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Bombyx mori Transcription Factor, ATFC Binds directly to the UPRE of Molecular Chaperones

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Objectives

We describe here the identification of a transcription factor, ATFC that regulates the UPR by binding to the UPRE only when the signaling pathway is activated. The data in this study cover the first set of results, showing that ATFC has a major role in the insect UPR.

Materials and Methods

Materials - Insect cell ilnes : Sf9 and Bm5

Gene : ATFC

Methods - Construction of Expression vector for ATFC and Western blot analysis

- Purification of ATFC-His6 fusion protein
- Electrophoretic mobility shift assay

Results and Discussion

Electrophoretic mobility shift assays were carried out to determine whether ATFC-His6 fusion proteins bind to the UPRE directly to activate transcription of genes encoding ER resident proteins. To this end, we incubated with ATFC-His6 fusion proteins a [α-32P] labeled 22 bp oligonucleotide containing the UPRE sequence. After incubation, the mixture was fractionated on nondenaturing polyacryamide gels. ATFC-His6 fusion proteins bound to the UPRE and retarded its migration in the gel, resulting in the formation of a new band (Fig. 4b). These results are consistent with the conjecture that ATFC itself is the transcription factor binding the UPRE in the promoters of ER resident proteins. Therefore, we conclude that the *B. mori* ATFC represents a major component of the putative transcription factor responsible for the insect UPR leading to the induction localized stress proteins.

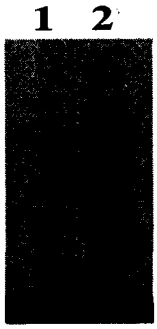

Figure 4. Specific binding of the ATFC gene product to UPRE. Electrophoretic mobility shift assay was carried out using purified ATFC-His protein and 32P-labelled, synthetic double-stranded oligonucleotides containing 22-bp element, the UPRE as probe. Probe (0.3ng) was mixed with ATFC-His fusion protein (0.5 μg). Sample was separated by electrophoresis

through a 5 % nondenaturing polyacrylamide gelB).

Reference

Mori, K. (2000). Tripartite management of unfolded proteins in the endoplasmic reticulum. *Cell* 101, 451-454.