Int. J. Indust. Entomol. Vol. 7, No. 2, 2003, pp. 145~149

International Journal of *Industrial Entomology*

Molecular Cloning of a cDNA Encoding Putative Apolipophorin from the Silkworm, *Bombyx mori*

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(Recived 22 September 2003; Accepted 2 October 2003)

ApolipophorinIII (apoLp-III) is a protypical exchangeable apolipoprotein that is abundant in hemolymph of many insect species. Its function lies in the stabilization of low-density lipophorin particles (LDLp) crossing the hemocoel in phases of high energy consumption to deliver lipids from the fat body to the flight muscle cells. But, recent studies with naive *Galleria mellonella*-apoLp-III gave first indication of an unexpected role of that protein in insect immune activation. In this research, we cloned a cDNA encoding putative apoLp-III from the silkworm, *Bombyx mori* injected with *E. coli* and characterized its role. We constructed a cDNA library using whole bodies of *B. mori* larvae injected with *E. coli*, carried out the differential screening, and selected the up-regulated clones. Among these clones, we focused on a cDNA showing a high sequence similarity to the apolipophorinIII from other insects and analyzed the nucleotide and deduced amino acid sequences. The pupative *B. mori* Jam123 apoLp-III cDNA contained 1,131 bp encoding 186 amino acid residues. Phylogenetic analysis revealed that the nucleotide and amino acid sequences of the *B. mori* apoLp-III cDNA formed a highly inclusive subgroup with Bombycidae. But, it was interesting that *B. mori* Jam123 is closer to *B. mandarina* than *B. mori* P50 and *B. mori* N4. Northern blot analysis showed a signal in the fat body, posterior silkgland and midgut.

Key words: Insect, *Bombyx mori*, ApolipophorinIII, cDNA sequence, Differential screening, Phylogenetic analysis

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Introduction

Exchangeable apolipoproteins play a critical role in plasma lipoprotein metabolism through their function as structural components of lipoproteins, activators of lipid metabolic enzymes and proteins or ligans for cell surface receptors. More than a dozen unique exchangeable apolipoproteins have been identified and characterized from vertebrate and invertebrate plasma and these generally possess a common structural motif: the class A amphipathic α-helix (Sergrest *et al.*, 1994). Such helics are characterized by well-defined polar and non-polar faces which are proposed to interact with the solvent and hydrophobic milieu, respectively, at the lipoprotein surface. In insects, lipid-transporting haemolymph lipoprotein, lipophorin, is considered to fulfill various functions of mammalian plasma lipoproteins. Lipophorin generally contains two apoproteins, apo-I and apo-II, and about a half of the molecular mass comprises the mixture of non-polar and polar lipids (Narayanaswami and Ryan, 2000).

ApolipophorinIII (apoLp-III) is an exchangeable apolipoprotein that is abundant in hemolymph of many insect species. It is an approximately 18 kDa globular protein with a high content of α-helix secondary structure (Narayanaswami and Ryan, 2000). In the adult stage, apoLp-III functions in transport of diacylglycerol (DAG) from the fat body lipid storage depot to flight muscles, a process which occurs in response to adipokinetic hormone-stimulted activation of fat body triacylglycerol lipase (Arresse and Wells, 1997; Ryan and Horst, 2000). The number of helices present in a given exchangeable apolipoprotein can be predicted by secondary structure analysis of apolipoprotein sequences (Segrest *et al.*, 1992) and, in four cases, high-resolution structural information is available (Borhani *et al.*, 1997; Wang *et al.*, 1997). Although each of these proteins exerts its biological

effects in a lipid associated state, the structures reported are in a lipid-free state. Thus, reaching the next level of understanding in the apolipopretein field hinges on acquisition of detailed structural knowledge of these proteins in their biologically active, lipid associated states. Several researchers have used avalilable information to design experiments which provide insight in changes in protein structure which accompany lipid interaction. Its function lies in the stabilization of low-density lipophorin particles (LDLp) crossing the hemocoel in phases of high energy consumption to deliver lipids from the fat body to the flight muscle cells. Recent studies with naive *Galleria mellonella* apoLp-III gave first indication of an unexpected role of that protein in insect immune activation (Niere *et al.*, 1999; Zakarian *et al.*, 2002).

