

A FURTHER ANALYSIS OF LOCI IN THE SO-CALLED
"INERT REGION" OF THE X CHROMOSOME
OF DROSOPHILA

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INTRODUCTION

THE so-called "inert region" of the X chromosome of *Drosophila* was originally supposed by MULLER and PAINTER (1932) to consist of a line of relatively inactive genes comparable in number per unit of length of the mitotic chromosome to those in the active region. But it was later shown by MULLER and GERSHENSON (1935) that the breaks in this so-called "inert region" took place preferentially in certain discrete positions, and they accordingly proposed the hypothesis that there were relatively few genes in this region, but that these genes, or at any rate certain individual genes amongst them, are represented by a relatively great amount of chromosome material during the mitotic stage of the chromosome. That is, the "inert region" of the mitotic chromosome was compounded of a small number of blocks, each of which resulted from an individual gene (cf the large chromatin nucleoli noted by Wenrich and others at definite points in prophase chromosomes). The latter hypothesis received strong support in the finding of MULLER and PROKOFYEVA (1935) that in the salivary glands the so-called "inert region" of the X (XI), as well as the whole Y chromosome, had a structure fundamentally similar to that of the so-called "active regions," consisting of rows of discs spaced at intervals similar to those in other regions, but that XI and Y were very short, containing relatively few discs, and therefore presumably relatively few genes. In the salivary chromosome the length of these "inert regions" was in fact not much greater than in the mitotic chromosomes, and so it might be supposed that the chromonema of these regions, or at any rate that part of it representing the genes which caused the blocks, did not become as much coiled as that of other regions during mitosis.

SEPARABILITY OF BOBBED AND BLOCK A

In the series of deleted and inverted X chromosomes reviewed by MULLER and GERSHENSON which led to their block hypothesis of the "inert regions," the locus of bobbed, whenever present, was found to be associated with a large block of chromosome material in the mitotic chromosome. Whenever the locus of bobbed was present, this block, which

in fact constituted the major portion of the "inert region" of mitosis, was present, and when bobbed was absent this block was absent. This block we may call "Block A," and we may provisionally presume it to be likely that it is the product of a single gene. It was accordingly suggested that the gene in question, the gene for Block A, might be identical with the gene of the bobbed locus itself. Having as one object a decision of this question, we have continued with an analysis of further cases of X chromosome rearrangements. The results of the work with deleted X chromosomes is being reported by Gershenson in another paper. In none of those yet analyzed has a separation between the locus of bobbed and of Block A been found. But in two inversions which we have analyzed, namely the inversions of scute-L8 and mottled-4, we have found that a point of breakage and reattachment of the inversion had come between the locus of bobbed and that of Block A. These loci are therefore separate ones. This accordingly increases the number of loci in the "inert region" that are genetically known to be separate.

The evidence for the above conclusion is derived from the study of chromosomes resulting from single crossing over between the above inversions and other inversions of the X. It had been shown by SEREBROVSKY and KAMSHILOV (1931) that crossovers having the left hand portion of the scute-4 inverted chromosome and the right hand portion of the scute-8 inverted chromosome were deficient for the bobbed locus and, as they pointed out, this meant that the righthand point of breakage of the scute-4 inversion was to the left of bobbed and that of the scute-8 inversion to the right of bobbed. GERSHENSON (1933) had found that these crossover chromosomes were considerably shorter than normal; as we would now say, they lacked one or more of the blocks, including certainly Block A. This meant that the righthand break of scute-4 was to the left of Block A, and that of scute-8 to the right of Block A and possibly of one or more smaller blocks besides. In neither of these cases then had there been a breakage between bobbed and Block A. By the same method of testing of crossovers, applied to combinations of scute-4 and scute-S₁, we find that the scute-S₁ chromosome, like that of scute-8, is broken to the right of both bobbed and Block A (at least). When, however, we use the scute-L8 inverted chromosome, securing the crossover having the lefthand portion of scute-4 and the righthand portion of scute-L8, we find that the righthand break of the scute-L8 chromosome must have occurred between bobbed and Block A. For the crossover chromosome just mentioned is deficient for the bobbed locus but is of normal length, showing that the breaks of scute-4 and scute-L8 were on different sides of the bobbed locus (left and right respectively), but on the same side (the left side) of Block A. The complementary crossovers, having the left portion from scute-L8 and

