Changes of yolk and haemolymph proteins during ovarian development in the miniature egg mutant, *emi*, of *Bombyx mori*

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The changes in the amount of yolk and haemolymph proteins were investigated during ovarian development in a mutant which produces small sized eggs and was named the miniature egg (*emi*). The ovary of this mutant possessed not only vitellin (H and L) and the 30 kDa proteins synthesized in extra-ovarian tissues but also the egg specific protein (ESP) synthesized in the follicular epithelial cells of the ovary. However, the contents of these proteins in the mutant ovary were considerably lower than in the normal ovary. On the other hand, large amounts of vitellogenin and the 30 kDa proteins were found to remain in the haemolymph of *emi* females at the late pupal and the adult stages. The *emi* gene is likely to affect neither incorporation of vitellogenin and the 30 kDa proteins into the ovary nor synthesis of ESP in the ovary. There is the possibility that the gene is expressed in the process of determination of egg size.

Key words: *Bombyx mori*, egg character mutation, miniature egg mutant, ovarian development, yolk proteins, haemolymph proteins

In *Bombyx mori*, there have so far been known numerous mutants which produce morphologically unusual eggs, such as kidney-shaped, spindle-shaped, long-elliptic, large-sized and small-sized ones (DOIRA et al., 1992). The egg shape is decided by the maternal genotype before fertilization, and therefore it is inherited in a pseudo-maternal manner.

A spontaneous mutant named the small egg (CHIKUSHI and DOIKA, 1970) is controlled by a recessive gene *sm* located on the 3rd linkage group and a similar mutant named the small egg-2 (DOIKA et al., 1974) is controlled by a recessive gene *sm-2* located on the 13th linkage group. The eggs of these mutants are lacking in proteinaceous large yolk spheres, and cannot undergo blastoderm formation and
fertilization (OHTSUKI, 1965). Another spontaneous mutant of egg character was found in 1989. Genetic analyses (KAWAGUCHI et al., 1991) have indicated that the relevant gene is recessive and linked with the outer-layer yellow cocoon (C) gene, a cocoon color mutant located on the 12th linkage group. Since the eggs were as small as those of sm and sm-2, the new mutant was named the "miniature egg" with the symbol emi. However, unlike sm and sm-2, the emi mutation allows fertilization and hatching (KAWAGUCHI et al., 1993). The mutant was thus considered to offer a beneficial material for the elucidation of gene expression during the process of egg-yolk formation.

In the present study, we investigate changes in the content of yolk and haemolymph proteins during oogenesis of the emi mutant. Also analyzed are the gel electrophoresis patterns of the mutant for vitellin, 30 kDa proteins and the egg specific protein (ESP), which are the major components of yolk proteins. All these proteins were found to be present in yolk of the emi ovaries, but their contents were lower compared to the normal yolk. On the other hand, the haemolymph of the mutant animals was rich in yolk precursor proteins.

Materials and Methods

Insects: The silkworm strain d36 (a stock of the Institute of Genetic Resources, Faculty of Agriculture, Kyushu University), which contains the emi mutant gene, was applied. This strain includes the C gene as a maker and has been maintained by the cross C +/- emi (female) x +/+ emi (male). The cross gave rise to individuals with yellow cocoons (C) and white cocoons (+) within the same batches. The C females produced normally sized eggs (Fig. 1A, C), while the + females produced small sized, emi eggs (Fig. 1B, D).

The ovaries and haemolymph from the C female pupae (and adults) were used as normal samples and those from the + female pupae (and adults) were used as emi samples.

Preparation of specimens for protein analyses: Ovaries were collected from pupae at 48 hr intervals during the pupal-adult development, and also from adults shortly after emergence (unmated), washed with distilled water, weighed and homogenized in ice-cold insect Ringer's solution using a Teflon-glass homogenizer. The homogenate was centrifuged at 15,000g for 15 min and the supernatant was subjected to the analyses of protein quantity and quality. At 48 hr intervals during the pupal-adult development, female pupae (and newly emerged, unmated female adults) were

Fig. 1. Features of ovarian eggs and deposited eggs. A and C, normal; B and D, emi. Arrow indicates unfertilized eggs.
each pricked with a pin to make a hole, through which clear haemolymph was dropped into a micro test tube containing a few crystals of phenylthiourea. The haemolymph was spun at 5,000g for 5 min at 5°C using a microcentrifuge and the supernatant was used for protein analyses.

