Bombyxin F1 Gene: Structure and Expression of a New Bombyxin Family Gene That Forms a Pair with Bombyxin B10 Gene

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Bombyxin F1 Gene: Structure and Expression of a New Bombyxin Family Gene That Forms a Pair with Bombyxin B10 Gene

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ABSTRACT—Bombyxin F1 gene, a new bombyxin family gene, has been identified. The F1 gene forms a pair with bombyxin B10 gene with an opposite transcriptional orientation and the gene pair F1/B10 is located between bombyxin gene pairs B9/C1 and A7/B7 in a bombyxin gene cluster. The nucleotide sequence of the F1 gene and its deduced amino acid sequence deviate moderately from those characterized previously for the family-A, family-B, family-C, family-D, and family-E bombyxin genes; the bombyxin F1 gene and preprobombyxin F1 share no more than 62% and 53% sequence identities with other bombyxin members, respectively. Harr-plot analysis indicated that the spacer of the F1/B10 gene pair has low sequence similarity with that of other bombyxin gene pairs characterized. The bombyxin F1 mRNA in *Bombyx mori* brain was shown to locate in four pairs of medial neurosecretory cells, which also produce other bombyxin family mRNAs. Genomic Southern hybridization indicated that the *Bombyx* haploid genome contains a single copy of the family-F bombyxin gene.

INTRODUCTION

The bombyxin gene, belonging to the insulin-related multigene family, comprises at least 30 gene copies per haploid genome of the silkmoth *Bombyx mori* (Kondo *et al.*, 1996; Tsuzuki *et al.*, 1997). The 30 bombyxin genes have been classified into five families, A, B, C, D, and E, according to their sequence similarity. The *Bombyx* genome includes 10 family-A genes, 12 family-B genes, six family-C genes, one family-D gene, and one family-E gene. The bombyxin genes except the family-E gene cluster in two genomic segments in unique distribution patterns (Kondo *et al.*, 1996). The arrangement of these genes has been classified into three categories: gene pairs, gene triplets, and single genes.

Bombyxin genes share structural features with the vertebrate insulin gene. Bombyxin genes encode precursor molecules which consist of four domains in the order of a signal peptide, B chain, C-peptide, and A chain (Iwami *et al.*, 1989). The C-peptide is flanked by the processing signals, indicating the excision of the C-peptide after translation (Iwami *et al.*, 1989) and the A and B chains of the mature molecules are linked together by disulfide bonds (Nagasawa *et al.*, 1988), like insulin (Steiner *et al.*, 1985). The bombyxin genes, however, possess some characteristic features markedly different from the vertebrate insulin gene. The copy number of bombyxin gene is more than 30 per haploid genome (Kondo *et al.*, 1996; Tsuzuki *et al.*, 1997). All bombyxin genes so far characterized lack introns, indicating that they are presumably the functional processed genes or genes derived from the processed genes (Iwami, 1990; Iwami *et al.*, 1989, 1990; Kawakami *et al.*, 1989; Kondo *et al.*, 1996; Tsuzuki *et al.*, 1997). Bombyxin genes are expressed predominantly in the brain (Kawakami *et al.*, 1989) and at low levels in a number of other tissues of *Bombyx* larvae (Iwami *et al.*, 1996b), in contrast to the vertebrate insulin gene which is expressed in the gastroenteromic organ and almost silent in the barin.

In the course of determination of nucleotide sequence of the upstream region of bombyxin B10 gene, we found a new bombyxin gene whose sequence deviates moderately from any of the previously identified bombyxin family genes. We therefore define this bombyxin gene group as family-F and named the presently isolated gene as F1 gene. We determined the sequences of two allelic forms of the gene, one from Japanese race, Kinshu, and the other from Chinese race, Showa. Genomic Southern hybridization indicated the presence of a single copy of the family-F bombyxin gene in the *Bombyx* haploid genome. We also localized the expression site to four pairs of the medial neurosecretory cells in the brain by whole-mount and section *in situ* hybridizations.

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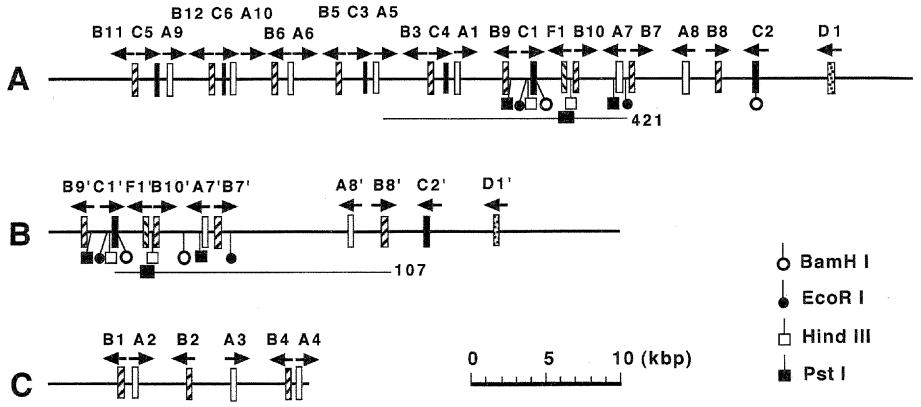

