A Maternal Effect Mutation Leading to Deficiencies of Organs and Homeotic Transformations in the Adults of Drosophila

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Summary. The temperature sensitive mutation \( fs(1)h \) is characterized at the restrictive temperature of 29°C by both a maternal effect responsible for the early embryonic lethality and pupal zygotic lethality. The two phenotypes are inseparable and map at a short deletion in the X chromosome (7D1, 7D5~5). At semi-permissive temperatures, hemizygous mutant females produce adults with morphological defects, such as organ deficiencies and homeotic transformations of haltere to wing and third leg to second leg. These defects depend on the maternal genotype and are governed by an early temperature sensitive period, which covers the end of oogenesis and the first hours of embryogenesis. Furthermore, this maternal effect mutation interacts with some dominant mutations of the bithorax system. These properties suggest that \( fs(1)h \) is somehow involved in segmental determination.

Key words: Maternal effect mutant – Homeotic-mutants – Pattern formation – Drosophila

Introduction

In Drosophila, during the first hours of embryogenesis (i.e., during nuclear multiplication), the development of the embryo depends on products synthesized by maternal genes. The zygotic nuclei appear inactive during this time and begin to produce RNA only at the blastoderm stage (Zalokar 1976; Lamb and Laird 1976; Anderson and Longay 1979). At this stage or shortly afterwards, segmental determination, which is probably dependent on positional information present in the egg (Wolpert 1971; Schubiger 1976; Sander 1976), occurs (Wieschaus and Gehring 1981). Maternal effect mutations provide a tool with which to analyse this information, since informative molecules in the egg must be supplied by the mother. In the past ten years, numerous mutations causing death or abnormal development of embryos have been isolated which depend on the genotype of the mother (Bakken 1973; Rice and Garen 1975; Gans et al. 1976; Mohler 1977).

Few, however, have been shown to have an effect on the process of determination. Among those that have are two mutations which severely disturb the basic embryonic pattern, bicaudal and dorsal; their characteristics suggest that this pattern is defined by two gradients, one antero-posterior and one dorso-ventral (Nüsslein-Volhard 1977, 1979, Nüsslein-Volhard et al. 1980).

Maternal effect mutants producing adults displaying homeotic transformations could be especially useful for studying imaginal determination. Very few are known and only two have been described in any some, tumorous-head (Gardner 1970; Bourdais-Vardiabasis and Bownes 1978) and Regulator of bithorax, discovered by Lewis (Garcia-Bellido and Capdevilla 1978) and its alleles (Ingham and Whittle 1980). Here we describe another maternal effect mutation which causes both organs deficiencies and homeotic transformations of a bithorax type. This temperature sensitive mutation, previously described by Gans et al. (1975) under the n° 1456 and here renamed \( fs(1)h \), is characterized at the restrictive temperature by an embryonic lethality, and at the semi-permissive temperature by the production of adults with missing metathoracic and abdominal structures. We have further observed that when the females are hemizygous for the mutation, some of their offspring have bithorax-like homeotic transformations. Furthermore, the mutation interacts with some dominant mutations of the bithorax system.

Materials and Methods

Stocks

The stocks used were: \( fs(1)h/FM3, M5 \) or \( FM6, se ec ec et g f \, cm.\) \( \text{cm}^2 \) m² (for description of mutations see Lindsley and Grell 1968), \( Df(1)me^{128} \) (Lefevere and Johnson, 1973) obtained from G. Lefevere and \( Ubx^{TM1}, Df(3)red/TM1, Df(3)red^{P93b} Shl/3\text{in}3L/P+\text{Sb}18\text{MeUbx}^{1} \), stocks obtained from A. Garcia-Bellido.

Culture Media

The Standard medium used was composed of corn flour, brewer’s yeast, agar and methylhydroxybenzoate. The egg laying medium contained fresh yeast, succarose, vinegar, agar and neutral red.

Temperature Shifts

The temperature sensitive periods for embryonic lethality and for the morphological abnormalities of the adults were determined by a single exposure of the females or embryos to a higher temperature.

For the study of the temperature sensitive period during embryogenesis, the eggs were either collected on laying medium then washed and placed on thin paper, or collected directly on paper covered with yeast laid on the laying medium so that they could be rapidly shifted from one temperature to another. For the study of the temperature sensitive period during oogenesis, females were transferred from one temperature to another, and the eggs laid during successive periods of a few hours were collected.

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Embryonic Lethality

The shifts were performed between 20°C (permissive temperature) and 25°C (restrictive temperature) and the eggs collected for 30 min periods.

Adult Abnormalities

The shifts were performed between 16°C and 23°C (semi-permissive temperature). The eggs were collected for 30 min periods at 23°C and for 1 h periods at 16°C.

The temperatures were controlled to within ±0.5°C.

Results

Genetic Localization

The mutant was characterized firstly by the failure of eggs laid by mutant females at 29°C to develop and secondly by a zygotic dependant lethality at the pupal stage at this temperature. In a previous experiment, both properties of the mutant had been localized to about 17 UC0 on the X chromosome; no recombinants could be recovered where the two mutant characters were separated. A more precise localization has been obtained first by using marked X chromosomes. The recombinants were tested by complementation with $fs(1)h$ for female sterility and zygotic lethality at 29°C. We were again unable to separate the two traits. The experiment yielded a localization between 0.1 map units to the left of and 3.7 map units to the right of $sn$.

