B31

Differential Regulation of Activities of Sorbitol Dehydrogenase and Glycerol Kinase in the Diapause Termination of *Bombyx mori*

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Diapause of the silkworm, *Bombyx mori*, occurs at the early embryonic stage. With the initiation of diapause, glycogen is converted into sorbitol, together with glycerol. Along with the diapause termination that is promoted by incubation at 5°C, sorbitol is converted into glycogen, and thereafter glycerol is also utilized. To understand the mechanism of how these polyols are utilized by incubation at 5°C, first we have examined the key enzymes controlling the catabolism of sorbitol and glycerol.

NAD-sorbitol dehydrogenase (SDH; the Km value for sorbitol = about 140 mM) was shown to be a rate-limiting enzyme for the utilization of sorbitol. Although SDH activity was not detected in diapausing eggs kept at 25°C, it appeared only in the eggs chilled at 5°C for 50~60 days. Western blotting experiments with an antiserum against SDH, showed that temporal pattern in amount of SDH protein paralleled that of SDH activity. Although the cDNA encoding a protein similar to mammalian SDH was isolated, the structure of substrate-binding domain was somewhat different from mammalian SDH. SDH mRNA occurs at yolk cells after exposure of diapause eggs to 5°C for 40~50 days. These results showed that SDH activation is regulated at a transcription level. Because the SilkBase suggested that there is an another gene encoding SDH in *Bombyx* genome, such a full-length cDNA was isolated. The deduced amino-acid sequence of this SDH-2 showed higher similarity to mammalian SDH than SDH-1. Expression of the *SDH-2* gene was activated by incubation at 5°C in parallel with the *SDH-1* gene.

For glycerol utilization, glycerol kinase (GK) was identified as a key enzyme. Although GK activity was not found in diapausing eggs kept at 25°C, it occurred after 5°C-chilling for 60 days. There was no difference in amount of GK mRNA between diapausing eggs incubated at 25°C and the eggs exposed to 5°C, and an increase in GK mRNA amount was not observed throughout 5°C-chilling. These results indicated that the regulation mechanism of GK activity occurs after the transcription level, differing from that of SDH activity.