Studies on the neurosecretory cell-specific expression of bombyxin gene,an insulin-related gene of the silkmoth Bombyx mori

メタデータ	言語: eng 出版者: 公開日: 2017-10-05 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	http://hdl.handle.net/2297/16356

氏　　名	SALAH ELDIN ABDEL RAOUF ABDEL AZIZ ABDEL SALAM
生年月日	
本　　籍	エジプト
学位の種類	博士（学術）
学位記番号	博甲第397号
学位授与の日付	平成13年3月22日
学位授与の要件	課程博士（学位規則第4条第1項）
学位授与の題目	Studies on the neurosecretory cell-specific expression of bombyxin gene, an insulin-related gene of the silkmoth *Bombyx mori*（ボンビキシン（カイコインスリン様ペプチドホルモン）遺伝子の神経分泌細胞特異的発現に関する研究）
論文審査委員(主査)	岩見　雅史（自然科学研究科・助教授）
論文審査委員(副査)	櫻井　　勝（理学部・教授）山口　正晃（理学部・助教授）
	山口　和男（遺伝子実験施設・教授）中西　義信（薬学部・教授）

学位論文要旨

ABSTRACT

Invertebrate insulin-related peptides play key roles in growth and metabolism. Genes encoding these peptides are specifically expressed in neurosecretory cells. Bombyxin genes of the silkmoth *Bombyx mori* encode an insulin-related peptide and are expressed in four pairs of the brain neurosecretory cells. No regulatory element has been identified to confer the neurosecretory cell-specific expression. By promoter-deletion analysis and *in vitro* electroporation, a 12 bp element was identified enough to confer the specific expression of bombyxin F1 gene, one of the bombyxin multifamily genes. In addition, an activator element was identified to be responsible for increasing the expression level.

Insulin-like activities are found in various phyla of invertebrate, indicating that insulin-related peptides are common in invertebrates. The peptides are considered as key hormones for the regulation of growth, metabolism, and longevity . Bombyxin of the silkmoth *Bombyx mori*, molluscan insulin-related peptide, and locust insulin-related peptide have been identified as insulin-related peptides. These peptides show remarkable

conservation of the basic insulin-like structure and precursor organization. In addition, many bombyxin-related genes with the insulin-like domain structure were discovered in various insect species. In despite of the structural similarities to insulin, invertebrate insulin-related peptides are produced predominantly in the neurosecretory cells. In particular, bombyxin gene expression is restricted to occur in only four pairs of medial group of the brain neurosecretory cells. This topological specificity led us to identify the element(s) that is responsible for the expression of bombyxin gene. For this end, we introduced reporter gene into the brain cells by an improved *in vitro* electroporation, which enabled to perform reporter gene assay in various animals which have no transgenic system.

Thirty-two copies of bombyxin gene are present in the *Bombyx* genome, and classified into 7 families, A to G, according to their sequence similarities . These genes are clustered on at least two genomic segments and arranged into three organizations: a gene exists singly (single gene), makes a pair with an another gene (gene pair), or with two other genes (gene triplet). Promoter-deletion experiment was used to analyze the promoter region of the bombyxin F1 gene that shares a pair with bombyxin B10 gene in an opposite transcriptional direction. When a construct consisted of the whole 5' upstream region of F1 gene as a promoter and the green fluorescent protein, GFP, gene as a reporter was introduced into *Bombyx* brain by *in vitro* eletropoporation (Fig. 1A), strong green fluorescence was detected in four pairs of the brain neurosecretory cells that had previously been identified as bombyxin-producing cells (Fig. 1B, C). The GFP expression levels were quantified approximately by immunodetection of GFP, and graded as high (+++), medium (++), and low (+) (Fig. 1D, E, and F).