Fig. 1. Maps of three *Bombyx* (Kinshu × Showa) genomic DNA segments (A, B, and C) carrying bombyxin genes. Rectangles on the maps represent the bombyxin genes. Arrows over the genes indicate their transcriptional directions. Restriction sites surrounding bombyxin F1 gene are indicated. Horizontal thin lines and right-hand numerals under the maps indicate the clones and their names, respectively. Filled boxes on the horizontal thin lines indicate the sequenced regions in this study. Segments A and B are presumed to be allelic in nature and have derived from the parental races Showa and Kinshu, respectively. Segment C is presumed to have no allelic variation. The topological relationship of map A (or B) to map C is unknown.

MATERIALS AND METHODS

Animals

Eggs of racial hybrid, Kinshu (Japanese race) × Showa (Chinese race), of the silkmoth *Bombyx mori* were obtained from Kanebo Silk Elegance (Kasugai, Aichi, Japan). Larvae were reared at 25 ± 1°C under a photoperiod of 12 hr light and 12 hr dark on artificial diet (Silkmate, Nihon Nosan Kogyo, Yokohama, Japan).

Clone isolation and sequencing of bombyxin F1 gene

Two phage clones (107 and 421) previously isolated (Kondo *et al.*, 1996) were used (Fig. 1). Clone 107 contains a part of bombyxin gene cluster derived from the *Bombyx* Kinshu race and clone 421 contains the allelic component of the same locus derived from the Showa race. The upstream regions, which contain the newly identified bombyxin F1 gene copies, of bombyxin B10 genes in both clones were sequenced; the nucleotide sequences of both allelic forms of bombyxin F1 gene and the spacer between genes F1 and B10 were determined. Both DNA strands were sequenced by the A. L. F. DNA sequencer (Pharmacia, Sweden) using an AutoRead sequencing kit (Pharmacia) or a Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham, UK). Nucleotide sequence data were assembled and analyzed using computer program in DNASIS (Hitachi Software Engineering, Yokohama, Japan).

In situ hybridization

For whole-mount *in situ* hybridization, brains of the fifth-instar *Bombyx* larvae were fixed for 40 min in a fixative containing 85% ethanol, 4% formaldehyde, and 5% acetic acid at room temperature, and washed in 10 mM phosphate-buffered saline (PBS), pH 7.4, containing 15% (w/v) sucrose at 4°C for 15 to 20 hr (Bandtlow *et al.*, 1987). After washing with PBT (PBS containing 0.05% Tween20), the brains were treated with proteinase K (0.05 mg/ml in PBT) at 37°C for 40 min, and fixed again with 3% (w/v) paraformaldehyde in PBS at room temperature for 20 min. The brains were washed three times with PBT and hybridized with 100 ng of a bombyxin F1 gene probe (5'-ATCAGCCGGGAGAAGTTCGGGCAT-3') labeled with digoxigenin at the 5'-end at 37°C for 20-48 hr in 100 µl solution of 50% formamide, 5 × SSC (SSC: 0.15 M NaCl, 0.015 M sodium citrate), and 5% (w/v) dextran sulfate. The probe was specific for the C-peptide domain of bombyxin F1 gene. We also used a digoxigenin-labeled probe whose sequence (5'-CGGAAATCACCAACAGGACGATAAC-3') was specific for the signal peptide domain of bombyxin F1 gene. The condition prevented cross-hybridizations among bombyxin family mRNAs. After washing with 50% formamide, 5 × SSC and then with PBT at room temperature, the brains were treated with 5% sheep serum (Cappel, USA) at 4°C for 15-20 hr and with an alkaline phosphatase conjugated anti-digoxigenin antibody (Boehringer Mannheim, Germany) at room temperature for 2 hr. The color development was done with 4-nitroblue-tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate. After dehydration, the brains were clarified with methyl salicylate.

Section *in situ* hybridization was carried out essentially the same as whole-mount *in situ* hybridization as described previously (Iwami *et al.*, 1996b). After fixation and serial dehydration, brains were embedded in paraffin wax (Paraplast, Oxford Labware, USA) and sectioned at 10 µm. The sections were then dewaxed, hydrated, and treated with 0.2 N HCl for 20 min followed by proteinase K (1 ng/ml in 20 mM Tris-HCl, pH 7.4, 2 mM $CaCl_2$) at 37°C for 25 min. The sections were fixed again with 4% paraformaldehyde for 15 min, washed with PBS, and hybridized with both of the F1 probes used for whole-mount *in situ* hybridization under the above condition. The color development was done in the presence of 1 mM levamisole, a potent inhibitor of lysosomal phosphatase.

