Regulation of Silk Genes by Hox and Homeodomain Proteins in the Terminal Differentiated Silk Gland of the Silkworm Bombyx mori

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Abstract: The silk gland of the silkworm Bombyx mori is a long tubular organ that is divided into several subparts along its anteroposterior (AP) axis. As a trait of terminal differentiation of the silk gland, several silk protein genes are expressed with unique regional specificities. Most of the Hox and some of the homeobox genes are also expressed in the differentiated silk gland with regional specificities. The expression patterns of Hox genes in the silk gland roughly correspond to those in embryogenesis showing “colinearity”. The central Hox class protein Antennapedia (Antp) directly regulates the expression of several middle silk gland–specific silk genes, whereas the Lin-1/Isl-1/Mec3 (LIM)-homeodomain transcriptional factor Arrowhead (Awh) regulates the expression of posterior silk gland–specific genes for silk fiber proteins. We summarize our results and discuss the usefulness of the silk gland of Bombyx mori for analyzing the function of Hox genes. Further analyses of the regulatory mechanisms underlying the region-specific expression of silk genes will provide novel insights into the molecular bases for target-gene selection and regulation by Hox and homeodomain proteins.

Keywords: Antp; Bombyx mori; fibroin; homeodomain; Hox genes; promoter element; sericin; silk gland; silkworm

1. Introduction

The pioneering genetic studies on homeotic mutations in the silkworm Bombyx mori, which change the identity of the larval thoracic segments and abdominal segments, were mostly carried out in the first half of the 20th century [1–3] and therein. More than 30 homeotic mutations in Nc and E loci have been described in these studies. Hox genes responsible for some of these homeotic mutations, and those corresponding to the genes in the Antennapedia (Antp) complex and bithorax complex of Drosophila, were subsequently mapped in the Bombyx genome structure [4–11].

Hox genes are well-known master regulators for determining regional identities along the anteroposterior (AP) axis in most multicellular animals [12–15]. Hox genes encode transcriptional factors, are expressed in different but often overlapping regions along the AP axis during embryogenesis, and regulate different sets of target genes, leading to a different identity of each region in animal bodies. However, the 60-amino-acid-long DNA-binding domains near the C-terminus of Hox proteins, termed homeodomains, are highly conserved and commonly bind to similar adenine (A) and thymine (T)-rich DNA stretches containing the ATTA core, at least in vitro [16–20].
This discrepancy is referred to as the “Hox paradox” [21–23]. The coordination of Hox proteins with another class of homeodomain proteins, Extradenticle (Exd)/Pbx and Homothorax (Hth)/Meis, increases their DNA-binding specificities [24–30]. However, these complexes still retain the ability to bind to divergent sequence elements [21,31]. Although genome-wide approaches using microarray or chromatin immunoprecipitation (ChIP) technologies have identified large numbers of Hox downstream gene candidates [23,32–35], the molecular mechanisms by which Hox proteins select and regulate these candidates have not been analyzed in detail. Furthermore, the mechanisms of those leading to functional differences in a Hox protein on target genes also have not been elucidated. Hox proteins can sometimes activate a set of target genes and otherwise repress another set of target genes. Various mechanisms including cofactor-mediated and cofactor-independent DNA-binding steps in addition to DNA-binding-independent steps appear to be involved in the functional regulation of Hox proteins [28,36–45]. The identification of more target genes and their regulatory elements that are actually recognized by Hox proteins will be useful for understanding more clearly the molecular bases underlying the in vivo functions of Hox proteins. We previously reported that the regional specific expression of silk protein genes in the fully differentiated silk gland of the silkworm Bombyx mori was directly regulated by several homeodomain-containing transcriptional factors including the Hox protein Antp, and identified their regulatory elements recognized by these homeodomain proteins [46–51].

Larvae of the silkworm Bombyx mori possess a pair of silk glands. The silk gland is a long tubular organ consisting of a single cell layer of approximately 1000 substantially large, polyploid cells. Silk gland placodes are induced in the ectoderm of the labial segment by Sex comb reduced (Scr) during embryogenesis, and the expression of Scr disappears from the invaginating silk gland with the fork head (fkh) and POU-1 genes being expressed there instead [52–55]. The expression of the fibroin-heavy-chain gene (fibH) is detectable immediately after the completion of silk gland development at around stage 25 of embryogenesis [56]. Silk glands in the silkworm extend from a spinneret on the head to the seventh abdominal segment, and are divided into several subparts along the AP axis (Figure 1) [57–62]. Silk fiber proteins are produced in the posterior silk gland (PSG), secreted into the lumen, and then transferred to the middle silk gland (MSG). Several glue proteins are produced in the MSG, secreted into the lumen, and then coat silk fiber proteins. Silk proteins are transferred forward through the cuticle-lined anterior part of the silk gland (ASG), and are spun as silk threads from the spinneret. The MSG is further divided into three subparts: the anterior (MSG-A), middle (MSG-M) and posterior (MSG-P) portions, based on natural turns and their functions [46,47,51,55,57,59,62,63].

