THE LOCALIZATION OF BASIC PROTEINS IN THE NUCLEI OF LARVAL DROSOPHILA SALIVARY GLANDS*

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The identification and specific localization of chemical constituents within the cell nucleus have lagged considerably behind the qualitative and quantitative characterization of these classes of substances, with the possible exception of one component, the deoxyribonucleic acid (DNA). In addition to the latter, the nucleus contains ribonucleic acid1, 2 (RNA), histone or basic protamine3 (BP), and at least one other type of protein, the chromosomin of Stedman and Stedman,4 the tryptophane-containing protein (Tr.Pr.) of Mirsky and Pollister,5 or the non-histone protein fraction which Hamer6 has subjected to comparative amino acid analysis. The localization of these substances in the live nucleus is dependent almost wholly on inference from a variety of analyses and observations made on fixed preparations. The much disputed and maligned Feulgen reaction points to the DNA as a major constituent of the chromosomes. Studies with ultraviolet absorption spectroscopy,1 enzymatic attack, and pyronin staining2 indicate that the RNA of the nucleus is predominantly nucleolar. The distribution of the BP seems to be much less precisely delineated. For example, Caspersson7 has reported its presence in the chromosomes and nucleolus of Drosophila salivary gland nuclei from his ultraviolet absorption studies. Vincent,8 from his chemical studies of the isolated nucleoli of starfish oocytes and of maize, could find no basic protein. Bloch and Godman9 have reported a proportional quantitative relationship between DNA and histone synthesis per rat fibroblast or liver cell. They have also shown cytochemically that, in a general manner, DNA and histone have the same nuclear distribution.

In the present study the Feulgen reaction for DNA localization, combined with the fast-green-staining method of Alfert and Geschwind10 for basic protein, has been employed with Drosophila salivary gland material to determine more precisely the localizations of BP with respect to DNA.

The method employed is essentially that of Bloch and Godman:9 salivary glands of D. virilis fixed with trichloroacetic acid (TCA) were partially crushed under a cover slip; these preparations provided some whole nuclei. After the slide was frozen on a block of "dry ice," the cover slip was flicked off and the preparation stained directly by a modified Feulgen technique following 24 hours' lipid extraction in 95 per cent alcohol. Other salivary squashes were prepared similarly, using the conventional acetic acid fixative that permits rupture of the nuclei, yielding fully extended chromosomes. After a Feulgen treatment in which TCA was substituted for HCl, photographs were taken of a number of chromosomes or of whole nuclei; the position of each was recorded on a calibrated mechanical stage. The preparations were then re-exposed, and, after TCA extraction (15 minutes at 90° C.) to remove the DNA, were stained by the fast-green technique of Alfert and Geschwind. The recorded fields were then rephotographed.
The results are illustrated in the accompanying figures. In both the ruptured (Figs. 1–3) and unruptured nuclei (Fig. 4) there was a clear band-for-band staining correspondence between the Feulgen-treated chromosomes (Figs. 1A, 2A, and 3A) and their DNA-extracted fast-green-stained remnants (Figs. 1B, 2B, and 3B). Diffuse heterochromatin of the chromocenter stained after both treatments. The nucleolus was completely unstained by the Feulgen reaction, although its presence was frequently indicated by the Feulgen-positive reaction of the nucleolus-organizing region (Fig. 1A, arrow). After the fast-green treatment, the nucleolus, which often appeared vacuolated, and its organizing region were clearly evident (Fig. 1B). Their position invariably was neither against the nuclear membrane nor in a central position but rather near the chromocenter.

The whole nuclei (Fig. 4) clearly showed a heavier fast-green reaction in the region of the nuclear membrane; the exact location of this concentration could not be determined, primarily because the gland cytoplasm also presented a strongly positive fast-green reaction. In unbroken nuclei the materials of the interchromosomal space gave no reaction with either procedure. The placement of the ends of chromosomes in whole nuclei showed no detectable pattern.