We then used deletion mapping and have localized the gene(s) governing female sterility as well as zygotic lethality in $Df(1)sn^{C128}$ to a short deficiency of five or six bands (7D1, 7D5-6) including and extending to the right of $sn$ (Lefevre and Johnson 1973).

It thus appears probable that both female sterility and pupal lethality depend on a single mutation; however, only the characterisation of new alleles can provide a proof of this assumption.

Influence of Temperature and Dosage of $fs(1)h$

on the Mutant Phenotype

Zygotic Lethality: The viability of the mutant progeny of heterozygous females: $fs(1)h/FM6$ and $Df(1)sn^{C128}/FM6$, crossed to $fs(1)h$ males, was tested at different temperatures (Table 1). The zygotic lethality at 28.5°C is complete for the $Df(1)sn^{C128}/fs(1)h$ females whereas 10% of the homozygous mutant females or males survive. Thus, $fs(1)h$ appears to be hypomorphic.

Maternal Effect: In the experiment reported in Table 2, the progeny at different temperatures of three types of females, $fs(1)h/FM6$, $Df(1)sn^{C128}/fs(1)h$, and $Df(1)sn^{C128}/FM6$, crossed to $fs(1)h$ males, were studied simultaneously. The females were of similar age and were raised at the test temperature, except for the females tested at 29°C. In this case, mutant homozygous and hemizygous females which eclosed at 25°C were shifted to 29°C four days before the test; under these conditions the whole temperature sensitive period takes place at 29°C.

Eggs laid by homozygous females at 29°C do not hatch and Zalokar et al. (1975) observed that they do not develop further than the gastrula stage. At 25°C, about 40% failed to hatch, and these died mostly as segmented embryos. Dead embryos and newly hatched larvae were reared for their segmentation pattern. More than 80% displayed a partial or complete deficiency of one or several segments in the thoracic and anterior abdominal regions.

Table 1. Viability of $fs(1)h$ at different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>20°C</th>
<th>25°C</th>
<th>28.5°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 $fs(1)h/fs(1)h$</td>
<td>177±0.95</td>
<td>222±1.09</td>
<td>19±0.09</td>
</tr>
<tr>
<td>2 $Df(1)sn^{C128}/fs(1)h$</td>
<td>113±1.13</td>
<td>92±0.80</td>
<td>0±0.00</td>
</tr>
<tr>
<td>3 $fs(1)h/y$</td>
<td>193±1.03</td>
<td>215±1.05</td>
<td>29±0.13</td>
</tr>
</tbody>
</table>

Flies 1 and 3 were obtained from the cross FM6/fs(1)h $\delta \times fs(1)h$ $\delta$. Flies 2 from the cross FM6/Df(1)sn$^{C128}$×fs(1)h $\delta$. The viability is expressed as the ratio: Number of flies 1, 2 or 3/Number of their FM6 sisters.

A large percentage of the hatched embryos died at the larval and pupal stages (50% and 12% respectively). Thus the lethality is not specific to a developmental stage and occurs earlier in development at higher temperatures.

At semi-permissive temperature (23°C and 25°C) the adult progeny show morphological defects: absence of parts of the metathorax, either the halteres or, less often, the third legs. These deficiencies are not due to a failure of evagination of the imaginal discs, since upon dissection fragments of non-eva
ginable disc material in the interior of the fly were observed in only 5% of the cases. Females are less viable than males and present a higher percentage of such defects as we observed regularly in other experiments. For example, in a first experiment at 23°C, the percentages of flies lacking halteres and third legs respectively were 9% and 5% among 450 females and 2% and 1% among 430 males. In a second experiment at 24°C, they were 16% and 4% among 159 females and 4% and 1% among 211 males. Very few malformations or partial deletions of halteres were observed (<0.5% in the preceding experiments). Partial deletions of the third leg were more frequent (<2%) and crippled legs were almost as frequent as complete leg deficiencies. Duplications of metathoracic structures were very rarely observed. The flies also have defects in the abdominal segments. These consist of totally or partially missing tergites or contralateral fusions between tergites (about 50% of the flies at 25°C), and of partial or complete absence of the second sternite (about 20% of the flies at 25°C).

Both the penetrance of the defects and the size of the abnormal area increase with higher temperatures. At 23°C, the defects are mainly restricted to the lack of a single haltere or leg. At 25°C, the two halteres and the third legs can be missing. Sometimes crippled or missing second legs or wings are noted and some of the unclosed adults show deficiencies of the first leg or of a part of the head.

All the mutant characters are also observed in the progeny of Df(1)sn$^{C128}$/fs(1)h females, and at identical temperatures are expressed more strongly than in the progeny of homozygous $fs(1)h$ females. The most striking feature is the appearance among the adults obtained at 25°C, of a relatively important percentage of flies carrying homeotic transformations of metathorax to mesothorax. The transformed areas are generally small and apparently restricted to the anterior compartment. Dorsal transformations of haltere to wing, appearance of fragments of notum, and ventral transformations of the sternopleura of the third leg to the second leg were scored (see Table 2). Distal transformations of the third leg into second leg, i.e., the presence