Figure 1. Schematic of the silk gland of Bombyx mori. ASG; anterior silk gland, MSG; middle silk gland, MSG-A; anterior portion of the MSG, MSG-M; middle portion of the MSG, MSG-P; posterior portion of the MSG, PSG; posterior silk gland, PSG-A; anterior half of the PSG, PSG-P; posterior half of the PSG. In order to avoid contamination of the neighbor regions, each portion was prepared as shown in this figure. The structural border is not observed in the PSG, and so the long tubular tissue was divided simply into the anterior and posterior halves.
Although the silkworms produce a large amount of silk proteins at the end of the last instar to make cocoons, a certain amount of silk proteins is produced constantly from the first instar, except during the molting stages, possibly to keep the larvae stable on mulberry leaves and other substrata. Therefore, the expression of silk protein genes is repeatedly turned on and off in a manner depending on the larval molting cycles [56,58,64–67]. The mechanisms of this stage-specific expression involve temporal-specific regulation of the activity of Hox and homeodomain-containing transcriptional factors by the insect hormones ecdysone and juvenile hormone, regardless of whether directly or indirectly. We herein describe and discuss the roles of Hox and other homeodomain-containing transcriptional factors in regulating the region-specific expression of silk genes in the terminally differentiated silk gland at the last instar.

2. Region-Specific Expression of Silk Genes in the Silk Gland

The fibroin-heavy-chain (fibH), fibroin-light-chain (fibL) and fibrohexamerin (fhx) genes for silk fiber proteins are expressed specifically in PSG cells (Table 1) [64,67–70]. Silk fiber proteins are assembled into a large elementary unit consisting of Fib-H, Fib-L and Fhx in a 6:6:1 molecular ratio [71], and are then secreted into the lumen. In contrast, the sericin-1 (ser1), sericin-2 (ser2) and sericin-3 (ser3) genes for glue proteins are expressed in MSG cells with sublocal specificities (Table 1) [46,47,51,55,57,59,62,63]. ser1 is expressed in the MSG-M and MSG-P at the last instar, ser2 is mainly expressed in the MSG-A, and ser3 is expressed in the MS-M and MSG-M. ser1 mRNAs are differentially spliced depending on the larval stages and the subparts of the MSG, and several isotypes of Ser1 proteins are produced [47,57,72]. ser2 mRNAs are also differentially spliced [57,62]. In addition to these silk genes, Tsubota et al. [51] recently found that other silk genes, fibrohexamerin-like 4 and 5 (fhxh4 and 5), are specifically expressed in the MSG-M and MSG-P as ser1. Fhxh4 and Fhxh5 were previously identified as abundant proteins in cocoons [73]. Thus, the expression of silk protein genes is regulated strictly in region-specific manners in the silk gland of Bombyx mori.

3. Region-Specific Expression of Hox and Several Homeobox Genes in the Fully Differentiated Silk Gland

Similar to the silk protein genes, Hox and several homeobox genes are known to be expressed with sublocal specificities along the AP axis of the silk gland (Table 1) [6,46,47,55,74–76]. The anterior Hox genes labial (lab) and Scr are mainly expressed in the ASG, whereas the expression of central Hox genes such as Antp, Ultrabithorax (Ubx) and abdominal-A (abd-A) is absent in the ASG [47]. Antp was previously found to be expressed in the MSG, and abd-A was expressed in the MSG and PSG. The expression level of Ubx was very low, at least at the last instar, but the RT-PCR products were barely detected in the MSG-P and PSG. The posterior Hox gene Abdominal-B (Abd-B) was shown to be expressed in the ASG and posterior half of the PSG [47]. Although we have not yet detected the structural border within the PSG, it may be divided into subparts with different functions. The segment polarity gene engrailed (en) and its paralogue invected (in) are strongly expressed in the ASG and posterior portions of the MSG [47,74,76]. The Pit/Oct/Unc-86 (POU)-homeodomain protein gene POU-M1 is also strongly expressed in the ASG and MSG-A [46,54]. The Arrowhead gene (Awh), which codes a transcriptional factor belonging to the Lin-1/Isl-1/Mec3 (LIM)-homeodomain family, was shown to be expressed specifically in the PSG [48,49]. Expression of the maternal homeobox gene in Drosophila embryogenesis, caudal (cad), was detected in ASG and MSG. These Hox and homeobox genes have been expected to serve in the regional specificity of the silk gland and the region-specific expression of silk protein genes.
Table 1. Region-specific expression of the genes for silk proteins, and Hox and some homeodomain-containing transcriptional factors in the silk gland.

