p260/270 Expressed in Embryonic Abdominal Leg Cells of Bombyx mori Can Transfer Palmitate to Peptides

Kohji Ueno† and Yoshiaki Suzuki
From the Department of Developmental Biology, National Institute for Basic Biology, Myodaiji, Okazaki 444, Japan

During the study on the mechanisms of abdominal leg development in the silkworm Bombyx mori, we found that a high molecular mass protein (p260/270) was expressed specifically in abdominal leg cells during early embryonic stages and disappeared by a late embryonic stage. p260/270 consists of two polypeptides with molecular masses of 260 and 270 kDa. We have established a purification procedure for p260/270 and have raised an antibody against p260/270. Immunoblot analysis of the Ec/Ec (additional crescent) and En/En (new additional crescent) mutants, which lack the Bombyx abdominal-A gene and therefore do not express abdominal legs, demonstrated that the two mutants lacked p260/270. Therefore, we speculate that the expression of p260/270 may be regulated by the Bombyx abdominal-A gene. cDNA cloning and sequencing demonstrated that p260 and p270 have structures similar to that of rat fatty-acid synthase, which synthesizes palmitate. Most of the enzymatic domains for palmitate synthesis were well conserved in the amino acid sequences of p260 and p270, while the thioesterase domains of p260 and p270 were less homologous to that of rat fatty-acid synthase. Purified p260/270 can transfer palmitate to cysteine residues of synthetic peptides in vitro. We propose that p260/270 may be involved in protein palmitoylation and may function in abdominal leg development.

Homeotic genes that specify segment identity regulate morphogenesis of segment-specific tissues and organs in the fruit fly Drosophila melanogaster (1), the flour beetle Tribolium castaneum (2), and the silkworm Bombyx mori (3). Molecular mechanisms of limb development have been well studied, and the genes involved in limb development were elucidated in Drosophila (4–6). In Drosophila, development of larval and adult thoracic limbs is thought to be repressed in abdominal segments by the bithorax complex (7, 8). In vertebrates, molecular cloning and sequencing demonstrated that p260 and p270 have structures similar to that of rat fatty-acid synthase. Most of the enzymatic domains for palmitate synthesis were well conserved in the amino acid sequences of p260 and p270, while the thioesterase domains of p260 and p270 were less homologous to that of rat fatty-acid synthase. Purified p260/270 can transfer palmitate to cysteine residues of synthetic peptides in vitro. We propose that p260/270 may be involved in protein palmitoylation and may function in abdominal leg development.

Homozygous alleles of the bithorax complex (Bx) and the Ultrabithorax (Ubx) genes are known to regulate the development (9). In Bombyx, the abdominal-A (Abd-A) gene is probably an essential gene for development of abdominal legs (3). We have reported that the E complex is similar to the Drosophila bithorax complex (10, 11). The E complex comprises three homeobox genes: Bombyx Ultrabithorax (Bm Ubx), Bombyx abdominal-A (Bm abd-A), and Bombyx Abdominal-B (Bm Abd-B). Analyses of two mutants that lack abdominal legs demonstrated that Bm Abd-A is deleted in the mutant chromosomes (10). Therefore, Bm abd-A is probably an essential gene for development of abdominal legs.

Many viral, cytoskeletal, and cell-surface receptor proteins, among others, have been reported to be modified with palmitate at their cysteine residues (17, 18). Some small GTP-binding, heterotrimeric G-protein, and G-protein-linked receptor proteins are known to be modified with palmitate via thioester linkages. Such modifications may be important in the regulation of signal transduction (19–21). However, the enzyme responsible for protein palmitoylation has not yet been elucidated. In this report, we describe the purification of p260/270 from embryos and show that p260/270 can transfer palmitate to peptides via cysteine residues. We propose that p260/270 may function in the regulation of signal transduction in abdominal cells by palmitoylation of proteins and, in this way, may regulate abdominal leg development.

EXPERIMENTAL PROCEDURES

Animals—Embryos of a commercial strain of B. mori (Kin-Shu × Sho-Wa; Kanebo Silk Co., Kasugai City, Japan) were incubated at 25 °C for 3–8 days to stage 19–24 (22) and dissected from eggs under a binocular microscope. The B. mori eggs of mutant strains f12, carrying the Ec chromosome, and f21, carrying the En chromosome, were provided by Dr. H. Doira (Institute of Genetic Resources, Kyushu University, Fukuoka, Japan).

