

Intranucleolar DNA in the Salivary Gland Cells of the Melonfly, *Dacus cucurbitae*

The occurrence of DNA in the nucleoli of plant and animal cells has been demonstrated by chemical analysis^{1,2}. On the other hand, cytochemical studies have not unequivocally confirmed the presence of DNA in the nucleolus. In cases where faint Feulgen reaction was observed in the nucleolus, it was considered to be due to the 'perinucleolar chromatin' or heterochromatin³⁻⁵, or chromosomal tags adherent to the nucleolar mass^{6,7}. It was also suggested that cytochemical techniques were not sufficiently sensitive to detect very small quantities of DNA that may be present in structures like the nucleoli⁸. Only recently definitive evidences for the presence of DNA in the nucleolus using fluorescent dyes, isotope labelling and electron microscopic techniques, is forthcoming⁹⁻¹¹. During the course of our studies on the salivary gland chromosomes of *Dacus cucurbitae*, it was possible to demonstrate cytochemically the DNA strands in the nucleolus, further confirming the recent reports.

Intranucleolar DNA in *Dacus* occurs in various morphological states (Figures 1-3). It is seen in the form of granules of various sizes (Figure 1) or as delicate strands running along the nucleolus (Figure 2) or in the form of long Feulgen positive strands radiating from a strongly Feulgen positive core (Figure 3). But these different morphological states are not associated with development, since all these are seen in different cells of the same gland. The sensitivity of the strands to the action of DNase and the fact that they are unaffected by RNase treatment further confirms their nature. Like the rest of the chromatin material of the salivary gland chromosomes, the nucleolar DNA is associated with histone proteins as revealed by ammoniacal silver staining (Figure 4).

Incorporation of ³H-thymidine into nucleolar DNA also follows the pattern seen in the chromosomal DNA (Figure 5). When isolated salivary glands were incubated in Ringer's solution containing ³H-thymidine (10 min, 10 μ C/ml, sp. act. 3.0 C/mM, Schwarz Bioresearch Inc.), the nucleoli incorporated the precursor only up to mid

third instar stage (144 h after oviposition), by which time the chromosomes had achieved their maximum ploidy¹².

Extensive examination did not reveal any direct association of the intranucleolar DNA with the chromosomal arms and thus the intranucleolar DNA in *Dacus* does not seem to have any structural continuity with the chromosomes.

The origin of the intranucleolar DNA can only be speculated. In an analogous situation as in the amphibian oocyte nucleoli, the extrachromosomal nucleolar DNA has been suggested to arise from a single chromosomal locus endoreduplicating independent of the rest of the genome¹³. In *Dacus* also, an organizer chromosome region might have migrated into the nucleolus and endoreplicate to the same extent as the rest of the genome.

The function of the intranucleolar DNA is far from being clear. There is evidence implicating the nucleolus in the synthesis of ribosomal RNA¹⁴ and the nucleolar organizer region has been described to be the site of

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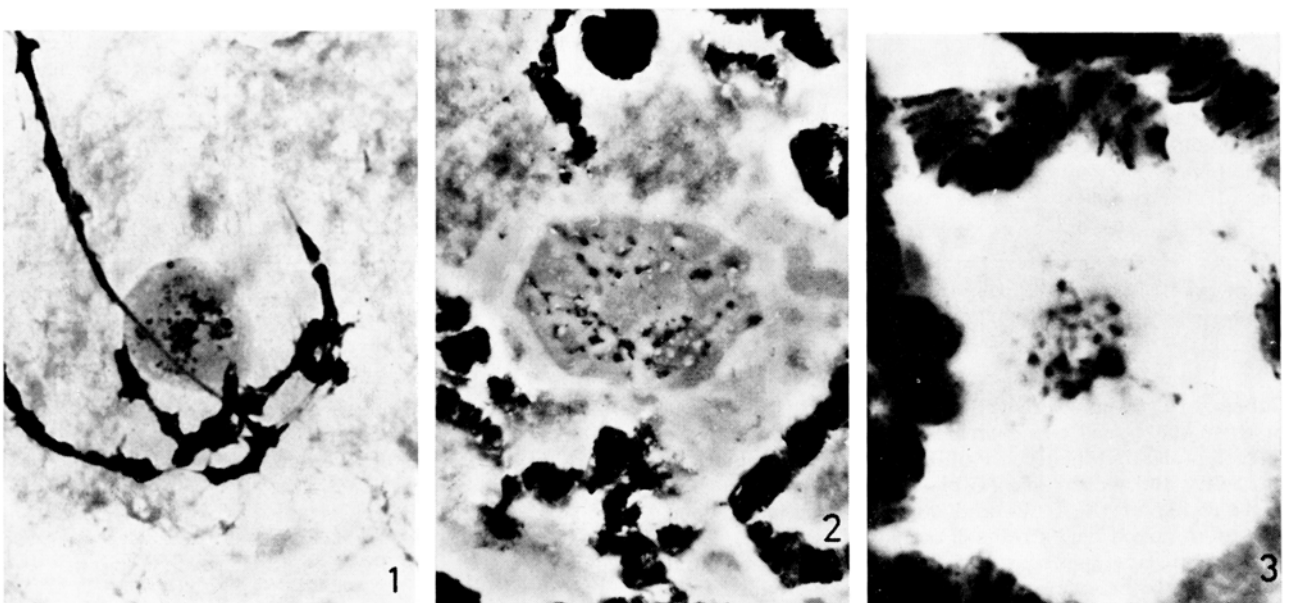
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Figs. 1-3. Nucleolus in the salivary gland cells of *D. cucurbitae* showing the various morphological states of the intranucleolar DNA. $\times 1700$.