A series of promoter-deletion constructs was prepared to identify the minimal region required for the cell-specific expression of the F1 gene (Fig. 2A). High GFP expression level was detected from deletions up to -190 bp upstream of the F1 translation start site. Medium and low expression levels were resulted from the deletion construct -174. Further deletions up to -146 and -120 bp resulted in abolishment of GFP expression. These results suggest the presence of an activator element which boosts the expression level high and a cell-specific element which is responsible for the basal expression levels (low and

medium) in the neurosecretory cells. The activator element may locate in the region from -190 to -174 bp, whereas the cell-specific element may locate in the region from -174 to -146 bp. To define the minimal sequence of the activator, a series of deletion constructs was prepared in the downstream of -190 bp (Fig. 2B). Deletions up to the construct -185 did not affect the high expression level, but the construct -184 resulted in the loss of the high level. This result indicates that the 5' start of the activator is at -185 bp. The deletion constructs between –184 and -180 were expressed in only medium and low levels, suggesting the possibility that the 3' end of the activator is located in this region. Mutations of the three nucleotides from -180 to -178 bp did not affect the high expression level, whereas mutations in the three nucleotides from -184 to -182 bp resulted in the loss of the high expression level. These results indicate that the 3' end of the activator is –181 bp and thus the minimal activator sequence is TCAAG from -185 to -181 bp upstream of F1 translation start site. Electroporation of the whole promoter construct with a double stranded oligonucleotide (AATCTTCAAGAATTTGTGCAAA) that contains the activator sequence as a competitor resulted in the loss of the high expression level. The activator element is similar to the core of the element TYAAGT (Y = C or T) in the gut-specific enhancer region of the mosquito *Anopheles gambiae* trypsin gene. The similarity of the elements indicates that *Bombyx* and *Anopheles* may share homologous transcription factor(s) that binds two different genes in different organs, and that the regulatory system of genes expressed in brain and gut is evolutionally conserved.

To determine the minimal sequence of the cell-specific expression element, many deleted and mutated constructs were prepared in the region from -174 to –146 bp (Fig. 2C). Deletions up to -170 bp resulted in maintaining the medium and low levels of expression, but the deletion constructs -169 and -168 completely abolished the GFP expression, indicating that the 5' start of the cell-specific expression element is at -170 bp. A set of constructs mutated at the sites, -167 to -165 bp, -164 to -163 bp, and -162 to -161 bp, resulted in no GFP expression, indicating that these mutated nucleotides are included in the cell-specific expression element. Point mutations in the nucleotides at -159, -158 or –157 bp were prepared to determine the 3' end of the element. Only the construct with a mutation at -159 bp resulted in no GFP expression, indicating that the 3' end of the cell-

specific expression element is at –159 bp. Mutations in the nucleotides from -153 to -151 bp and -149 to - 147bp did not affect the expression levels. Therefore, the cell-specific expression element, which is responsible for the basal expression levels (low and medium) in the neurosecretory cells, is a 12 bp segment from -170 to -159 bp with a sequence of AAACCTACACAC. Electroporation of the whole promoter construct with a double stranded oligonucleotide (AAACCTACACACTGTCGAAACAGTT) that contains the 12 bp cell-specific element as a competitor resulted in the shutoff of all expression levels. No protein that would bind to the 12 bp element was found by searching the transcription factors database. Thus, the cell-specific element appears to be a novel regulatory element. We therefore named the 12 bp element as BOSE, Bombyxin gene-Specific Element.

The tissue specificity conferred by cis-acting regulatory elements is examined for the rat insulin I gene whose tissue-specific expression is not resulted from any single minimal sequence but from an interaction of multiple sequence elements. In the rat insulin II gene, an element as small as 41 bp is capable of regulating pancreatic temporal and spatial gene expression. In the present study, BOSE is enough to confer the brain cell-specific expression of bombyxin F1 gene. Genes of all 7 bombyxin families have been proved to be expressed in the four pairs of neurosecretory cells in the brain. Sequence comparisons of the bombyxin F1 cell-specific expression element with the identified sequences of the presumed promoter regions of other bombyxin family genes revealed the sequence identity and relative location with some genes and high similarities with others. Present study first revealed an element involved in molecular mechanism underlying the neurosecretory cell-specific gene expression and may provide important suggestions for the mechanisms that control the neurosecretory cell-specific expression in other invertebrates as well as vertebrates.