Purification of p260/270—4 g of B. mori eggs containing stage 22 embryos was added to 50 ml of buffer A (10 mM Tris-HCl (pH 8.0), 200 mM NaCl) and was homogenized in a Dounce glass homogenizer. The homogenate was centrifuged at 10,000 × g for 10 min. Protein in the supernatant was incubated in 25 °C for 30 min and was applied to a hydroxylapatite column (GIGAPITE, Seikagaku Kogyo Co., Tokyo, Japan). After washing away unbound proteins, p260/270 was eluted in a gradient of 10–150 mM sodium phosphate. p260/270 was

† To whom correspondence should be addressed. Tel.: 81-564-55-7565; Fax: 81-564-55-7566.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U67866 (p260) and U87867 (p270).

The abbreviations used are: FAS, fatty-acid synthase; PAGE, polyacrylamide gel electrophoresis; CAPS, 3-cyclohexylamino)propanesulfonic acid; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; mBHA, 4-methylbenzyldrylamine.
detected by electrophoresis of eluted proteins on a 0.1% SDS, 5% polyacrylamide gel (PAGE, Atto Corp., Tokyo, Japan). The p260/270-containing fraction was concentrated using Centriprep-30 (Amicon, Inc.), applied to a 50 × 1.0-cm Sephacryl S-300 column (Pharmacia), and eluted in buffer A. Then, the p260/270-containing fraction was again concentrated with Centriprep-30 (Amicon, Inc.) and again purified by column chromatography using Sephacryl S-300.

**Immunological Methods**—p260/270 was resolved by SDS-PAGE. The bands containing p260 and p270 were excised from the gels and were used to prepare an anti-p260/270 polyclonal antibody in mice (23).

Indirect immunofluorescence microscopy of whole mount B. mori embryos was used in a method developed by Mitchison and Sedat (24). Embryos were fixed for 30 min in 4% paraformaldehyde in phosphate-buffered saline and permeabilized in phosphate-buffered saline containing 0.2% Triton X-100. Embryos were stained with a 5,000-fold dilution of anti-p260/270 antibody followed by fluorescein-labeled goat anti-mouse antibody (Cappel) in phosphate-buffered saline containing 0.2% Triton X-100 and 5% goat serum.

Immunoblot analysis was done using a method described by Glenney (25). Ten embryos each of the wild type, E"p270", and E"p260" were dissected from B. mori eggs and homogenized in 25 µl of a buffer containing 2% SDS, 50 mM Tris-HCl (pH 8.0), 1% β-mercaptoethanol, and 5% glycerol. 0.5 µl of each homogenate was applied to 4–15% gradient polyacrylamide gel (PhastSystem™, Pharmacia). Electrophoresed proteins were transferred to nitrocellulose membrane. The membrane was stained with India ink and then treated with a 5,000-fold dilution of anti-p260/270 antibody and with horseradish peroxidase-labeled anti-mouse antibody (Cappel). p260/270 was detected using an ECL™ system (Amersham Corp.).

p260 and p270 cDNA Clones—A cDNA library was constructed using mRNA extracted from embryos at stage 20 (22). A QuickPrep™ mRNA purification kit (Pharmacia) was used to extract mRNA from 200 embryos. Purified mRNA was used to synthesize cDNA using a Time-Saver™ cDNA synthesis kit (Pharmacia). cDNAs were inserted into Agt11 and transformed into Escherichia coli Y1088 or Y1090.

Immunoscreening of a cDNA library with an antibody against p260/270 was done using an ECL™ system, DNA was sequenced with a T7 dye primer cycle sequencing kit and a T7 DyeDecoy™ terminator cycle sequencing kit of an Applied Biosystems 373 DNA analysis system. To isolate flanking cDNAs, we prepared DNA probes by polymerase chain reaction and screened an embryonic cDNA library. Polymerase chain reaction (26) was used to determine DNA sequence at the 5' end of the p270 cDNA as described. BLAST and FASTA were used for DNA homology analysis.