| Silk Gene | ASG (1) | MSG | PSG | Reference |
|-----------|---------|-----|-----|-----------|
| Silk Fiber Protein | | | | |
| fibH | – | – | – | +++ (1) | +++ [48] |
| fibL | – | – | – | +++ | +++ [48] |
| fhx | – | – | – | +++ | +++ [48] |
| Glue Protein | | | | |
| ser1 | – | – | +++ | +++ – – | [46,47,51] |
| ser2 | – | +++ | ++ | – – | [51] |
| ser3 | – | +++ | ++ | – – | [51] |
| fhxh4 | – | – | ++ | +++ – – | [51] |
| fhxh5 | – | – | ++ | +++ – – | [51] |
| Hox Gene | | | | |
| lab | ++ | +/- | + | + ++ | [47] |
| Scr | ++ | +/- | – | – – | [47] |
| Antp | – | ++ (4) | ++ | – – | [47] |
| Ubx | – | – | +/- | ++ +/- | [47] |
| abd-A | – | + | + | + ++ | [47] |
| Abd-B | + | +/- | – | – – | [47] |
| Homeobox Gene (5) | | | | |
| Awh | – | – | – | ++ | ++ [46] |
| En | + | +/- | + | + – | [47] |
| in | ++ | – | + | ++ – | [47] |
| Pou-m1 | ++ | + | + | – | [46,47] |
| cad | ++ | +/- | +/- | – | [47] |
| exd | ++ | ++ | ++ | ++ ++ | [47] |
| hth | + | +/- | ++ | ++ + | [47] |

(1) Total RNA prepared from each region of the silk gland at two days or three days (in the reference [51]) of the fifth instar was used for RT-PCR; (2) The structural border is not observed within the PSG. The long tissue was divided simply into the anterior and posterior halves; (3) Results of RT-PCR are summarized. (+++) detectable with less than 25 cycles, (++) detectable with 30 cycles, (+) detectable with 35 cycles, (+/-) barely detectable with more than 35 cycles, and (–) undetectable. Expression levels were not be compared between different genes; (4) The Antp gene is efficiently transcribed, whereas the Antp protein was not detected in the MSG-A with Western blotting; (5) Genes encoding homeodomain-containing proteins except for the Hox genes.

4. Antp Regulates the MSG-Specific Expression of ser1

The expression of ser1 is restricted to the MSG-M and MSG-P in the last instar [46,47,55]. We attempted to identify the promoter elements responsible for the region-specific expression of ser1. By using a gene gun, reporter constructs containing the ser1 promoter were introduced into silk glands dissected out from the last instar larvae, and silk glands were then transplanted into other silkworms at the same stage. After being cultivated for one or two days, the transplanted silk glands were removed from the recipient larva, and the promoter activity of each construct was assessed. We found that the –70 region of the ser1 promoter (the transcription start site is denoted +1) was essential for its MSG-specific expression [50]. A protein factor or factor complex binding to the –70 element was detected in extracts prepared from the intermolt MSG-P using the electrophoretic mobility shift assay (EMSA), but not in extracts from the PSG or MSG-P at the molting stage, which is consistent with the spatio-temporal specificity of ser1 expression. This factor was designated as the MSG-intermolt-specific complex (MIC). The MIC also bound regions further upstream (–1350, –320 and –180) of the ser1 promoter, and these MIC-binding elements were found to commonly contain the ATTA-core AT-rich sequences recognized by homeodomain proteins (Table 2) [50].
Since Antp, en and in are specifically expressed in the MSG [47,74,76], we prepared recombinant proteins of Antp and En, and determined whether these proteins bind to the −70 element. Although both proteins bound to the −70 element of the ser1 promoter, Antp but not En showed a similar DNA-binding specificity to that of MIC [47]. The apparent molecular size of the Antp-probe DNA complex detected with EMSA was markedly smaller than that of the MIC-probe complex. Hox proteins are known to make a complex with the TALE class homeodomain proteins extradenticle (Exd) and homothorax (Hth) via the conserved hexapeptide motif that resides on the N-terminal side of the homeodomain of Hox proteins [28,29]. The addition of recombinant Exd and Hth proteins together with Antp into EMSA reactions led to the formation of a MIC-like complex with a similar size to that of MIC. Using antibodies specific to Antp, Exd or Hth in EMSA, we confirmed that the MIC in MSG-P extracts included these Hox and homeodomain proteins [47], though the −70 element of ser1 does not contain apparent binding sequences for Exd or Hth, except the ATTA core commonly recognized by