the right portion from scute-4 are, as expected, also found to have normal length and they are not deficient for bobbed. These conclusions are checked by the study of crossovers between the scute-S₁ and scute-L8 chromosomes. In this case we have the reverse findings since the righthand breaks of both chromosomes are on the same side of bobbed (to the right of it), and hence neither type of crossover chromosome is deficient for bobbed, but since the breaks of these two chromosomes are on opposite sides of Block A, one crossover class of chromosome is short, like the scute-4 scute-8 combination, being deficient for the block, and the opposite type of crossover is correspondingly more elongated than the normal, having Block A (at least) represented at both ends. The short chromosome is that having the left portion of scute-L8 and the right portion of scute-S₁, while the long chromosome is the complementary type of crossover. The scute-8 chromosome, of course, gives the same results in these respects as the scute-S₁ chromosome when taken in its crossover combinations with scute-L8.

Similar studies of crossovers between the mottled-4 inversion and the scute-8 inversion give the same results with respect to bobbed and chromosome length as do the studies of scute-L8 and scute-8. That is, mottled-4 also is broken between bobbed and Block A. (In order to obtain viable individuals containing the crossover chromosome having the left portion of mottled-4 and the right portion of scute-8, an extra fragment of the left end of the X chromosome derived from the mottled-5 translocation was inserted by crossing, since the crossover chromosome in question lacks the so-called viability gene.)

PROPERTIES OF BLOCK A

In view of the fact that bobbed is due to a separate locus from that of the large chromatin block, the question of the function of the latter locus becomes more prominent. The short crossover chromosome having the lefthand portion of scute-4 and the righthand portion of scute-8 was known not to be deficient for any genes necessary for life whose place could not be taken by genes in the Y chromosome, since both males and homozygous females containing this short X and also a Y lived and were apparently normal (except for the expected characters dependent upon the scute genes present). On the other hand such individuals died if they lacked a Y chromosome. But this was to be expected, owing to their deficiency for the locus of bobbed, and so it could not be determined whether they were at the same time deficient in any other locus necessary for life or for a normal appearance. With the use of scute-L8, however, the question could now be attacked whether the locus of Block A or some other locus associated with it and separated from that of bobbed was necessary for life or for a normal

phenotype. Accordingly, crosses were made to secure females homozygous for the short crossover chromosome having the lefthand portion of the scute-L8 inversion and the righthand portion of the scute-8 inversion. It was found that such homozygous females were in fact viable and normal in appearance, except, of course, for the expected scute characteristics. Since, however, there was a possibility that these females might contain a Y chromosome covering their deficiency in the "inert region" of the X, a number of them were tested individually to ascertain whether they contained an extra Y. The test was made by crossing them to males heterozygous for Curly and for a dominant allele of brown eye, that called "A" by DUBININ, which he has shown to give a normal eye color in the presence of an extra Y. It was found by this means that no extra Y had been present in a number of these females. In others, however, it had been present and had given the expected results, thus proving the validity of the test. Hence we may conclude that Block A is neither necessary for life nor fertility, nor for a normal external phenotypic appearance. In fact, as the same tests showed, it does not seem to have a decided effect even on the phenotypic expression of the characters of so-called "eversporting displacements," like the dominant alleles of brown (cf NOUJDIN'S 1935 finding of such an effect by the "inert region" of the X in general). The question of the possible function of the block in the synapsis and segregation of the sex chromosomes has been investigated by GERSHENSON and is being discussed in a parallel paper.

