Studies of Maternal - Effect Mutations Affecting Embryonic Segmentation in *Drosophila melanogaster*

An Honors Thesis (ID 499)

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INTRODUCTION

A mutation that alters the spatial organization of embryonic structures can be utilized to study the early stages of embryonic pattern formation. Many of these mutations affecting the spatial pattern and thus the embryonic fate map, are present in the embryo by way of the maternal genome (Schubiger and Newman, 1980; Nusslein-Volhard and Wieschaus, 1980). These maternal - effect mutants may have an integral role in the explanation of the actual patterning process. In an attempt to explain this process, Meinhardt (1977) proposed a model in which the mutant phenotypes could be explained by the presence or absence of a morphogenetic gradient in which the autocatalytic "activator", in conjunction with a long ranging, more diffusable "inhibitor", establishes a morphogen concentration that supplies positional information. If this model is correct, then a single mutation could affect the entire embryonic pattern. This is in contrast to the theory of localized determinants which states that there should exist a whole class of maternal - effect mutants, each causing a single formation failure while the rest of the embryo is unaltered. Recent research on segmental mutants appears to support Meinhardt's proposal (Nusslein-Volhard et. al., 1980; Meinhardt, 1977).

Meinhardt believed that the important interval for embryonic organization was between egg deposition and blastoderm completion. It is in this period that the proposed morphogenetic gradient based on the kinetics of molecular reaction and movement is established. The

morphogen source is suggested to be located at the posterior pole of the embryo, the site where the activating substance stimulates production of itself and the faster diffusing inhibitor. This inhibitor diffuses and suppresses activator production outside the activated area. At the active site, the activator concentration increases by autocatalysis until the loss by diffusion equals the net production. Thus, in a steady state condition, the inhibitor is made primarily in the region of the sharp activator peak. The morphogen production is controlled by the localized activator concentration, but it is the inhibitor that actually provides the positional information; the individual embryonic structures are formed over a particular range of inhibitor concentration. Meinhardt states that the determination of cells is a sequence of steps with all cells beginning in a determined state corresponding to the most anterior embryonic structure. Determination of other structures involves passing irreversibly through lower levels of determination until the final level, controlled by the local morphogen concentration, is reached. This adaptation of the level of determination to increasing morphogen concentration is the rate limiting process in organization. Molecularly, the inhibitor drives the biochemical oscillation of an enzyme capable of advancing the determination. This oscillation terminates at the level of determination correlated with the inhibitor. Thus Meinhardt's model is based on two assumptions: that a morphogen source is activated at the posterior pole by autocatalysis and a long range inhibitor, with the inhibitor itself acting as a morphogen; and that determination proceeds stepwise and irreversibly until it corresponds to the level of morphogen concentration.

Many mutations affecting embryonic patterning have been induced in the laboratory in order to investigate the phenomenon of spatial

organization and its possible explanation by a morphogenetic gradient. Kalthoff and Sander (1968), and Sander (1961) used ultraviolet irradiation of Smittia and Euscelis eggs to observe organizational defects such as double abdomen formation and the omission of segments. Meinhardt (1977) reviewed their results and explained all of the irregularities by the formation or repression of new activator peaks in the morphogenetic gradient that could cause multiple structures (a result of a second activator peak forming), or a reduced number of segments (a result of the repression of the activator which causes a lesser amount of inhibitor to be made and diffused). These type of segment omissions were also found as a result of ligations performed in the work of Schubiger and Newman (1980). Nusslein-Volhard and Wieschaus (1980) have identified fifteen loci which, when mutated, alter larval segment patterns. These include three classes of mutants: segment polarity mutants, pair rule mutants, and gap mutants. L.E. Engstrom, in the process of related research, identified a type of segment mutation in which the number of latter dorsal segments was greatly reduced. The tissue in this case did not remain undifferentiated, but rather appeared as one or more oversize segments. A mutant similar to this is the dorsal mutant classified by Nusslein-Volhard et.al. (1980). This mutant is defective in structures derived from the ventral region of the blastoderm, including muscles and part of the ventral hypoderm. Nusslein-Volhard suggested that the dorsal mutant, rather than eliminating a localized determinant, affects a continuous property such as a morphogenetic gradient that defines the entire dorso-ventral coordinate. This appears to be in agreement with Meinhardt's theory except that Meinhardt dealt exclusively with the antero-posterior coordinate and did not develop the concept of a dorso-ventral gradient.