**Determination of protein concentration:** The ovary and haemolymph supernatants were each mixed with 2 vols. of cold 10% trichloroacetic acid. The precipitate was collected by centrifugation, washed twice with cold 5% trichloroacetic acid and once with ethanol : ether (1 : 1, v/v) and dissolved in 0.1 N NaOH. The solution was measured for protein concentration by a modified Lowry's method using filter paper tips (Hayashi, 1983) with bovine serum albumin as a standard.

**Polyacrylamide gel electrophoresis of proteins:** Aliquots of the supernatants were examined for protein constituents by polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate (SDS-PAGE) at 8.5% gel concentration (Laemmli, 1970). Proteins were stained with Coomassie Brilliant Blue R-250 (Sigma Co. Ltd.). The size markers (Sigma Co. Ltd) myosin (205 kDa), galactosidase (116 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa) and carbonic anhydrase (29 kDa) were co-migrated. The bands for vitellin-H, vitellin-L, ESP and the 30 kDa proteins were allocated according to previous gel patterns (Ono et al., 1975; Izumi et al., 1981; Irie and Yamashita, 1983; Zhu et al., 1986). The gels were subjected to densitometry using a Beckman DU-8 spectrometer, and the protein contents were calculated on the basis of the relative peak areas.

**Results**

**Ovarian development and changes of yolk proteins**

The values of body weight during the progress of pupal life scarcely differed between the normal and the emi animals (Fig. 2A, B). The ovaries of the normal individuals grew rapidly on days 3 to 9, whereas the ovaries of the emi individuals exhibited a significantly slower weight gain than the normal (Fig. 2C). The weight ratio of ovary vs. pupal body was more than 50% in the day-9 normal pupae, whereas it was below 25% in the emi pupae at the same age (Fig. 2D). The rate of increase in protein content was very marked in the normal but not in the mutant (Fig. 3). Upon analysis of protein constituent by SDS-PAGE (Fig. 4), the ovaries showed intensive vitellin-H and vitellin-L bands on days 5 to 9 in both normal and emi animals. Also 30 kDa protein bands in the mutant ovaries were as strong as those in the normal ovaries. ESP bands were clear on day 5 and thereafter both in the normal and the emi ovaries. The ovaries (mostly composed of matured eggs) taken out of unmated female adults showed bands for vitellin-H, vitellin-L, the 30 kDa proteins and ESP upon SDS-PAGE (Fig. 5A). The staining intensity of bands seemed to be low in the mutant. The gels shown in Fig. 5A were subjected to densitometry and the tentative contents of the proteins were calculated. All vitellin-H, vitellin-L, the 30 kDa proteins and ESP were less abundant in the mutant than in the normal (Fig. 5B).

**Changes of haemolymph proteins during pupal development**

Fig. 6 shows the quantitative changes of haemolymph proteins in female pupae during the pupal-adult development until emergence. In the normal females, there was a rapid decrease in concentration during the period from day 3 to emergence. On the other hand, the emi
Fig. 2. Changes in weight of body and of ovary during pupal-adult development. A and B, body weight of normal and emi, respectively, (circles, females; triangles, males); C, wet weight of ovary (open circles, normal; closed circles, emi); D, ratio of ovary weight per pupal body weight (open columns, normal; shadowed columns, emi). 1 to 9, day 1 to day 9 of pupal stages; AS, adult shortly after emergence. Vertical bar, standard deviation.
Fig. 3. Changes in content of ovarian proteins during pupal-adult development. Open circles, normal; closed circles, emi. For abscissa see Fig. 2.

Fig. 4. SDS-PAGE analyses of yolk proteins in developing ovaries. The crude extracts (10 μl, ca. 10 μg protein per lane) were subjected to SDS-PAGE (8.5%). The positions of markers, with sizes in kDa, are given along the left margin. P-1 to P-9, day 1 to day 9 of the pupal-adult development. A, normal; B, emi. ESP, the egg specific protein; 30K, the 30 kDa proteins.