In this paper we described an apoLp-III cDNA up-regulated upon *E. coli* infection from the domestic silkworm, *B. mori* Jam123 and compared the nucleotide and amino acid sequences of the gene to known other insect apoLp-III.

Materials and Methods

Insect, differential screening and nucleotide sequencing

The silkworms, *B. mori* Jam123, were reared on an artificial diet at 24 – 27°C and 70 – 90% humidity. Silkworms were immunized with injection of *Escherichia coli* K12 strain DH1, grown in LB liquid medium at 37°C for overnight with shaking. Differential hybridization was carried out to select inducible genes which were quickly expressed at high level after immunization. We constructed cDNA library from *B. mori* larvae injected with *E. coli* using ZAP-cDNA Synthesis Kit (Stratagene Co.) to synthesize the native and immunized *B. mori* poly(A)$^+$ RNA and screened approximately 600 plaques using [α-^{32}P]dATP labeled probes. A number of plasmids were isolated from bacterial colonies, and transferred onto the duplicates of nylon membrane, and hybridized with radiolabelled 1st strand cDNA of poly(A)$^+$ RNA from immunized and naive *B. mori* in Rapid Hybridization Solution (Amersham Co.). We selected cDNA clones showing stronger signals in immunized blots compared to the controls. For putative identification of selected cDNA clones, partial sequence of these clones was done by a dye terminator cycle sequencing method using an automatic DNA sequencer (Perkin Elmer Co., ABI 377). For sequencing, double-stranded DNA was prepared using Wizard Plus SV Minipreps DNA Purification System (Promega Co.). DNA (300 – 500 ng) was mixed with T3 and T7 primers (3.2 pmole), respectively, and 8 μl of Terminator Reaction Mix (Perkin Elmer Co.), and was reacted by Polymerase Chain Reaction (PCR) method. Twenty-five cycles of PCR were performed at 96°C for 30s, 50°C for 15s, and 60°C for 4 min. The resulting PCR products were electrophoresed on the 4.5% denatured polyacrylamide gel and analyzed by DNA Sequencing Analysis Software (Perkin Elmer Co.).

Data anlaysis

The sequences were compared using the DNASIS and BLAST programs provided by the NCBI (www. ncbi. nlm.nih.gov). GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program (www.ncbi.nlm.nih.gov/BLAST). Clustal W (http://clustalw.genome.ad.jp/) program was used to align the nucleotide and amino acid sequences of apoLp-III. Phylogenetic analysis was performed using PAUP (Phylogenetic Analysis using Parsimony) version 4.0 (Swofford, 2000). The tree was obtained by bootstrap analysis with the option of heuristic search (1,000 replications). The accession numbers of the sequences in the GenBank are: *B. mori* Jam123 (this study, AY341912), *B. mori* P50 (U59244), *B. mori* N4 (U59243), *B. mandarina* (AF-001289), *Manduca sexta* (M17286), *Spodoptera litura* (AF094582), *G. mellonella* (AJ006975), *Epiphyas postvittana* (AF314181), *Locusta migratoria* (J03888), and *Acheta domesticus* (L25277).

RNA isolation and Northern blot analysis

Total RNA was isolated from whole body, fat body, posterior silkgland, midgut, testis and ovary of the *B. mori* Jam123 using Total RNA Extraction Kit (Promega Co.). Total RNA (5 μg/lane) from the *B. mori* Jam123 was separated on 1.2% formaldehyde gel and transferred onto a nylon blotting membrane (Schleicher & Schuell Co.). The Hybond-N membrane blot was hybridized with [α-^{32}P] dATP-labeled *B. mori* apoLp-III cDNA probe. A hybridization solution dontaining 50% (w/v) formamide, 5 × SSC, 10 × Denhardts solution (0.2% each of bovine serum albumin, Ficoll and polyvinylpyrrolidone), 25 μg ultra-sonicated salmon sperm DNA ml^{-1}, and 50 mM sodium phosphate (pH 7) was used. After hybridization, membranes were washed for 30 min at 65°C with increasing stringency, from 2 × SSC and 0.1% SDS to 0.1 × SSC and 0.1% SDS.

