Control of Protein Synthesis in the Female Pupa of *Bombyx mori*

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Synthesis of specific proteins of silkmoth pupae has been studied, but an investigation on the production of general classes of proteins would be equally important for understanding mechanisms of insect metamorphosis. Synthetic rates and contents of total RNA and proteins change markedly in the female pupa of *Bombyx mori*. We have, therefore, attempted to find what is the limiting step for the synthesis of the bulk of proteins during adult development of the female pupa.

Several female pupae of a hybrid strain (N122 × C115) were taken at each of stated periods and homogenized in a mortar-driven, Teflon-glass homogenizer in 3 volumes of buffer A (20 mM Tris-HCl, pH 7.8, 5 mM MgCl₂, 100 mM KCl, 6 mM β-mercaptoethanol, 1 mM dithiothreitol, 0.1 mM EDTA, and 0.25 M sucrose). If necessary, meconium was removed from the pupae before homogenization. Ribosomal fraction was prepared from the homogenate essentially according to a previously reported procedure, except that 1 M sucrose cushion, made in buffer A without sucrose, was used for pelleting ribosomes. The pellets were resuspended in buffer A to a final concentration of 50 A₂₆₀/ml. Aliquots of the ribosome suspensions were measured for incorporation of leucine depending on endogenous mRNA (a) and incorporation of phenylalanine directed by added poly U (b). The reaction mixture contained the following (per ml); 4 A₂₆₀ units of ribosome preparations, 6 mg of mouse liver high-speed supernatant proteins which was prepared as previously described, 4 mM MgCl₂ (10 mM for (b)), 60 mM KCl (90 mM for (b)), 1.6 μCi ³H-leucine for (a) or 1.6 μCi ³H-phenylalanine for (b) (51 Ci/m mole or 1 Ci/m mole, respectively; Radiochemical Centre, Amersham), 0.025 μmoles each of other amino acids (omitted for (b)), 1 μ mole of ATP, 0.3 μmoles of GTP, 10 μmoles of phosphocreatine, 50 μg of creatine phosphokinase, 50 μg of mouse liver tRNA, 100 μg of poly U (omitted for (a)), and 20 mM Tris-HCl, pH 7.8.

![Figure 1. Incorporation of Leucine (Endogenous mRNA) and Phenylalanine (Poly U) by Ribosomes Prepared from the Female Pupae at the Periods Indicated.](image)

Each incubation was carried out at 30°C for 30 min in a reaction mixture of 100 μl, supplied with mouse liver supernatant proteins and other substances as described in text. Acid-insoluble radioactivity was assayed as previously described. Data were corrected for non-specific incorporation. Data for phenylalanine were also corrected for endogenous incorporation measured without poly U.

Figure 1 shows that the incorporation of leucine due to endogenous mRNA is the largest in 9–10 days pupae of the samples studied. This result is in agreement with that of an in vivo incorporation of a radioactive amino acid into proteins of the female pupae. On the other hand, poly U-directed phenylalanine incorporation, which depends on a mRNA-binding activity of ribosomes, exhibited a maximum on the 7th day, and then completely dropped on the 9–10th day, indicating that ribosomes were saturated with natural mRNA at the latter period. It is likely that available mRNA rapidly increases at the time when a
massive protein synthesis takes place.

We suggest that the synthesis of proteins during the late adult development of the female silkworm is controlled at the level of mRNA. The similar conclusion has also been described about the production of total proteins in a fibroblast, α-fetoprotein in the developing mouse liver, total proteins and histones in developing sea urchins and muscle proteins in the tobacco hornworm.

As seen in Fig. 1, the increase of ribosomes which were active to bind mRNA preceded the appearance of available endogenous mRNA. This increase of active ribosomes may at least partly be attributed to a neogenesis, since the total ribosome content rises on 5–7 days in the female pupae, and the incorporation rate of RNA precursors is extremely high about the middle of the pupal period. The increase of active ribosomes may be attributed also to a possible “run-off” of previous ribosomes, which have probably originated in the larval tissues. It is conceivable that such a neogenesis or a run-off serves as a less direct control for the protein synthesis during metamorphosis of Bombyx mori.

The production of mRNA and ribosomes involves several steps and further studies are needed to determine which of these steps are more limiting. An investigation on tissue specific controls in the silkworm pupae is in progress in our laboratory, using homogeneous pupal systems without supplying mouse liver proteins.

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