Fig. 5. SDS-PAGE profiles of yolk proteins (A) and the contents of vitellin (H plus L), the 30 kDa proteins and ESP (B) in normal and emi eggs. The position of size marker, in kDa, are given along the right margin in A. Vertical bars in B, standard deviation.

females showed a slower decrease. Consequently, the haemolymph protein concentration in the adults of emi remained considerably high. The electrophoretic profiles of haemolymph proteins after larval-pupal
ecdysis until emergence are shown in Fig. 7. The bands for vitellogenin-H, vitellogenin-L and the 30 kDa proteins were recognized as the major components on day 1 in both normal and emi samples. Vitellogenin (H and L) was detected sex-specifically and the 30 kDa proteins were bisexual. In the normal pupae, these components seemed to be decreased during the pupal-adult development (Fig. 7A). In the mutant pupae, however, vitellogenin (H and L) and the 30 kDa proteins were detected as the intensive bands throughout the stages tested (Fig. 7B). The putative concentration of vitellogenin (H and L) in the haemolymph at the middle to late pupal stages, calculated on the basis of the densitometric analysis of the gels, reduced rapidly in the normal females while it rather increased in the emi females (Fig. 8A). As to the 30 kDa proteins, a quick decline in concentration was seen in both normal and emi, but its rate was slower in the latter than in the former (Fig. 8B).

**Discussion**

The fact that homozygous females for the recessive mutant gene of emi produce eggs which are considerably small in size but are able to be fertilized and hatch might have relevance to the present finding that the weights of homozygous pupae of emi did not differ from the normal values, and the ovaries of the mutant pupae matured according to the same time schedule as the normal ovaries. However, the rate of increase in ovarian weight in the mutant was significantly lower than that of the normal. All of vitellin H, vitellin L and the 30 kDa proteins (derived from the respective haemolymph precursors) and ESP (synthesized in the ovaries per se) were accumulated also in the emi eggs, implying that the nutritional materials required for embryogenesis are prepared in the emi eggs in spite of their small size. The finding indicates that the mutant ovaries can take up vitellogenin and the 30 kDa proteins from the haemolymph into the ooplasm normally, but the amounts of these proteins are repressed. It is worth noting here that the number of mature eggs produced per individual were clearly fewer in emi than in normal (KAWAGUCHI et al., 1993). As a result, large amounts of vitellogenin (the precursor of vitellin) and the 30 kDa proteins were retained in the haemolymph of emi females at the late pupal stages. We suggest that the synthesis of vitellogenin and the 30 kDa proteins in the fat body, as well as their incorporation into the ovaries, and the synthesis of ESP in the follicular epithelial cells are scarcely

![Fig. 7. SDS-PAGE profiles of haemolymph proteins during pupal-adult development. Haemolymph specimens (10 μl, ca. 8 to 10 μg protein per lane) were subjected to SDS-PAGE (8.5%). A, normal; B, emi mutant. 30 K, 30 kDa proteins. For abscissa see Fig. 4. The positions of markers, with sizes in kDa, are given along the left margin.](image-url)
affected by the emi mutant gene. In view of these results it is possible to infer that the emi gene functions as an egg size determinant. Further studies on this mutant is in progress in our laboratories.

References


Seikagaku, 55, 257-258.


ONO, S., NAGAYAMA, H. and SHIMURA, K.


ZHU, J., INDRASITH, L. S. and YAMASHITA, O.


河口 豊・伴野 豊・古賀克己・土井良 宏: カイコ卵小卵における卵黄タンパク質の形成と体液タンパク質

受精卵と孵化能を有するカイコ小卵突然変異、卵小卵 emi の卵巣発達に伴う卵黄と体液タンパク質の変動について正常卵との比較分析を行った。emi 卵卵果のタンパク質含量は発育の経過に伴って上昇するものの正常に比べ約１／３と低くなっていた。しかし、卵黄タンパク質組成には変化はみられなかった。一方、体液タンパク質については孵化初期では顕著と組成に差異は認められなかったが、孵化期と羽化直後では emi 雌の体液中に卵黄タンパク質前駆成分が多量残存していた。これらの結果から、emi 遺伝子は卵サイズ決定のみに関与しているものと推察した。