Genomic Southern hybridization

Large molecular mass DNA was prepared from the first-instar larvae, according to the method of Weeks *et al.* (1986). A 10 µg aliquot of the *Bombyx* genomic DNA was digested with *Bam*HI, *Eco*RI, or *Hin*dIII, separated by electrophoresis on 0.7% (w/v) agarose gel, and transferred to nylon membrane filter with 0.4 N NaOH, 0.6 M NaCl. Hybridization was done with the ^{32}P-labeled bombyxin F1 gene fragment (position 421-682 in Fig. 2) at 65°C in the presence of 6 × SSC. The filter was washed at 65°C with 2 × SSC, 0.1% SDS and then with 0.2 × SSC, 0.1% SDS. The condition did not allow cross-hybridization among bombyxin families.

RESULTS

Structure of bombyxin F1 gene

In the upstream region of bombyxin B10 gene (Fig. 1), we identified a gene which forms a pair with bombyxin B10 gene. The gene had sequence similarity with the previously

characterized bombyxin family genes but was found to belong to none of the five bombyxin families (Iwami *et al.*, 1989, 1990; Kawakami *et al.*, 1989; Kondo *et al.*, 1996; Tsuzuki *et al.*, 1997) because its sequence similarity with any of other family genes was 62% at most. The bombyxin genes belonging to the same family have more than 85% similarity in nucleotide sequence with each other with no exceptions (Kondo *et al.*, 1996). We thus establish a new bombyxin gene group as family-F and designate the isolated gene from the segment A as gene *F1* and that from the segment B as gene *F1'*, since we previously designated the bombyxin genes carried by the segment B, which is allelic to segment A, with prime (') (Kondo *et al.*, 1996). Genes *F1* and *F1'* therefore represent the allelic forms from Chinese race and Japanese race, respectively (see DISCUSSION).

Figure 2 shows the nucleotide sequences of genes *F1* and *F1'*. The nucleotide sequence of gene *F1'* is identical with that of gene *F1*, except for the position 625 of the sequence

