THE GENETIC AND CYTOGENETIC LOCALIZATION OF THE THREE STRUCTURAL GENES CODING FOR THE MAJOR PROTEIN OF DROSOPHILA LARVAL SERUM

DAVID B. ROBERTS AND SUSAN EVANS-ROBERTS

Genetics Laboratory, Biochemistry Department, South Parks Road, Oxford OX1 3QU England

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ABSTRACT

The α, β and γ polypeptides that make up Drosophila Larval Serum Protein-I seem to be coded for by genes that have evolved by duplication of a common ancestral gene. We have found variants of all three polypeptides, and these are variants of the coding sequences. The α-chain variant mapped to 39.5 on the X chromosome and to the polytene interval 11A7-11B9. The β-chain variant mapped to 1.9 on chromosome 2L and to 21D2-22A1. The γ-chain variant was mapped as 0.13 map units from the tip of chromosome 3L or to −1.41 with respect to ru, which has been defined as 0.0, and to 61A1-61A6.

THE larval serum proteins (LSP-1 and LSP-2) are the major protein components of the haemolymph of Drosophila larvae shortly before pupariation (ROBERTS, WOLFE and AKAM 1977). These proteins are being studied in order to understand the mechanism of their control. As part of this program, we have mapped the coding sequence for LSP-2 to 37.0 and 68E2-4 on chromosome 3 (AKAM et al. 1978); in this paper, we describe the genetics of the coding sequences for the LSP-1 polypeptide chains.

LSP-1 is a family of proteins made up of an apparently random association into hexamers of three polypeptides that have similar amino acid compositions and share common antigenic sites (WOLFE, AKAM and ROBERTS 1977). The common antigenic sites must be due to common amino acid sequences as this protein has no associated carbohydrate (CRUMPTON, personal communication) or lipid (ROBERTS, unpublished results). The three polypeptides differ slightly in molecular weight, and the heaviest is designated as the α chain, the middle molecular weight form the β chain, and the lightest the γ chain. The similar amino acid composition and the common antigenic sites suggest that these three chains have evolved by duplication of an ancestral gene, followed by mutation. The similarity of both one- and two-dimensional peptide maps of the subunits following partial and complete digestion of the α, β and γ polypeptides support this view. Brock and Roberts, in preparation).

Our first step was to identify variants of the three polypeptide chains. These were shown to be variants of the coding sequence by quantitative studies (Rob-
ERTS and EVANS-ROBERTS 1979; AKAM et al., in preparation). The variant alleles have been mapped using standard genetic techniques and have been mapped cytogenetically, using existing deficiencies or by generating segmental aneuploids (LINDSLEY et al. 1972).

MATERIALS AND METHODS

Stocks: The Drosophila strain Oregon-R was used as the reference wild-type strain in this work and, unless indicated otherwise, the LSP-1 of all stocks was the same as Oregon-R. Stocks were maintained on standard yeast-cornmeal-agar medium in half-pint milk bottles at 25°. The other wild-type stocks were either caught locally or obtained from stock centers around the world as listed in Drosophila Information Service. The wild-type stocks that eventually yielded variants were obtained as follows: Bacup, Genetics Institute, Groningen University, The Netherlands; Formosa, Zoological Institute, Munich University, Germany; Kochi-R, Department of Genetics, Umeå University, Sweden; Ponza, Biological Institute, Vienna University, Austria; Strömsvretten-11 caught in Åkersberga, Sweden.

