Proc. Natl. Acad. Sci. USA
Vol. 76, No. 5, pp. 2385–2389, May 1979
Genetics

Mutation generating a fragment of the major heat shock-inducible polypeptide in *Drosophila melanogaster*

(heat shock puffs/abnormal polypeptide/chromosomal rearrangements)

C. CAGGESE, R. CAIZZI, M. MOREA, F. SCALENGHE, AND F. RITOSSA

Institute of Genetics, University of Bari, Via Amendola 165 A, Bari, Italy

Communicated by M. S. Meselson, February 22, 1979

ABSTRACT *Drosophila melanogaster* tissues carrying a third chromosome with the deletion *Df(3R)kar*^{D2} make a 40,000-dalton (Dal) heat shock protein not made by wild type. The unusual polypeptide was inducible in every tissue examined. Tryptic peptide fingerprints showed it to include part of the 70,000-Dal major heat shock protein. Mapping experiments placed the mutation responsible for the 40,000-Dal protein at or close to the *kar*^{D2} deletion. One break point of the deletion is in subdivision 87A, close to or at a heat shock locus that codes for the 70,000-Dal protein. The results are consistent with the possibility that this break point is within a gene for the 70,000-Dal protein, leaving only the initial portion of its coding sequence. This would specify the direction of transcription of the mutant gene as proximal to distal on the normal chromosome. The 87A heat shock locus should contain at least two genes for the 70,000-Dal protein, because embryos homozygous for the *kar*^{D2} deletion and lacking the heat shock locus at 87C, which also codes for the 70,000-Dal protein, nevertheless produced both the 40,000-Dal and the 70,000-Dal proteins upon temperature elevation. Using the presence of the 40,000-Dal protein to monitor chromosome segregation, we found that embryos homozygous for deletions of the heat shock puff site at 93D exhibited a normal electrophoretic pattern of heat shock proteins.

Heat shock (HS) and certain other treatments induce puffs at specific sites on the polytene chromosomes of *Drosophila* (1–3) and induce the synthesis of specific polypeptides (4–6). The response is not tissue specific (7) and occurs also in cultured cells (6). From induced cultured cells, messenger RNAs have been purified that hybridize *in situ* at the heat shock puff sites (6, 8–10). The presence of DNA sequences coding for the major heat shock polypeptide [70,000 daltons (Dal)] at both regions 87A and 87C is indicated by the finding that messenger RNA that codes for the 70,000-Dal polypeptide in a cell-free system (11, 12) cosediments with RNA that hybridizes to both regions (11). This conclusion has been verified by showing that cloned DNA fragments that hybridize at 87A and 87C also hybridize to messenger RNA for the 70,000-Dal polypeptide (13, 14). Such localization is also supported by the observation that embryos homozygous for deletions of both 87A and 87C fail to produce the 70,000-Dal polypeptide (15). Both 87A and 87C appear to contain multiple copies for the genes for the 70,000-Dal HS polypeptide (16).

We report here a mutation that leads to the induction after HS of an unusual polypeptide whose sequence is part of the major HS polypeptide. The mutation maps at region 87A and appears to result from a deletion that removes part of a gene for the 70,000-Dal HS protein.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

***Drosophila melanogaster* Strains.** We obtained the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome in two steps. The inverted chromosome *In(3R)AFA, e* was selected as an ebony (*e*) mutation after x-irradiation (17). The *kar*^{D2} deletion was found in the inversion after further x-ray treatment and selecting for *kar* mutations (18). *In(3R)AFA, e Df(3R)kar*^{D1} obtained in the same experiments appears to be identical to *In(3R)AFA, e Df(3R)kar*^{D2} and presumably owes its separate designation to a stocking error. *In(3R)AFA, e* and *Df(3R)kar*^{D2} have been described previously (19) and are further analyzed here. Deletions *Df(3R)e*^{D7}, *Df(3R)e*^{F1} and *Df(3R)e*^{F3} were obtained after x-ray treatment. Details of their cytology are given elsewhere (19, 20). The lethal mutation *l(3)R12* was found after ethyl methanesulfonate treatment and is not complemented by the *kar*^{D2} deletion. The mutations *l(3)AFA7* and *l(3)AFA17* were found in *In(3R)AFA, e* chromosomes after ethyl methanesulfonate treatment. Neither of them is complemented by the deletions *e*^{D7}, *e*^{F1}, or *e*^{F3}. Flies were cultured as described (19).