**Amino Acid Sequences of p260 and p270—p260 and p270 were separated by preparative SDS-PAGE as follows. Purified p260/270 was applied to 3% polyacrylamide gel (PAGEL) containing 0.1% SDS. p260 and p270 were separated with a ChromaPhor® protein visualization system (Promega). After excising the bands corresponding to p260 and p270, these proteins were eluted in 100 mM CAPS (pH 11.0) with SDS sample buffer (50 mM Tris-HCl, 1% β-mercaptoethanol, 10% glycerol, and 5% β-mercaptoethanol containing 2% SDS, 5% glycerol, and 5% β-mercaptoethanol). p260 and p270 were separated with a ChromaPhor® protein visualization system (Promega).

Approximately 15 µg each of purified p260 and p270 were fixed to polyvinylidene difluoride membrane by ProSpin (Applied Biosystems) and was used to determine the N-terminal amino acid sequences. To determine the internal amino acid sequences of p260 and p270, ~20 µg of each fragment was fragmented in 70% formic acid containing 70 µg/ml bovine serum albumin (BSA) at room temperature for 24 h. The reaction mixture was dried in vacuo and applied to a reverse-phase column (300C4-HG-5, Nomura Chemical Co., Seto, Japan). Amino acid sequencing was done using Applied Biosystems 473A and 492 peptide sequencers.

In **In Vitro Peptidyl Protein Palmitoylation Activity of p260/270—**Approximately 0.05 µg of purified p260/270 and 0.1 µg of synthetic peptides were incubated in 10 µl of reaction solution (40 mM sodium phosphate (pH 6.5), 10 mM acetyl-CoA, 0.01 µCi of [2-14C]malonyl-CoA, 0.5 mM NADPH, and 200 µM bovine serum albumin) at 37 °C. After 1 h, reactions were stopped by the addition of SDS sample buffer (50 mM Tris-HCl (pH 6.8), 2% SDS, 5% glycerol, 0.001% bromophenol Blue), and labeled peptides were separated on a 15–20% polyacrylamide gradient gel (Dai-ichi Co., Tokyo, Japan) at 40 mA of constant current for 40 min in Tris/Tricine buffer (Dai-ichi Co.) containing 0.1% SDS. The electrophoresed proteins could separate low molecular mass peptides ranging from ~2 to 16 kDa. After electrophoresis, the gel was dried and analyzed with a Bio-Image analyzer (BAS 2000, Fuji Photo Film Co., Ltd.).

Fatty acid-peptide binding was analyzed using a synthetic peptide linked to 4-methylbenzhydrazide (mBHA) resin. Approximately 0.5 µg of purified p260/270 and 1 mg of mBHA-linked peptide, which fixes ~200 µg of peptide, were incubated in 100 µl of reaction solution. After 1 h, the mBHA-linked peptide was centrifuged at 5,000 × g for 2 min and washed twice in methanol. Fatty acid bound to peptide was released as described (27). The washed mBHA-linked peptide was incubated in 300 µl of 0.1 M KOH/methanol for 30 min at room temperature, and released fatty acid was recovered into the organic phase. After methylation of fatty acid in 5% HCl/methanol, fatty acids were analyzed by TLC as described (28). [1-14C]Myristylate, [1-14C]Palmitate, and [1-14C]Steareate were purchased from DuPont NEN. Various peptides for palmitoylation assay as shown in Fig. 7A and the mBHA-linked peptide were synthesized with an Applied Biosystems 431A peptide synthesis system.

**RESULTS**

**Isolation of p260/270—**Our primary interest was to identify proteins that are expressed specifically in abdominal leg cells. As shown in Fig. 1A, early in embryogenesis, a wild-type B. mori embryo expresses three pairs of thoracic legs in thoracic segments (T1 to T3) and four pairs of abdominal legs in abdominal segments (A3 to A6). We dissected and divided embryos...
into six body regions: the head, the thoracic legs, the thoracic body, the abdominal legs, the abdominal body, and the caudal region (Fig. 1A). Proteins from each body region were solubilized in SDS sample buffer and resolved by SDS-PAGE. Two high molecular mass polypeptides of ~260 kDa (p260) and ~270 kDa (p270) were detected only in abdominal legs (Fig. 1B).