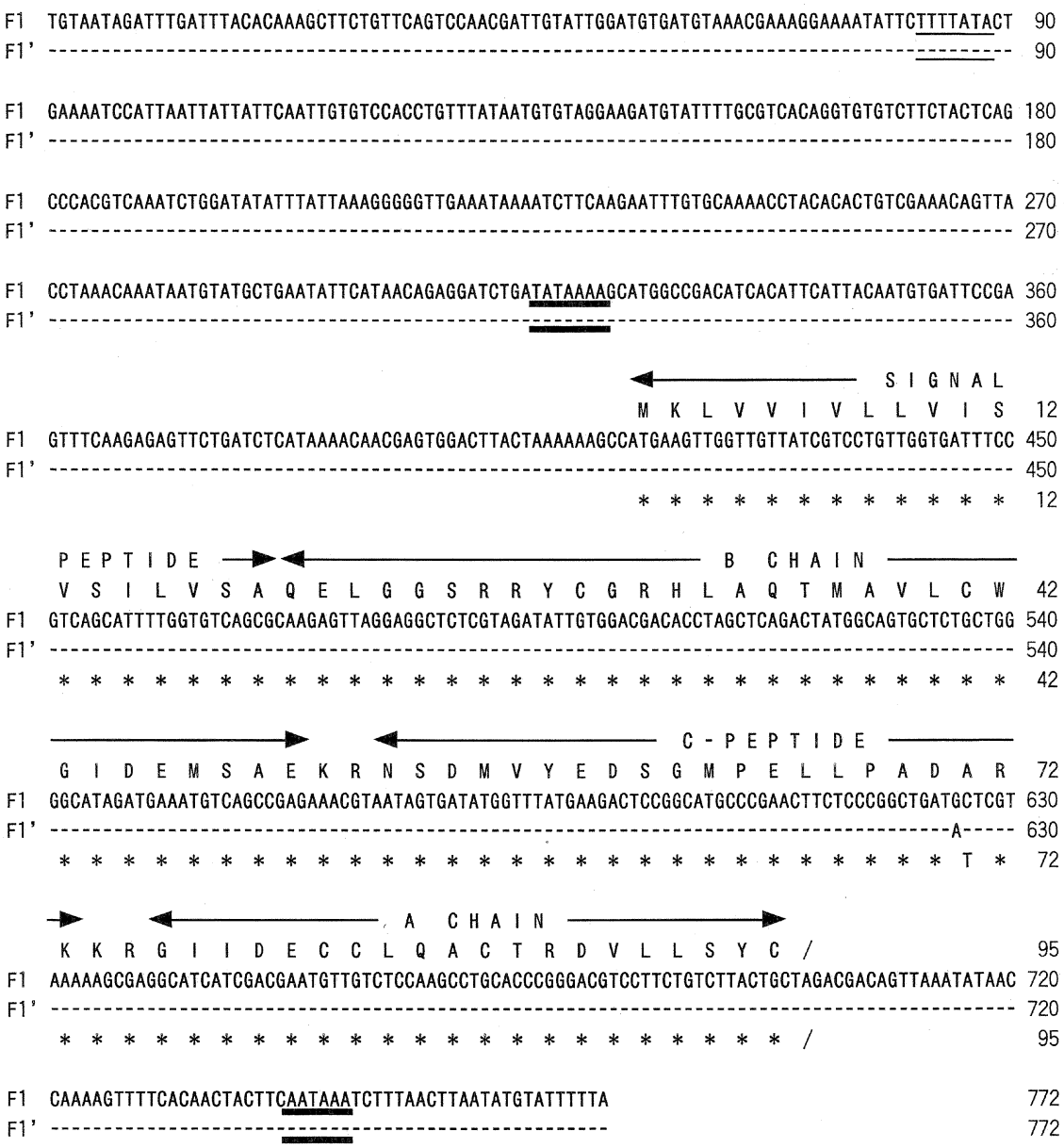

Fig. 2. Nucleotide and deduced amino acid sequences of bombyxin F1 gene and the spacer between bombyxin F1 and B10 genes. F1 and F1' represent the genes carried by the Showa and Kinshu genome, respectively. Signal peptide, B chain, C-peptide, and A chain are indicated. Slash indicates the termination codon. Dashes (–) in the F1' sequence represent the nucleotides identical to those of the F1 sequence. Amino acid sequences are shown in single-letter code and asterisks (*) in the preprobombyxin F1' sequence represent the amino acids identical to those of the preprobombyxin F1 sequence. One amino acid residue in the C-peptide domain is substituted between preprobombyxins F1 and F1'. TATA box-like sequence and polyadenylation signal for the genes *F1* and *F1'* are indicated by thick underlines. TATA box-like sequence for the B10 gene is located at position 82–88 and indicated by thin underline.

where G is replaced by A. The nucleotide sequences have been deposited in the DDBJ/GenBank/ EMBL DNA data base under accession numbers AB001049 (gene *F1* and the spacer between genes *F1* and *B10*) and AB001050 (gene *F1*' and the spacer between genes *F1*' and *B10*'). The transcritional orientation of bombyxin F1 gene is opposite to that of the B10 gene. The open reading frame of the F1 gene apparently encodes preprobombyxin F1 with four domains in the order of signal peptide/B chain/C-peptide/A chain, as in other preprobombyxins (Iwami *et al.*, 1989, 1990; Kawakami *et al.*, 1989; Kondo *et al.*, 1996; Tsuzuki *et al.*, 1997), preprobombyxin-related peptides (Kimura-Kawakami *et al.*, 1992; Iwami *et al.*, 1996a), and preproinsulins (Steiner *et al.*, 1985). A TATA box-like sequence (TATAAAA) and a polyadenylation signal sequence (AATAAA) are observed 99 bp upstream from the translation initiation site and 41 bp downstream from the termination site, respectively. The coding region of the bombyxin F1 gene lacks introns and no splicing signal is found in the untranslated regions as in other bombyxin genes (Iwami, 1990; Iwami *et al.*, 1989, 1990; Kawakami *et al.*, 1989; Kondo *et al.*, 1996; Tsuzuki *et al.*, 1997).

