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MUTATIONAL ANALYSIS OF DEVELOPMENT

IN DROSOPHILA MELANOGASTER

Edward B. Lewis, Principal Investigator

Professor of Biology,

Division of Biology, California Institute of Technology

Pasadena, California 91109

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ABSTRACT

Our work uses X-rays and chemical mutagens to analyze a small region of the third chromosome of *Drosophila* known as the bithorax gene complex. We are interested in this region because we find that it controls the course of development of certain major segments of the body. Hence our work is related to the problem of how genes control the course of development of an organism. We hope to find out to what extent, if any, the kinds of genetic control mechanisms found in microorganisms apply to a complex higher organism like *Drosophila*. The work in progress has three phases: 1) genetic analyses of the bithorax cluster itself and of regulator-like genes in the vicinity of that cluster; 2) the use of fluorescent chromosome staining to identify and follow chromosomal fragments carrying the bithorax gene cluster; and 3) biochemical studies of a possible nonsense suppressor in *Drosophila*; namely, the suppressor of Hairy-wing mutant.

PROGRESS REPORT

Progress has been made in deriving rearrangements which will enable the regulator of postbithorax mutant (Rg-pbx) to be contained in a small chromosomal fragment carrying a Y centromere. This approach to studying gene regulation by being able to vary the number of doses of a regulator-like mutant now appears especially promising in the light of our findings that the *Drosophila* Y chromosome stains differentially with the acridine dye, quinacrine.

Several investigators, starting with Zech, a colleague of Caspersson, noticed that quinacrine mustard and later quinacrine itself specifically stains the human Y chromosome (see brief review in Nature 226:897, June 6, 1970). We decided therefore to undertake a study of the staining of *Drosophila* cells with quinacrine, which is the more readily available dye.

We have found (in collaboration with Loring Craymer, an undergraduate student at Caltech) that in *Drosophila melanogaster* not only the Y chromosome but the fourth chromosome as well shows intense yellow fluorescence after staining with quinacrine and examining under the microscope with UV illumination. The Y chromosome contains at least three brightly fluorescing regions in the long arm and at least one fluorescing region in the short arm. The fourth chromosome shows three fluorescing regions: one corresponding to a band in the middle of the chromosome; another corresponding to bands 101 F1-2 of the salivary gland chromosomes; and a third region proximal to the 101 F1-2 bands. These observations are based on analyses of chromosomes in larval neuroblast and imaginal disc nuclei as well as in salivary gland chromosome nuclei.

The following kinds of aberrant chromosomes have been analyzed: attached X-Y-short (Stern); attached-X-Y-long; ring Y-long (Muller); attached Y-short-X-Y-long (Lindsley and Novitski); and translocations between Y and the third

chromosome; and between the third and the fourth chromosomes.

We have also studied the staining of somatic resting nuclei of larval and adult tissues with quinacrine to determine if it would be possible to distinguish cells with a Y chromosome from those without a Y chromosome at a time when the chromosomes are not visibly distinguishable. The impetus for this was the realization that staining with quinacrine might provide a method of correlating internal with external tissue compositions of gynandromorphs. The results make it likely that it will be possible to "sex" most cells of the body in this way. Thus, adult *Drosophila* brain cells show a single bright fluorescing body which is much brighter in the XY male than it is in the XX female. We interpret this body in the resting nucleus as the normal chromocenter, which is known to be formed by the fusion of the proximal heterochromatic regions of all of the chromosomes. We have observed that the basal heterochromatic regions of the X, second and third chromosomes (particularly 3R) contain weakly fluorescing bodies which evidently coalesce with the fourth chromosomes to form a single fluorescent body in resting nuclei of adult XX females. The much brighter body in the corresponding nuclei of adult XY males is evidently the result of inclusion within the chromocenter of the heterochromatic and highly fluorescent Y chromosome. Sex per se is not involved since the XY male is indistinguishable in this regard from the XXY female; and XO male resting nuclei are indistinguishable from XX female resting nuclei.

Remarkably, the resting nuclei of imaginal disc cells have a single fluorescing body in XX or XO tissues but tend to have two such bodies, of approximately equal fluorescent intensity, in XY or XXY resting nuclei. We interpret this to mean that in these rapidly dividing tissues the Y often does not have time to fuse with the chromocenter. We have also extended this observation by studying males carrying an extra Y-long arm attached to X in addition to a normal Y.

Such males show three bodies in the resting nuclei of their imaginal wing discs instead of two suggesting that extra Ys do not tend to fuse with one another in imaginal disc tissue.

We are preparing a publication with photographs illustrating these findings. Several of these photographs are appended to this application.

We propose to continue attempting to construct a selective system which will enable us to do fine structure mapping of the Ultrabithorax locus of the bithorax complex. We have failed to obtain sufficient fertility from the complex strains already derived for this purpose to have a practical method of pursuing this problem. Therefore no progress on this phase of our work can be reported this year.

Dr. K. Asano has been isolating and characterizing *Drosophila* transfer-RNA from the adult flies. Preparations of tRNA have been isolated not only from wild-type but also from flies homozygous for the mutant, suppressor of Hairy-wing. This suppressor was one of the first suppressors found in *Drosophila* (C. B. Bridges in 1923). Although this suppressor was lost, we isolated a second occurrence in 1948. We have shown that this second occurrence, symbolized su^2-Hw , suppresses a wide variety of alleles at many loci. Since this mutant is sterile in females we have devised a special system of deriving large numbers of mutant flies for chemical analysis. The specific cross we use is as follows: $Df-su^2-Hw$ Sb / $In(3L)P, Mé + In(3R)Ubx^{P18}$ males are mated to su^2-Hw Ubx / Tm1, Mé females (Sb = Stubble; Mé = Moiré; Ubx = Ultrabithorax). The only progeny from this mating which survive to the adult stage are the su^2-Hw / $Df-su^2-Hw$ class; since Ubx / Ubx; Tm1 / Sb and Mé / Mé zygotes die at very early stages of embryonic or larval development. Inversions in the Tm1 chromosome prevent recombination from breaking up the recessive lethals combinations in the parental females.

A control group of flies of genotype $su^2-Hw / +$ have been derived in a similar way, by starting with parental males which were identical to those just described except that they lacked the deficiency for su^2-Hw in the Sb-bearing chromosome. In order to achieve an approach to isogenicity in these experiments, we induced during the contract year the deficiency for su^2-Hw in this Sb-bearing chromosome and have maintained it in balanced form since its inception over the $Mé Ubx^{P18}$ inversion complex.

Fig. 1. Prophase chromosomes of a larval neuroblast from an attached X-Y male. The small fourth chromosomes appear as a brightly staining pair of dots. The X-Y attached chromosome consists of a long arm of the Y chromosome with three bright staining regions followed by a faintly staining X chromosome terminating in a weaker fluorescent body corresponding to the short arm of Y.

Fig. 2. Early prophase chromosomes of a larval neuroblast from a male of the ring Y-long strain of Muller. Note two widely separated bright bodies corresponding to the fourth chromosomes and one circular chromosome containing five or six fluorescent regions corresponding to the ring Y-long chromosome. The thread-like chromosome with a terminal weakly fluorescent region is the X-Y-short chromosome of this strain. The other thread-like chromosomes are the remaining two pairs of autosomes.