The mutant stocks used in this work were obtained as follows: Cy/Pm; Sb/D, v P f w, wY, Df(1)psa+ ras f/v+b-ro Y+ +/v w f=, Df(1)A26/FM7, Df(1)N12 ras v/FM6, Df(1)C246/FM6, Df(1)HA92/FM7, Df(1)HFS8/FM7, Df(2L)ai/In(2L)Cy, In(2R)Cy, E/S/Cy c8n, Df(2L)S1/In(2L)Cy In(2R)Cy, E/S/Cy c8n, Df(2L)S+ S/SM1, T(1;2)v85b lz506/y w f=, T(Y;3)P3, T(Y;3)H141 (California Institute of Technology), sc ec cv ctiv g8 f, ru h th st cu sr e8 ca (rucuca, Bowling Green, Ohio), y; Dp(1)scJ~iu ira red/TM1 mrvh (MRC Cambridge), and the segmental aneuploid stocks: R132, R108, B71 and S50 (LINDSLEY et al. 1972) (UCSD, La Jolla, California). The other stocks used have been in our possession for over five years. For details of all mutant stocks, see LINDSLEY and GRELL (1968).

Haemolymph collection: In order to collect haemolymph for analysis from individual larvae, flies were allowed to lay eggs on yeast-glucose-agar medium (10% yeast, 10% glucose, 3% agar + 0.1% Nipagin) seeded with fresh yeast suspension. Haemolymph was collected from larvae reared on this medium, as described previously (ROBERTS, WOLFE and AKAM 1977). In some experiments it was necessary to analyze the haemolymph of female larvae only, in which case the larvae were sexed according to the size and the gonads (BODENSTEIN 1950). For the experiments described here, wandering larvae (within 12 hr of pupariation) were used.

Cellogel electrophoresis: Cellogel electrophoresis was carried out according to the manufacturers' instructions (Reeve Angel Scientific, Limited). The buffers used were: 0.05 M pH 7.0 phosphate; and 0.05 M pH 9.3, barbitone/HCl. A potential difference of 6v/cm was applied to the gel for 3 hr at 4°. The gels were stained with amido schwarz 10B and washed in 5% acetic acid.

Polyacrylamide gel electrophoresis: Dodecyl sulphate polyacrylamide gel electrophoresis was carried out according to the technique described by LAEMMLI (1970) with a 1 cm 3% acrylamide stacking gel and 8% acrylamide resolving gel in the BioRad model 220 vertical slab gel apparatus. The apparatus was cooled during electrophoresis by running tap water, and the gels were run at a constant current of 10mA per gel until the bromophenol blue tracking dye reached the end. They were stained with 0.1% coomassie blue in 50% trichloracetic acid in 47.5% methanol.

For two-dimensional electrophoresis, haemolymph samples were first electrophoresed in cellogel. A strip of the cellogel 2 mm wide was cut out and the remaining frame stained to show the distribution of bands in the first dimension. The strip was layered in molten agar (12%) on top of the stacking gel in the slab gel apparatus and electrophoresis was carried out as described above.
RESULTS

Search for variants: When wild-type larval haemolymph is electrophoresed on Cellogel at pH 7.0, LSP-1 migrates as a single band (ROBERTS, WOLFE and AKAM 1977), but at pH 9.3 it migrates as a triplet of bands that can be identified by cross electrophoresis into a dodecyl sulphate polyacrylamide gel (Figure 1). The most rapidly migrating band on cellogel is the α chain, and the most slowly migrating band the γ chain, and we have shown that, at high pH, LSP-1 dissociates into monomers (see below and BROCK and ROBERTS, unpublished results).

We analyzed haemolymph from late third-instar larvae of 48 different wild-type stocks on Cellogel in 0.05 M pH 7.0 phosphate buffer (LSP-1 a single band) and in 0.05 M pH 9.3 barbitone buffer (LSP-1 a triplet). Because of the likelihood that the stocks were heterogeneous, four larvae from each stock were tested at each pH. In the first analysis, a number of stocks gave patterns unlike Oregon-R, but on retesting only five stocks consistently gave some larvae that did not have the Oregon-R pattern in one or other of the buffer systems. In no case did all the larvae from a variant stock give the same pattern, so that it was necessary to inbreed the variant stocks. This was done by setting up a number of pair matings and keeping the sibs of ten larvae, all of which gave the same variant pattern. These stocks were retested many times before they were actually established and used for further study. In this way we established six variant stocks, Ponza G8, Ponza Gla, Stömsvreten 11A, Formosa M, Bacup 3D and Kochi-RB1.