Analysis of the HS Polypeptides. The method of Tissières *et al.* (4) was followed as described (20). Larvae were shocked at 36.5°C for 20 min and 1–10 salivary glands were incubated for 20 min at 23°C in 5–10 μl of Ringer's solution containing 200–600 μCi of [^{35}S]methionine per ml (200–1000 Ci/mmol) (1 Ci = 3.7 × 10^{10} becquerels). In mapping experiments HS protein patterns were also studied on single adult males. Testis and ejaculatory ducts were excised in Ringer's solution under oil, shocked for 15 min at 36.5°C, and then incubated with [^{35}S]methionine as described above. After incubation, the tissues were successively washed with cold 10% trichloroacetic acid, absolute ethanol, and chloroform/methanol (1:1, vol/vol), 20 min each. After drying, the polypeptides were extracted at 90°C for 1.5 min with a solution containing 2% (vol/vol) sodium dodecyl sulfate ($NaDodSO_4$)/2% (vol/vol) 2-mercaptoethanol, 0.02 M Tris·HCl at pH 7.5, and 10% (vol/vol) glycerol. Electrophoresis was done on $NaDodSO_4$/acrylamide gels (12, 20, 21), which were then stained with Coomassie blue and autoradiographed (20). For preparative purposes, about 90 salivary glands were used in groups of 15. Larvae were shocked as above, but incubations with [^{35}S]methionine were for 1 hr at 23°C. The stained bands were cut from the gels and the polypeptides were eluted overnight into dialysis bags (12). The polypeptides were precipitated in the cold with 10% trichloroacetic acid, and the precipitate was collected and washed with absolute ethanol and ether. Further purification was accomplished by a second $NaDodSO_4$/acrylamide gel.

Abbreviations: HS, heat shock; Dal, dalton(s); $NaDodSO_4$, sodium dodecyl sulfate.

Pulse Labeling of Embryos. The technique of Ish-Horowicz *et al.* (15) was followed. Adults were maintained on yeast-enriched medium. Eggs laid over a period of 1 hr were collected and after 18–20 hr the embryos were collected and washed. For the analysis of crosses, embryos were placed in plastic wells and incubated 20 min with 20 μl of Ringer's solution at 23°C (controls) or at 37°C (HS). The solution was removed and 2.5 μl of Ringer's solution containing [^{35}S]methionine was pipetted over the embryo, which was then punctured and its membranes were removed. Incubation was for 45 min at 23°C. When selected embryos were used, the chorions were first removed in 2% NaOCl for 1 min. After washing, the embryos were selected under the dissection microscope and treated as described above. After incubation each embryo was extracted with 40 μl of extraction solution. Electrophoretic analysis was done as described for salivary glands.

Partial Digests and Fingerprinting. The precipitated polypeptides were dissolved in 0.5% $NaDodSO_4$/0.05 M Tris·HCl at pH 7.5/10% glycerol and digested with α-chymotrypsin (Serva, Heidelberg, W. Germany; three times crystallized) according to Cleveland *et al.* (22). After digestion, $NaDodSO_4$ was adjusted to 2% and mercaptoethanol was added to a concentration of 5%. The samples were kept at 100°C for 1.5 min and electrophoresed on $NaDodSO_4$/acrylamide gels. Fingerprinting was as described by Allet *et al.* (23) and Mirault *et al.* (12). Precipitated polypeptides were washed with ethanol and ether. The dried precipitate was oxidized with 0.1 ml of performic acid on ice. Performic acid was removed by two washes with 0.2 ml of water followed by lyophilization. Samples were dissolved in 0.1 ml of 0.05 M ammonium bicarbonate at pH 8.5 containing trypsin at 100 μg/ml (Worthington, 241 units/mg) and kept 1 hr at 37°C. After the addition of another 10 μg of trypsin, incubation was continued overnight. Bidimensional chromatography was done on cellulose (20 × 20 cm Polygram Cel 300), eluting with pyridine/isoamyl alcohol/water (7:7:6, vol/vol) in the first dimension and with butanol/pyridine/acetic acid/water (5:4:1:4, vol/vol) in the second dimension. Kodirex plates were used for autoradiography.

