Cytological Evidence that Both RNA and DNA May Form a Complex with the Same Protein*,‡

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PLATES 93 AND 94

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ABSTRACT

Deoxyribonucleic acid can be added back to protein sites from which the original nucleic acid, ribo- or deoxyribo-, is removed. If sections of frozen-substituted ovarian follicle cells of a leafhopper are first extracted by hot trichloracetic acid to remove nucleic acids and then immersed in a solution of a commerical preparation of deoxyribonucleic acid, the nucleic acid becomes attached to nuclear and cytoplasmic sites and can be rendered visible by the Feulgen reaction. The addition occurs in certain other tissues as well.

The results are discussed in relation to biochemical and other cytochemical investigations of the nucleoprotein complex.

INTRODUCTION

The nature of the complex between nucleic acids and protein has been explored biochemically by Mirsky and Pollister (1946), Kirby (1957), and Crampton and Chargaff (1957), to mention only a few of the many works in this field. Cytochemical investigations involving extraction methods were made by Kaufmann, Gay, and McDonald (1951), while other kinds of cytochemical evidence relevant to the problem have been considered by Alfert and Geschwind (1953), Alfert (1955), and Swift (1953). The present paper describes cytochemical evidence that deoxyribonucleic acid (DNA) can be made to form a complex with the same protein that binds ribonucleic acid (RNA).

Methods

Ovarian follicle cells of the leafhopper *Macrosteles fascifrons* Stål (Homoptera, Cicadellidae) were used, because these cells are rich in cytoplasmic RNA. The tissues were fixed by hot ethanol after freeze-substitution (Woods and Pollister, 1955), or in Carnoy's acetic-ethanol fluid. Observations also were made on eggs, sperm, and Malpighian tubule cells of this insect.

The experimental procedure was as follows: sections were deparaffined and brought to water. Both RNA and DNA were removed by extraction with boiling 5 per cent trichloracetic acid (TCA) for 15 minutes (Schneider, 1945). The sections were subsequently immersed at room temperature for 2½ hours in a 0.1 per cent solution of a commercial preparation of calf thymus DNA (Worthington). After washing in 5 changes of distilled water, the sections were hydrolyzed in normal hydrochloric acid for 12 minutes at 60°C. and stained by the Feulgen reagent for DNA. In the reverse experiment, 0.1 per cent or 1.0 per cent calf thymus RNA (Mann Research Laboratories, New York) was substituted for DNA and stained with azure B instead of the Feulgen reaction.

Other extractive procedures included 10 per cent perchloric acid at 70°C. for 15 minutes and 0.02 per cent ribonuclease (Worthington) at 35°C. for 1¾ hours. The basic dye azure B was used to stain nucleic acids or protein according to the methods developed by Flax and Himes (1952). Acetylation of protein amino groups was tried in an attempt to increase basic dye binding by nucleic acids, as suggested by Alfert (1952) and Deitch (1955).

OBSERVATIONS

The pronounced cytoplasmic basophilia of the follicle cells and egg cytoplasm resulted from the presence of RNA, as shown by digestion with

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ribonuclease and staining with azure B (Figs. 1 and 2). That protein remained after extraction with hot TCA was shown by staining with the basic dye azure B at two different pH values (Flax and Himes, 1952): at pH 4.0 protein is not stained and, since the nucleic acids were removed, there was no staining in the follicle cells or other tissues. Protein is stained at pH 5.0, however, and in Fig. 3 it can be seen that staining attributable to protein occurs in the cytoplasm and nuclei of the follicle cells and the egg cytoplasm. The same conclusion was reached by Brachet and Shaver (1948), who obtained positive tests for arginine and tyrosine after hot TCA extraction of mouse ovary sections.

After extraction with TCA, addition of DNA, and the Feulgen reaction, test slides gave a positive reaction for DNA in both the cytoplasm and nuclei of the follicle cells as well as in basophilic sites in the egg cytoplasm (Fig. 4). Controls, in which either the hydrolysis with hydrochloric acid or the treatment with DNA was omitted, did not stain (Figs. 5 and 6). Evidently the removal of RNA and DNA by hot TCA freed sites on the proteins to which the added DNA became attached. The DNA also could be added to extracted nuclei of Malpighian tubule cells and sperm.

To test whether DNA can displace RNA from protein, sections were immersed in the DNA solution without any previous TCA extraction and then hydrolyzed and stained with the Feulgen reagent as before. While the cytoplasm remained unstained, the nuclei gave a normal positive reaction, which indicated that RNA must first be removed before DNA can form a complex with the protein.

