Abstract

The cholionergic neuron uses acetylcholine as neurotransmitter. Acetylcholine is widely preserved among species, and function on mainly parasympathetic nerves and motor neurons in vertebrate, while on central nervous system in insect. Choline acetyltransferase (ChAT) is an enzyme to synthesize acetylcholine and expressed specifically in cholinergic neuron. Therefore, identification of the cholinergic neuron is defined expression of ChAT as indicator. It is known that structure of the cholinergic locus encodes ChAT and vesicular acetylcholine transporter (VAChT) is conserved in *Bilateria*. In insect, studies on the localization of cholinergic neuron and expression patterns of ChAT gene have been analyzed in *Drosophila melanogaster*. However, there is little information about other insect species. Insects have varying body plans, which affect the layout and organization of cholinergic neurons. In this study, I demonstrated the structure of cholinergic locus and localization cholinergic locus-expressing cells in the silkworm, *Bombyx mori*.

B. mori ChAT (BmChAT) and *VAChT (BmVAChT)* cDNAs share the 5′-region corresponding to the first and second exon. This genomic structure is conserved among other species. However, the number of the shared exon is two only in silkworm, and this feature is likely to relate with silkworm specific gene expression system. *BmChAT* cDNA encodes a deduced polypeptide including a putative choline/carnitine *O*-acyltransferase domain and a conserved His residue required for catalysis.

Spatial gene expression analyses revealed that *BmChAT* and *BmVAChT* were specifically in the brain and segmental ganglia. Temporal gene expression analyses indicate that *BmChAT* was expressed from 3 days after oviposition and continued until adult stage. However, *BmChAT* expression levels were decreased temporally in the early pupal stage. Next, I demonstrated the localization of neurons expressed cholinergic locus using a baculovirus-mediated gene transfer system. The recombinant viruses were contained an EGFP under control of a cholinergic locus promoter. EGFP signals were observed medial neurosecretory neurons and lateral cortex region in larval brain and segmental ganglia. These results suggested that cholinergic neuron has a variety role in *Bombyx* central nervous system. In the adult brain, EGFP signals were detected strongly medial lateral neurons, optic lobe, antennal robe and calyx of mushroom body.