RESULTS

We observed that various *Drosophila* tissues carrying the rearranged chromosome *In(3R)AFA, e Df(3R)kar*^{D2} synthesized an unusual polypeptide after heat shock. This polypeptide has an apparent molecular weight of 40,000 on $NaDodSO_4$/acrylamide gels (Fig. 1). The rearranged chromosome is usually stocked with markers *cu kar Sb* (24). Homozygous ebony (*e*) females were crossed to *cu kar Sb/In(3R)AFA, e Df(3R)kar*^{D2} males. Because the *cu kar Sb* chromosome carries a wild allele of ebony while the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome carries ebony, progeny larvae that carry either of the parental chromosomes can be selected because ebony larvae have dark trachael spiracles. Several e^+ and *e* larvae from the cross were collected and heat shocked, and the excised salivary glands were incubated with [^{35}S]methionine. As shown in Fig. 2, only tissues from the ebony larvae exhibit the 40,000-Dal polypeptide. Thus, the mutation leading to the synthesis of the 40,000-Dal polypeptide after HS is associated with the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome.

Analysis of the Polypeptide. The partial α-chymotrypsin digest of the 40,000-Dal polypeptide contained a series of fragments that comigrated with fragments obtained, after

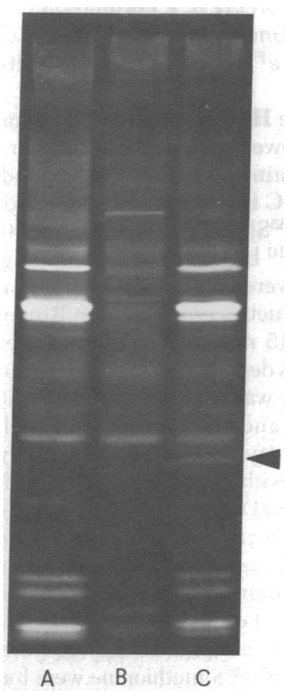

FIG. 1. Electrophoretic analysis of HS polypeptides of tissues carrying the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome. Five mature larvae were kept 20 min at 36.5°C. Salivary glands were excised and incubated with [^{35}S]methionine. Protein extracts were electrophoresed on $NaDodSO_4$/10% acrylamide gels (12) and autoradiographed. Samples contained about 6 × 10^5 cpm. Exposure was for 6 hr. Lane A, heat-shocked *In(3R)AFA, e/+*. Lane B, *cu kar Sb/In(3R)AFA, e Df(3R)kar*^{D2} uninduced control. Lane C, heat-shocked *cu kar Sb/In(3R)AFA, e Df(3R)kar*^{D2}. An arrow indicates the 40,000-Dal HS polypeptide.

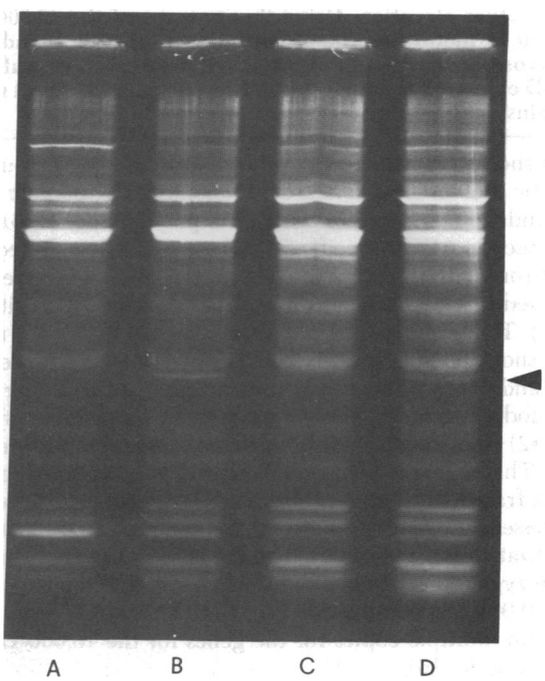

FIG. 2. Association of the mutation leading to the 40,000-Dal HS polypeptide with the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome. Homozygous *In(3R)AFA, e* males were crossed to *cu kar Sb/In(3R)-AFA, e Df(3R)kar*^{D2} females. Selected larvae were heat shocked and their salivary glands were pulsed with [^{35}S]methionine. Protein extraction, electrophoresis, and autoradiography were done as for Fig. 1, but $NaDodSO_4$/10% acrylamide gels were as described by Laemmli (21). Lane A, ebony$^+$ larvae that were *cu kar Sb/In(3R)AFA, e*. Lane B, ebony larvae that were *In(3R)AFA, e/In(3R)AFA, e Df(3R)kar*^{D2}. Lane C, homozygous *In(3R)AFA, e*. Lane D, *In(3R)AFA, e Df(3R)-kar*^{D2}*/cu kar Sb*.

