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Endoplasmic Reticulum Stress Response of *Bombyx mori* Calreticulin

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Objectives

To further understanding of the role of calreticulin in insects, we have isolated a cDNA of calreticulin from silkworm, *Bombyx mori*. The cDNA encodes a 398 amino acid residues of *B. mori* calreticulin with endoplasmic reticulum retentional HDEL motif at its C-terminus, and a predicted molecular mass of 45801 Da. The *B. mori* calreticulin shows high protein homology with those of *G. mellonella* (88%), *A. aegypti* (71%) and *H. sapiens* (63%). Relatively high level of mRNA expression of *B. mori* calreticulin was exhibited only in the fat body. Although the expression of *B. mori* calreticulin was susceptible to intracellular calcium disturbance, other ER stress conditions such as inhibition of intracellular protein transport, reducing of disulfide formation, glycosylation inhibition, heat shock and oxidative stress did not inducible. In addition, we have examined that the interaction of calreticulin with other ER chaperones (BiP, ERp29, PDI, ERp72 and carexin) under without or with ER stress inducible conditions.

Experimental Procedures

- cDNA library was constructed using poly(A)$^+$ mRNA isolated from the whole body of *B. mori* larvae
- Sequence of each cDNA clone was determined using an automatic sequencer
- The sequences were compared using the DNASIS and BLAST programs provided by the NCBI
- Total RNA was isolated from fat body, midgut, silk gland, ovary, and testis of the *B. mori* and approached to Northern botting
- Immunoblot analysis was done to know induction of *B. mori* calreticulin under ER stress conditions

Results and Discussion

B. mori calreticulin encoding for a protein of 398 amino acids, in addition a typical ER retentional HDEL motif is shown at its C-terminus that means *B. mori* calreticulin also localized in the ER. The deduced amino acid sequence of *B. mori* calreticulin is 88% identical with that of *G. mellonella*, 71% with *A.*

aegypti, 69% with *A. gambiae* and *D. melanogaster,* but it shows a relatively low identity of 61% with *H. sapiens.* The result of comparative sequence analysis of *B. mori* calreticulin is suggested that it is highly conserved in evolution. No obvious signal of transcripts was observed in the tissues examined, exceptionally the only dominant expression of *B. mori* calreticulin level was detected only one species of 1.9 kb mRNA in fat body. There is no evidence for alternative mRNA splicing. Another, 3.76 kb mRNA has been reported but the detail identity of this larger mRNA is unclear. *B. mori* culture cell line of BM5 was treated with ER stress inducible drugs for indicated times such as brefeldin A (inhibition of intracellular protein transport from ER to Golgi complex), DTT (reducing of disulfide formation in protein), tunicamycin (glycosylation inhibitor of glycoprotein), heat shock and H_2O_2 (oxidative stress). *B. mori* calreticulin may play a important role on the UPR associated with intracellular calcium mainly, but not in cases of accumulation and/or aggregation of unfolded proteins in ER by preventing protein escape from ER and dysformation of disulfide band in a protein, glycosylation inhibition, protein denaturation by heat shock and oxidative condition. Calcium may affect both function and ability of calreticuiln which recognize and /or was recognized target proteins in the lumen of ER for the correct UPR. We are now particularly interested to establish the functional importance of *B. mori* calreticulin to maintain of intracellular calcium homeostasis that give correct protein folding and assembly in ER. The study of *B. mori* calreticulin may give very valuable clue to understand calreticulin because *B. mori* has basically very good classical genetics through long cultural history. We think finally we hope that the molecular characterization of *B. mori* calreticulin in this study will expand the understanding of insect calreticulins.