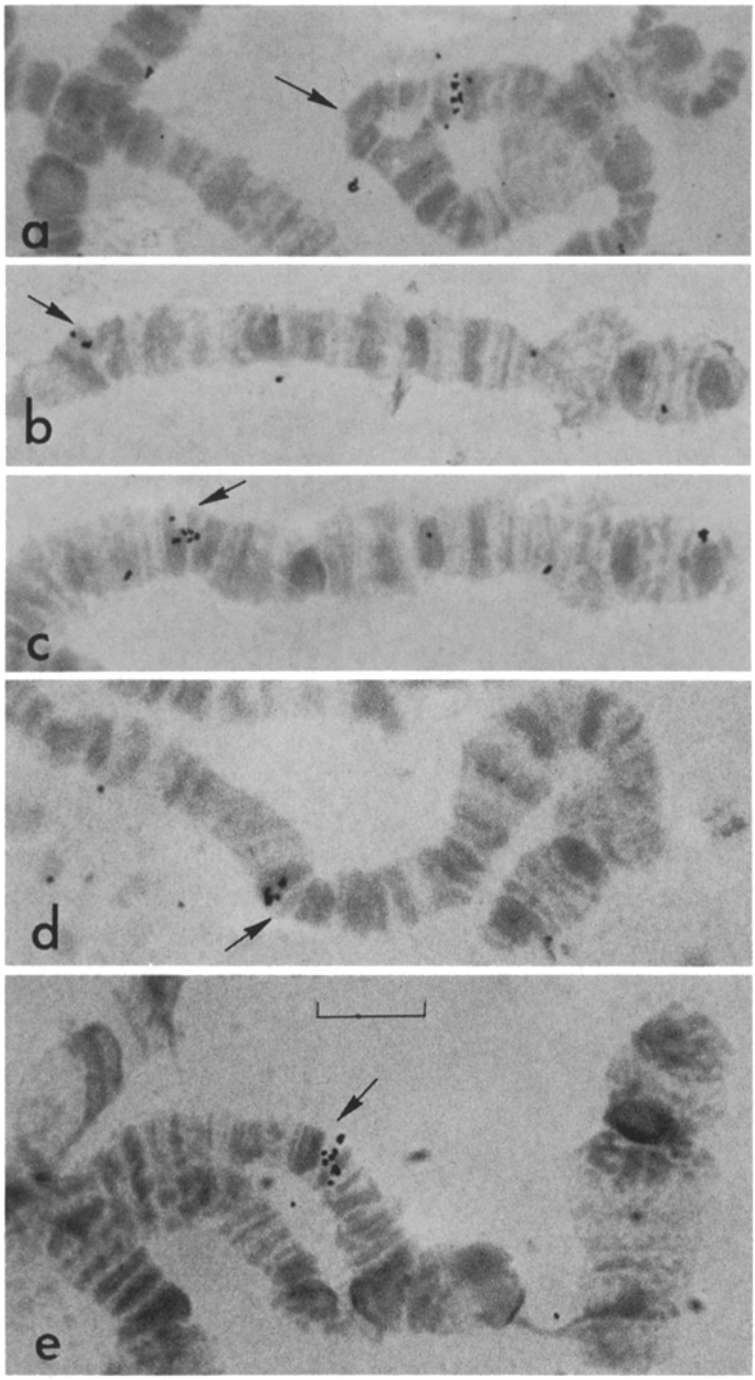

Fig. 2a–e. Autoradiographs of the distal ends of X chromosomes following hybridization with a ^{3}H-labelled actin coding sequence. The arrows point to the Df^{+} strand in each case. Bar represents 10 μm. **a** Silver grains span the 5C region lying to the right of the large Df^{+} loop in a $Df^{+}/Df(1)HF366$ chromosome. **b–d** $Df^{+}/Df(1)C149$ chromosomes showing silver grains associated with the Df^{+} homologue. When the Df^{+}/Df is squashed to clearly display the Df^{+} bands, the silver grains are localized to the right end of this region (**b**). **e** Asynapsed homologues of a $Df^{+}/Df(1)N73$ chromosome with silver grains on the Df^{+} strand

chromosomes similar to those described above for *DfC149*. We also scored 20 preparations with silver grains associated with the wedge-shaped Df^+ homologue.

Our analysis confirms that the hybridization site for λDmA2 is 5C. Furthermore, this site must reside within the region where *DfC149* and *DfN73* overlap since hybridization of the probe was restricted to one homologue of heterozygous deficient chromosomes for either *DfC149* or *DfN73*. The distribution of the silver grains to the left end of the Df^+N73 homologue (Fig. 2e) and at the right end of the Df^+C149 homologue (Fig. 2b) also agrees with this conclusion. According to Bridges' (1938) map of the X chromosome the 5C region consists of 10 bands; Dr. G. Lefevre (personal communication) places the right break of *DfC149* at 5C4± and the left break of *DfN73* at 5C3. Thus, the position of the actin gene is narrowed to band 5C3 or 5C4.

This analysis does not, unfortunately, indicate coincidence between a known morphological lesion and the actin gene. We were originally encouraged to undertake this study by the fact that 3 of the 6 hybridization sites for actin genes are located at or near loci for *crossveinless* (*cv*) mutants: 5C and *cv* on the X chromosome; 57A and *cv-2* on the second chromosome; 87E and *cv-c* on chromosome 3. The *C149* chromosome is deficient for *cv* whereas the *N73* chromosome is not. The actin gene is located within the overlap region of these two deficiencies, thus precluding a correlation between it and the *cv* locus which lies within the region of the chromosome covered by the *C149* deficiency but outside the region of its overlap with *DfN73*. On the other hand, the proximity of *cv* to the hybridization site for λDmA2 suggests that this mutant will be useful for screening deletions in the vicinity of the 5C actin gene. Direct analysis of the transcriptional activity at 5C as well as the kind and amount of actin found in the *cv* mutant may provide a better assessment of a possible interaction between these two loci at 5C.

The results presented here constitute an improvement of the in situ hybridization technique for the actin genes. We think that the use of a DNA probe with lower specific activity resulted in low backgrounds. Use of a DNA probe also reduced the amount of manipulation of the chromosome preparations since the post-hybridization RNase step was eliminated. Furthermore, the resolving power of in situ hybridization was increased by cytogenetic analysis so it was unnecessary to use *giant* larvae to obtain large chromosomes to visualize the hybridization site. Combination of cytogenetic and molecular genetic approaches will no doubt be useful for assigning DNA probes to specific bands of the salivary gland chromosomes.

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