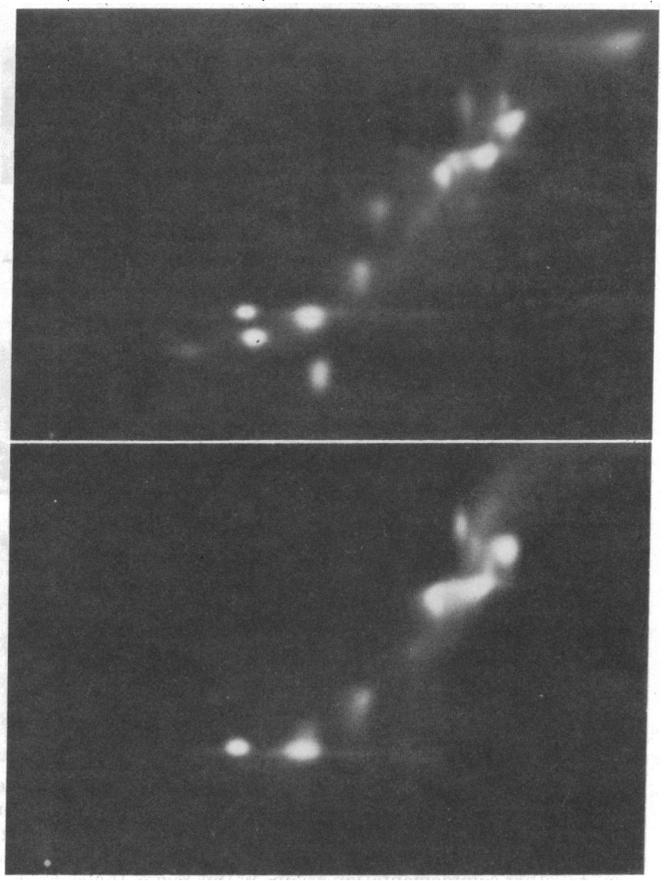

FIG. 3. Tryptic digests of the 70,000-Dal and 40,000-Dal HS polypeptides. ^{35}S-Labeled polypeptides from *cu kar Sb/In(3R)AFA, e Df(3R)kar*^{D2} larvae were digested and chromatographed as described in the text. (*Upper*) The 70,000-Dal HS polypeptide, 10^5 cpm, exposure 7 days. (*Lower*) The 40,000-Dal polypeptide, 2×10^4 cpm, exposure 35 days.

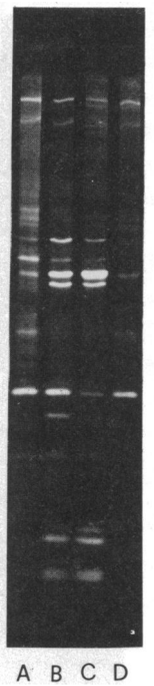

FIG. 4. Induction of the 40,000-Dal HS polypeptide in embryos homozygous for the *kar*^{D2} deletion. Polypeptides synthesized by single embryos were labeled with [^{35}S]methionine and electrophoresis on $NaDodSO_4$/10% acrylamide gels was followed by autoradiography. Lane A, uninduced *In(3R)AFA, e Df(3R)kar*^{D2} homozygotes. Lane B, heat-shocked *In(3R)AFA, e Df(3R)kar*^{D2} homozygotes. Lane C, heat-shocked Canton S embryos. Lane D, Canton S uninduced controls.

similar treatment, from the major 70,000-Dal HS polypeptide (data not shown). Fingerprints of the [^{35}S]methionine peptides obtained after trypsin digestion also showed similarity between the 40,000-Dal and the 70,000-Dal HS polypeptides (Fig. 3). The fingerprint of the 40,000-Dal polypeptide showed the complete absence of some of the methionine peptides of the 70,000-Dal HS polypeptide, while spots corresponding to all the major [^{35}S]methionine fragments of the 40,000-Dal polypeptide were present in the fingerprint of the major HS polypeptide.