To study p260 and p270, we purified them from whole embryos by ammonium sulfate fractionation, hydroxylapatite column chromatography, and two rounds separation on a Sephacryl S-300 gel filtration column (Fig. 1C). p260 and p270 coeluted from the gel filtration columns, and the stoichiometry of p260 and p270 was equimolar (Fig. 1C). The native molecular mass of the protein was estimated as ~430 kDa by gel filtration column chromatography (Fig. 1D). Therefore, we inferred that p260 and p270 form a heterodimer complex; hence, the protein was named p260/270.

Expression of p260/270 in Wild-type Embryos—To localize p260/270 within embryos, we made a polyclonal antibody against p260/270. Immunohistochemical analyses were done using whole mount B. mori embryos.

p260/270 was not detected in embryos at stage 19, when abdominal legs are not yet developed. Significant amounts of p260/270 were detected in very few cells in segments A1 to A8 of embryos at stage 20, when abdominal legs begin to develop (Fig. 2A). The cells expressing p260/270 appeared in clusters consisting of ~50 cells. At stage 21A (~1 day after stage 20), the cells expressing p260/270 increased in number to ~100 in each cluster (Fig. 2B), and small abdominal legs began to develop. One more day later (stage 22), these clusters consisted of ~200 cells (Fig. 2C). Abdominal legs continued to develop in segments A3 to A6 from stage 21A to stage 22, although abdominal leg development stopped in segments A1, A2, A7, and A8. During stage 22, cell clusters in segments A3 to A6 lengthened more than those in the other abdominal segments. p260/270 was most concentrated in the cells at the distal ends of the cell clusters in the eight abdominal segments. At stage 24 (2 days after stage 22), p260/270 had disappeared from the embryo (Fig. 2, D and E).

Immunoblot Analysis of E Complex Mutants—We performed immunoblot analysis to determine whether mutants that lack abdominal legs express p260/270. Embryos homozygous for the E^cm (additional crescent) mutation express three pairs of normal thoracic legs, but lack abdominal legs in the abdominal segments (29). Embryos homozygous for the E^n (new additional crescent) mutation express many thoracic-type legs in would-be abdominal segments (29). Thus, embryos homozygous for E^cm and E^n lack abdominal legs in the abdominal segments (10).

Proteins extracted from 10 wild-type and 10 mutant embryos were resolved by gradient SDS-PAGE (0.1% SDS, 4–15% polyacrylamide). India ink staining of nitrocellulose blots showed no qualitative difference between wild-type and mutant proteins, although the amount of total protein in the mutant embryos was slightly less than that in the wild-type embryos (Fig. 3A). Antibody staining detected p260/270 in wild-type embryos, but not in embryos homozygous for E^cm or E^n (Fig. 3B). Although p260 and p270 could not be separated here by gradient SDS-PAGE, the polyclonal antibody recognizing p260/270 detected both p260 and p270 individually; hence, they could be distinguished on 5% polyacrylamide.

cDNA Cloning of p260 and p270—A cDNA expression library was constructed from stage 20 embryos because we first detected p260/270 at that stage. The library was screened with the antibody against p260/270. Inserts in one cDNA clone of p260 (260-4) and two cDNA clones of p270 (270-2 and 270-7) were sequenced (Figs. 4A and 5A). Then, to obtain clones containing flanking sequences, we prepared DNA probes corresponding to the ends of these insert DNAs and rescreened the embryonic cDNA library.

The p260 cDNAs (Fig. 4) represented a long open reading frame encoding 2342 amino acids (Fig. 4B). We purified p260 by preparative SDS-PAGE (described under "Experimental Procedures") to determine the N-terminal and internal amino acid sequences. The N-terminal amino acids (TMVNPPEVK) corresponded to the deduced amino acids at positions 8–16, implying that the amino acids from methionine to arginine (residues 1–7) are removed during maturation of p260. The amino acid sequences of 260(2), 260(12), 260(5), and 260(6) (revealed by analysis of CNBr-fragmented peptides) were found at amino acids 146–164, 1455–1466, 1563–1574, and 2169–2184, respectively. Therefore, p260 comprises the amino acid sequence.

