Pattern Comparison of in Vivo Protein Synthesis between Normal and "Kidney" Eggs of *Bombyx mori* (Lepidoptera: Bombycidae)\(^1\)

Akira Saito,\(^2,3\) Eiichi Kosegawa,\(^2\) Si Kab Nho,\(^2,4\) Katsumi Kooga,\(^2,5\) Bungo Sakaguchi\(^5\) and Yasushi Sugimoto\(^6\)

\(^a\)Laboratory of Sericulture, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan
\(^b\)Department of Food and Nutrition, Seinan-Jogakuen Junior College, Kitahara-Kitakata, Kitakyushu 803, Japan

(Received July 15, 1991)
(Accepted September 21, 1991)

**Key words:** protein synthesis, *Bombyx mori*, organogenesis, ki mutant

The mutant of *Bombyx mori* called "kidney-shaped egg (ki)" is known to exhibit peculiar genetic effects on the morphology of chorions; the eggs are distorted into a kidney-like appearance because of some abnormality in the ovarian follicular epithelial cells by which the chorion proteins are secreted (Sakaguchi et al., 1982; Kawaguchi et al., 1987), and the embryo differentiates neither mesodermal nor endodermal organs but only aberrant ectodermal tissues (Suzuki and Ichimaru, 1955; Miya, 1985). In a study on developmental changes in activity of alkaline phosphatase (Koga et al., 1988), the ki eggs exhibited marked diversity from the normal pattern after day 5 (the blastogenesis stage) of embryogenesis. This paper deals with two-dimensional gel analyses of the prevalent class of newly synthesized proteins in the ki and normal eggs. On day 5 after oviposition mutant-dependent defects in protein spots were apparent.

**MATERIALS AND METHODS**

The stock d4l, which contains the *ki* gene as well as the E\(^k\)s gene as a marker, was used to obtain the maternally segregated *ki* and normal eggs (Sakaguchi et al., 1982; Kawaguchi et al., 1987). Eggs deposited within 10 to 30 min were collected at 24 h of development and treated with hot HCl (spec. grav. 1.07, 47°C, 5 min) to break diapause and allow further development at 25°C. Embryogenetic staging was done according to Takami and Kizazawa (1960). Our unpublished results indicated that the blastokinesis occurred in the *ki* eggs on a normal time schedule.

Eggs glued onto a paper substrate were each punctured twice with a sharp tungsten needle on the dorsal side of the chorion, and 1.2 \(\mu\)Ci/0.04 \(\mu l\) of \(\text{L-[35S]}\)methionine (1,225 Ci/mmole, Amersham) was injected through one of the holes with a thin glass needle. Forty eggs were used for one analysis. After storage at 25°C, the injected eggs were dehydrated with acetone and cut with a razor blade in lysis solution containing 9.5 M urea, 10% sodium dodecyl sulphate, 2% (w/v) Triton X-100, 2% (v/v) Ampholine (Pharmacia, pH range 3.5–10 plus 5–7, 1:4) and 5% 2-mercaptoethanol. The contents of the eggs were thoroughly homogenized with a glass pestle in a glass homogenizer. The mixtures were centrifuged at 10,000 \(\times\) g for 10 min at 0°C and the resulting supernatants were subjected to two-dimensional gel electrophoresis (Shoikawa et al., 1989).

---

\(^2\) Present address: National Institute of Agrobiological Resources, Tsukuba, Ibaraki 305, Japan
\(^3\) Present address: Department of Sericulture, College of Agriculture, Kyungpook National University, Daegu, Korea
\(^5\) To whom correspondence should be addressed.
1985). The gels were stained with Coomassie brilliant blue in 45% methanol-10% acetic acid. Radioactive spots were then visualized by fluorography as described previously (Shiokawa et al., 1985). The amounts applied were adjusted to contain roughly equal radioactivity in all the samples. In the second dimensional migration, mol. wt. marker proteins (Shiokawa et al., 1985) were run in parallel.