HS Proteins of Homozygous *In(3R)AFA, e Df(3R)kar*^{D2} Embryos. Embryos homozygous for the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome die before hatching. They can be recognized because of a series of abnormal traits, including the almost complete absence of visible treacheae. If heterozygous females and males carrying the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome are crossed, 25% of the progeny are expected to show no 40,000-Dal polypeptide after HS and the remaining 75% are expected to exhibit its induction. Of 113 embryos from a cross of *cu kar Sb/In(3R)AFA, e Df(3R)kar*^{D2}, 28 had no 40,000-Dal polypeptide after HS, while it was induced in the remaining 85. Thus, the rearranged chromosome segregates regularly. All the embryos that showed induction of the 40,000-Dal polypeptide also showed induction of the 70,000-Dal polypeptide. From a cross of *In(3R)AFA, e Df(3R)-kar*^{D2}*/In(3R)AFA, e*, 30 nonhatching embryos deficient in visible tracheae were individually heat shocked and labeled with [^{35}S]methionine. They all synthesized the 40,000-Dal polypeptide. None of the known HS polypeptides was missing, although, in comparison with wild type, the amount of the 70,000-Dal polypeptide was consistently reduced relative to most other HS proteins. We noted that while salivary glands (Figs. 1 and 2), epidermis, imaginal discs, and 18-hr embryos (Fig. 4) did not show detectable amounts of radioactive 40,000-Dal polypeptide in the uninduced controls, a polypeptide of similar molecular weight was observed in young embryos of various genotypes.

HS Polypeptides of Embryos Homozygous for Deletions of 93D. Previously, we reported (20) that embryos lacking the region at 93D where a large puff is inducible by HS do not show induction of the major 70,000-Dal HS polypeptide. This conclusion was based on the analysis of HS proteins from randomly chosen embryos generated by crossing flies heterozygous for deletions in this region, without knowledge of their genotypes. The presence of the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome can be monitored because of the inducibility of the 40,000-Dal polypeptide. Embryos generated by crossing *In(3R)AFA, e Df(3R)kar*^{D2}*/Df(3R)e*^{D7} (the e^{D7} deletion lacks the HS puff site at 93D) were heat shocked and labeled with [^{35}S]methionine. Embryos from this cross that show no 40,000-Dal polypeptide are *Df(3R)e*^{D7}*/Df(3R)e*^{D7}. In these embryos the 70,000-Dal polypeptide was unambiguously inducible. None of the usual HS polypeptides seemed missing (Fig. 5). Thus, we must retract our earlier conclusion that the HS locus at 93D is essential for induction of the 70,000-Dal HS protein (20).

Cytology of the *In(3R)AFA, e Df(3R)kar*^{D2} Chromosome. The left breakage point of the inversion in the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome is in 86C (17, 19). Its right breakage point is between bands 93D2 and 93D4, with this last band not included in the inversion (Fig. 6 *C* and *D*). The right breakage point is just proximal to the HS puff inducible in 93D and is

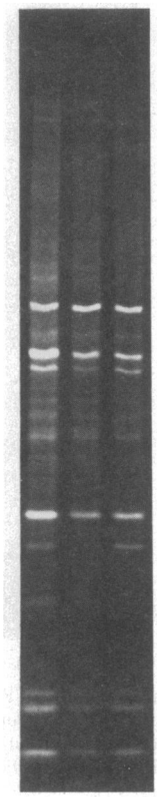

FIG. 5. HS polypeptides of three unselected single embryos from a cross between *In(3R)AFA, e Df(3R)kar*^{D2}/*Df(3R)e*^{D7} flies. The embryo analyzed on lane B had no 40,000-Dal HS polypeptide, showing that it was homozygous for the *Df(3R)e*^{D7} deletion. Nevertheless, the HS polypeptide pattern was normal, as may be seen by comparison with lane C of Fig. 4.