![Fig. 2. Localization of p260/270 in wild-type embryos.](image)

**Fig. 2. Localization of p260/270 in wild-type embryos.** A–D, indirect immunofluorescence microscopy of whole mount B. mori embryos and localization of p260/270 in cell clusters of abdominal segments. A, stage 20; B, stage 21A; C, stage 22; D, stage 24; E, visible image of the same embryo in D. Scale bars represent 100 μm.

![Fig. 3. Immunoblot analysis of proteins from wild-type embryos and embryos homozygous for E^cm and E^n mutations.](image)

**Fig. 3. Immunoblot analysis of proteins from wild-type embryos and embryos homozygous for E^cm and E^n mutations.** A, nitrocellulose-blotted proteins stained with India ink; B, antibody staining with an antibody against p260/270. Lanes 1, wild-type embryos; lanes 2, E^cm/E^n embryos; lanes 3, E^n/E^n embryos.
acid sequence from residues 8 to 2342 (Fig. 4). The molecular mass of p260 estimated by SDS-PAGE coincides with the molecular mass (261,608 Da) of the amino acid sequence deduced from the open reading frame.

To obtain a clone containing the 5'-end of p270, we performed polymerase chain reaction (26) to generate 270-PCR8 (Fig. 5A). Analysis of p270 cDNAs yielded a long open reading frame encoding 2422 amino acids (Fig. 5B). The sequenced N-terminal amino acid sequence (APTTVVED) was encoded by the DNA sequence corresponding to the position one amino acid after the first methionine residue. The amino acid sequences of 270(2) and 270(4) (revealed by analysis of CNBr-fragmented peptides) corresponded to amino acids 729–740 and 1675–1686, respectively, in the translated cDNA sequence. From this we concluded that p270 comprises the complete amino acid sequence from residues 2 to 2422 of the translated cDNA (Fig. 5B). The molecular mass of p270 estimated by SDS-PAGE approximated the deduced molecular mass (268,017 Da) of the peptide translated from the open reading frame.

Amino acid sequence comparison between p260 and p270 revealed sequence homology of 38% and no region of common sequence between the two polypeptides. Therefore, it is un-
likely that either polypeptide is a modified or processed product of the other.

Homology Comparisons of p260 and p270 with Rat FAS—Amino acid sequence analyses of p260 and p270 showed that these proteins have structures similar to that of rat FAS (30), which synthesizes palmitate from acetyl-CoA and malonyl-CoA (Fig. 6). Fatty-acid synthase possesses seven enzymatic domains: ketoacyl synthase, malonyl/acetyltransferase, dehydrase, enolreductase, ketoreductase, acyl carrier protein, and thioesterase. Amino acid homologies between rat FAS and p270 ranged from 43 to 59% in all but the dehydrase and thioesterase domains. That ketoacyl synthase, malonyl/acetyltransferase, enolreductase, ketoreductase, and acyl carrier protein catalyst sites of rat FAS are Cys161 for ketoacyl synthase (31), Ser 581 for malonyl/acetyltransferase (31), His 878 for dehydrase (32), 1670GXXGXXG for enolreductase (33), 1886GXXGXXG for ketoreductase (33), Ser2302 for thioesterase (34), and Ser 2151 for the 4'-phosphopantetheine-binding site in acyl carrier protein.
tein domain amino acid sequences are conserved suggests that, like rat FAS, p270 may synthesize palmitate from acetyl-CoA and malonyl-CoA.

Although amino acid homologies between rat FAS and p260 in the ketoacyl synthase, malonyl/acyltransferase, and enolreductase domains ranged from 37 to 42%, homologies in the ketoreductase, acyl carrier protein, and thioesterase domains were considerably lower (22–26%) (Fig. 6). The amino acid sequence of p260 contained catalytic sites for enolreductase, malonyl/acyltransferase, dehydrase, enolreductase, and thioesterase, but a catalytic site for ketoreductase and a binding site for 4'-phosphopantetheine in acyl carrier protein could not be found. The four C-terminal domains of p260 (enolreductase, ketoreductase, acyl carrier protein, and thioesterase) were less homologous to those of rat FAS than were those of p270.