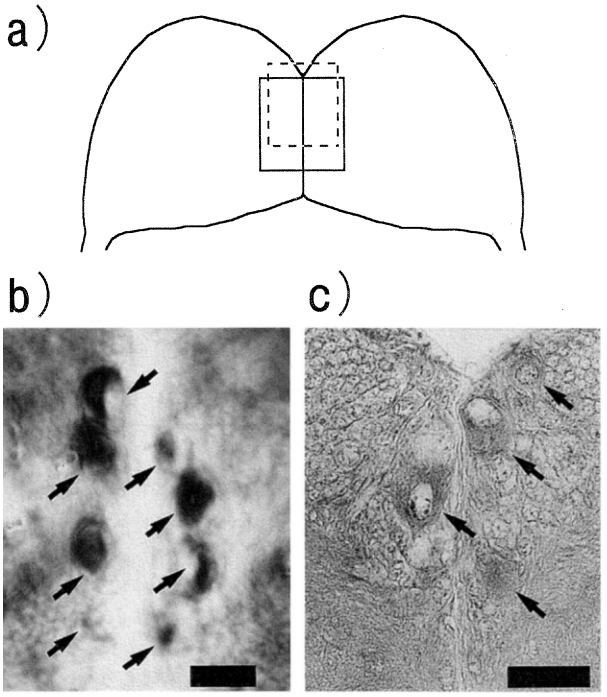

Fig. 3. (**a**) Schematic drawing of a *Bombyx* brain. The portions boxed with solid and broken lines represent the areas shown by photographs in (**b**) and (**c**), respectively. (**b**) Whole-mount *in situ* hybridization of a *Bombyx* brain. The bombyxin F1 mRNA was detected using the C-peptide specific oligonucleotide as a probe. Arrows indicate the neurosecretory cells which produce the bombyxin F1 mRNA. The same cells were also positive when the signal peptide specific probe was used. The photograph shows the medial portion of the brain. Scale bar = 50 μm. (**c**) Section *in situ* hybridization. A section of a brain was hybridized with the F1 probes. Four medial neurosecretory cells (arrows) are positive here, but serial sections (not shown) demonstrate eight (four in each brain hemisphere) positive cells. Scale bar = 50 μm.

Brain neurosecretory cells that produce bombyxin F1 mRNA

Figure 3 shows the cellular localization of bombyxin F1 mRNA in the brain of *Bombyx* larva. The F1 mRNA was localized to four pairs of medial large neurosecretory cells which are classified as M1 subgroup-a cells (Ichikawa, 1991). The M1 subgroup-a cells also produce the family-A (Iwami, 1990), family-B (Iwami, 1990), family-C (unpublished), family-D (unpublished), and family-E (Tsuzuki *et al.*, 1997) bombyxin mRNAs. We previously localized the expression site of the bombyxin-related genes of *Samia* (Inoue *et al.*, unpublished) and *Agrius* (Iwami *et al.*, 1996a) to the medial neurosecretory cells in the brain. The expression of bombyxin family genes in the medial neurosecretory cells is therefore an intriguing feature, in contrast to the vertebrate insulin gene which is expressed in the gastroenteromic organ and is almost silent in the brain.

The *Bombyx* genome contains a single copy of the family-F bombyxin gene

To estimate the number of the family-F gene copy in the *Bombyx* genome, the genomic DNA prepared from the racial hybrid, Kinshu × Showa, was subjected to Southern hybridization analysis (Fig. 4). Two bands (13.5 kbp and 4.5 kbp)

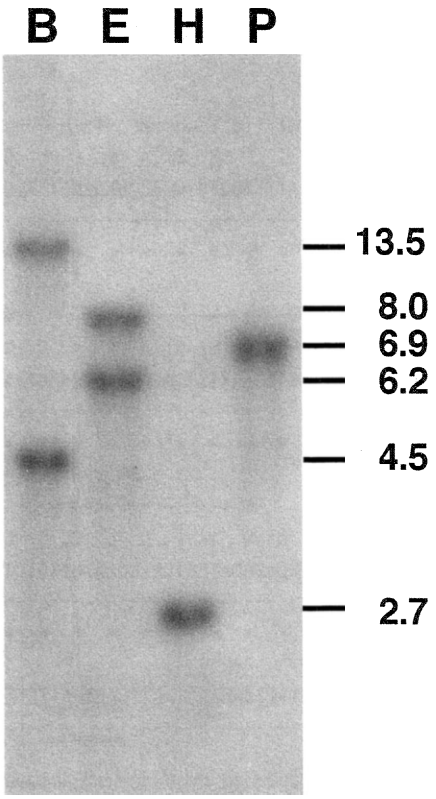

Fig. 4. Genomic Southern hybridization analysis of the family-F bombyxin gene. Genomic DNA from the *Bombyx* racial hybrid (Kinshu × Showa) digested with *Bam*HI (lane B), *Eco*RI (lane E), *Hin*dIII (lane H), or *Pst*I (lane P) was analyzed. The sizes of DNA fragments containing the family-F bombyxin genes are shown at the right in kbp.

were observed when the DNA was digested with *Bam*HI. The 13.5 kbp and 4.5 kbp bands correspond in size to a *Bam*HI fragment bearing bombyxin gene *F1* and another *Bam*HI fragment bearing gene *F1'*, respectively (Fig. 1). The 8.0 kbp and 6.2 kbp bands in the *Eco*RI-digested DNA correspond in size to *Eco*RI fragments bearing genes *F1'* and *F1*, respectively. The size of the *Hin*dIII fragment bearing gene *F1* or *F1'* is 2.7 kbp when calculated from the restriction maps. The 2.7 kbp band were observed with about double intensity. When the DNA was digested with *Pst*I, the 6.9 kbp band with about double intensity was observed. The 6.9 kbp band contained two DNA fragments, one for gene *F1* and the other for gene *F1'*. The sizes of the hybridized fragments digested with *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I therefore exactly matched those of the expected fragments from the restriction maps and no extra fragment bearing another copy of the family-F gene could be detected. We thus concluded that the *Bombyx* haploid genome contains a single copy of the family-F gene.

