STUDIES ON THE *SAL* LOCUS IN *DROSOPHILA PSEUDOOBSCURA*. II. MODES OF REGULATION OF THE *SAL* BANDING PATTERN¹

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ABSTRACT

From a consideration of genetic and chromosomal alterations which affect the banding pattern of a specific salivary chromosome region, it is concluded that (1) a regulator locus, *sal*, is closely linked with the region in question but separated from it spatially by a few bands; (2) either mutation of the *sal* locus or transfer of the region to a heterochromatic region affects the banding pattern similarly; and (3) the change probably consists of a compaction of DNA and a concomitant inactivation of genetic loci.

THE existence in Diptera of regulatory genes which control the transcription of RNA from other structural genes has been inferred by ASHBURNER (1970) from the non-autonomous or semi-autonomous puffing behavior of the salivary gland chromosomes of *Drosophila* larvae heterozygous for certain puffs. The change in banding pattern at the tip of salivary chromosome 3 determined by the mutant *salivary* (*sal*) in *Drosophila pseudoobscura* has been shown to behave in a semi-autonomous manner (GERSH 1972). The *sal* banding pattern behaves genetically as if it were the result of a single mutation (LEVINE and VAN VALEN 1962). Yet the difference in banding pattern between *sal*⁺ and *sal* involves a change in several bands (Figure 1A, B), suggesting that the *sal* locus regulates the activity of several genes, directly or indirectly. Data presented in this paper further suggest that *sal* is separated distally in the chromosome from the genes or bands that it controls. In addition, the similarity of this control and that exercised by heterochromatic regions in V-type position effects (LEWIS 1950) is illustrated.

LEVINE and VAN VALEN suggested that *sal* was closely linked with, if not actually in, the region of affected bands near the tip of chromosome 3. It would then be fairly closely linked with crossveinless (*cv*), the most distal marker gene in chromosome 3 (TAN 1936). Translocation(3; 4)190 (*T*(3; 4)190), a reciprocal translocation between the tips of chromosomes 3 and 4, has been used to determine the approximate location of *cv*. Figures 1A and C show the positions of breaks in chromosomes 3 and 4 respectively: chromosome regions are numbered following TAN (1937). Chromosome 3 is broken between sections 80 and 81 and the bands in the proximal part of section 81 (i.e., those affected by *sal*) and the

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FIGURE 1.— $\times 2800$. A, B: Comparison of paired tips of chromosome 3 in the homozygous CH sequence. A. Homozygous *sal*⁺ (arrow shows position of break in *T(3;4)190*. P, puff in section 80. B. Homozygous *sal*. a, b, c, dark bands characteristic of *sal* pattern. C. Tip of chromosome 4 showing position of break in *T(3;4)190* (arrow). D. *T(3;4)190(CH)/or Sc pr cv* (ST), showing interchanged tips of chromosomes 3 and 4 (T3, T4) paired with their normal homologues (N3, N4). The proximal part of chromosome 3 is heterozygous for the CH and ST sequences. 3P, 4P, proximal parts of chromosomes 3 and 4. E. "Deficiency" (3) *cv⁶⁸¹(CH)/sal⁺(ST)*. Tips of 3 paired, bracket shows region in ST sequence chromosome that appears to be deficient in the homologue. F. Same tips unpaired, *sal*⁺ (ST) left, *Df(sal)* (CH) right.

bands distal to them are exchanged with the tip of chromosome 4, which is broken in the distal part of section 98. Chromosomes of flies heterozygous for this translocation are shown in Figure 1D. If they carry the recessive *cv* allele in their normal chromosome 3, these flies are wild type in respect of crossveins of the wing. By mating such heterozygotes with *cv* males whose chromosomes are structurally normal, one can obtain among the offspring flies which are hypo- or hyperploid for the tip of chromosome 3 (and hyper- or hypoploid, respectively, for the tip of 4). The former have only that translocation chromosome consisting of the main part of chromosome 3 with the tip of 4 attached (3^4). The other chromosome 3 (with *cv*) is normal and two normal fourth chromosomes are present. These flies are phenotypically *cv*, showing that the *cv*⁺ allele was carried by the missing, translocated tip of chromosome 3. The complementary type of fly has the translocated chromosome (4^3) consisting of most of 4 with the tip of 3 attached. The second chromosome 4 is normal and there are two normal third chromosomes. These flies, of genotype *cv*⁺/*cv*/*cv*, have *extra* crossveins (Figure 2). These two hypo-/hyperploid types were observed cytologically in addition to being identified genetically by the use of markers on the normal third and fourth chromosomes.