Peptide Palmitoylation Activity of p260/p270—Many proteins have been reported to be modified with palmitate at cysteine residues (17, 18). Protein palmitoylation may be an important regulation mechanism for signal transduction (20, 21). However, the structure of the enzyme responsible for protein palmitoylation is not yet known.

Comparison of p260/p270 with rat FAS revealed some interesting differences with respect to catalytic activity. Rat FAS comprises two identical multifunctional polypeptide chains. The two polypeptides align head-to-tail, and two centers for fatty acid assembly form at the subunit interface (15, 16). Since p260/p270 comprises two different polypeptides, one of which (p260) lacks the catalytic site for ketoreductase and the binding site for 4'-phosphopantetheine in acyl carrier protein, we hypothesized that one center formed at the p260/p270 interface may synthesize palmitate. Although the ketoacyl synthase and malonyl/acyltransferase domains of p260 and p270 were highly homologous to those of rat FAS, the thioesterase domains of p260 and p270 were only 26 and 22% homologous to that of rat FAS, respectively (Fig. 6). Because the thioesterase domain catalyzes palmitate synthesis and releases palmitate from the enzyme, we further hypothesized that p260/p270 transfers synthesized palmitate to target polypeptides. To test this hypothesis, we determined whether p260/p270 could palmitate peptides at cysteine residues in vitro.

Insect cells are thought to possess protein palmitoylation activity since palmitoylated peptides can be obtained using baculovirus expression systems (35, 36). However, there is no known endogenous acceptor peptide that is modified with palmitate in insect cells. The C-terminal regions of yeast and mammal Ras proteins are known to be modified with palmitate in vivo (37) and in vitro (38). Cys181 is palmitoylated, and Cys186 is polyisoprenylated in the C-terminal region of p21Hs-Ras (37). Therefore, we inferred that the C-terminal regions of the Drosophila ras1 and ras2 gene products (39) would be suitable acceptors for in vitro protein palmitoylation by p260/270. Fig. 7A shows the amino acid sequences of synthetic peptides used in our experiments. C1 and C2 are 20-mer peptides of the C-terminal regions of the Drosophila ras1 and ras2 gene products, respectively.

Peptides were subjected to palmitoylation in vitro by p260/p270 in the presence of acetyl-CoA and malonyl-CoA. The reaction products were resolved by gradient SDS-PAGE (0.1% SDS, 15–20% acrylamide) in Tris/Tricine buffer. The labeled product was detected based on the presence of p260/p270, acetyl-CoA, and NADPH (data not shown). The labeled products moved faster than unlabeled peptides. We assumed that the increase in hydrophobicity causes the increase in SDS gel mobility. As shown in Fig. 7B, C1, C2, C1SC, and C1CS were palmitoylated by p260/p270, whereas C1SS, C2SS, and E-C1 were not. Palmitoylation apparently occurred only at cysteine residues, as long as they were located in a basic region. A single substitution of serine for cysteine, as in C1SC or C1CS, resulted in reduction of the radioactivity of the labeled peptides to ~70% that of C1 (Fig. 7B), implying that each cysteine residue in C1 is palmitoylated about equally. C2 was also palmitoylated to ~70% the degree of palmitoylation of C1 (Fig. 7B, lane 6). This lesser degree of palmitoylation of C2 may have resulted from steric hindrance between fatty acids at adjacent cysteine residues. The absence of cysteine residues, as in C2SS, resulted in no detectable labeled peptide (Fig. 7B, lane 7).

Basic amino acid residues in the palmitoylated region are thought to be necessary for modification with palmitate (21, 40). To test this hypothesis, we synthesized E-C1, in which basic amino acids (lysine and arginine) were substituted with acidic amino acids (glutamate). Despite the presence of cysteine residues in E-C1, this peptide proved to be unsuitable for palmitoylation by p260/p270 (Fig. 7B, lane 2). The basic amino acid sequence may be necessary for recognition of an acceptor peptide by the enzyme.