Results and Discussion

To identify novel genes that are expressed specifically or preferentially in immunized- *B. mori*, we constructed a cDNA library using whole bodies of *B. mori* larvae injected with *E. coli*, carried out the differential screening using cDNA synthesized with total RNA from *B. mori*

injected with *E. coli* and without injection, respectively, and selected the up-regulated clones. Among these clones, we focused on a cDNA showing a high similarity with apoLp-III from other insects, and sequenced its full cDNA (Fig. 1). The pupative *B. mori* apoLp-III cDNA (GenBank Acc. No. AY341912) contained 1,131 bp encoding 186 amino acid residues. The amino acid size of *B. mori* Jam123 apoLp-III was all same in the silkworm species (*B. mori* and *B. mandarina*), but variable in other insect species (ranged from 180 – 189 amino acid residues; Table 1). A multiple sequence alignment of the deduced amino acid sequence of *B. mori* Jam123 apoLp-III gene with other insect apoLp-III sequences is shown in Fig. 2.

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GGCACGAGTCGAGTTTTTTTTTTTTTTTTTTAGATTTTTAAGGTTTTTATTTAAATTTAAGCCCTAAAA   70
TTCACTTTGTAGCCTTCTTTGCCGACTTAATGCTCGCCTGTTGTGCCTTGAGGACCTTCACAATTTTTTG  140
TTCTTCAATGAGGAAGGCTCTGACAATGCGTTGCTTGACACATTTATGGCAGAGGACACCACCATAAACA  210
CGTTTCACTGTTTTCTTACGGTAGCAAAGACGGGAACGTTCAGCAGGTCTAGCTGGCTGGATACCACGGA  280
GTTTGCTCTTGCACTGACCACACCTTGGGATCTTCTTGGGCTTTTTGACATACTGATAAACCAAGCGGCC  350
ACCCGGTGTCCTTACTATTCTTCTTTGATTTGATTTTGTGTTGTACGACAGTCGTCGCCTGAATGTAAGC  420
CGCTGCACCATTTTCAAGCTTTTTGCTCGTGCCGAATTCGGCACGAGCCGGTTCGCATCTCTCGCATCAG  490
TCTCTCGACAGTACATCATGGCCGCCAAGTTCGTAGTTCTCTTCGCCTGCATCGCTCTGGCCCAAGGAGC  560
                M  A  A  K  F  V  V  L  F  A  C  I  A  L  A  Q  G  A
GATGGTGCGACGCGACGCTCCCGACTTCTTCAAGGACATCGAACACCACACCAAGGAGTTCCATAAGACT  630
  M  V  R  R  D  A  P  D  F  F  K  D  I  E  H  H  T  K  E  F  H  K  T
TTAGAACAACAGTTTAACTCGCTCACCAAGTCAAAGGACGCACAGGACTTCAGCAAGGCTTGGAAGGACG  700
  L  E  Q  Q  F  N  S  L  T  K  S  K  D  A  Q  D  F  S  K  A  W  K  D
GCTCCGAGTCCGTGCTGCAACAGCTCAACGCCTTCGCCAAGAGTCTCCAGGGAGCGCTCGGAGACGCGAA  770
G  S  E  S  V  L  Q  Q  L  N  A  F  A  K  S  L  Q  G  A  L  G  D  A  N
CGGCAAGGCCAAGGAGGCTTTGGAACAGTCGAGGCAGAACATCGAGCGCACGGCCGAGGAGCTCCGCAAG  840
  G  K  A  K  E  A  L  E  Q  S  R  Q  N  I  E  R  T  A  E  E  L  R  K
GCCCACCCTGACGTCGAGAAGAACGCCACCGCCCTCCGCGAGAAGCTGCAGGCCGCCGTGCAGAACACCG  910
  A  H  P  D  V  E  K  N  A  T  A  L  R  E  K  L  Q  A  A  V  Q  N  T
TGCAGGAATCCCAGAAGTTAGCGAAGAAGGTGTCCTCGAACGTGCAGGAGACTAATGAGAAACTGGCGCC  980
V  Q  E  S  Q  K  L  A  K  K  V  S  S  N  V  Q  E  T  N  E  K  L  A  P
CAAGATCAAGGCCGCCTACGACGACTTCGCGAAGAACACCCAGGAGGTGATCAAGAAGATCCAGGAGGCC 1050
  K  I  K  A  A  Y  D  D  F  A  K  N  T  Q  E  V  I  K  K  I  Q  E  A
                                  ***
GCCAACGCCAAGCAGTGAGCGTCGATATTGAACTCTCACACTTAATAAATATTTCTTTCATATAAAAAAA 1120
  A  N  A  K  Q
AAAAAAAAAAA                                                            1131
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Fig. 1. The nucleotide and deduced amino acid sequences go *B. mori* Jam123 apolipophorin III. The start codon ATG is boxed and the termination codon is shown by asterisks. The polyadenylation sites are underlined.