The probable location of the *cv* locus (and also that of *sal*) was narrowed down by cytological analysis of *cv*^{68f}, a mutant obtained by irradiating *cv*⁺*sal*⁺ males with X rays (4000r) and mating them with *or Sc pr cv* (orange eye, Scute bristles, purple eye, crossveinless) females. The chromosome 3 of this *cv* mutant appears to be deficient for two bands in the distal part of section 81 (Figure 1E,F). An alternative possibility is that material (presumably including DNA) between the two bands bordering the apparent deficiency has been condensed into

FIGURE 2.—Right wing of fly with a duplication of the *cv* locus and heterozygous deficiency for the tip of chromosome 4. Extra crossveins marked by arrows. The posterior crossvein between longitudinal veins L4 and L5 is in its normal position, but has an extra branch (double-headed arrow). $\times 60$.

FIGURE 3.—A-C Translocation (*Y*; 3; 4)190 *cv*^{70f}. $\times$ ca. 2800. A. Left, normal 4 (N4) paired in proximal part (4P) with a homologue capped by part of the *Y* chromosome (TY); right, normal *sal*⁺ 3 (N3) paired proximally with its homologue (3P) to the point where the tip of 4 (T4) is attached. p, puff. B. A normal *Y* chromosome at the chromocenter at two different focal levels, from a *sal*(CH)/or *Sc pr cv* (ST) male larva. C. Left, tip of 3 at chromocenter (T3) with dark proximal bands (arrow); right, normal *sal* 3 (N3) with dark bands (a, b, c), tip of 4 translocated to 3 (T4), normal 4 (N4).

these two bands. Because of this doubt I shall refer to *cv^{esf}* as a "deficiency." *cv^{esf}* is lethal when homozygous. It will be noted that in Figure 1E where the tips of chromosome 3 are paired, the bands in the proximal part of section 81 are dotted as in *sal*⁺ though somewhat heavier than usual. In Figure 1F, showing unpaired chromosomes, the normal 3 shows the confused *sal*⁺ banding while the "deficient" chromosome shows the heavy bands characteristic of *sal*. Thus not only the *cv* locus but the *sal* locus also had been affected in the altered chromosome, and it is reasonable to suppose that both loci are normally present in the region that appears deficient in the *cv^{esf}* chromosome. In such case, the locus which controls bands of the proximal part of section 81 would be distal by a few bands from the loci it controls.

The semi-autonomous behavior of the banding pattern (or pattern of gene activation) indicates that the influence of the *sal*⁺ allele on the proximal bands of section 81 can be transmitted intrachromosomally or from one synapsed homologue to the other, but not across an intervening nucleoplasmic region. While ASHBURNER (1970, p. 324) considered this unlikely when a regulatory gene is separated from the structural genes it controls, it is probable that he had in mind a situation where the regulator gene was much further removed from its target structural genes, perhaps even in a non-homologous chromosome.

The compact form of the proximal bands in section 81 can also be caused by juxtaposition of these bands to a heterochromatic region. Irradiation with X rays of the *T(3; 4)190* chromosomes resulted in a relocation of the tip of chromosome 3 to a proximal heterochromatic region in the chromocenter of the salivary nucleus. In its new position, the tip of 3 with the *cv*⁺ allele was inherited only by males, showing that it was reattached to the Y chromosome, part of which now occupied its former position at the distal end of chromosome 4 (Figure 3A, B, C). The tip of 3 had been broken at its previous attachment to chromosome 4, immediately preceding section 81. It is shown in Figure 3C, at the left, with a group of strongly compacted bands corresponding to some or all of the dense bands of the *sal* pattern (3C, right). The adjacent heterochromatic material thus exercises a control over the banding pattern similar to that of the mutant *sal* (*cf.* Figure 1B and Figure 3C, right), by effecting a reduction of puffing (similar to that described by SCHULTZ 1965 and RUDKIN 1964) and a compaction of bands.

On the basis of autoradiographic work (FUJITA and TAKAMOTO 1963; KEYL and PELLING 1963; PELLING 1964) on DNA and RNA synthesis in salivary gland chromosomes, FUJITA (1965) deduced that "inactivated positions in the chromosome correspond to bands and heterochromatic regions where the DNA chains are folded and packed densely." Many instances of V-type (variegated) position effect are known where the activity of specific genes is inhibited in some cells in which those genes would normally be active. SCHULTZ (1936; 1939) showed that such position effects are associated with transfer of genes to heterochromatic regions and that the rearrangement is often accompanied by compaction of bands of the salivary gland chromosome adjacent to the break-point like that described here. The effects of similar rearrangements on some puffs have already been noted. It is reasonable to suppose that the control of banding, both

by heterochromatic material and by a regulator such as *sal*, is effected by means of the folding or coiling of interband or puff DNA which causes accretion of the bands, and that the highly compact form of this DNA is associated with genetic inactivity.

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