Proteins of Microdissected Polytenic Cells

II. Analysis of the Proteins from Chromosomes, Nuclear Sap and Nucleoli of Chironomus tentans Salivary Glands

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Abstract. Chironomus tentans salivary glands were incubated in vitro with 3H-leucine for three hours. After fixation of the glands and microdissection, the labeled proteins from chromosomes, nucleoli and nuclear sap were analyzed by SDS-polyacrylamide gel electrophoresis. The nucleoli display a very distinctive profile with many well resolved low molecular weight bands. The chromosomes and nuclear sap may share common proteins since similarities are found in their profiles. Both profiles are specific, however. A highly labeled protein at about 65,000 D is the main characteristic of the chromosomes. Some of the major species of the nuclear sap, at 165,000, 135,000 and 50,000 D, are very minor components of the chromosomes. The nuclear sap was further microdissected into inner and outer zones. The outer zone, which also includes the nuclear envelope, shows a 210,000 band which is absent from the inner zone.

Introduction

The polytene chromosomes of salivary gland cells of Diptera offer an almost unique opportunity to study regulatory proteins operating at the pretranscriptional level. In Chironomus tentans, the salivary glands are specialized in the synthesis of a group of secretory proteins (reviewed by Grossbach, 1977). Cytogenetic evidence (Beermann, 1961; Grossbach, 1969) demonstrates that the information for the synthesis of these proteins is encoded in specific regions of chromosome IV harboring the large Balbiani rings. These giant puffs can be induced or repressed by various physical (Beermann, 1952; Yamamoto, 1970) or chemical (Beermann, 1973; Clever, 1966) treatments. On the other hand, the micromethods developed by Edström and coworkers (Edström, 1964; Lambert and Daneholt, 1975) allow one to analyze the macromolecular components of single genetic loci such as the Balbiani rings. In the accompanying article, we have described how these micromethods originally devised for the study of RNA synthesis and maturation can be applied to the analysis of nuclear
proteins. Prior to the study of specific regulatory proteins at the level of the Balbiani rings, we have analyzed by SDS-polyacrylamide gel electrophoresis the proteins from microdissected nuclear compartments of *C. tentans* salivary gland cells: chromosomes, nuclear sap and nucleoli. A preliminary report of this work has been presented elsewhere (Tanguay and Nicole, 1977).

**Experimental**

Materials and methods relevant to the rearing of the larvae, the incorporation of a labeled amino acid by explanted glands, the fixation and microdissection of the glands, the extraction and electrophoresis of proteins on SDS-polyacrylamide gels as well as the fluorography, have been described in the accompanying article. Thus, only a few supplementary details will be given in this section. Up to seven explanted salivary glands were incubated together in 50 μl of Cannon’s medium supplemented with 3H-leucine. The ethanol-acetic acid (3:1, v/v) mixture was the only fixative used in the present study. Nuclear components (Fig. 1) were isolated under a phase contrast microscope at a magnification of 200 ×. Proteins used as molecular weight markers for SDS-polyacrylamide gel electrophoresis included myosin heavy chain (220,000 D), phosphorylase A (94,000 D), γ-globulin heavy chain (50,000 D) and α-chymotrypsinogen (25,700 D).

**Results**

*C. tentans* salivary gland cells contain two nucleoli or sometimes a single fused nucleolus. In this study, the nucleoli were dissected free of visible pieces of chromosomes. Therefore, contamination by proteins from extranucleolar chromatin is kept minimal. Though the nucleoli constitute a relatively minor portion of the nuclear mass, they contain about the third of the nuclear radioactivity after 45 min of incubation with 3H-leucine, as illustrated in Table 1. After longer periods, the nucleolus-associated radioactivity declines relative to the chromosomes and nuclear sap. This will be expected if the nucleolar proteins as a bulk, or some of them, have a higher turnover rate than the majority of the proteins of other nuclear compartments. As shown in Figure 2A and Table 2, the nucleolar proteins demonstrate a distinctive electrophoretic pattern. Many