DISCUSSION

Organization of bombyxin F1 gene

We identified the bombyxin F1 gene which forms a pair with the bombyxin B10 gene. The gene pair F1/B10 is located between bombyxin gene pairs B9/C1 and A7/B7. We previously characterized the allelic components of the genomic DNA segment which includes multiple copies of bombyxin gene (Kondo *et al.*, 1996). One genomic segment (map A in Fig. 1) derived from Chinese race, Showa, and the other (map B in Fig. 1) derived from Japanese race, Kinshu. The right-hand part of segment A was very similar to the majority of segment B with respect to the distribution patterns of bombyxin genes and restriction sites. These two segments were thus concluded to represent the heterozygous components of the same chromosomal segment (Kondo *et al.*, 1996). Segment C was presumed to have no allelic variation between the two races. The haploid genome therefore contains segments A (or B) and C. Now the segment A contains 24 bombyxin gene copies and the segment C contains six bombyxin gene copies (Fig. 1). Bombyxin E1 gene, a unique family-E gene, has not been localized on these segments (Kondo *et al.*, 1996). Total number of the bombyxin gene copy is thus revealed to be 31 in the haploid *Bombyx* genome. The family-A, family-B, and family-C bombyxin genes have 10, 12, and six copies in the genome, respectively. The family-D, family-E, and family-F bombyxin genes exist in single copy in the genome.

The arrangement of the bombyxin gene copies on these segments can be classified into three categories: gene pairs, gene triplets, and single genes, as mentioned previously (Kondo *et al.*, 1996). Sixteen out of the 31 bombyxin genes form pairs, 12 form triplets, and two lie singly. (Bombyxin E1 gene has not been known to form a pair or triplet or to exist as single. See Tsuzuki *et al.*, 1997). In the pairs, two genes, each of which belongs to different families, are apposed with an opposite transcriptional orientation. Most gene pairs are composed of family-A and family-B members, while gene pairs B/C and F/B exist singly. All triplets are composed of the family-A, family-B, and family-C genes and the three genes are apposed to each other. The transcriptional direction of the family-A and family-C genes is opposite to that of the family-B gene. All the family-C genes in the triplets are pseudogenes. The triplets have probably been generated by an unequal crossing-over between two gene pairs, B/A and B/C (Kondo *et al.*, 1996). Bombyxin C2 and D1 genes are present singly, without forming either a pair or triplet but there is the possibility that they forms pairs or triplets with undetected bombyxin genes because of the presence of molecules whose primary structures resemble, but deviate appreciably from, the six bombyxin families characterized so far (Nagasawa *et al.*, 1984; Jhoti *et al.*, 1987). Further studies expect to disclose more bombyxin and bombyxin-related gene copies in *Bombyx*.

Nucleotide sequence of the spacer between bombyxin F1 and B10 genes

The bombyxin gene pair F1/B10 is located between the gene pairs B9/C1 and A7/B7 (Fig. 1). The distance between genes F1 and B10 is 0.4 kbp, which is shorter than that between the spacer of any other bombyxin gene pairs (0.5 to 2.3 kbp; Kondo *et al.*, 1996). The spacer regions of a similar gene pair organization have been extensively studied for the silkmoth chorion genes (Goldsmith and Kafatos, 1984). The expression of various chorion gene pairs are coordinately regulated in a developmental-stage-spacific manner by the spacer sequences (Spoerel *et al.*, 1986, 1993). It is thus probable that the spacers of bombyxin gene pairs play important role(s) in regulating gene expression.

Figure 5 compares the nucleotide sequence of the spacer of gene pair *F1*/*B10* with that of other gene pairs, *B1*/*A2* (map C in Fig. 1), *A7*/*B7* (map A in Fig. 1), and *B9'*/*C1'* (map B in Fig. 1), by Harr-plot analysis. The spacers of these three gene pairs have different nucleotide sequence each other and therefore are presumed to contain different regulatory units. The upstream region of the gene *B10* contains a TATA box-like sequence (Fig. 2) and the sequence identity extends to about 150 bp from the translation initiation site. The upstream region of gene *F1* except for a TATA box-like sequence, however, does not show sequence similarity with that of genes *A2*, *A7*, and *C1'*. In addition, the central region of the spacer of *F1*/*B10* also has no significant sequence similarity with that of gene pairs *B1*/*A2*, *A7*/*B7*, and *B9'*/*C1'*. We previously suggested that two gene pairs *B1*/*A2* and *B2*/*A3* (map C in Fig. 1) must have been generated by duplication of an original pair and may share a transcriptional regulation mechanism because of their high sequence similarity (Kawakami *et al.*, 1989). On the other hand, the central region of spacer of *A7*/*B7* had no significant sequence similarity with that of gene pairs *B1*/*A2* and *B2*/*A3* (Kondo *et al.*, 1996), and therefore gene pair *A7*/*B7* could be under the control of a regulation mechanism different from that of *B1*/*A2* and *B2*/*A3*. Similarly, the regulatory mechanism of gene pair *F1*/*B10* may be also different from that of any of bombyxin gene pairs characterized previously.