| Table 2. Nucleotide sequence around the regulatory elements of the silk gene promoter. |
|---|
| **Silk Fiber Protein** |
| fBH | −1620 | − | SGF-2 | CTTG CAATTA AGC/CTATTC | [77] |
| −200 | + | SGF-2 | GAT CAATTA AAT CATAATTA ATC | [48,49,78] |
| −110 | + | SGF-2 | GATA CAATTA CATAG AAATA ATC | [48,49,78] |
| −270 | − | Fkh | GTAA TATTTAAAAA AATTTA ATC | [78] |
| −130 | − | Fkh, FMBP-1 | ATCT TTTTATTCAT AAAAAA | [79,80] |
| −70 | + | Fkh | TAGA TTTTACTT CTCTT | [78] |
| −60 | − | Fkh | GACG TATTTACTT CAGT | [78] |
| −130 | + | POU-M1 | TTGT ATTTAAA AAAAA | [78] |
| −110 | + | POU-M1 | TTCT ATTTGAA ATTC | [78] |
| **Glue Protein** |
| ser1 | −1350 | + | MIC | TAATGC AATATTAA ATC | [50] |
| −310 | + | MIC | AATTC AATATTAA ATC | [50] |
| −180 | + | MIC | GAAATC AATATTAA ATC | [50] |
| −70 | − | MIC | ATTTA TTTTACTT TTCC | [50] |
| −160 | − | Fkh | ATTTT TTTTACTT TTCC | [50] |
| −120 | + | Fkh | ATTAA TTTTACTT TTCC | [50] |
| −90 | + | Fkh | ACAT TTTTACCA AATTT | [84–86] |
| −200 | + | POU-M1 | ATCT ATTTAAA AAAAA | [85,86] |
| −140 | − | POU-M1 | ATCT ATTTAAA AAAAA | [85,86] |
| ser3 | −90 | − | MIC, Fkh | AAATAAATTTATTTATTTATTTATTAG | [51] |
| −100 | − | Fkh | TAAT TTTTATTTATTTATTAG | [51] |
| −30 | − | Fkh | GC/CTATTTAATTTATTTATTAG | [51] |
| −70 | − | POU-M1 | ATCT ATTTAAA AAAAA | [51] |
| **fsh34** | −1660 | − | MIC | AAATGATTTATC AAGA | [51] |
| −180 | − | Fkh | TTTT TTTTATTTATTTATTAG | [51] |
| −160 | − | Fkh | TTTT TTTTATTTATTTATTAG | [51] |
| −60 | + | Fkh | TAAA TTTTATTTATTTATTAG | [51] |
| −40 | − | Fkh | ACTT TTTTATTTATTTATTAG | [51] |
| −30 | + | Fkh | AATTAAATTTATTTATTTATTAG | [51] |
| **fsh5** | −300 | − | MIC | AAATGATTATC AATTTAATTTATTTATTAG | [51] |
| −200 | + | Fkh | CTTT TTTTATTTATTTATTAG | [51] |
| −130 | + | Fkh | ATATTTATTTATTTATTAG | [51] |
| −60 | − | Fkh | GTTTAATTTATTTATTTATTAG | [51] |
| −30 | − | Fkh | AAATGATTTATC AATTTAATTTATTTATTAG | [51] |

(1) Presumption from consensus sequences; (2) Takiya, unpublished results; (3) Paired SGF-2-binding element; (4) The FMBP-1-binding element “ATNWNTTA” or its variations overlapped; (5) The Fkh-binding element and FMBP-1-binding element overlapped with the TATA box; (6) The incomplete paired SGF-2-binding element overlapped with the TATA box; (7) The Fkh-binding element overlapped with the TATA box; (8) The TATA box of fsh4 might be a weak Fkh-binding element. Affinity of Fkh to various sequence elements was estimated experimentally in reference [87].
homeodomain proteins. Although a hexapeptide-independent interaction between Exd and several Hox proteins has been reported [88,89], the YPWM motif of Antp appeared to be necessary for the formation of MIC with the cofactors Exd and Hth on the –70 element of the ser1 promoter.

