B24

Overexpression and Purification of Y-box Family Protein of the Silkworm, *Bombyx mori*

Kohji Yamamoto, Taro Okada, He Ningjia, Yutaka Banno and Hiroshi Fujii

Laboratory of Insect Genetic Resources, Graduate School of Bioresource and Bioenvironmental Science, Kyushu University, Japan

The Y-box proteins are a family of nucleic acid binding proteins, which interact with the particular nucleotide sequence, so-called Y-box element and are thought to be involved in both transcriptional and translational regulation. Previously, we identified RNPs (ribonucleoprotein particles) including maternal mRNA in an unfertilized egg of *Bombyx mori* and demonstrated that protein synthesis was fluctuated during embryonic development (1). It is also reported that Y-box proteins have been found to be a core responsible for the formation of RNPs (2). Taken together, it has been postulated that Y-box family proteins may be associated with embryogenesis in silkworm egg.

To elucidate the function of Y-box protein, we have cloned gene encoding silkworm Y-box protein using RT-PCR with primers based on the Silkbase. We have also sequenced the gene and identified the open reading frame containing cold shock domain (CSD) at N-terminal region and RGG repeat at C-terminal region. CSD is known to be required for sequence-specific recognition of RNA and highly conserved among Y-box proteins from bacteria to human. Here, we constructed overexpression and purification system of Y-box protein of the silkworm, *Bombyx mori*. The structural gene was ligated into pET11b expression vector and the resultant plasmid was transformed into *Escherichia coli* BL21 (DE3), which was grown at 37 $_{600}$ of 0.7. Y-box protein was induced by the addition of IPTG to a final concentration of 1 mM for 3hr. The overproduced protein was migrated at about 31 kDa in SDS-polyacrylamide gel corresponding to a predicted molecular weight of Y-box protein. Cultures were harvested by centrifugation and cell pellet was sonicated. Recombinant Y-box protein was detected in the supernatant after centrifugation. After ammonium sulfate precipitation, the pellet was dialyzed against 10 mM phosphate buffer (pH 6.5) with 0.1 M KCl and loaded onto an anion exchange chromatography equilibrated with the same buffer. The column was run with a linear gradient of KCl from 0.1 to 0.4 M. The silkworm Y-box protein was purified to near homogeneity on SDS-PAGE by ammonium sulfate fractionation and an anion exchange chromatography.