In a search for variation in the amount of LSP-1 produced by different stocks (ROBERTS, BLACKWELL and AKAM, unpublished results), a low LSP-1 producer was found to lack the γ chain. This stock carried the recessive chromosome 3 marker, eyg. During the course of this study, we found that the balancer chromosome TM6 in the stock S50/TM6 also carried the γ-chain null allele. When tested, both these stocks behaved, with respect to the γ chain, like the Ponza G8 stock.

The electrophoretic pattern of the variant stocks and of the variant Oregon-R heterozygotes are shown in Figures 2, 3 and 4 compared with the reference pattern of Oregon-R. With the exception of Bacup 3D, all of the variant stocks heterozygous with Oregon-R gave a pattern on Cellogel unlike the variant parent. The pattern of the Bacup/Oregon heterozygote is, under optimum conditions, unlike either parental pattern, but as the two parental patterns are similar under the standard gel conditions, it is frequently difficult to distinguish the heterozygote in anything other than the best gels. Thus, the alternative tris-borate acrylamide gel was developed to analyze the Bacup variant.

The Kochi-RB1 and Stömsvreten 11A stocks carry slow electrophoretic variants of the α chain, while Formosa M carries a fast electrophoretic variant. The Ponza G8 stock clearly lacks the γ chain; it is a γ-chain null allele. Ponza G8/Oregon-R heterozygotes synthesize only 50% of the γ chain synthesized by Oregon-R (unpublished results). Ponza G8 also carries a fast allele of the α chain, but this is not analyzed here. The Ponza Gla stock is complicated, and we believe that it might be heterozygous for the γ-chain null allele, the heterozygotes being
Figure 1.—Correlation of LSP-1 bands on Cellogel with LSP-1 bands on SDS acrylamide gels. Haemolymph samples from larvae were run on Cellogel at pH 9.3 and cross electrophoresed into SDS gels. The α, β and γ polypeptides of LSP-1 and the LSP-2 polypeptide are indicated. (a) Oregon-R; (b) Kochi-RB1. (Note the reduced amount of the slow-migrating α chain of Kochi-RB1.)

maintained by a balanced lethal system. We do not consider this stock further here.

The β chain in Bacup 3D is a fast-moving allele of the wild-type β chain, and in the tris-borate gel system the Bacup/Oregon heterozygote shows both β chains (Figure 3).

Genetic localization of the α-chain structural gene: Using standard genetic
crosses, the α-chain variants of Kochi-RB1, Strömsvreten 11A and Formosa M were localized on the X chromosome (Figure 4). For the more precise localization of the Kochi-RB1 variant, the protocol described in Figure 5 was used. The first experiment made use of a series of recombinants in which the recombination event had taken place in different intervals. The results are shown in Figure 5. This established that the variant locus was between \( v \) and \( g^2 \). In the second experi-
FIGURE 3.—Electrophoretic pattern of the Bacup 3D variant. Haemolymph from Oregon-R, Bacup 3D and Oregon/Bacup heterozygous larvae was electrophoresed in the tris-borate gel system. Under these conditions, LSP-1 is dissociated into its three polypeptides. The figure shows the fast allele of the \( \beta \) chain in Bacup 3D and both forms of the \( \beta \) chain in the heterozygote.