Alignment of the *B. mori* Jam123 apoLp-III sequences with those of apoLp-IIIs from several other lepidopteran and orthopteran species indicates the extent of the identity

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B. mori Jam123      -MAAKFVVLFACIALAQGAMVRRDAP---DFFKDIEHHTKEFHKTLEQQFNSLTKSKDAQ
B. mandarina        -MAAKFVVLFACIALAQGAMVRRDAP---DFFKDIEHHTKEFHKTLEQQFNSLTKSKDAQ
B. mori P50         -MAAKFVVLFACIALAQGAMVRRDAP---DFFKDIEHHTKEFHKTLEQQFNSLTKSKDAQ
B. mori N4          -MAGKFVVLFACISLAQGAMVRRDAP---DFFKDIEHHTKEFHKTLEQQFNSLTKSKDAQ
M. sexta            MAAKFVVVLAACVALSHSAMVRRDAPAGGNAFEEMEKHAKEFQKTFSEQFNSLVNSKNTQ
E. postvittana      MVAKLVVFVAFVALAHG--MVRRDAPAQS-PLQEVEKHAAEFQKTLSEQWNALVNSKNTQ
G. mellonella       MAAKYVFVVAACSALAQAGIVRRDA---STPLQDLEKHAAEFQKTFSEQLNAFTNSKDTK
S. litura           -MVAKLFVLVACIALSHAAMVRRDAPPANTLLQDIEKHAAEIHKTFSEQLNSIANSKNTQ
A. domesticus       MKVIIALVAALACMQVMEARVRRDAGTTGADFNSLFEAAQRHFQNLTATIQNALPSQ--E
L. migratoria       -----MNTLLAVLMLAVAAQARPDAAG-HVNIAEAVQQLNHTIVNAAHELHETLGLPTPD
                         .* **  :  .  .  .  :  .  .  .  :  .  .  .  :  .

