CYTOCHEMICAL STUDIES ON CYTOPLASMIC RNA-ASSOCIATED BASIC PROTEINS IN OOCYTES, SOMATIC CELLS, AND RIBOSOMES* **

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Summary. Cytochemical studies on oocytes from representatives of several animal groups, of somatic tissues known to possess high levels of cytoplasmic RNA, and of isolated ribosomes indicated that basic proteins associated with the ribosomes may be stained by the methods currently in use for demonstrating histones. These proteins were resistant to weak acid extraction in fixed material and were labile to acetylation, but pretreatment with hot 5% TCA caused a measurable increase in staining with a modified Sakaguchi reaction. Such proteins were not confined to oocytes, but could be demonstrated with the TCA-alkaline fast green method in mouse pancreas and liver cells, and by the ammoniacal-silver method in all cells with high levels of cytoplasmic RNA. Microspectrophotometric studies on Ascidia nigra and Clavelina picta oocytes indicated that the highest concentration of cytoplasmic basic proteins was found in smaller oocytes, but the concentration of alkaline fast green stainable cytoplasmic RNA-associated basic proteins decreased in a pattern that differed significantly from that of stainable levels of cytoplasmic RNA. The implications of these findings to current concepts in developmental biology were discussed.

Nucleoplasmic basic proteins with high levels of arginine which are not associated with nucleic acids were encountered in oocytes of all the echinoderm species studied and in the mouse.

Introduction

In the wake of the biochemical findings of Huang and Bonner (1962) and others, considerable attention has been focused on the possible role of histones in development. Cytochemical studies of Bloch and Hew (1961), Horn (1962), Moore (1963) and Bäckström (1965) have indicated that cytoplasmic RNA-associated basic proteins are present in blastomeres, and that these proteins disappear or become unstainable as differentiation progresses. Earlier studies of Cowden (1958, 1962, 1963), and Seshachar and Bogga (1962) indicated that the patterns of staining intensity of cytoplasmic basic proteins in oocytes stained by methods for histones were similar to those obtained for RNA; i.e., cytoplasmic staining intensity was highest in previtellogenic oocytes and diminished as vitellogenesis progressed. Davenport and Davenport (1965a and b) and Bäckström (1965) demonstrated that these cytoplasmic proteins were associated with RNA, and that they were labile to both acetylation and deamination.

In the light of these findings it seemed desirable to investigate a broad spectrum of oocytes to establish whether this is a general finding, to examine some somatic tissues known to possess high cytoplasmic levels of RNA and isolated bacterial and animal ribosomes with staining procedures for demonstrating

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histones, to compare the patterns obtained with the published cytochemical methods for demonstrating histones, and to quantitate the relationship between levels of RNA staining and staining for basic proteins in growing oocytes.

Materials and Methods

Ovaries of the following species were investigated in this study: *Deroceras reticulatum*, *Arion* sp., *Littorina zinzac*, *Pecten zinzac* and *Modiolus americana* (molluscs); *Lytechinus verigatus*, *Echinometra leucunter*, *Euclidaris tribuloides*, and *Stichopus badionotus* (Echinoderms); *Ascidia nigra* and *Clavelina picta* (Ascidians); *Branchiostoma caribaeum* (Cephalochordate); *Rana pipiens* (Amphibian); and *Mus musculus* (Mammal). All of the marine material with the exception of *Branchiostoma* was collected near the Bermuda Biological Station, St. George’s West, Bermuda. The latter was obtained by dredging near the Sea Horse Key Marine Laboratory of the University of Florida. Terrestrial species were obtained locally or purchased from dealers. In order to compare the patterns of staining in oocytes with those of somatic tissues, the following organs were subjected to identical procedures: mouse liver, pancreas and cerebellum, *Amphiuma* liver, and central nervous systems of the roach, *Nauphoeta cinerea*. Free ribosomes were isolated from *Shigella*, and by deoxycholate treatment of the microsomal fraction from rat livers. Ribosomes from both sources were resuspended in a smaller volume of fluid, drops were placed on slides, and these were allowed to dry. The dried preparations were subsequently fixed in neutral formalin and subjected to the same cytochemical procedures used on tissues.

Ovaries and the other tissues studied were fixed in neutral buffered formalin or ethanol-acetic acid (3:1). These were embedded in a paraffin embedding mixture in the routine manner and sectioned serially at 5 µm. Ethanol-acetic acid fixed material was stained for RNA using DNase pretreated sections followed by either the methylene blue method of Deitch (1964) or the azure B method of Flax and Himes (1952). Similarly fixed material was stained by the Sakaguchi reaction for protein arginine as modified by Deitch (1961), both with and without prior treatment with 5% trichloroacetic acid (TCA) at boiling water bath temperatures for 15 minutes. The latter procedure has been used to evaluate the extent to which removal of nucleic acid unmasks reactive groups of arginine (Backstrom, 1965).

Formalin fixed sections were employed for staining with the alkaline fast green method of Albert and Geschwind (1963) and its variants. In each series of experiments, the following pretreatments were employed: a distilled water control, hot 5% TCA for 15 min, hot TCA followed by two hours' acetylation in 5% acetic anhydride in glacial acetic acid (Deitch, 1955), RNase, and DNase. Sections were then stained for 30 min in 0.1% fast green FCF at pH 8.1. Similar experiments were conducted with the acid dye biebrich scarlet at pH levels of 8.0, 8.5, 9.5 and 10.5. Both formalin fixed and ethanol-acetic acid fixed material was used in conjunction with the ammonical-silver method of Black and Atchley (1964). As they stipulated, ethanol-acetic acid fixed sections received a brief treatment with formalin prior to staining. The metaphosphate-gallocyanin-chromalum method of Jobst and Sandritter (1964) was also employed. Ethanol is the fixative of choice and formalin fixed material can not be used. Since most of this material had been collected prior to the publication of their method, ethanol-acetic acid fixed sections were employed, but this probably does not represent optimal fixation.

Attempts were made to extract the cytoplasmic basic proteins with 0.2N HCl by treatment of sections for up to six hours at room temperature, and by digestion in 0.3% trypsin (Worthington 3 x recrystalized) at 37°C for 30 min according to the method of Moses and Coleman (1964). Since it was considered certain that the rather rigorous treatment with hot 5% TCA removed some protein as well as nucleic acids, this was evaluated by examining sections mounted on quartz slides with a Zeiss ultraviolet microscope using 2600, 2800, and 3100 Å monochromatic light before and after treatment.

Microspectrophotometric measurements were undertaken using the E. Leitz, Inc. (New York, New York) commercial version of the microspectrophotometer developed by Pollister and Ris (1947). Measurements on oocytes were made using a 45× objective, and three measurements were taken at different sites in the oocyte cytoplasm and averaged. The major and minor axis of oocytes were measured with a Leitz 12.5× filar micrometer ocular