Figure legends

Fig. 1. (**A**) Schematic representation of the plasimd pB10/F1::EGFP. The plasmid consists of the whole 5' upstream region of bombyxin F1 gene including its translation start codon and the reporter GFP gene in CMV-promoter-deleted plasmid pEGFP-N3. The amino acid sequences of bombyxin F1, GFP, and the bridge between them are shown in orange, green,

and purple, respectively. The positions of the TATA-boxes for F1 and B10 genes are indicated with red. **(B)** The cell-specific expression of pB10/F1::EGFP in the four pairs of the medial neurosecretory cells of *Bombyx* brain as detected with UV microscope with NIBA cube. Scale bar, 100μm. **(C)** NUA cube was used to examine background of the same brain in (B). No fluorescence was detected, indicating very weak background in the brain in (B). The immunodetected levels of GFP expression were graded as high +++ **(D)**, medium ++ **(E)**, and low + **(F)**.

Fig. 2. Promoter-deletion analysis of bombyxin F1 gene. **(A)** **(Left)** The whole promoter region, that is the spacer between the bombyxin gene pair B10/F1 (414 bp), was serially deleted in the F1 transcriptional direction to identify the minimal promoter region that includes the element(s) required for the cell-specific expression of F1 gene. Red boxes show the position of TATA-box for each gene. Black arrowheads indicate the 5' start of each deletion construct related to the F1 translation start site at +1. Blue box represents the region from -190 to -174 bp that may include an activator element. Green box represents the region from -190 to -174 bp that may include the cell-specific expression element. **(Right)** The percentage of brains that showed one of four expression levels is shown against each expression level. n, total number of electroporated brains. **(B)** **(Left)** Nucleotide sequence of the region from -197 to –168 bp showing the activator element sequence. The activator element is indicated in blue with underline. Several deleted and/or mutated constructs were prepared in the region downstream of -190 bp to define the minimal activator element sequence. Sequences in red represent *Bam*HI site or mutated nucleotides and those in blue show the activator element. Red arrowheads show the position of the mutated nucleotides. **(Right)** Same as in (A). **(C)** **(Left)** Serial deletion of nucleotides from the region from -178 to -146 bp reveals the cell-specific expression element in the region from -170 to -159 bp. Deleted and mutated constructs in the region from -172 to -146 bp were prepared to determine the cell-specific expression element. The cell-specific element is indicated in green with underline. Sequences in red represent *Bam*HI site or mutated nucleotides and sequences in green show the cell-specific element. **(Right)** Same as in (A).