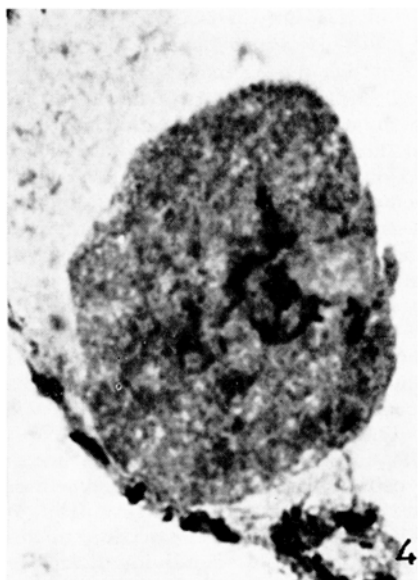


Fig. 4. Nucleolus in the salivary gland cells of *D. cucurbitae* showing the basic proteins. Ammoniacal silver. $\times 2000$.

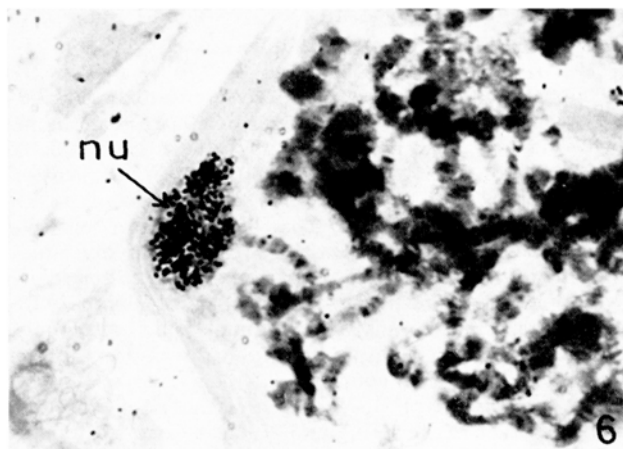


Fig. 6. Autoradiograph showing the incorporation of ^3H -cytidine preferentially into the nucleolus of the salivary gland cells of *D. cucurbitae* ($50 \mu\text{C}/\text{ml}$, 30 sec, 15 days exposure). $\times 600$.

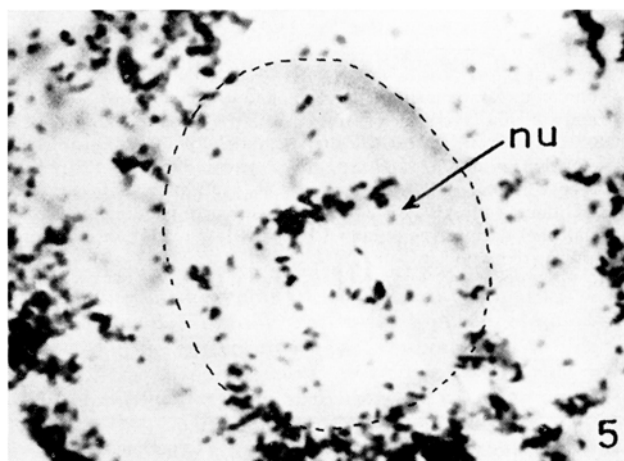


Fig. 5. Autoradiograph showing the incorporation of ^3H -thymidine into the nucleolus of the salivary gland cells of *D. cucurbitae* ($10 \mu\text{C}/\text{ml}$, 10 min, 15 days exposure). $\times 2000$.

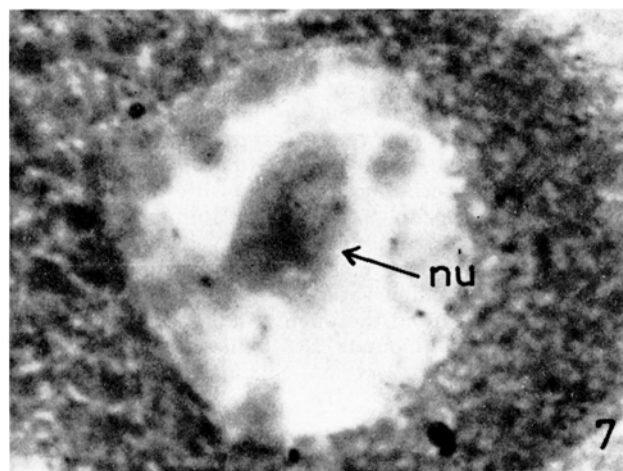


Fig. 7. Section of an actinomycin D ($20 \mu\text{g}/\text{ml}$, 10 min) treated salivary gland showing the complete inhibition of the incorporation of ^3H -cytidine ($50 \mu\text{C}/\text{ml}$, 5 min, 15 days exposure). $\times 1500$.

ribosomal cistrons¹⁵. In short term autoradiographic experiments using H^3 -cytidine it was observed that the nucleolus incorporated the precursor earlier than the chromosomes (Figure 6), and the incorporation is suppressed by actinomycin D treatment (Figure 7). This suggests the role of intranucleolar DNA in the synthesis of ribosomal RNA^{16,17}.

Zusammenfassung. Es wurde DNA nachgewiesen, das nicht in direkter Verbindung steht mit der chromosomalen DNA. Histologische Untersuchungen wurden an der Speicheldrüse einer Fliege (*Dacus cucurbitae*) durchgeführt. Die intranukleäre DNA findet sich in Form von Granula verschiedener Grösse, teilweise auch in Form von Bändern oder Zapfen. Die Kern-DNA scheint an Zell-

proteine gebunden. Wird H^3 -Cytidin einverleibt, ergibt sich, dass der Kern früher als die Chromosomen intranukleäre DNA in ribosomale RNA synthetisieren kann.

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¹⁷ We are indebted to Prof. B. R. SESHACHAR for the interest and providing facilities.