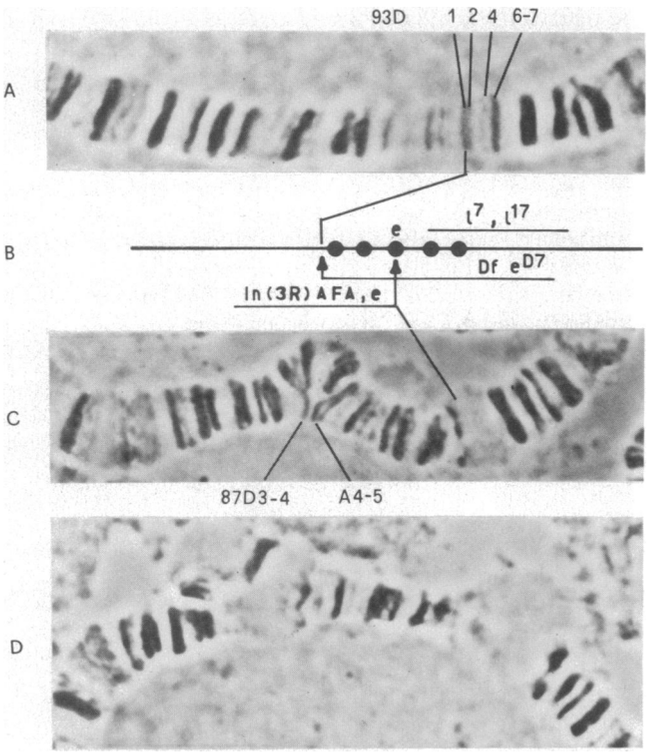

FIG. 6. Cytology of the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome. (×1900.) (*A*) Wild-type Canton S; (*B*) some genetic details of region 93D. The left break point of *Df(3R)e*^{D7} and the right point of breakage of *In(3R)AFA, e* are indicated relative to the site of ebony (*e*) and several other complementation groups in this region (black knobs). The approximate positions of *l(3)AFA7* and *l(3)AFA17* are also indicated. (*C*) *In(3R)AFA, e Df(3R)kar*^{D2}/*In(3R)AFA, e*; (*D*) chromosome from larva of the same genetic composition as in *C* but HS puffs are induced.

associated with a mutation at the ebony locus. This ebony mutation is not complemented by the *e*^{D7} deletion, whose left breakage point is between bands 93D1 and 93D2. As depicted in Fig. 6*B*, there are at least two lethal complementation groups to the left of the *e* locus that are not complemented by *Df(3R)e*^{D7}. The *kar*^{D2} deletion, located within the *AFA, e* inversion, includes the locus *kar*, many lethal complementation groups, and the HS puff site at 87C. The breakage points of the deletion are close to 87D3-4 and 87A4-5, although these bands are not deleted. The latter breakage point is close to the inducible puff at 87A, which is just outside of the deletion (Fig. 6*D*).