A number of these observations merit further comment. The precise correspondence of histone and DNA in the banded portions of the salivary gland chromosomes confirms Caspersson’s conclusion drawn from ultraviolet absorption studies on Drosophila salivary glands. Furthermore, Caspersson reported finding histone in the nucleolus, a discovery also confirmed by our data. The failure of the chromosomal interband regions to stain by either cytochemical reaction does not, of course, prove the absence of nucleic acid or basic protein in these positions but certainly indicates a lower concentration. Vincent’s argument relating what appears to be an unusually high protein content (40–90 per cent) reported for the nucleolus with its staining properties does not seem to apply to these studies. He argues that with such concentrations the abundance of diamino acids per unit volume will be greatly increased and that therefore any reaction based on the detection of the amino groups (e.g., Sakaguchi) will always be a relatively strong one. Alpert and Geschwind indicated that the intensity of their fast-green-staining reaction seemed less dependent on the absolute number and type of basic group involved than on the over-all balance of acid to basic groups. The positive reaction of the nucleolus, then, in these studies is interpreted to indicate the presence of BP; the concentration would be a foolhardy guess.

Vacuolated nucleoli have been reported (see Vincent for review) under other circumstances. It should be noted here that the fast-green-stained material shows vacuolation of the nucleolus particularly clearly in preparations of the whole nuclei. The vacuoles vary in size and in distribution throughout the nucleolus.

The similarity of staining reaction demonstrated by the diffuse heterochromatin of the chromocenter to that of the euchromatin lends support to the notion that these two types of chromatin are fundamentally alike chemically, differing perhaps only physiologically and temporally.

Since the interband regions must be accounted for chemically and since the remaining ill-defined protein fraction (chromosomin, Tr.Pr., acid-insoluble protein, residual protein, non-histone protein) remains unaccounted for in the nucleus, at least these portions of the chromosomes might by inference be thought to consist
Figs. 1-4.—Photomicrographs of *Drosophila* salivary gland preparations stained first with the Fu
glgen reaction (Figs. 1A, 2A, and 3A) and then, following DNA extraction, with fast green at pH 8 (Figs. 1B, 2B, and 3B). Figure 4 is of a whole nucleus prepared with fast green at pH 8 after DNA extraction. The arrow in Fig. 1A indicates the nucleolus-organizing region.
of this fraction. Such a scheme is not meant to imply limitation of this protein fraction to the interband region but rather should lend credence to a chromosome structure consisting of a "backbone" constructed of this protein along which the nucleoprotein complex is distributed finitely with spatial and chemical irregularity.

The large interchromosomal spaces seen in fixed intact nuclei would be predicted from the work of Buck and Boche on living salivary glands. They observed simultaneous chromosome shrinkage and nuclear swelling in live material as it was fixed with acetic acid. The present material cannot under the circumstances provide evidence for or against the existence of "nuclear sap." One should be reminded, however, that Buck and Boche characterized the living salivary chromosome as "behaving chemically somewhat like a sponge, in that it is capable of taking in and giving out an astonishingly large proportion of its fluid content, without permanent alteration of its fundamental internal structure." If at fixation, with its concurrent shrinkage of the chromosomes, materials are "given out," then they must not be Feulgen-positive nor fast-green-positive; in other words, at fixation neither DNA nor histone is released to the interchromosomal space in quantities detectable by the cytochemical techniques employed.

The fast-green-positive reaction of the cytoplasm of these glands is especially puzzling. The Alfert and Geschwind technique has been applied by us to a wide variety of tissues, both embryonic and adult, but never with the strongly positive cytoplasmic reaction seen for salivary glands. It is possible, of course, that some wholly unrelated substance elaborated by these larval structures produces a positive reaction following the application of the fast-green technique (the results are identical whether or not the Feulgen reaction precedes the fast green). A modified Sakaguchi reaction for the estimation of arginine showed the cytoplasm to be strongly positive, suggesting by inference that the positive reaction of the cytoplasm to the basic fast green was due to the presence of histone outside the nucleus.

**Summary.**—The successive treatment of *Drosophila* salivary gland nuclei with the Feulgen reaction for DNA and the fast-green technique of Alfert and Geschwind for basic protein has demonstrated an exact correspondence in position of these two chromosomal constituents. The nucleolus also appears to contain some basic protein.

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1. T. Caspersson and J. Schultz, these PROCEEDINGS, 26, 507–515, 1940.
8. W. S. Vincent, these PROCEEDINGS, 38, 139–145, 1952.