To account for this type of dorsal segment mutation, Deak (1980) suggested that once the antero-posterior gradient is formed and the inhibitor gradient has been registered by a series of operons, a dorso-ventral gradient develops, leading to the activation of new determination steps across the dorsoventral axis of the egg. The anterior-posterior gradient is established shortly before or at the blastoderm stage, while the dorsoventral gradient is not formed until the first few hours of embryogenesis. Thus each area of the hypoderm has both an anterior-posterior and dorsoventral morphogen value. Interactions at the gradient interfaces maintains the gradients. Deal then proposes that this maintenance of the two gradients leads to the growth of imaginal discs, the cells of which, at the time of metamorphosis, interpret the two gradients as positional information. In this way, the unit of localized determination, the imaginal disc, may be incorporated into a gradient system.

This study attempted to map the mutation responsible for the observed lack of segments of a Drosophila melanogaster embryo and to show that such a single maternal-effect point mutation could cause the patterning defect.

MATERIALS AND METHODS

The mutation was mapped by scoring the embryonic progeny of single recombinant males backcrossed to heterozygous females from the original mutant stock. The mutant stock was found and isolated by a screen for embryonic lethal mutants (i.e. female steriles) on the first chromosome. The balanced stocks were established from individual males heterozygous for the (.0125 M) ethyl methane sulphonate treated first chromosome. FM7 was used as a balancer for the individual first chromosomes. The bar eye mutant was used as a marker for the mutant stock.

Stocks: + - To be treated with EMS

y cv v f car - Markers on the first chromosome to be used to find map position

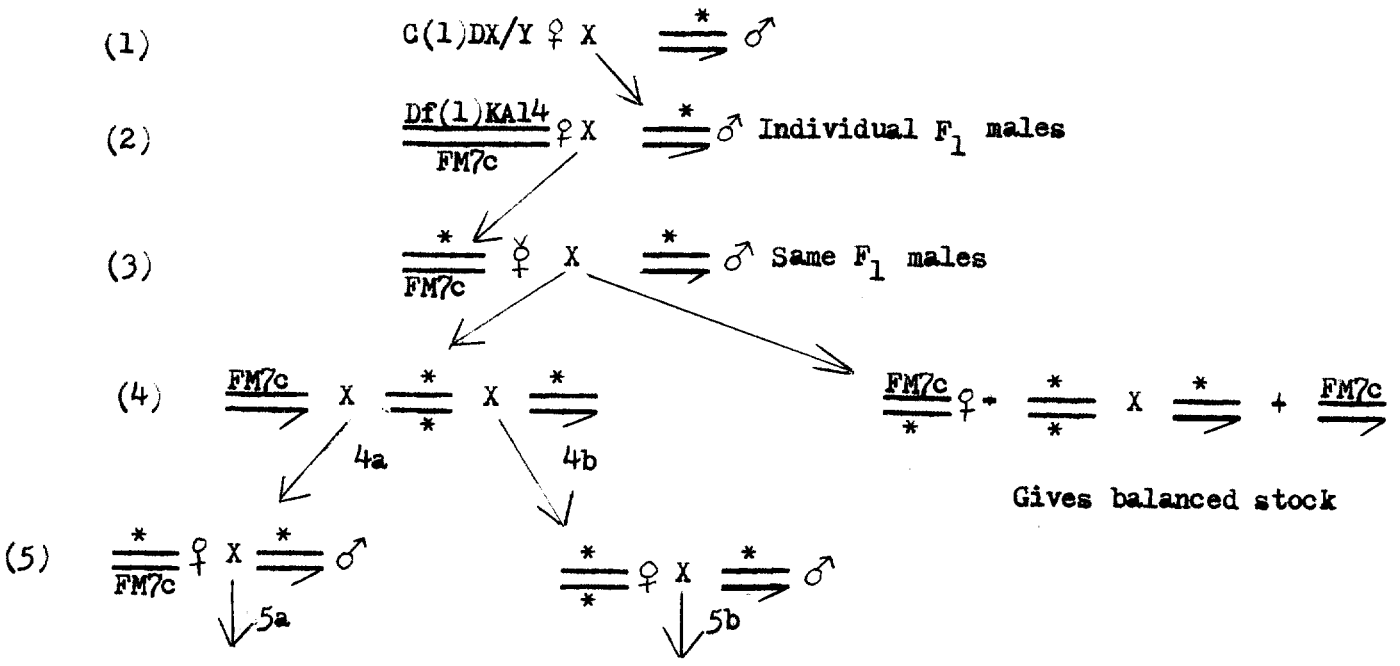
C(1)DX - To be mated to heterozygous males

Df(1)KA14 - To be used in screen

All stocks were originally obtained from the Mid-American Stock Center in Bowling Green, Ohio. Mutant genes are described in Lindsley and Grell (1968).