Mapping of the Mutation. In crosses such as those described in Fig. 2 we observed no segregation of the mutation leading to the 40,000-Dal HS polypeptide from the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome. The mutation is not carried by the original *In(3R)AFA, e* chromosome and is absent in other derivatives of this chromosome: that carrying the *Df(3R)kar*^{D3} deletion, which removes the puff sites at 87A and 87C (18, 19), and that carrying the *Df(3R)kar*^{D4} deletion, which removes neither of the puff sites (18). The possibility exists that the *kar*^{D2} deletion itself generates the 40,000-Dal HS polypeptide. Mapping experiments were done to test this possibility. Recombination was studied by associating the *In(3R)AFA, e Df(3R)kar*^{D2} chromosome with *In(3R)AFA, e* chromosomes, so that recombinants in the regions neighboring the *kar*^{D2} deletion could be recovered. Given the location of the *kar*^{D2} deletion (Fig. 6 *C* and *D*), to test whether the mutation leading to the 40,000-Dal HS polypeptide maps to the right of the *kar*^{D2} deletion, the *In(3R)AFA, e l(3)AFA7* chromosome was used. The lethal mutation *l(3)AFA7* belongs to a complementation group that maps from 0.19 to 0.4 units to the right of the ebony locus in normal chromosomes (data not shown). Thus, *l(3)AFA7* maps to the right of the right point of breakage of the *AFA, e* inversion, because this break point is associated with an *e* mutation (17). *In(3R)AFA, e Df(3R)kar*^{D2}/*In(3r)AFA, e* (*l(3)AFA7* females were crossed to *l(3)R12 H Df(3R)e*^{F3}/Ubx^{130}, *e*^{s} males. *l(3)R12* is lethal in association with the *kar*^{D2} deletion (25) and *Df(3R)e*^{F3} is a deletion of region 93D and is lethal with *l(3)AFA7*. After the cross, flies were selected that were not *Ubx* and survived with the *l(3)R12 H Df(3R)e*^{F3} chromosome. They were *H* and *e* in phenotype and are expected to be recombinants between *kar*^{D2} and *l(3)AFA7*. The outcome of the cross was 26,411 *Ubx e* and 166 *H e*. The putative recombinant chromosomes were singly stocked. Larvae carrying them were heat shocked and their salivary glands were pulsed with [^{35}S]methionine. Of the 166 *H e* individuals obtained, 54 were tested. All but one had apparently normal *AFA, e* chromosomes and none carried the mutation leading to the 40,000-Dal HS polypeptide. One *H e* recombinant appeared to be a product of unequal exchange: it carried a duplication of the 87A region, the *kar*^{D2} deletion, and showed the 40,000-Dal polypeptide after HS. The genetic distance between the *kar*^{D2} deletion and the *l(3)AFA7* mutation computed from the cross data and taking into account that *In(3R)AFA, e*/*l(3)R12 H Df(3R)e*^{F3} controls have only 50% survival, is 1.25 ± 0.13 units (95% confidence). Because none of the 53 recombinants showed the mutation leading to the 40,000-Dal HS polypeptide, we conclude that this mutation is not located to

the right of the kar^{D2} deletion at a distance greater than 0.07 units (95% confidence) under our experimental conditions. To test whether the mutation leading to the 40,000-Dal HS polypeptide maps to the left of the kar^{D2} deletion, *In(3R)AFA, e* $Df(3R)kar^{D2}$/*In(3R)AFA, e* females were crossed to *kar* males. Progeny males that were phenotypically either *kar* (*AFA, e* kar^{D2}/*kar*) or kar^{+} (*AFA, e*/*kar*) were selected and used to test whether the *AFA, e* chromosome they carried contained the mutation leading to the 40,000-Dal HS polypeptide. The inducibility was directly tested on the excised male apparatus of these flies. Alternatively, they were singly crossed to ebony females and the analysis was made on the salivary glands of the ebony larval progeny. If the mutation leading to the 40,000-Dal HS polypeptide is located to the left of the kar^{D2} deletion, recombinational events may unlink the two mutations and the 40,000-Dal HS polypeptide may be observable in kar^{+} males after the cross described above. Of the 178 *kar* males tested, all carried the mutation. Of the 154 kar^{+} males also tested, none carried the mutation. Thus, the mutation leading to the 40,000-Dal HS polypeptide does not map to the left of the kar^{D2} deletion at a distance higher than 0.9 units (95% confidence). The mapping experiments are consistent with the possibility that the mutation leading to the 40,000-Dal HS polypeptide is associated with one of the breakage points of the kar^{D2} deletion.

DISCUSSION

If a deletion extends part way into the coding sequence of a gene from some point beyond its distal end, one expects transcription to initiate normally but to terminate abnormally. If the abnormal message reaches the cytoplasm and can be translated, it is expected to direct the synthesis of a polypeptide of abnormal size, either longer or shorter than the normal polypeptide, the size being dependent upon the location of the break point within the coding sequence and the occurrence of a transcription stop, an RNA processing termination, or a nonsense codon in the abnormal message. The 40,000-Dal HS polypeptide synthesized by genomes carrying the *In(3R)AFA, e* $Df(3R)kar^{D2}$ chromosome clearly contained part of the 70,000-Dal HS polypeptide (Fig. 3). At least most of the DNA sequence coding for the unusual 40,000-Dal HS polypeptide appeared to be identical to part of a DNA sequence that specifies the 70,000-Dal HS polypeptide. The finding that the mutation that leads to the 40,000-Dal HS polypeptide maps at region 87A is in accord with the known location in this region of some of the genes for the 70,000-Dal HS polypeptide (11, 13–15). The mutation that leads to the 40,000-Dal HS polypeptide seems to be associated with the kar^{D2} deletion, making it likely that the abnormal polypeptide arises as described above. This remains to be proven, however, by a direct examination of DNA and messenger RNA. If our interpretation of the mutation is correct, the direction of transcription of the partially deleted HS gene in region 87A is leftward on the rearranged chromosome shown in Fig. 6, that is, proximal to distal on the normal chromosome. The observation that both the 70,000-Dal and the 40,000-Dal HS polypeptides are made by embryos homozygous for the kar^{D2} deletion, which removes the puff at 87C, suggests that the puff inducible at region 87A contains at least two genes coding for 70,000-Dal polypeptide (16).

