ABSTRACT

Silk is a protein product of immense industrial and commercial importance. Enhancement of its quality and quantity is highly relevant in this context and hence necessitates the molecular and cellular studies on its biosynthesis. Towards this direction, a development of a cDNA probe for fibroin the major protein component of silk would be useful for evaluating the silk gland tissue samples in vitro by monitoring the transcriptional and translational processes that may be controlled by different conditions including action of growth factors and hormones and other environmental factors. Such an understanding is necessary prior to the application of these factors in the silkworm farms.

To obtain cDNA probes for fibroin light and heavy chain, the posterior silk gland tissue from the local silkworm strain NB4D2 was used for the construction of cDNA / λzapII expression library for screening and characterisation of fibroin gene inserts. cDNA / λzapII recombinant plaques were screened by a validated anti-fibroin antibody raised in the rabbit. The immuno-positive recombinant cDNA / λzapII clones were further screened using PCR employing a primer set designed from the known template sequence coding for the heavy chain repetitive unit cDNA. In spite of screening several immunopositive clones by the PCR method, a clone containing the heavy chain cDNA insert could not be identified.

However, arbitrary PCR amplification was observed with some of the cDNA inserts. These arbitrarily amplified PCR products in the form of a smear or small nondiscrete bands was found to be due to uneven extension of the reverse primer alone annealed to one or more sites at the complementary stretch(es) in the corresponding cDNA template. The cDNA inserts of two clones, pBS7b and pBS52b, that were selected on the
basis of the above mentioned PCR (alternative primers) screening procedure, were sequenced. One full length light chain cDNA insert (1165 bp) in clone pBS52b was found to be 97% homologous to the published genbank database sequence for the putative full length fibroin light chain cDNA from 25 bp to 1175 bp. The other light chain cDNA clone (pBS7b) was found to be a partial clone of the light chain covering 490 bp - 1170 bp. Since full length light chain sequence was present in the clone pBS52b, the insert of this clone was preferred as the cDNA probe for detecting the fibroin light chain gene or mRNA. A third cDNA clone (pBS16c) with immunoreactivity to anti-fibroin antibody and positive in PCR screening has not been characterised completely.

To validate this cDNA probe (pBS52b), it was used to detect the fibroin mRNA expressed *in vivo* in the posterior silk gland during the fourth and fifth instar larval stages. The site and stage specific expression detected by the cDNA probe correlated well with the established expression pattern of fibroin gene in different strains of mulberry silkworm *Bombyx mori*. Southern analysis indicated that the probe is specific to silk worm DNA.

In both *in vivo* and *in vitro* applications, this cDNA probe for the fibroin light chain gene (pBS52b) could aid in the fibroin mRNA quantitation which inturn will aid in the identification of factors that stimulate the production of fibroin *in vitro*. These factors may be applied and evaluated in the silkworm farms. Since it was reported that light and heavy chain mRNA occur in equimolar concentration, it is also possible to calculate the quantity of the heavy chain mRNA from the quantity of light chain mRNA.