RESULTS AND DISCUSSION

Eggs were labeled with L-[35S]methionine, kept for 2 h and analyzed for newly synthesized proteins by two-dimensional gel electrophoresis followed by fluorography. The distribution patterns of radioactive spots were superimposed with stained gels, and marks were drawn on the fluorograms at the positions of the following major endogenous, non-radioactive yolk proteins: the heavy subunit of vitellin (H), the light subunit of vitellin (L), the heavy and light subunits of egg specific protein (E) and 30 kDa proteins (T), whose molecular weights and pIs have been previously reported (Irie and Yamashita, 1983; Zhu et al., 1986).

![Fluorography after two-dimensional gel electrophoresis of extracts from day-3 eggs. A, normal eggs; B, ki eggs. Eggs were injected with L-[35S]methionine, kept at 25°C for 2 h, extracted and analyzed as described in text. The first-dimensional run was from left to right and the second-dimensional run from top to bottom. The positions of marker proteins are drawn along the left margin (size in kDa). Refer to text for the symbols written on the photographs.](image-url)
About 300 radioactive spots were detected in the fluorography. Many spots, like those represented by the marks u to z, appeared constantly at all the stages tested for the normal eggs. The spot called x (mol. wt. 46,000 daltons and pI about 5.4) was tentatively identified to be actin, and the spots around y and z (mol. wt. 54,000 daltons and pI 4.5 to 5.2) seemed to make a cluster of tubulin, because their respective size and pIs were similar to those of actin and tubulin of different organisms (e.g. BEDARD and BRANDHORST, 1983). Other components including those with the numbers 1 to 16 showed temporal modulation in labeling.

The fluorographic patterns for the samples at 4 h after oviposition (as well as for unfertilized eggs) appeared to be between the normal and ki eggs (results not illustrated). These findings indicate that the ki mutation has little influence on the dominant class of proteins synthesized at the stages close to fertilization.

The distribution patterns of protein spots in the normal and ki eggs on day 3 after oviposition (at an early organogenetic stage) are shown in Fig. 1. Virtually all the spots found in the normal eggs (A) could be detected in the ki eggs (B). Some differences were seen in intensity of corresponding spots between the normal and ki eggs, but repeated experiments revealed the difficulty of drawing definite

---

Fig. 2. Fluorography after two-dimensional gel electrophoresis of extracts from day-5 eggs. A, normal eggs; B, ki eggs. Each of the spots with arrowheads in A had no counterpart in B (see the open circles). For other comments see the legend to Fig. 1.
specificity of the \( ki \) eggs; only Spot 13 was consistently weaker in the \( ki \) eggs than in the normal eggs. We therefore conclude that the \( ki \) eggs synthesize a very similar set of major proteins to that of the normal eggs at this stage.

On day 5 after oviposition, i.e., at the mid-organogenetic, blastokinesis stage, the components including 1, 3, 4, 8, 15 and 16 found in the normal eggs (see the arrowheads in Fig. 2A) were no longer detected in the \( ki \) eggs (see the open circles in Fig. 2B); Spots 10 and 11 were newly found ones in normal eggs, but their counterparts were absent in \( ki \) eggs. Spot 13 was still fainter in \( ki \) eggs than in normal eggs. Even the component named y, a part of the tentative tubulins, was lacking. These results for day-5 eggs were reproducible in duplicated series of experiments.

As a whole, we suppose it to be on day 5, or a bit earlier, that the \( ki \) eggs exhibit the first appreciable aberration in protein synthesis. In the analyses of total egg protein composition, a difference between the normal and \( ki \) eggs becomes obvious again on day 5 (Kawaguchi et al., 1987). In contrast, the effects of the \( ki \) mutation in morphology have already emerged at the onset of invagination (Miya, 1985), i.e., at the stage of early gastrulation. Thus, the \( ki \)-dependent defects in terms of synthesis and metabolism of proteins become manifest later than those of the first morphological symptoms. This implies the polypeptides that were specifically affected in the \( ki \) eggs include the products of the genes that would normally be expressed after the differentiation of mesodermal and endodermal organs.

ACKNOWLEDGEMENTS
We thank Professor H. Doi and other members of the Institute of Genetic Resources, Kyushu University, for their help in preparing the silkworms.

REFERENCES