In a third experiment involving the Formosa M variant, nineteen recombinant males were tested as before, and the results are also given in Figure 5. These results are consistent with the suggestion that both Kochi-RB1 and Formosa M are variants of the same structural gene so that the data from all three experiments have been grouped to give the map position for the variant locus as 39.5 on
Figure 4.—Localization of the $\alpha$ chain variant on the $X$ chromosome. Kochi-RB1 females were crossed to Oregon-R males. Haemolymph from male and female larvae was electrophoresed on Cellogel at pH 9.3. Male larvae had the Kochi-RB1 pattern, establishing the $X$-linkage of the $\alpha$ chain. The $X$ chromosome, with 95% confidence limits from the binomial distribution of 37.8 and 41.2 map units, taking the map positions of $\nu$ and $g^2$ to be 33.0 and 44.4 map units, respectively (Lindsley and Grell 1968).

Cytogenetic localization of the $\alpha$-chain structural gene: A series of overlapping deficiencies of the region between $\nu$ and $g^2$ was supplied by the stock center at Pasadena from the collection of $X$-chromosome deficiencies deposited by G. Lefevre. These were tested to see whether the Df/Kochi-RB1 heterozygote uncovered the variant pattern or gave the heterozygous pattern. The protocol was as shown in Figure 6.

In the case of Df(1)m21b-1, flies of the genotype Df(1)m21b-1 ras f/v+B Y+ were crossed to Kochi-RB1 females. The female progeny will have the Kochi-RB1 pattern if the variant locus is uncovered by the deficiency; otherwise they will have the heterozygous pattern. Of the six deficiencies tested, only JA26 and HF368 uncovered the variant locus.

We analyzed Kochi-RB1 against the deficiencies generated in the two insertional translocation stocks, T(1;2)$\nu^{\delta b}$ and T(1;3)$v^{+74c}$. T(1;2)$\nu^{\delta b}$ did not uncover the variant locus, but T(1;3)$v^{+74c}$ did. The results giving the cytological position of the $\alpha$-chain structural gene are shown in Figure 7. We placed the $\alpha$-chain structural gene between 11A7 and 11B9, an interval of 14 bands.

In a second series of experiments, the variants Str"omsvreten 11A and Formosa M were tested against Df(1)HF368, and both were uncovered by this deficiency.

Genetic localization of the $\beta$-chain structural gene: Using standard genetic crosses, the $\beta$-chain variant in the Bacup 3D stock was localized on chromosome 2. Using techniques similar to those described for mapping the $\alpha$-chain variant, we mapped the $\beta$-chain variant between $al$ and $dp$. For a more precise localization,
the protocol described in Figure 8 was used. Twenty-eight crossovers in the \(al-dp\) region were crossed to Bacup 3D females, and 10 larvae from each cross were examined on Cellogels. The results of these crosses are given in Figure 8. The \(\beta\)-chain variant was mapped to 1.86 on the left arm of chromosome 2, with 95% confidence limits from the binominal distribution of 0.52 and 4.24 map units, taking the map positions of \(al\) and \(dp\) as 0.1 and 13.0 map units, respectively (LINDSLEY and GRELL 1968).

**Cytogenetic localization of the \(\beta\)-chain structural gene:** Deficiencies close to the \(\beta\)-chain structural gene (based on the mapping data) were made heterozygous with the variant chromosome, and ten larvae were analyzed on tris-borate gels to see whether a variant or heterozygous pattern was formed. Deficiencies \(Df(2L)S^2\)
**Figure 6.**—Protocol for the cytogenetic localization of the α-chain gene. Haemolymph from the larvae was tested on Cellogel at pH 9.3.

and Df(2L)S5 both uncovered the variant gene. This placed the β-chain structural gene between 21D2 and 22A1, an eleven band interval (Figure 9.)

**Genetic localization of the γ-chain structural gene:** The null allele of the γ chain in the variant Ponza G8 stock was localized on chromosome 3, using standard genetic crosses. For a more precise localization, the protocol described in Figure 10 was used.

