210. *Development of Chromogranules in the Larval Skin of the Silkworm*^{*)}

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In our preceding papers, studies have been reported on the structure and chemical composition of chromogranules produced in larval skin cells of the silkworm (in press). An analysis of the secretion products stored in the vesicle of chromogranules was carried out and a part of the experimental results were reported (Tsujiita and Sakurai, 1964 a, b, c).

The present paper deals with our studies of developmental processes of the chromogranules in larval skin cells of the silkworm carried out by electron-microscopical observation of thin sections.

Materials and methods. Two normal strains (YD₁ and B₁) and two mutants (*E-lem* and *w^{oz}* oily) were used as materials. The dorsal skin of 6-7 segments of larvae in 3rd-5th instar were cut off and fixed. The materials were fixed in 4% formic acid containing Millonig's phosphate buffer, 1% osmic acid containing s-Collidine buffer or 2% osmic acid containing Millonig's phosphate buffer. After fixation, the materials were dehydrated and embedded in Epon-resin. Sections were stained with uranyl acetate or double stained with uranyl acetate and Reynold solution (Reynold 1963). Among those fixatives 2% osmic acid containing Millonig's phosphate buffer gave better results than the other two.

Observation. The larval skin cells of the silkworm larvae have generally the shape of short cylinders and the elliptical nucleus occupies a central position. Figs. 1 and 2 represent sections through a part of a hypodermal cell immediately after the 3rd moulting showing a portion of the nucleus and cytoplasm. An elliptical mitochondrion with double membrane and inner tubular structure can be seen in the cytoplasm (Fig. 1. cell M). There are numerous ribosomes, 150-200Å in diameter, and they assemble here and there in the cytoplasm forming ribosomal aggregates. A small spherical vesicle appears in the center of each aggregate. This well delimited formation is the precursor of the chromogranules; the vesicles gradually increase in size and spherical or elliptical granules are formed. The ribosomes arrange themselves uniformly in a layer surrounding the granular membrane and some proteinous substance

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can be seen inside.

It was substantiated (Tsujita and Sakurai 1964) that soluble proteins combining either with pteridine pigments or with uric acid are present in the chromogranules. It may be safely said that these compounds are contained in the granular vesicles and

Fig. 1. Section of a larval skin cell of the normal (YD₄) strain.

Fig. 2. Section of a larval skin cell of the normal (YD₄) strain.

that when the chromogranules are pressed upon, they are squeezed out. According to another experiment described in our preceding paper (in the press), the chromogranules contain lipids and RNA. It seems that the lipids are contained in the membrane of the granules and RNA is an essential component of the ribosomes associated with the granular membrane.

Fig. 3. Procedure used to separate mitochondria from the cytoplasmic layer of larval skin cells.

The well delimited precursor of the chromogranules can be precipitated by a procedure leading to the separation of the mitochondrial fraction as shown in Fig. 3. Fig. 4 shows a section through the mitochondrial fraction. In this figure numerous chromogranules precursors are seen, each of them surrounded by ribosomal particles. It was stated before that the process of chromogranule development from initial to mature stage can be followed up in the cytoplasm of hypodermal cells. Almost the same developmental process, as Fig. 5 shows, can be observed on the sections through the mitochondrial fraction. Among a to k stages of this

Fig. 4. Section of the mitochondrial fraction from larval skin cells of the lemon strain.

DC: developing chromogranule M: mitochondria
(Fixative: 2% osmic acid containing Millonig's phosphate buffer,
magnification: 20,000).

Fig. 5. Developmental process of chromogranules in hypodermal cells of the silkworm.

a: initial stage, b-k: developmental stages

(a-i: from section of the mitochondrial fraction; j-k: from section of the chromogranule fraction).

Fig. 6. Section of the mitochondrial fraction from larval skin cells of the *w^{ox}* strain.

M: mitochondria, DC: developing chromogranules

(Fixative: 2% osmic acid containing Millonig's phosphate buffer, magnification: 20,000).

figure j and k sections through the granules were picked up after precipitation by the procedure described in our preceding paper (in the press), because they had much higher density than the granule precursors and could not be precipitated by the procedure used to separate the mitochondrial fraction.

It was reported (Tsujiita 1959) that w^{oz} gene is located on the 10th chromosome and that no chromogranules are produced in the hypodermal cells of the homozygous larvae in which pteridine compounds and uric acid are scarcely present. However, when the procedure to collect mitochondria was carried out using the cytoplasmic layer gathered from larval skin cells, the chromogranule precursors were precipitated together with the mitochondria in the mitochondrial fraction as shown in Fig. 6. Therefore, it is considered, that owing to the presence of w^{oz} gene, the development of the precursors into mature chromogranules containing soluble proteins is completely suppressed in the hypodermal cells of w^{oz} homozygotes.

Discussion. It is well known that the cell sap of many cell types (e.g. liver) contains a system of interconnected membrane-bounded compartments collectively known as the endoplasmic reticulum (ER). To the surface of some portions of the endoplasmic reticulum particles of about 150 Å diameter known as ribosomes are attached. The membranes of the reticulum have a glandular appearance in the electron photograph, hence it was called rough surface endoplasmic reticulum. The rough surfaced membrane is the site of synthesis of proteins which are dispatched from here, for example such digestive compounds as the enzymes produced in the pancreas and albumin produced in the liver. Other portions of the endoplasmic reticulum are free from ribosomal particles and are correspondingly termed smooth surfaced endoplasmic reticulum.

The granule membrane with attached to it numerous ribosomal particles resembles the rough surfaced endoplasmic reticulum. But, it is not identical, because the membranes found in the present study surround closed pouches for secretion and storage. Eleven loci involved in the manifestation of transparent larval hypodermis which are distributed on nine chromosomes have so far become known in the silkworm. It is assumed that among them at least several loci including w^{oz} locus, are concerned with the formation of chromogranule membrane which probably plays some important role in the secretion of soluble proteins combining with pteridine pigments and with uric acid and also in the storage of those

products. And when genes which are related to the development of granular membranes are normal, the vesicles have complete or sufficient ability to secrete and to store, and abundant chromogranules packed compactly with secretion products are formed in the larval skin. However, if either of the two functions of the granular membrane, secretion and storage, is defective owing to mutation of one of the normal genes, no chromogranules can be produced or the density of each mature granule as well as their amount are reduced and consequently highly transparent or semi-transparent skin is produced.

So far as our experimental results are concerned it seems that uric acid production and pteridine metabolism are normally carried out in the w^{ox} mutant larvae. Therefore, a hypothesis that some critical error might occur in the secretion of soluble proteins combining with these products into the glandular vesicle surrounded by ribosomes or in the storage of these products within the chromogranules is presented in an attempt to explain, why the further development of chromogranule precursors is entirely suppressed, in homozygotic w^{ox} mutant larvae.

References

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