B. mori Jam123      DFSKAWKDGSESVLQQLNAFAKSLQGALGDANGKAKEALEQSRQNIERTAEELRKAHPDV
B. mandarina        DFSKAWKDGSESVLQQLNAFAKSLQGALGDANGKAKEALEQSRQNIERTAEELRKAHPDV
B. mori P50         DFSKAWKDGSESVLQQLNAFAKSLQGALGDANGKAKEALEQSRQNIERTAEELRKAHPDV
B. mori N4          DFSKAWKDGSESVLQQLNAFAKSLQGALGDANAKAKEALEQSRQNIERTAEELRKAHPDV
M. sexta            DFNKALKDGSDSVLQQLSAFSSSLQGAISDANGKAKEALEQARQNVEKTAEELRKAHPDV
E. postvittan       AVNKALKEGSDSVLQQLSQLSTSLQGAINDANGKAKEALETSRANIEKAAAELRKAHPEV
G. mellonella       EFNTALKEGSDSVLQQLNALASSLQKALNDANGKAKEALEQTRTNLERTAEELRRAHPDV
S. litura           EVNKAIKDGSDSVLQQLSALSSSLQSAMTDANAKAKTALEQARQNLEKTAEDLRKSHPDV
A. domesticus       EVRTQLQTHAQTFANNLQAAATQFNEKAAELSGDAQTAVRQAAQQLEQQVSNLRQQFPDG
L. migratoria       EALNLLTEQANAFKTKIAEVTTSLKQEAEKHQGSVAEQLNAFARNLNNSIHDAATSLNLQ
                       .  :::.  ::  :.::  . .... :.  :::.  :

B. mori Jam123      EKNATALREKLQAAVQNTVQESQKLAKKVSSNVQETNEKLAPKIKAAYDDFAKNTQEVIK
B. mandarina        EKNATALREKLQAAVQNTVQESQKLAKKVSSNVQETNEKLAPKIKAAYDDFAKNTQEVIK
B. mori P50         EKNATALREKLQAAVQNTVQESQKLAKKVSSNVQETNEKLAPKIKAAYDDFAKNTQEVIK
B. mori N4          EKNATALREKLQAAVQNTVQESQKLAKKVSSNVQETNEKLAPKIKAAYDDFARNTQEVIK
M. sexta            EKEANAFKDKLQAAVQTTVQESQKLAKEVASNMEETNKKLAPKIKQAYDDFVKHAEEVQK
E. postvittan       EQQASQLRDRLQAAVQSGVQESQKLAKEVAAHVDETNKKLEPQIKKAYDDFVKQAEEVQK
G. mellonella       ERQAGALRDRLQTAVQATVQETQKLAKTVGANLEETNKKLAPQIKSAYDDFVKQAQEVQK
S. litura           ERQAGELRTKLQAAVQNTAQEVQKLAKEVASNVEETNEKLAPKLKEAVENFSKHVEEVQK
A. domesticus       AQAADKLKASIESALAEEARRVQEAVQPHADAVAES---LKTAARTAVEQATVITNQVQQ
L. migratoria       DQLN-----SLQSALTNVGHQWQDIATKTQASAQEAWAPVQSALQEAAEKTKEAAANLQN
                    :          :::*:  :. *. .        *:  :  . : * :.  . :: :

B. mori Jam123      KIQEAANAKQ-
B. mandarina        KIQEAANAKQ-
B. mori P50         KIQEAANAKQ-
B. mori N4          KIQEAANAKQ-
M. sexta            KLHEAATKQ--
E. postvittan       KLHEAATKQ--
G. mellonella       KLHEAASKQ--
S. litura           KVHEAASKQ--
A. domesticus       SVQQAANAH--
L. migratoria       SIQSAVQKPAN
                    .::.*.
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Fig. 2. Multiple sequence alignment of the insect apolopophorinIIIs. The amino acid sequences for apolipophorinIIIs from *B. mori* Jam123, *B. mori* P50, *B. mori* N4, *B. mandarina*, *M. sexta*, *S. litura*, *G. mellonella*, *E. postvittana*, *L. migratoria* and *A. domesticus* have been aligned by using Clustal W. Identical residues (*), conserved substitutions (:) and semi-conserved substitutions (.) between the ten sequences are indicated below the sequences. The five putative amphipathic α-helics (Smith *et al.*, 1994) are underlined.