The first experiment used a series of recombinant males in which the recombination event had taken place in different intervals. The results placed the variant locus to the left of h. In a second experiment, forty-three recombinants in the

**Figure 7.**—Cytological positions of the α-chain gene and of the breakpoints of the deficiencies (LeFevre, personal communication). (After Bridges 1938.)
interval *hu−h* were examined. Ten of these were of the class *+ h th st sr e⁺ ca* and all carried the variant allele. The remaining thirty-three were all *ru + + + + + +* and none carried the variant allele. *ru* maps at 0.0 and *h* at 26.5 map units on the left arm of chromosome 3 ([Lindsley and Grell 1968](#)), which places the variant locus either to the left of *ru* or, with 95% confidence limits from the binomal distribution, with 2.1 map units to the right of *ru*. 

**Figure 8.—** Protocol for mapping the β-chain variant. Haemolymph from the larvae was tested on Cellogel at pH 9.3.

![Diagram](image)
Although \textit{ru} is defined as 0.0 on the standard chromosome 3 map, it is known from cytological and genetic data to be some distance from the tip of 3L, a minimum of 23 bands and four map units, respectively (Lindsley and Grell 1968). In order to map the variant locus to the left of \textit{ru}, we constructed the stock \( y^{+}; Dp(1)sc^{ld}ve/TM1 mwh \), which marks the tip of 3L with \( y^{+} ve \). In constructing this stock, the frequency of crossovers between the tip of 3L and \( ve \) was 1.7%.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9.png}
\caption{Cytological positions of the \( \beta \)-chain gene and of the breakpoints of the deficiencies. (After Bridges 1938.)}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure10.png}
\caption{Protocol for mapping the \( \gamma \)-chain variant. Haemolymph from the larvae was tested on Cellogel at pH 9.3.}
\end{figure}
(51/3004). The distance between the tip and mwh from the same study was 1.24 map units, and between mwh and ve, 0.46 map units. (cf., 0.5 map units, ROBERTSON and RIVIERA 1972). Combining the data of ROBERTSON and RIVIERA with the present data allows us to redraw the tip of 3L as in Figure 11. This result, while in good agreement with ROBERTSON and RIVIERA, does not agree with the distance of 4.0 map units between the tip of 3L and ru cited by LINDSLEY and GRELL (1968).

The protocol for mapping the γ-chain variant to the left of ru is shown in Figure 12. These results place the γ-chain structural gene at 0.13 map units from the tip of 3L, with 95% confidence limits of 0.01 and 0.41 map units. The cytological position for ve is given as 61E2–62A6, but as it maps to the right of ru, whose cytological position is 61F5–62A3, the true cytological position of ve must be between 61F5 and 62A6. The recombination frequency in this region lies between 0.043 and 0.053 map units per band. This compares with a value of 0.1 map units per band calculated for the ru to h interval from the data given in LINDSLEY and GRELL (1968).

Cytogenetic localization of the γ-chain structural gene: To localize the γ-chain structural gene cytologically, we made use of the segmental aneuploid stocks gen-

![Figure 11](image1)

![Figure 12](image2)

**Results of 27 recombinants**

<table>
<thead>
<tr>
<th>Parental phenotype</th>
<th>Heterozygous pattern</th>
<th>Ponza pattern</th>
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<tbody>
<tr>
<td>y+ve+</td>
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<tr>
<td>y ve</td>
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10 larvae examined

FIGURE 12.—Protocol for mapping the γ-chain variant to the left of ve. Haemolymph from the larvae was tested on Cellogel at pH 9.3.
erated by LINDSLEY et al. 1972. This approach was made particularly easy by the fact that heterozygous deficiencies for a considerable length of 3L are viable. The technique used for this analysis is illustrated in Figure 13. All of the segmental aneuploid stocks used, including the stock generating the shortest deletion of the tip of 3L, S50, uncovered the γ-chain null allele. This places the structural gene between the tip (61A1) and 61A6, which is in the 6-band interval, and is in good agreement with the genetic localization. These results are summarized in Figure 14.