To verify the identity of the fatty acid joined to C1, palmitoylation was done using C1 bound to mBHA resin (C1-mBHA). In this way, the fatty acid could easily be released from C1 and purified. The palmitate-peptide thioester bond was cleaved by treatment with 0.1 M KOH/methanol (27). TLC separation of fatty acids released from C1-mBHA yielded palmitate and a small amount of stearate (Fig. 7C). We assumed that p260/p270 can synthesize not only palmitate, but also a small amount of stearate. We concluded that p260/p270 transferred palmitate to C1 via thioester linkages.

We performed a standard fatty acid synthesis assay with p260/p270 as described (41) and compared rates versus peptide acylation with the enzyme. Since the rate of peptide acylation was approximately one-third of that of fatty acid synthesis, we concluded that the peptide acylation activity of p260/p270 was an endogenous function of the enzyme.
We supposed that Bm Ubx might be mutated in the E Bm abd-A lane 4, C1SC; and/or Ubx and A8. For example, the segments must have the potential to express abdominal legs until late embryonic stages. Therefore, all eight abdominal legs in all eight abdominal segments. Whereas abdominal legs in segments A1, A2, A7, and A8 stop developing at legs in all eight abdominal segments. Whereas abdominal legs by repressing expression of Bm abd-A might affect the development of abdominal legs and repress the development of abdominal legs. To elucidate cell division or maturation of cell clusters. To elucidate the mechanisms of p260/270 regulation of cell division, an enzyme for protein palmitoylation using a membrane fraction was described (28, 38), and partial purification of a palmitoyl acyltransferase was accomplished (45, 46). These mechanisms of peptide palmitoylation occurred at cysteine residues. Basic amino acids within the vicinity of these cysteines were necessary for palmitoylation, although an acceptor peptide consensus sequence for palmitoylation is not yet known (21, 40). We found that the p260/270 was transferred to cysteine residues. Basic amino acids within the vicinity of these cysteines were necessary for palmitoylation and repressed the development of abdominal legs. To elucidate cell division or maturation of cell clusters. To elucidate the mechanisms of p260/270 regulation of cell division, an enzyme for protein palmitoylation was described (28, 38), and partial purification of a palmitoyl acyltransferase was accomplished (45, 46). These mechanisms of peptide palmitoylation occurred at cysteine residues. Basic amino acids within the vicinity of these cysteines were necessary for palmitoylation, although an acceptor peptide consensus sequence for palmitoylation is not yet known (21, 40). We found that the thioesterase domains of p260 and p270 contained highly acidic regions at amino acids 2200–2219 and 2280–2296, respectively. The content of glutamate and aspartate residues was 45 and 31%, respectively, in these regions of p260 (Fig. 4B) and p270 (Fig. 5B). These acidic regions may interact with and recognize the basic regions of acceptor peptides.

In this work, we showed that p260/270 is a soluble protein that can palmitoylate peptides in the presence of acetyl-CoA and malonyl-CoA. We supposed that palmitate synthesized by p260/270 was transferred to cysteine residues of acceptor peptides. Many studies have been done to determine the molecular mechanisms of peptide palmitoylation in vitro. For example, a cell-free system for protein palmitoylation using a membrane fraction was described (28, 38), and partial purification of a palmitoyl acyltransferase was accomplished (45, 46). These authors used labeled palmitate as the fatty acid substrate for palmitoyl acyltransferase and found that a palmitoyl acyltransferase was localized in the membrane fraction. Since the fatty acid substrate and the enzyme source differed between our work and theirs, it would be interesting to compare the structure and function of p260/270 to those of a purified palmitoyl acyltransferase.

The cells that expressed p260/270 during embryogenesis increased in number, and the cell clusters changed shape during abdominal leg development. It seems therefore likely that p260/270 regulates protein palmitoylation in a small number of abdominal leg cells and that a palmitoylated protein may regulate cell division or maturation of cell clusters. To elucidate the mechanisms of p260/270 regulation of cell division, an endogenous acceptor for protein palmitoylation in the embryo must be identified. Using the in vitro protein palmitoylation
method described herein, we should be able to identify an endogenous acceptor protein and to elucidate the molecular mechanism of p260/270 regulation of abdominal leg development in *B. mori*.

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