Short Communication

Stage-dependent Synthesis of a Protein during Post-diapause Development of *Bombyx mori*

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Silkworm eggs enter diapause at the gastrula stage if they have been conditioned to hibernate. During diapause the rate of protein synthesis is suppressed,1) but it rises by 3 to 4 times after the onset of post-diapause development.2) Most of the increase is during the first 20- to 24-hr period,3) which is morphologically a preparatory stage preceding the burst of organogenetic processes.3) When total RNAs were extracted at the onset of, and at 10 and 20 hr of, post-diapause development and their translation products in an *in vitro* system were compared, no marked differences in sizes of the products were observed, indicating that the higher rate of protein synthesis is established without overall changes of translatable mRNA species.4) However, this does not exclude the possibility of temporal appearance of a specific mRNA population. Here, we describe a translation product with a size of 73 kDa that is detected when RNAs are extracted within a limited duration of post-diapause development. A material with the same size was also found in proteins labeled *in vivo.*

Eggs of a Chinese-Japanese hybrid had diapause ended and post-diapause development started by long-term chilling and hot-HCl treatment (spec. grav. 1.10, 48°C, 6 min).2) Total RNAs were extracted and translated *in vitro* in a rabbit reticulocyte lysate cell-free system mixed with L-[35S]methionine (80 μCi/10 embryos) at 25°C for 2 hr. Then the embryos were washed with fresh medium, collected by centrifugation at 1800 rpm for 5 min, and extracted with the sodium dodecyl sulfate sample buffer.2) The solution was electrophoresed and fluorographed as above.

As shown in Fig. 1, the translation products of RNAs extracted at zero (shortly after the hot-HCl treatment), and at 10 and 20 hr, of post-diapause development were substantially more intense in Lane 2 compared to Lanes 1 and 4, which had RNAs from 5 and 20 hr post-diapause development. A material with the same size was also found in proteins labeled *in vivo.*

![Fig. 1. Products of Translation *in vitro* of RNAs in Rabbit Reticulocyte Lysate.](image)

Electrophoresis and fluorography were done as described in the text. RNAs were extracted at zero, 5, 10, and 20 hr after the onset of post-diapause development (Lanes 1, 2, 3, and 4, respectively). Lane 5, endogenous incorporation. The bars on the left stand for the positions of marker proteins (76, 67, and 60 kDa, from top to bottom). The solid triangle by Lane 2 points the stage-dependent 73-kDa protein. The band below the latter (72 kDa) was sometimes very intense but this was not reproducible.
 Electrophoresis and fluorography were done as in Fig. 1. Embryos were taken at zero, 5, 10, and 20 hr after the onset of post-diapause development (Lanes 1, 2, 3, and 4, respectively). The amount of total label increased as development proceeded and the intensities of bands were made as even as possible among different lanes by controlling the time of fluorographic exposure. See Fig. 1 for the bars and solid triangle. The open triangle along Lane 1 shows the 54-kDa band which was selectively strong in early stages.

similar to each other, in agreement with our previous results.\(^4\) However, an additional band the molecular mass of which was estimated to be 73 kDa was consistently seen when 3- to 9-hr RNAs were translated. Figure 1 shows a typical pattern at 5 hr. A band with this size was scarcely detected in samples at other ages. Also in the newly synthesized proteins labeled in the cultures of cut embryos, a 73-kDa band was observed (Fig. 2). This band was very intense only when the above-mentioned stage-specific translation product appeared. In this case a co-migrating band was weakly seen in the samples at zero, 10, and 20 hr (Fig. 2). Whether this is the same molecule or not is unclear. At any rate, we conclude that at least one protein species, and its mRNA, appear (or increase) only during a limited stage after the onset of post-diapause development. It was recently reported that a molecule which was similar to this 73-kDa protein in size and in mode of appearance has also been found in the eggs of European races of the silkworm and that its production was dependent upon the heat treatment; thus this substance may be a kind of heat-shock protein (M. Coulon, personal communication).

REFERENCES