In a second series of experiments, we used two duplications, \( T(Y;3)H141 \) and \( T(Y;3)P3 \), which have 61B–62D and tip of 3L to 61E–F inserted into the Y chromosome. The experimental design is illustrated in Figure 15. In this experiment, we demonstrated that the γ-chain coding sequence must lie between the tip and 61E–F,—on \( T(Y;3)P3 \)—but not in the region 61B–62D, i.e., not in \( T(Y;3)H141 \), which agrees with the results using deficiencies. These results are summarized in Figure 14.

DISCUSSION

The genetic data presented here show that the genes for the LSP-1 α, β and γ chains map to different chromosomes, although we believe that these genes have descended from a common ancestral gene. Moreover, quantitative studies on the synthesis of these proteins suggest that they are coordinately synthesized and that in the female they are produced in equimolar amounts (POWELL, SATO and ROBERTS, in preparation), whereas in the male only 50% of the amount of the X-
linked α chain found in females is produced (Roberts and Evans-Roberts 1979).

Since gene duplication was first observed in Drosophila (Bridges 1936; Muller 1936), its significance in evolution has been discussed on many occasions (Horowitz 1945; Ohno 1970). Duplicated genes fall into two classes, those that have been duplicated by some mechanism such as unequal crossing over and have remained closely linked, forming a gene cluster, and those that have been duplicated but are scattered through the genome. The scattering of these duplicates may have been brought about by translocation after duplication, or they may have been scattered as a consequence of the duplication, for example by the incorporation of a duplicated part or all of a chromosome into the genome. Another possible mechanism for gene duplication in Drosophila would be if a gene were moved in association with a transposing element (Ising and Ramel 1976) to a second chromosome; from this fly, a stock with four copies of the gene could be selected. As there is no obvious similarity in the banding pattern of the regions carrying the LSP-1 genes, it is unlikely that gross chromosomal translocations or duplications account for this scattering. Thus, this last suggestion involving transposing elements may be particularly appropriate in this case.

Gene clusters have been studied extensively (see review by Bodmer 1979); perhaps the best example at the molecular level is the cluster of genes coding for the β, γ, δ and ε chains of haemoglobin. Haemoglobin is in fact the best example for comparison with the α, β and γ chains of LSP-1 because the gene coding
Figure 15.—Protocol for the cytogenetic localization of the \( \gamma \)-chain gene using duplications. Haemolymph from larvae was tested on Cellogel at pH 9.3.

for the \( \alpha \) chain of haemoglobin, which associates with one of the other chains to form an active molecule, is in a different linkage group. In discussing this, Bodmer (1979) pointed out that “in all those cases where the genetics of dimeric proteins that are made up of different subunits is sufficiently well advanced, the genes for the two different chains are on different chromosomes, or at least very loosely linked.” The \( \alpha \), \( \beta \) and \( \gamma \) chains of LSP-1 fall into this category.

We have suggested that the dispersal of the LSP-1 genes may be “recent,” as the \( \alpha \)-chain gene on the \( X \) chromosome has not yet acquired dosage compensation (Roberts and Evans-Roberts 1979), and we are studying the evolution of these proteins by comparing the proteins of different Drosophila species and comparing the distribution of their coding sequences by in situ hybridization with cDNA prepared from the mRNAs.

It is interesting to speculate why the coding sequences for polypeptides that associate to form an active protein are dispersed. Especially interesting is the case
of the LSP-1 $\alpha$, $\beta$ and $\gamma$ chain genes, as we believe that their polypeptides are coordinately synthesized. The coordination of synthesis of these polypeptides is not achieved as in bacteria by the close association of their coding sequences. Bodmer (1979) has observed that in many cases only one gene in a cluster is expressed at any one time, and that for two related genes to be expressed simultaneously, the strategy for control in higher eukaryotes may demand that they be unlinked (or no more than loosely linked). If this is so, then a study of the control of the LSP-1 polypeptides in Drosophila, an organism that is amenable both to classical genetic analysis and to techniques involving recombinant DNA, will go a long way to test this hypothesis.

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