Finally, it should be noted that the results presented here, making use of a deletion of the 93D region, in accord with recent data (11, 13, 14) rule out the possibility we formerly advanced, that the puff inducible at that region contains structural sequences for the 70,000-Dal HS polypeptide (20).

We thank Miss A. M. Contini, Mr. N. Di Turi, and Mrs. L. Gentile for help in selecting mutations and stock-keeping, and Mr. V. Di Turi for photographic work. Results similar to ours have been obtained with embryos carrying the *In(3R)AFA, e* $Df(3R)kar^{D2}$ chromosome by Dr. D. Ish-Horowicz. We thank him for this communication. The present work was supported by Consiglio Nazionale delle Ricerche Contract N77/01490.

1. Ritossa, F. (1962) *Experientia* **18,** 571–573.
2. Berendes, H. D., van Breugel, F. M. A. & Holt, Th. K. H. (1965) *Chromosoma* **16,** 35–46.
3. Ashburner, M. (1970) *Chromosoma* **31,** 356–376.
4. Tissières, A., Mitchell, H. K. & Tracy, U. M. (1974) *J. Mol. Biol.* **84,** 389–398.
5. Lewis, M., Helmsing, P. J. & Ashburner, M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3604–3608.
6. McKenzie, L. S., Henikoff, S. & Meselson, M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1117–1121.
7. Ritossa, F. (1964) *Exp. Cell Res.* **35,** 601–607.
8. Spradling, A., Penman, S. & Pardue, M. L. (1975) *Cell* **4,** 395–404.
9. Bonner, J. J. & Pardue, M. L. (1976) *Cell* **8,** 43–50.
10. Spradling, A. & Pardue, M. L. (1977) *J. Mol. Biol.* **109,** 559–587.
11. McKenzie, S. L. & Meselson, M. (1977) *J. Mol. Biol.* **117,** 279–283.
12. Mirault, M. E., Goldschmit-Clermont, M., Moran, L., Arrigo, A. P. & Tissières, A. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 819–827.
13. Schedl, P., Artavanis-Tsakonas, S., Stewart, R., Gehring, W. J., Mirault, M. E., Goldschmidt-Clermont, M., Moran, L. & Tissières, A. (1978) *Cell* **14,** 921–929.
14. Livak, K. J., Freund, R., Schweber, M., Wensink, P. C. & Meselson, M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5613–5617.
15. Ish-Horowicz, D., Holden, J. J. & Gehring, W. J. (1977) *Cell* **12,** 643–652.
16. Henikoff, S. & Meselson, M. (1977) *Cell* **12,** 441–451.
17. D'Alessandro, A., Ritossa, F. & Scalenghe, F. (1977) *Drosophila Information Service* **52,** 46.
18. Costa, D., Ritossa, F. & Scalenghe, F. (1977) *Drosophila Information Service* **52,** 140.
19. Scalenghe, F. & Ritossa, F. (1976) *Atti Acad. Naz. Lincei* **13,** 439–528.
20. Scalenghe, F. & Ritossa, F. (1977) *Chromosoma* **63,** 317–327.
21. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
22. Cleveland, D. W., Fisher, S. G., Kirschner, W. M. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252,** 1102–1106.
23. Allet, B., Katagiri, K. J. & Gesteland, R. F. (1973) *J. Mol. Biol.* **78,** 589–600.
24. Lindsley, D. L. & Grell, E. J. (1968) *Genetic Variations of Drosophila melanogaster,* Carnegie Institution of Washington Publication No. 627 (Carnegie Inst. of Washington, Washington, DC).
25. Cascitelli, R. (1977) Dissertation (Univ. of Bari, Bari, Italy).