It is being shown by PROKOFYEVA and MULLER in another paper in which salivary gland chromosome studies are reported of scute-4 and scute-8 chromosomes, that the region including bobbed and Block A and probably also block B (see below) is represented by only about two of the faint discs of the chromocentral region of the X chromosome, as seen by our optical methods. A considerable portion of the chromocentral region, including of course the spindle fiber locus, lies to the right of this, and another portion lies to the left of it, that is, to the left of bobbed. This narrows down considerably the region of the salivary gland chromosome responsible for the greater bulk of the "inert region" seen in the mitotic chromosome, leaving little or no more than one faint disc, as seen by our methods, for the whole of Block A. Taking only this portion of the salivary gland chromosome, then, we find that it is not even several times longer than the same region in the metaphase chromosome, and we may conclude that in the metaphase chromosome this particular portion has not become coiled or shortened at all as compared with its condition in the salivary gland. This means that most of the rest of the "inert region" has become much shortened, that is, probably coiled, in much the same fashion as the "active" region. The non-coiling then is apparently confined to certain

particular loci, those which form the blocks. The functions of the other loci of the "inert region" largely remain to be determined, but there seems little reason to suppose that they are really "inert," even though it was found that Block A itself could be dispensed with.

OTHER LOCI IN THE "INERT REGION"

That "block B," or at any rate two blocks, are contained in the tiny section between the righthand breaks of scute-4 and scute-8, was shown by another experiment. In this a cytological study of the mitotic chromosomes was made in the case of a mutual translocation between the scute-8 chromosome and the fourth chromosome. A combination chromosome having the lefthand portion of the scute-8 chromosome, not including bobbed or the main block, and the righthand (spindle fiber) portion of the fourth chromosome, which had been broken to the right of bent, was found to be about twice as large as a normal fourth chromosome. This could only mean that it contained a small block of "inert region" that had been present in the lefthand end of the scute-8 chromosome, and that normally must lie to the right of the main block. (This is probably the same as "block B" of the deleted X chromosomes studied by GERSHENSON). The presence of even this locus, in addition to that of Block A and of bobbed, in the small region between the scute-4 and scute-8 breaks, emphasizes the smallness of the space occupied in the salivary chromosome by Block A itself, that is, by the major portion of the "inert region" of the mitotic chromosome. It thus becomes reduced to approximately a single disc, together perhaps with the internodal region bordering the latter on one or both sides.

The above then illustrates the multi-locus character of the "inert region," and the fact that most of its bulk at mitosis is due to a very few particular genes that form a minority of all the genes within it. The "inertness" seems to be due to these genes only, that is, to the relatively large bulk which they form at mitosis. Most of its other peculiar characteristics depend in large measure at least upon the other genes. For example, the attraction of this region to the chromocenter in the cells of the salivary glands depends upon genes scattered throughout the length of this region. One or more of the latter genes even lie to the left of bobbed and of the righthand break of the scute-4 chromosome, since PROKOFYEVA'S studies show that the left end of the scute-4 chromosome is attracted to the chromocenter, causing this chromosome to form a loop in the salivary gland. And all the peculiarities of morphological structure that go with this chromocentral position are to be found in this portion of the scute-4 chromosome also. It would perhaps be better then, provisionally, to designate the whole region in question as the "chromocentral region" rather than the "inert region." The chief objection to this term is that in some species,

which no doubt contain chromosome regions homologous to this, a real chromocenter is not formed. The same tendencies are probably present however, though to a lesser degree.

The above considerations bring to prominence one of the main questions that remains to be investigated concerning the chromocentral region. That is, why are there so many peculiar properties crowded together in this one region, including: attraction to chromocenter, formation of "blocks," effects on the expression of "eversporting displacements," sensitivity of crossing over processes to temperature and other influences, breakability and presence of the fiber locus. Especially noteworthy too is the fact that several of these functions depend upon a number of genes that can exhibit these properties independently of one another, and that in these cases all the genes of this kind are to be found lying close together, within this minute region.