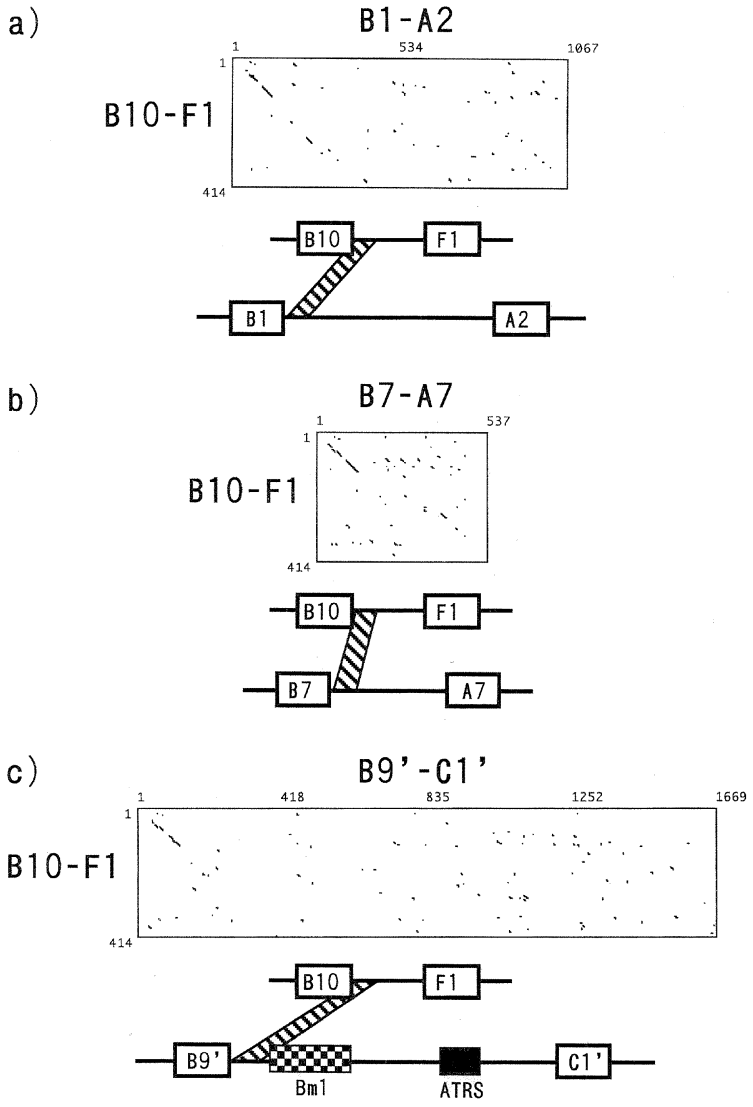

Fig. 5. Comparison of the spacer sequence of bombyxin gene pair *F1/B10* with that of other bombyxin gene pairs by Harr-plot analysis. (**a**) *F1/B10* vs *B1/A2*, (**b**) *F1/B10* vs *A7/B7*, (**c**) *F1/B10* vs *B9'/C1'*. Upper, Harr-plot analysis. Check size, 15. Number of match bases, 11. Lower, schematic representation of the result of the Harr-plot analysis. Homologous regions between the spacers are indicated by hatched parallelograms. The spacer of *B1/A2* has been sequenced by Kawakami *et al.* (1989) and that of *A7/B7* and *B9'/C1'* by Kondo *et al.* (1996). ATRS, A+T-rich repetitive sequence (Kondo *et al.*, 1996). Bm1, Bm1-related sequence (Kondo *et al.*, 1996).

Amino acid sequence of preprobombyxin F1

Figure 6 compares the deduced amino acid sequence of preprobombyxin F1 with that of preprobombyxins (Iwami *et al.*, 1989, 1990; Kawakami *et al.*, 1989; Kondo *et al.*, 1996; Tsuzuki *et al.*, 1997), prepro-bombyxin-related peptides of the saturniid moth *Samia cynthia ricini* (Kimura-Kawakami *et al.*, 1992), prepro-bombyxin-related peptides of the hornworm *Agrius convoluvuli* (Iwami *et al.*, 1996a), and human preproinsulin (Bell *et al.*, 1980). The amino acid sequence of preprobombyxin F1 deviates considerably from that of any of other preprobombyxins. Preprobombyxins within the same family have at least 73% identical sequence with each other, whereas preprobombyxin F1 has no more than 53% identical sequence with other preprobombyxins and 31% with preproinsulin.