Azure B staining (pH 4.0) did not reveal the presence of any cytoplasmic or nuclear DNA in sections treated with hot TCA and the DNA solution, probably because the basophilia of the DNA was too weak to be visible; even in untreated sections the DNA of nuclei is often only faintly stained. In an effort to increase the affinity of the added DNA for the basic dye, the sections were acetylated between the DNA step and the staining with azure B; no increase in staining was observed, however.

Substitution of perchloric acid for TCA as the nucleic acid–extracting agent was unsuccessful in this procedure; no added DNA was demonstrated in the tissue by the Feulgen reaction. Furthermore, hot perchloric acid removed not only nucleic acids but also abolished the metachromatic basophilia of the gut lining, which did remain after hot TCA treatment. Digestion by ribonuclease showed that this basophilia was caused by acidic substances other than RNA. In a further attempt to use perchloric acid, acetylation was employed with azure B in the following scheme: hot perchloric acid extraction, addition of DNA solution, acetylation, and staining with azure B. As with the TCA procedure, no added DNA was rendered visible by the use of this basic dye. A possible reason for the lack of success with hot perchloric acid may be found in Pearse's (1953) statement that even cold perchloric acid will extract various protein materials as well as RNA.

When RNA was added to sections which had been extracted with hot TCA, no azure B staining was observed. Again, if some RNA were bound but the amount was small, it may not have combined with sufficient dye to be visible.

Information about nucleoprotein complexes as they normally occur in cells after fixation has been obtained by the application of various extraction procedures to cytological material. Among these we may consider the hydrochloric acid hydrolysis used prior to the application of the Schiff reagent in the Feulgen reaction. Hydrolysis not only creates aldehyde groups on DNA but also extracts virtually all RNA, while it leaves all, or at least most, of the DNA. This differential extraction was shown in the leafhopper follicle cells by staining with azure B at pH 4.0 after a 12 minute hydrolysis in normal hydrochloric acid at 60°C. Cytoplasmic basophilia (except gut lining metachromasy) was more easily extracted and was abolished, while mature sperm nuclei were still basophilic, although less strongly so than in unhydrolyzed sections.

DISCUSSION

The observations described above, which showed that proteins that have been freed from nucleic acids can react with an acidic substance such as DNA, are in agreement with the results of other workers. Kaufmann, Gay, and McDonald (1951) found that hot TCA extraction of RNA from onion root tip cells increased the affinity of cytoplasmic proteins for acid dyes. Similarly, Deitch (1955) used microspectrophotometric methods to demonstrate an increase in the binding of the acid dye naphthol yellow S by newt liver

nuclei following the removal of DNA by deoxyribonuclease or hot TCA. The same sort of process undoubtedly occurred in the leafhopper tissues, except that DNA took the place of an acid dye and was subsequently located in both cytoplasm and nuclei by the Feulgen reaction.

It has been suggested, on the basis of biochemical evidence from differential extraction of RNA and DNA, that the two nucleic acids may be joined to protein by fundamentally different types of bonds (Kirby, 1957). Indeed, as was mentioned above, the Feulgen hydrolysis with hydrochloric acid, which removes RNA and DNA from sections at different rates, is an example of differential extraction at the cytological level. While the present experiments did demonstrate that it was possible to add DNA to certain proteins if RNA were first removed, the bonding was not necessarily the same. On the other hand, since it was shown that DNA can be added to cytoplasmic (non-histone) protein, these results are compatible with the observation of Mirsky and Ris (1951) that DNA in isolated chromosomes was combined with non-histone "residual protein."

The "reconstituted" DNA-protein complex in the nuclei was qualitatively and probably also quantitatively different from that originally present. The fact that it gave a much less intense Feulgen reaction argued that less DNA was present. There are several possible explanations: (1) calf thymus DNA was used instead of DNA from the same or a closely related species; (2) the treatment with TCA eliminated some potential binding sites; (3) the experimental conditions of concentration, pH, and temperature were not optimum.

Adsorption of the added DNA to the sections probably was not a significant factor since the sections were washed thoroughly in water after the DNA treatment and then subjected to hydrolysis in hydrochloric acid at 60°C.

SUMMARY

Deoxyribonucleic acid was added to sections of leafhopper ovarian follicle cells from which all nucleic acid had been removed by hot trichloracetic acid; the DNA became attached to protein in the cytoplasm as well as the nuclei. Previous extraction of RNA was a condition for formation of the complex between DNA and cytoplasmic protein. The added DNA could be visualized by the Feulgen reaction but not by the basic dye azure B. The experimentally formed DNA-protein complex in nuclei stained less intensely than did normal nuclei, which suggested that less DNA was bound. Adsorption probably did not play a significant role in the binding of the DNA to the tissues.