Procedure A: Mating scheme of screen for female steriles

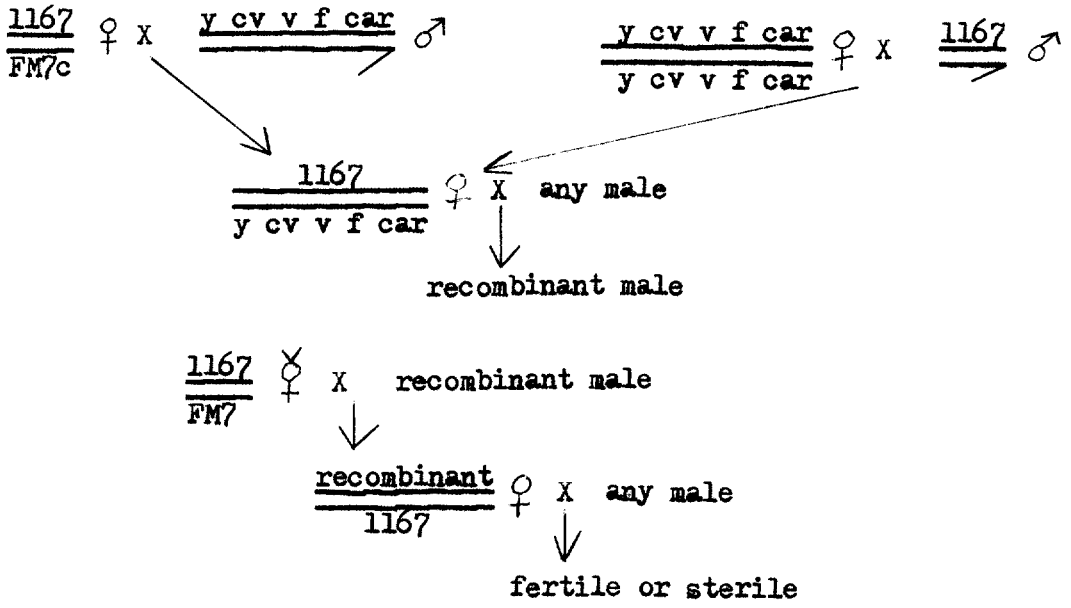
The mutagenized males now heterozygous for the first chromosome are mated to virgin C(1)DX/Y females.



The progeny from cross 4a are either sterile or nonsterile while those from 4b are sterile but possibly rescuable. Cross 5a progeny may have been male-rescued or else are grandchildless. Cross 5b progeny will be grandchildless and possibly agametic.

Procedure B: Crosses to obtain single recombinant males

Females of the balanced stock obtained from cross 3b of the previous mating scheme (now labeled as 1167 stock), and now carrying a Bar eye marker, are crossed to y cv v f car males. At the same time, y cv v f car females are crossed to 1167 males. The recombinant males obtained will be backcrossed to virgin 1167 females and the progeny scored according to the fertility or sterility of the parent. The map position of the mutation can then be determined.



RESULTS

Scoring of embryonic progeny of recombinant males backcrossed to heterozygous females. Due to knowledge obtained in a preliminary study that indicated the location of the mutation to be in the y cv v region of the chromosome, the f and car markers were disregarded.

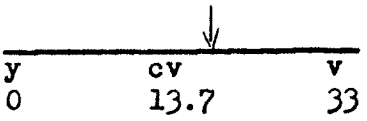
Recombination Phenotype	Number Fertile	Number Sterile
y + +	113	0
y cv +	97	0

RESULTS cont.

Recombination Phenotype	Number Fertile	Number Sterile
y + v	2	0
+ cv v	2	36
+ cv +	0	7
+ + v	1	103
	215	146

Total number of crosses is 361.

Number of double crossovers is 3 3/361 equals .83 map units
The position of the mutation on the chromosome is thought to be .83 map units to the right of the crossveinless marker.



DISCUSSION

The data obtained from this mapping procedure may not be considered very reliable because of the relatively small number of recombinants involved and the strong possibility of improper classification of recombinants. Since the general position of the mutation on the chromosome is known, further mapping using stocks deficient in regions of the general area should be done.

The mechanics of how the mutation actually causes the omission of segments remain to be determined. Although some evidence contradicting Meinhardt's gradient proposal has been found (Bumow, 1980), the elaborated gradient system suggested by Deak may present an explanation of this type of segmental mutation. Since the anterior-posterior development of the mutant embryo seems fairly normal, possibly the gradient defining the dorsoventral coordinates has been disturbed. Meinhardt (1977) explained

segmental deficiencies in anterior-posterior coordinates as a result of a shifting of the activating substance anteriorly, thus suppressing the original activator peak and giving a shallower gradient of inhibitor. Since the inhibitor concentration is known to supply the positional information, a change in its distribution could cause the lack of segment formation. If these "activator" and "inhibitor" substances could be biochemically identified, quantitative studies could be undertaken on the various mutations and the legitimacy of the proposed gradient could be determined.

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