Table 1. Apolioophorin III from various insect species

Common name	Species	Amino acid size	GenBank accession number
Lepidoptera			
Domestic silkworm	*Bombyx mori* Jam123	186	AY341912
Domestic silkworm	*Bombyx mori* P50	186	U59244
Domestic silkworm	*Bombyx mori* N4	186	U59243
Wild silkworm	*Bombyx mandarina*	186	AF001289
Tobacco hornworm	*Manduca sexta*	189	M17286
Common cutworm	*Spodoptera litura*	188	AF094582
Greater wax moth	*Galleria mellonella*	186	AF006975
Light brown apple moth	*Epiphyas postvittana*	183	AF314181
Orthoptera			
Migratory locust	*Locusta migratoria*	180	J03888
House cricket	*Acheta domesticus*	184	L25277

Table 2. Distance of the nucleotide (A) and amino acid (B) sequences among *B. mori* and other insects apolipophorins. Above and below diagonals indicate total character differences and mean character differences, respectively

A

		1	2	3	4	5	6	7	8	9	10
1	*B. mori* Jam123	–	0.01420	0.00161	0.00323	0.28485	0.32641	0.33575	0.34457	0.55047	0.60120
2	*B. mori* N4	9	–	0.01284	0.01124	0.27603	0.31388	0.32492	0.34548	0.55829	0.58962
3	*B. mandarina*	1	8	–	0.00161	0.24839	0.30000	0.30484	0.33063	0.54790	0.58199
4	*B. mori* P50	2	7	1	–	0.25000	0.29839	0.30645	0.33225	0.54958	0.58039
5	*M. sexta*	188	175	154	155	–	0.32117	0.29607	0.31176	0.56495	0.57872
6	*S. litura*	220	199	186	185	220	–	0.32000	0.30259	0.57360	0.58960
7	*G. mellonella*	232	206	189	190	196	216	–	0.29575	0.56872	0.58445
8	*E. postvittana*	265	218	204	205	212	210	202	–	0.54656	0.57849
9	*L. migratoria*	349	340	326	327	374	378	360	358	–	0.61286
10	*A. domesticus*	401	375	362	361	408	408	391	398	410	–

B

		1	2	3	4	5	6	7	8	9	10
1	*B. mori* Jam123	–	0.00000	0.00000	0.02094	0.32461	0.44503	0.39696	0.37173	0.79581	0.84817
2	*B. mori* N4	0	–	0.00000	0.02094	0.32461	0.44503	0.39696	0.37173	0.79581	0.84817
3	*B. mandarina*	0	0	–	0.02094	0.32461	0.44503	0.39696	0.37173	0.79581	0.84817
4	*B. mori* P50	4	4	4	–	0.34031	0.45550	0.39267	0.38220	0.80105	0.85340
5	*M. sexta*	62	62	62	65	–	0.28796	0.30366	0.29843	0.78534	0.86387
6	*S. litura*	85	85	85	87	55	–	0.31414	0.39791	0.80628	0.85864
7	*G. mellonella*	72	72	72	75	58	60	–	0.34031	0.79058	0.83770
8	*E. postvittana*	71	71	71	73	57	76	65	–	0.76440	0.83246
9	*L. migratoria*	152	152	152	153	150	154	151	146	–	0.84293
10	*A. domesticus*	162	162	162	163	165	164	160	159	161	–

that exists. In the nucleotide level, *B. mori* Jam123 apoLp-III nucleotide sequences show a very high degree of similarity among Bombycidae: *B. mori* P50 (99.7%), *B. mori* N4 (98.6%) and *B. mandarina* (99.8%). However, sequence similarity to other insects were moderate to low (39.9 – 71.5%). Among all insect apoLp-III, nucleotide sequence divergence ranged from 99.9 – 39.9%. In terms of amino acid, *B. mori* Jam123 apoLp-III showed 100 –97.9% among Bombycidae and 67.5 – 15.2% among all insect apoLp-III (Table 2). The degree of similarity between these five mature Lepidopteran apoLp-III amino acid sequences is particularly high in the five putative amphipathic-helices identified by Smith *et al.* (1994), as shown in Fig. 2. The very high degree of similarity between these five putative amphipathic-helices lends further support for their importance in the function of apoLp-III, as proposed by Breiter *et al.* (1991), which was elucidated in the basis of X-ray structure of ApoLp-III from *L. migratoria.*