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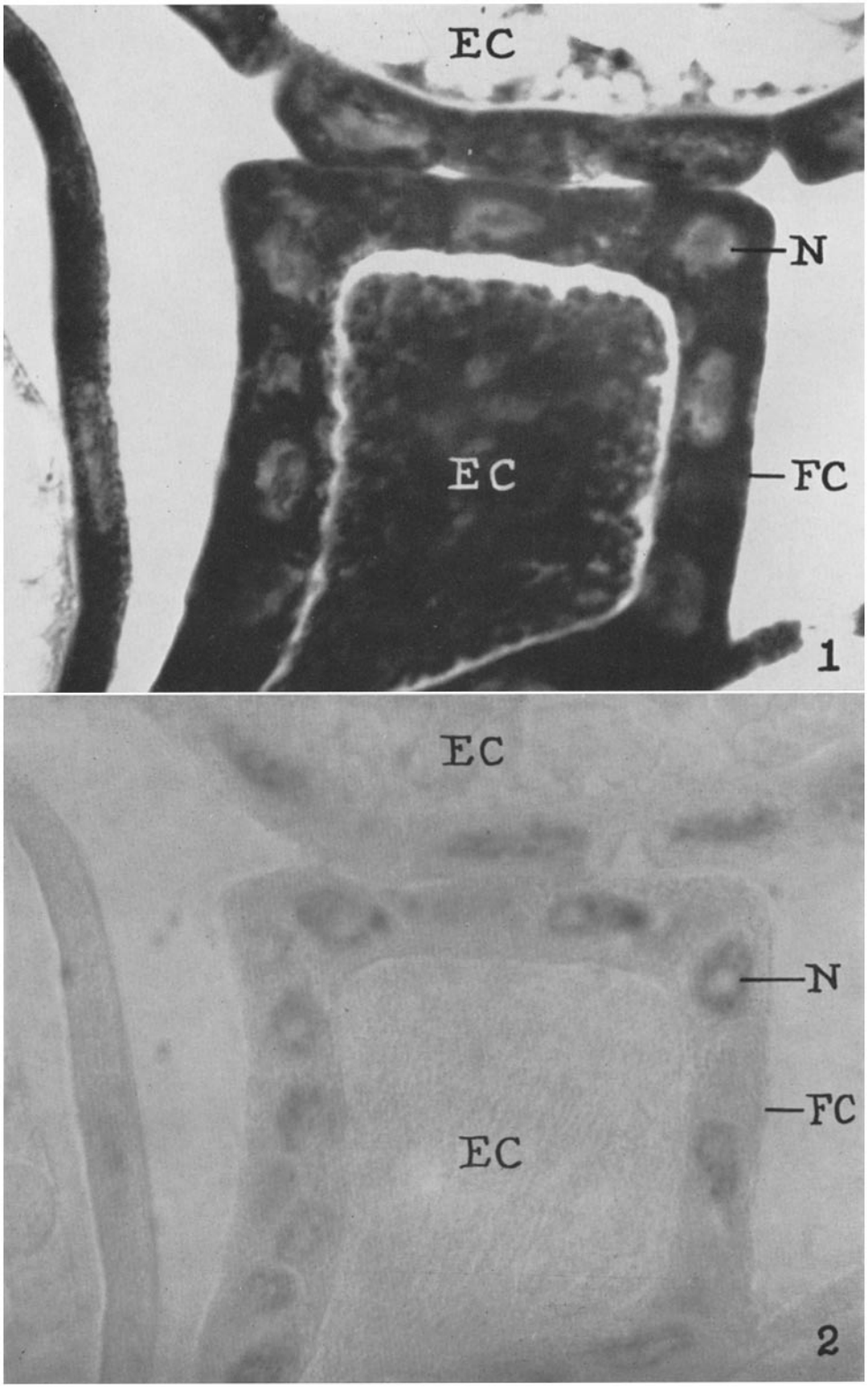
Explanation of Plates

Figs. 1 to 6. Ovaries of *Macrosteles fascifrons*. Follicle cells (*FC*), nuclei (*N*), and egg cytoplasm (*EC*) are designated. Photographs by J. A. Carlile.

Plate 93

Fig. 1. Material frozen-substituted and fixed in hot ethanol; stained with the basic dye azure B at pH 4.0. This is the control slide (no enzyme) for Fig. 2. Cytoplasm and nuclei of the follicle cells and some parts of the egg cytoplasm were basophilic. × 1420.

Fig. 2. Same as Fig. 1. The sections were digested by ribonuclease at 35°C. and stained with azure B at pH 4.0. Only the deoxyribonucleic acid of the nuclei stained; nucleolar and cytoplasmic basophilia was abolished by the enzyme. × 1420.