Similarities were found between residues of bombyxin F1 and other insulin family members that contribute to the tertiary structure of insulin (Blundell *et al.*, 1970). All cysteins (positions 6, 7, 11, 20 of A chain and positions 7 and 19 of B chain; taking the amino termini of the A and B chains of insulin as position 1) are conserved. All residues responsible for the hydrophobic core formation are conserved or substituted by hydrophobic residues (positions 2, 3, 16, 19 of A chain and positions 2, 6, 11, 15, and 18 of B chain). Glycines (position 1 of A chain and position 8 of B chain), which contribute to the main chain conformation, are also conserved. It therefore seems highly probable that bombyxin F1 has an insulin-like globular structure (see Jhoti *et al.*, 1987 and Nagata *et al.*, 1995). Interestingly, the sequence of the central part of B chain (position 6-20 of B chain) is conserved or conservatively substituted between bombyxin F1 and other bombyxin family

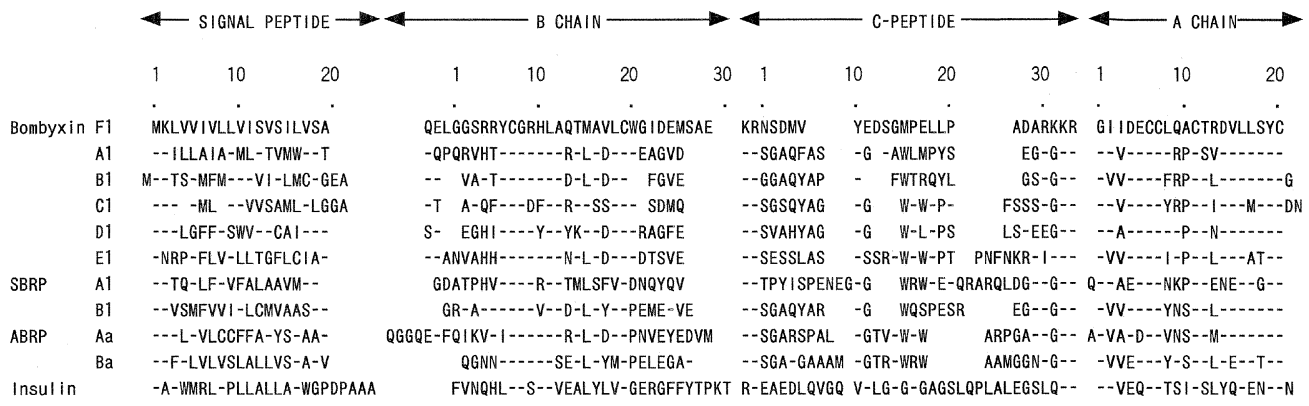

Fig. 6. Amino acid sequence comparison among bombyxins, bombyxin-related peptides, and insulin. Aligned are preprobombyxin A1 (Iwami *et al.*, 1989), B1 (Kawakami *et al.*, 1989), C1 (Iwami *et al.*, 1990), D1 (Kondo *et al.*, 1996), and E1 (Tsuzuki *et al.*, 1997), prepro-*Samia* bombyxin-related peptides A1 and B1 (SBRP; Kimura-Kawakami *et al.*, 1992), prepro-*Agrius* bombyxin-related peptides Aa and Ba (ABRP; Iwami *et al.*, 1996a), and human preproinsulin (Bell *et al.*, 1980). Dashes represent the residues identical to those of preprobombyxin F1. Amino acids are numbered from the amino terminus of each domain of insulin.

members. The central part of the B chain of bombyxin is of critical importance for the recognition by the bombyxin receptor (Nagata *et al.*, 1995). Thus, bombyxin F1 also probably binds to the bombyxin receptor. It should be noted that the conservation of amino acid sequence of A chain is high throughout the bombyxins and bombyxin-related peptides. This conservation suggests that the A chain of the bombyxin family peptides is critically important in keeping the tertiary structure and/or binding to receptors. The presence of paired basic residues (Lys-Arg) flanking both termini of the C-peptide suggests that preprobombyxin F1 is modified to form a mature molecule through the excision of C-peptide by a trypsin-like endopeptidase, as preproinsulins (Steiner *et al.*, 1985). In the signal peptide, a very low degree of similarity is observed between bombyxin F1 and other bombyxins. The signal peptide of preprobombyxin F1, however, consists of a stretch of hydrophobic residues and fulfills the general feature of signal peptide (Watson, 1984).

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