Constitution of the Salivary Gland Chromosomes of *Chironomus*

SINCE the discovery of giant salivary gland chromosomes in dipterous larvæ[1], cytophysiologists have regarded them as the most favourable material for studying the chemical composition and fine structure of chromosomes, especially the leptonic relation between nucleic acid and protein. The view that chromosomes are chiefly composed of nucleoprotein is now generally accepted. In salivary gland chromosomes, the bands represent regions of relatively high nucleic acid content, while the interbands carry very little, if any, nucleic acid. The results obtained by Feulgen staining[2], histochemical tests[3] and ultraviolet microscopy[4] have all substantiated this conclusion. Digestion experiments by Caspersson and by Mazia and Jaeger[5] clearly indicate that the salivary gland chromosomes possess a continuous protein framework the integrity of which is independent of the presence of nucleic acid molecules. In a more definite way, Astbury and Bell[6] and Schmidt[7] concluded from their X-ray diffraction and birefringence data that the most important elements in the molecular organization of the chromosomes are the partially folded and partially extended polypeptide chains, together with the parallel fitting of thymonucleic acid on to the extended portion of the chains. Based on this structural principle, Pfeiffer[8] and especially Calvin and Kodani[9] have proposed a probable structure for the salivary gland chromosome.

More recently, Stedman and Stedman[10] announced the isolation of an acid protein which was named 'chromosomin'. They considered this to be the chief protein constituent of the chromosome, since its staining behaviour was the same as that of the chromosome. These authors also suggested that nucleic acid, instead of being attached to the chromosome, is present mostly in the nuclear sap. They questioned the reliability of the Feulgen technique as a means of demonstrating the actual location of thymonucleic acid, and interpreted the results of its application on a basis quite different from the hitherto accepted interpretation, which appeared to be supported by the success of staining plant chromosomes with 'developed nucleal stain'[11]. These novel ideas at once attracted the attention of cytogeneticists, and an interesting discussion of the problem began[12].

Since the work of the Stedmans was done on vertebrate tissues, and since they made no comment on the structure of salivary gland chromosomes, which are large enough to permit of separation from the nuclear sap, we have made experiments on such chromosomes in *Chironomus* larvæ. (1) Instead of producing aldehyde groups by the hydrolysis of thymonucleic acid *in situ*, we allowed 5 per cent aqueous formaldehyde solution to react previously with leucobasic fuchsin *in vitro*, thus producing a 'developed nuclear stain'. The dye thus obtained stained the bands without hydrolysis in exactly the same way as in the standard Feulgen reaction. Nor was there any difference with respect to the differentiation between bands and interbands, if we first impregnated the gland cells with formaldehyde and then immersed them in leucobasic fuchsin. (2) When hydrolysis in normal hydrochloric acid at 60° was prolonged beyond 15 minutes, the chromosomes gradually lost their staining capacity toward the Feulgen reagent and eventually became entirely negative after 20–30 minutes hydrolysis. (3) Salivary gland chromosomes in smear preparations were first excised and then thoroughly washed with insect Ringer to remove as completely as possible the surrounding materials. Such 'naked' chromosomes gave the same Feulgen reaction as when present in the intact nuclei. (4) In the 'naked' chromosomes, formaldehyde-developed Feulgen reagent produced typical banded structures without acid hydrolysis. Likewise, a pretreatment with formaldehyde followed by leucobasic fuchsin yielded essentially similar results. (5) Prolonged hydrolysis also made such 'naked' chromosomes behave negatively toward the Feulgen reagent.

In order to explain these results, we assume that the Chironomus salivary gland chromosome has a structure something like that proposed by Calvin and Kodani. The ability of chromosomes to take up basic dyes is normally due to the formation of a salt-like compound of the latter with nucleic acid. The recent investigations of Kelley[13] on the dye-nucleoprotein reaction has offered confirmatory, though somewhat indirect, evidence on this point. The results of our experiments (1) and (4) might seem at first sight to be in accordance with the Stedmans' view that the chromosomes consist only of acidic protein; though the existence of banded structure in the chromosomes is difficult to explain. But these two experiments can equally well be interpreted by assuming nucleic acid to be present on the band; especially if the perpendicular attachment of plate-like nucleoside molecules to the bands results in a structure of relatively high porosity and consequently in a greater imbibition of formaldehyde or developed fuchsin in these regions. The loss of staining power toward Feulgen reagent after prolonged hydrolysis (our experiments (2) and (5)) may well be due, as Bauer[14] and Hillary[15] have suggested, to the gradual splitting of a bond between the ribose and phosphoric acid within a nucleotide. But it is the result of our experiment (3) which is particularly hard to reconcile with the Stedmans' view. We certainly do not contend that the 'naked' chromosomes as prepared above are completely free from nuclear sap. But it seems certain that the quantity present would not be sufficient to produce the intense chromosome-coloration by the standard Feulgen reaction, if nucleic acids were really present in the sap rather than on the chromosomes. Our experiments, at least those with 'naked' salivary gland chromosomes of *Chironomus*, strongly suggest the presence of nucleic acid in the chromosome.

J. CHU.
S. PAI.

Department of Biology,
National University of Chekiang,
Meitan, Kweichow, China.

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