(Littau: RNA and DNA complex with protein)

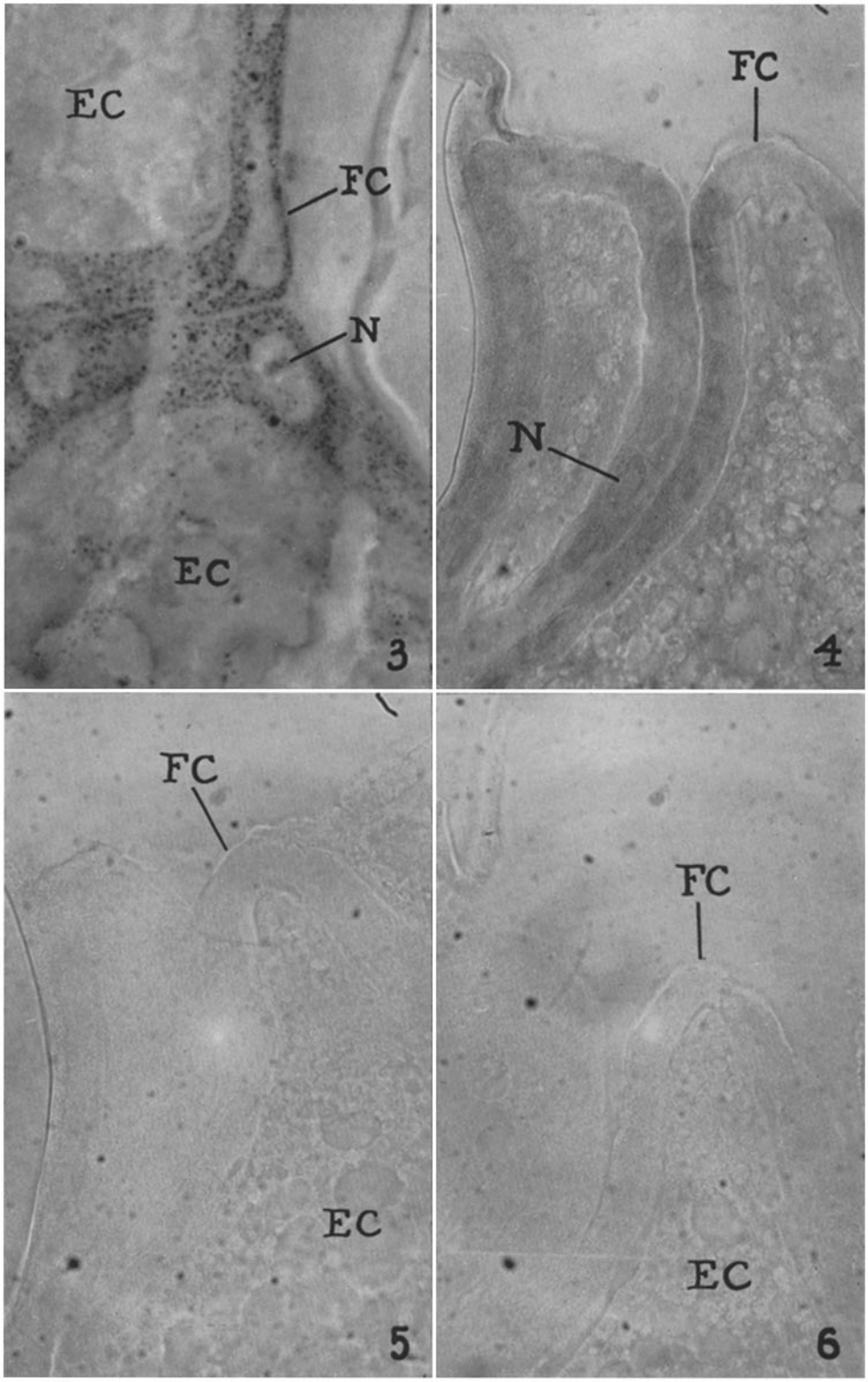
PLATE 94

FIG. 3. Material fixed in Carnoy's fluid; extracted with hot trichloracetic acid and stained with azure B at pH 5.0. Nucleic acids were removed; protein was stained. × 4595.

FIG. 4. Ovary frozen-substituted and fixed in hot ethanol; treated successively with hot trichloracetic acid, 0.1 per cent deoxyribonucleic acid, and the Feulgen reaction. A positive reaction (pink color) was given by both nuclei and cytoplasm of the follicle cells and by the egg cytoplasm. Green filter. × 710.

FIG. 5. Same as Fig. 1, except that the hydrochloric acid hydrolysis for the Feulgen reaction was omitted. Photographic conditions the same as in Fig. 1. No red or pink color was observed in these cells. × 710.

FIG. 6. Same as Fig. 1, except that the deoxyribonucleic acid step was omitted. Photographic conditions the same as in Fig. 1. Nuclei and cytoplasm are Feulgen-negative. × 710.



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