SUMMARY

1. Two cases are reported in which a combination of genetic and cytological analysis shows that breakage and inversion of the X chromosome had occurred between the loci of bobbed and of the main block, "Block A," which forms most of the bulk of the so-called "inert region" seen at the time of mitosis. In itself the locus of bobbed produces little if any of the bulk of the "inert region" at mitosis.

2. "Blocks A" and "B" in the salivary gland chromosome form a relatively small portion (not more than one "disc" each, as seen by our methods) of the whole of the so-called "inert region" seen at that stage, a portion little if any longer than the length of these regions in the mitotic chromosome. Hence the rest of the "inert region," excepting the loci of these blocks, probably becomes coiled at mitosis, while these loci themselves remain elongated, forming a bulky mass of chromatin.

3. Individuals lacking "Block A" but containing the locus of bobbed are viable, fertile, and normal in their external appearance, even if they do not have a Y chromosome to compensate for the absence of this locus.

4. The problem arises as to why a number of peculiar properties dependent upon different loci occur crowded together in one region of the X chromosome, and probably of other chromosomes, and why, in the case of certain of these properties, there are even several loci of a similar kind in this same region.

5. The most characteristic property of this region seems to lie in the attraction of its loci for other loci so as to result in the tendency to form a chromocenter, rather than in the formation of chromatin blocks, and it is hence suggested that it would be better at present to term these regions "chromocentral regions" rather than "inert regions," recognizing however

that most of the chromatin of these regions, as seen at mitosis, is in fact genetically inert, in the sense of containing relatively few genes.

LITERATURE CITED

- GERSHENSON, S. M., 1933 Studies on the genetically inert region of the X chromosome of *Drosophila*. I. Behavior of an X chromosome deficient for a part of its inert region. *J. Genet.* **2**: 297-313.
- MULLER, H. J. and GERSHENSON, S. M., 1935 Inert regions of chromosomes as the temporary products of individual genes. *Proc. Nat. Acad. Sci.* **21**: 69-75.
- MULLER, H. J. and PAINTER, T. S., 1932 The differentiation of the sex chromosome of *Drosophila* into genetically active and inert regions. *Z. i. A. V.* **62**: 316-365.
- MULLER, H. J. and PROKOFYEVA, A. A., 1935 The structure of the chromonema of the inert region of the X chromosome of *Drosophila*. *C. R. Acad. d. Sci. USSR* **1**: 658-660 (Russ. and Eng.).
- NOUJDIN, N. I., 1935 An investigation of an unstable chromosome in *Drosophila melanogaster* and of the mosaicism caused by it. Russ. with Eng. summary. *Zool. J. (Russ.)* **14**, 2: 317-352.
- 1936 Influence of the Y chromosome and of the homologous region of the X on mosaicism in *Drosophila*. *Nature* **137**: 319-320.
- OFFERMANN, C. A. and MULLER, H. J., 1932 Regional differences in crossing over as a function of the chromosome structure. *Proc. Sixth Int. Congress Genetics* **2**: 143-145.
- PROKOFYEVA-BELGOVSKAYA, A. A., 1935 Y chromosome in salivary glands of *Drosophila*. *C.R. Acad. d. Sci. USSR* **3**: 365-366.
- 1935 The structure of the chromocenter. *Cytologia* **4**: 438-443.
- SCHULTZ, J., 1936 Variegation in *Drosophila* and the inert chromosome regions. *Proc. Nat. Acad. Sci.* **22**: 27-33.
- SEREBROVSKY, A. S. and KAMSHILOV, M. M., 1931. "Breakage of the gene scute in the inversions scute-4 and scute-8." Public report at session of Timiriazev Biological Institute, Moscow, August 1931 (unpublished).