Drosophila “elastin-type” gene identified with antibody to Bombyx PTTH

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Abstract. Screening of two Drosophila nervous system cDNA libraries with DNA probes derived from lepidopteran prothoracicotropic (PTTH) did not yield any hybridizing clone. Monoclonal antibody to Bombyx PTTH recognized in the expression cDNA library several clones but their sequencing revealed relationship to elastins and collagens rather than to PTTH. We conclude that Drosophila PTTH cannot be identified with probes derived from lepidopteran PTTH.

There is good evidence that many neuropeptides are shared, in slightly modified forms, by animals belonging to distinctly related taxa. Assuming that this was also true for insect prothoracicotropic (PTTH), we employed nucleic acid probes and a monoclonal antibody specific for lepidopteran PTTH to search for a homologous neuropeptide in a dipteran, Drosophila melanogaster. Two Drosophila cDNA libraries were prepared, each from 5–10 µg poly(A)+RNA that was extracted with the aid of Mimi-QD™ Rapid Poly(A)+ isolation system (5 Prime – 3 Prime, Inc.) from 800 adult heads and from about 1,800 larval ganglia (brain plus ventral nerve cord), and primed with oligo d(T). Larval cDNA was cloned into non-directional vector Lambda gt10 (Amersham) and adult cDNA into directional expression vector Uni-Zap (Stratagene). Initial library titres were 4 × 10⁵ pfu/ml and 3.7 × 10⁶ pfu/ml, respectively.

The libraries were screened with 770 bp long EcoRI/Hind III cDNA fragment of Bombyx mori PTTH (Kawakami et al., 1990) and with two degenerate oligoprobes that were designed on the basis of Bombyx and Samia cynthia PTTH amino acid sequences (Samia sequence was kindly provided by Prof. H. Ishizaki): 40-mer (T/C)TIGA(T/C)AA(T/C)(T/A)(G/C)(G/A)TIGA(A/G)ACI(C/A)GIAC(C/A)GIAC(C/A)GIAA(G/A) (C/A)GIIGG designated PT1 and 41-mer (PT3)TTT(T/C)ATG(G/A)TIGA(A/G)GA(T/C)CA(G/A)ACI(C/A) GIAC(C/A)(A/G)AA(G/A)(T/A)TCAT(T/A)AA(T/C)TA corresponding to 103–116 and 38–51 amino acid positions of Bombyx PTTH, respectively. About 3 × 10⁵ independent clones were analyzed in each round of screening. Hybridization was performed in 5 × SSPE, 5 × Denhardt’s solution, 0.5% SDS and 100 mg/ml sonicated heterologous DNA, with a probe activity 1–5 × 10⁶ cpm/ml, and at temperatures shown in the Table.

<table>
<thead>
<tr>
<th>mRNA source</th>
<th>Vector</th>
<th>Probe</th>
<th>Hybridization (°C)</th>
<th>Washing (°C)</th>
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No positive signals were found in the screening. Since we also failed to detect any hybridization on genomic Southern, we concluded that Drosophila PTH antibody has either no or very low homology with Bombyx PTH at the nucleic acid level. We chose immunoscreening of the expression library as our next approach. Monoclonal antibody to Bombyx PTH (Mizoguchi et al., 1990), which recognizes specific neurons in the central nervous system of Drosophila (Zhuan et al., 1993), was first tested on the Western blots of proteins extracted from Drosophila nervous system and separated by SDS PAGE. Extracts of the larval brains of Galleria mellonella (Lepidoptera) were used for comparison. In Galleria the antibody reacted with two bands corresponding to cca 12 kDa and 30 kDa, in Drosophila larvae with three bands (17, 33 and 35 kDa), and in Drosophila adults with a single band of cca 31 kDa. Heating of samples before PAGE resulted in a considerable decrease or loss of immunoreactivity.

Protocol for the immunoscreening of the expression cDNA library was provided with the picobue™ Immunoscreening kit (Stratagene). Conditions were optimized using Galleria brain extracts spotted on nitrocellulose membranes: 1:500 dilution of the anti-PTH antibody combined with 1:8,000 dilution of the goat anti-mouse antibody (conjugated to alkaline phosphatase; BCIP/NBT was used as substrate) gave best results and detected antigen in 0.1 brain aliquot. However, both primary and secondary antibodies also reacted with the E. coli phage lysate. To prevent this unspecific reaction, six nitrocellulose membranes 4 x 4 cm were submerged in the lysate (1 : 10) for 30 min under gentle shaking, dried, and incubated in 5% skim milk for 1 hr. After 3 washings in TBST, 4 membranes were placed in 50 ml anti-PTH antibody (1 : 500) and 2 membranes in 50 ml of the secondary antibody (1 : 8000). Antibodies incubated with the membranes for 20 min were subjected to the dot blot test and proved to be devoid of any reaction with the E. coli phage lysate. Then they were used for the screening of cDNA library.

Among 7 x 10^2 independent colonies we detected 22 that gave positive reaction with the anti-PTH antibody. The colonies were isolated, grown in suspension culture, and their protein extracts were analyzed by Western blotting. Six clones proved false-positive in this assay, all others contained an immunoreactive band of cca 30 kDa. To verify specificity of the immunoreaction, protein suspension of clone #1 was analyzed in parallel with the extracts of Bombyx brain and Drosophila central nervous system. We was confirmed that the PTH antibody reacts exclusively with the fusion protein and with certain fractions of the nervous extracts. In Bombyx extracts, the reactivity was confined to 19 kDa as expected, but in Drosophila extracts it occurred in a band of 95 kDa; we assume that Drosophila immunoreactive fractions of 17, 33 and 35 kDa ran as a single 95 kDa band because sample buffer without mercaptoethanol was used.

Fig. 1. Nucleic acid sequence of putative LacZ fused protein of clone #1. LacZ part of the fused protein is given in italics and the fusion site of LacZ gene and inserted ORF is boxed. Presumptive polyadenylation signal is underlined.
The cDNA insert of clone #1 was isolated and sequenced (Fig. 1). The longest open reading frame (ORF), 267 bp long, is in frame with the LacZ gene ORF and obviously encodes a LacZ fused protein. Downstream, there is a 188 bp long non-coding region that includes putative polyadenylation site and is followed by 43 bp long poly(A) tail. Deduced translation product consists of 128 amino acids and its molecular weight is 13,450 kD. This is considerably less than the size (30 kDa) of the immunoreactive fusion protein on the Western blots. Another discrepancy was found when radiolabelled plasmid clone #1 was used as hybridization probe in the Southern analysis of 12 other immunopositive clones that were treated with EcoR1 to liberate the insert. Surprisingly, the result revealed that the size of hybridizing cDNA inserted varied from 400 to 1,200 bp.

Deduced amino acid sequence of the immunopositive clone shows no homology with Bombyx PTTH. Search in databanks indicated relation to about 50 elastins and collagens. Highest homology, amounting to 44.9% identity in a 49 amino acids overlap (residues 54 to 93 in Fig. 1) was found with tropoelastin of chicken aorta (Bressan et al., 1987; Tokimitsu et al., 1987). On the other hand, the only fully identified Drosophila collagen (Blumberg et al., 1988) was not detected as homologous. We assume that our immunopositive clones encode various fragments of Drosophila elastin(s). We cannot explain apparent correlation between the presence of these inserts and the occurrence of a “PTTH-like” antigen in the phage E. coli lysate.

Our attempts to identify Drosophila PTTH using information on Bombyx PTTH failed with both nucleic acid probes and the antibody, suggesting that Drosophila neurohormones controlling endocrine production must be isolated as proteins active in a bioassay.

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REFERENCES