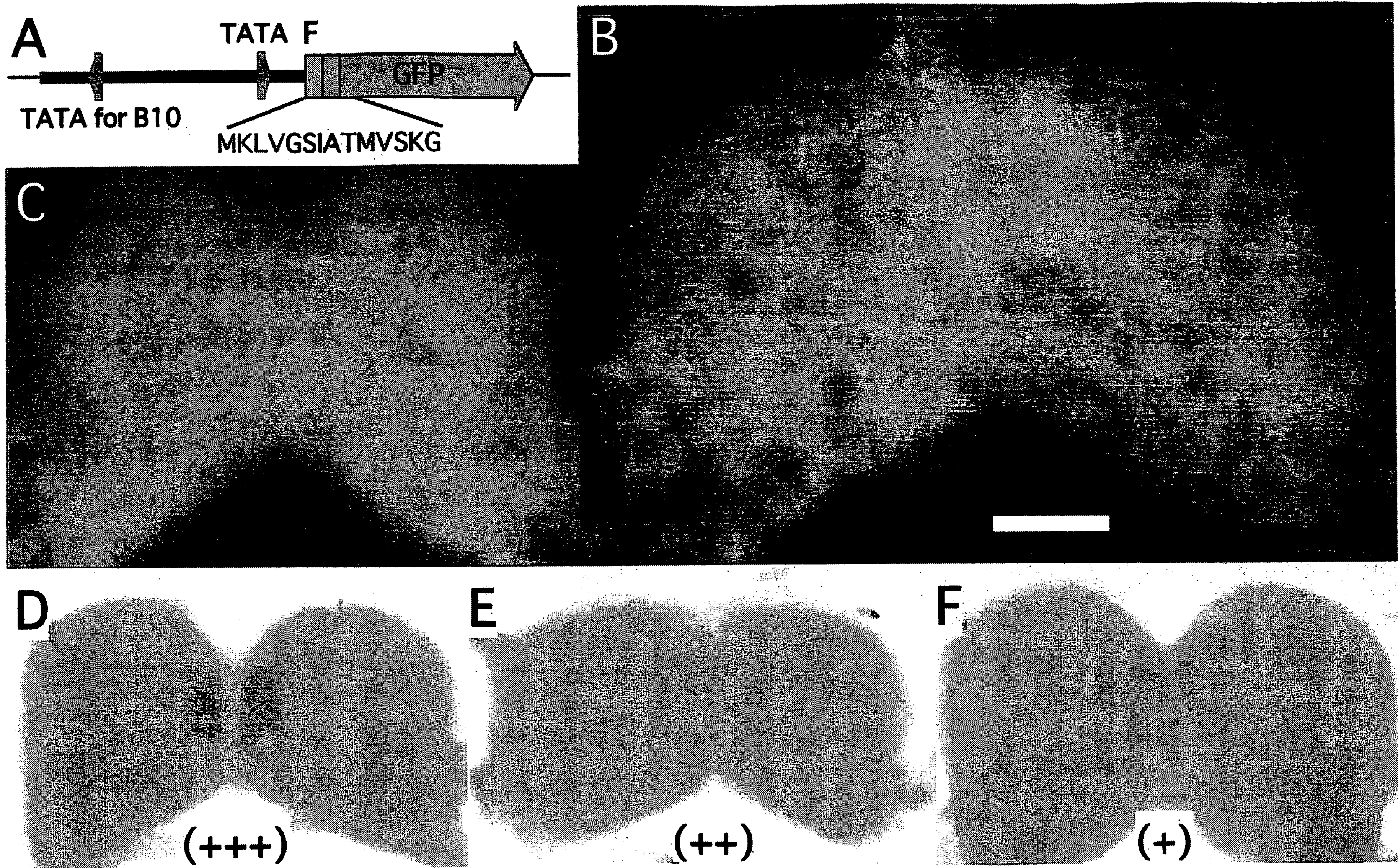

Fig. 1

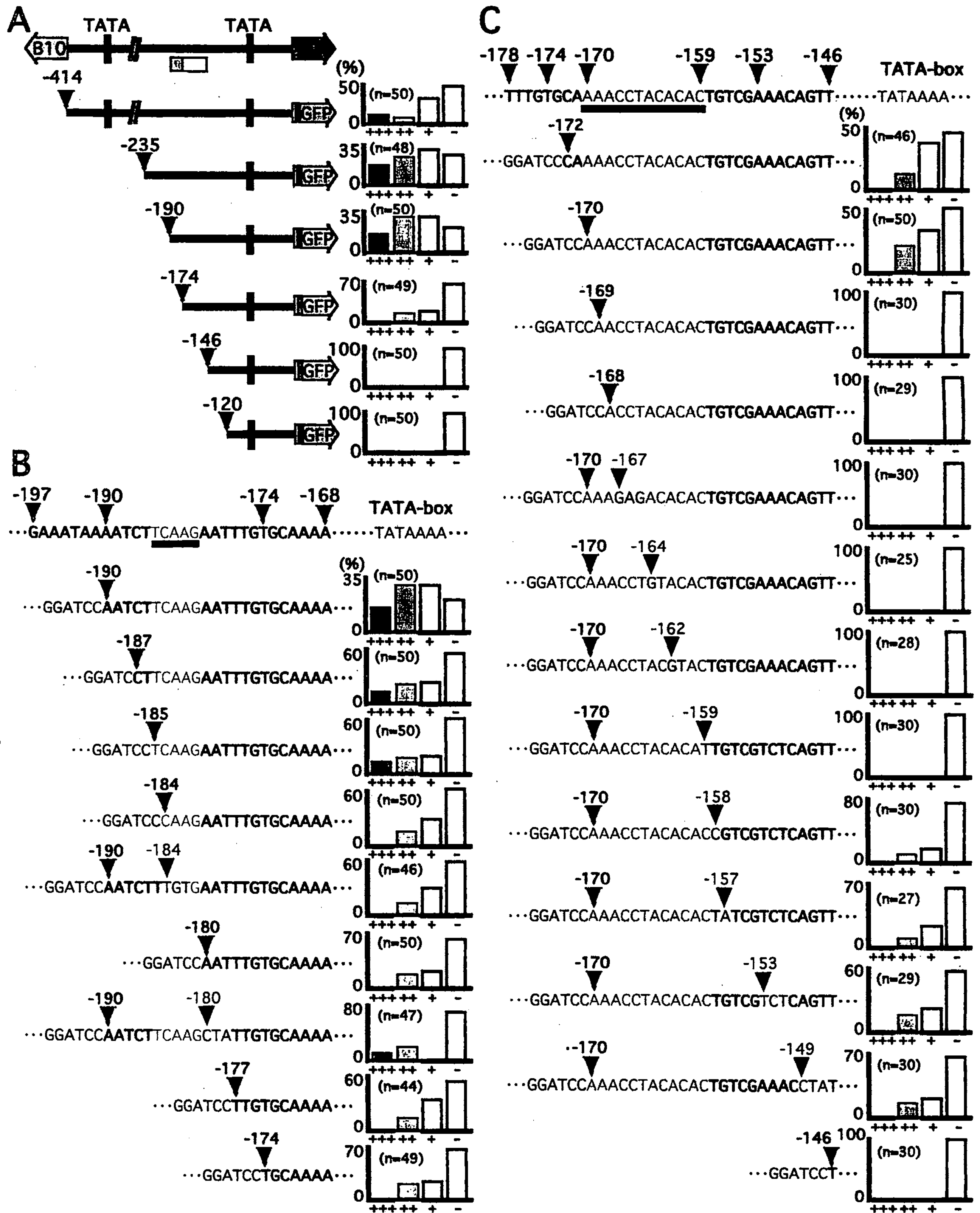

Fig. 2

学位論文審査結果の要旨

提出論文の評価と平成13年2月1日に行われた学位申請者の口頭発表および質疑応答の結果をふまえて審査を行い，同日開催の審査委員会において以下の通り判定した。

本論文は，昆虫脳への遺伝子導入法を応用し，神経分泌細胞特異的転写をもたらすボンビキシン遺伝子プロモーターの解析を記述したものである。特に，ボンビキシン遺伝子の神経分泌細胞特異的転写エレメントを塩基配列レベルで明らかにした意義は大きい。本論文による，具体的な研究成果は以下の通りである。

カイコガ幼虫脳への遺伝子導入により，ボンビキシンF1遺伝子プロモーターの神経分泌細胞特異的転写エレメントの解析に関して，以下の1)～3)の成果が得られた。

1) 6種類のボンビキシンF1遺伝子上流域を有するGFP(緑色蛍光蛋白質)レポーター遺伝子をカイコガ幼虫の脳に導入した結果，F1遺伝子の翻訳開始点上流－174～－146bp領域にボンビキシン細胞特異的転写(ボンビキシン遺伝子はカイコガ脳の背側中央部の4 対の神経分泌細胞で転写されている)のためのエレメントが存在することを示した。さらに，翻訳開始点上流－190～－174bp領域に発現量を増大させるactivator 配列が存在することを示した。
2) ボンビキシン細胞特異的転写エレメント(BOSE;BOmbyxin gene-Specific Element)は、AAACCTACACACの12塩基対からなる配列であることを，点突然変異や欠失を有する12の変異体によるレポーター解析で明らかにした。このエレメントと同一または高い相同性を有する配列が，他のボンビキシン遺伝子上流域に見出されたが(ボンビキシン遺伝子は少なくとも32コピーよりなる多重遺伝子族である)，既知の転写エレメントとの相同性は見出されなかった。
3) 発現量を増大させるactivator配列は，TCAAGであることを，点突然変異や欠失を有する7つの変異体による解析で明らかにした。このactivator配列はカ(Anopeles gambiae)のtrypsin遺伝子の腸での発現に不可欠な配列，およびカイコガのchorion遺伝子の発現に不可欠な配列と相同性を有していた。

以上，本論文は神経分泌細胞特異的転写エレメントの配列を初めて明らかにしたものであり，高く評価される。したがって，博士(学術)に値すると判定した。