Fig. 3 shows a tree based on the nucleotide-derived (a) and the amino acid-derived (b) distances. These trees shows that the apoLp-III of the Bombycidae formed a strong subgroup. One interesting aspect is that *B. mandarina* apoLp-III grouped together with *B. mori* apoLp-III

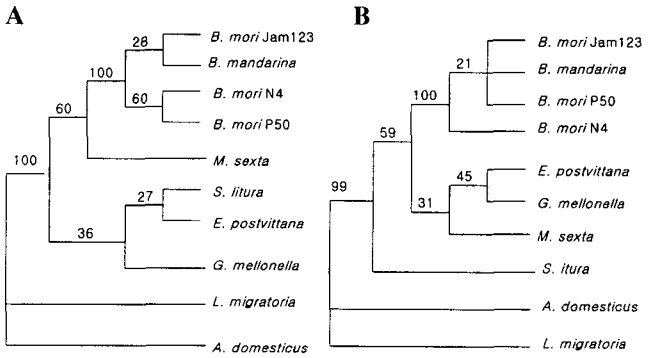

Fig. 3. Relationships among nucleotide (A) and amino aicd (B) sequences of the *B. mori* Jam123 and the known apolipophorinIIIs. The information of apoLp-III sequences extracted from GenBank is described in Table1. The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1,000 replicates.

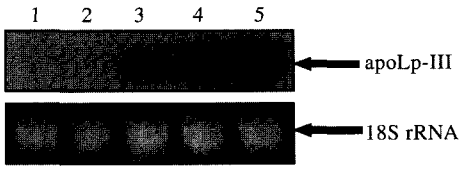

Fig. 4. Nortehrn blot analysis of the *B. mori* Jam123 apolipophorinIII transcripts. Total RNA was isolated from the testis (lane 1), ovary (lane 2), fat body (lane 3), posterior silkgland (lane 4) and midgut (lane 5) of *B. mori* larvae. The RNA was separated on1.2% formaldehyde agarose gel (lower panel), transferred onto a nylon membrane, and hybridized with [α-^{32}P]dATP-labeled *B. mori* apoLp-III cDNA probe (upper panel).

without any distinction among them in both nucleotide and amino acid-based analysis (Fig. 3). Particularly, in nucleotide-based analysis, *B. mori* Jam123 grouped together with the wild silkworm, *B. mandarina*, although the support for the node was weak (bootstrap value 28%; Fig. 3a). This possibly suggests that the *B. mori* Jam123 might have originated from *B. mandarina*, although this aspect can be illustrated in the taxonomy-oriented study.

To confirm the expression of the *B. mori* Jam123 gene at transcriptional level, Northern blot analysis was performed using total RNA prepared from the testis, ovary, fat body, posterior silkgland and midgut of *B. mori* Jam123 (Fig. 4). Among them, signals were detected from fat body, posterior silkgland and midgut. Signal was stronger in the midgut than fat body and posterior silkgland, indicating that the midgut might be the main site for the synthesis of the *B. mori* Jam123 apoLp-III protein.

In conclusion, we have cloned a novel cDNA encoding putative apolipophorin III from the domestic silkworm, *B. mori*. Up-regulation of *B. mori* Jam123 apoLp-III gene upon *E. coli* infection is consistant with the recent studies that naive *G. mellonella* apoLp-III played an unexpected role in the insect immune activation (Niere *et al.*, 1999; Zakarian *et al.*, 2002) and relationship between changes in lipophorins and activation of phenoloxidase in the haemolymph of *L. migratoria* in response to injection of immunogens (Mullen and Goldsworthy, 2003). We hope that this study expands an understanding of *B. mori* Jam123 apoLp-III gene.

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