When the recombinant Antp protein was added to extracts from the PSG, the formation of a MIC-like complex was observed via EMSA. In addition, the genes for Exd and Hth were expressed in the PSG as in the MSG. We investigated whether the misexpression of Antp in the PSG induces ectopic expression of ser1. The expression of ser1 was induced in the PSG of transgenic silkworms expressing Antp. When the binding of MIC to the –70 element was disturbed by two-nucleotide mutations, the promoter activity of ser1 was mostly lost [50]. Thus, the central Hox protein Antp is an essential activator of ser1 expressed specifically in the middle portions (MSG-M and MSG-P) of the fully differentiated silk gland of normal silkworms [47], and the activation function of Antp is achieved through binding to the –70 element of ser1 promoter.

5. Antp Activates Other MSG-Specific Silk Genes

Tsubota et al. [51] recently found the novel Antp-induced silk genes fhxh4 and fhxh5. fhxh4 and fhxh5 are specifically expressed in the MSG-M and MSG-P as ser1 in wild-type silkworms, whereas the ectopic expression of these genes was induced in the PSG when Antp was misexpressed in transgenic silkworm lines. The ectopic expression of ser3 in the PSG induced by Antp was also observed. Consensus MIC-binding sequences have been identified in the upstream regions of these genes (Table 2), and the binding of MIC to these elements was confirmed with EMSA, suggesting that these MSG-specific silk genes are direct targets of Antp, similar to ser1 [51]. Antp appears to function as a master regulator for MSG-specific gene expression. The expression of ser3 is restricted to the MSG-A and MSG-M in wild-type silkworms. mRNAs for Antp are detected in the MSG-A, but the Antp protein was not detected with Western blotting, and even with the addition of recombinant Antp into MSG-A extracts, the MIC-like complex was not formed in EMSA [47]. Therefore, the expression of ser3 in the MSG-A appears to be regulated by an Antp-independent mechanism.

6. The LIM-Homeodomain Protein, Arrowhead, Regulates the PSG-Specific Expression of Silk Fiber Protein Genes

Of the PSG-specific silk fiber protein genes, the promoter of fibH has been analyzed in detail using cell-free transcription systems prepared from PSG and other tissues of the silkworm [79,90–96]. The activity of natural DNA of the fibH promoter obtained from the PSG without cloning technology has also been assessed in cell-free systems [97]. Regulatory elements bound by various transcription factors such as Fork head/silk gland factor-1 (Fkh/SGF-1), silk gland factor-2 (SGF-2), POU-M1/SGF-3 and fibroin-modulator-binding protein-1 (FMBP-1) were detected (Table 2) [78–80,84,92,98]. In these studies, the SGF-2-binding elements present from –238 to –73 were found to be essential for efficient transcription of fibH in PSG extracts [78,91,94,95]. Furthermore, Shimizu et al. [77] demonstrated that the combination of two far-upstream enhancer elements with promoter elements is indispensable for the full activation of fibH in vivo using a gene gun system, and an SGF-2-binding sequence at approximately –1620 was a key element in the far-upstream enhancer.

The LIM-homeodomain protein Arrowhead (Awh) was found to be an essential factor regulating PSG-specific expression of fibH, fibL and fhx. SGF-2 was purified from PSG extracts by Ohno et al. [49], and it was shown to be composed of Awh, LIM-domain binding protein (Ldb) and a member of a single-stranded DNA-binding protein family (LIM-homeodomain and Ldb complex associated factor; Lcaf). In transgenic Drosophila, a reporter gene having Bombyx fibH or the fhx promoter are expressed in a restricted region around the imaginal ring of the salivary gland, which resembles the expression region of the Drosophila Awh gene [99–101]. The salivary gland of Drosophila develops from the labial segment under the regulation of Scr, as does the Bombyx silk gland; however, the Bombyx salivary gland is derived from the mandibular segment. In Bombyx mori, Awh is expressed in a strictly specific manner in the PSG, but not in the salivary gland in the last instar, whereas Ldb and Lcaf are expressed...
in most tissues [48,49]. The misexpression of Awh in transgenic silkworms has been shown to induce the ectopic expression of fibH, fibL, and fibx in the MSG, and SGF-2-binding elements have also been detected around the promoter of fibL and fibx (Table 2). We previously reported that the core promoter of fibx itself is an SGF-2-binding element, while the core promoter of fibL has the ability to function as a binding element for both Fkh and FMBP-1. The core promoter of fibx and fibL may act as a tissue-specific regulatory element.

Ldb and Lcaf are expressed in most tissues in last-instar larvae, and the misexpression of Awh was induced in the whole body from a heat-shock promoter in transgenic silkworms. However, the ectopic expression of fibH was induced only in the MSG. Under the same conditions, ectopic expression of fibL and fibx was induced in the ASG in addition to the MSG [48]. The findings suggest that SFG-2 directly regulates the expression of these PSG-specific silk genes, and that coordination with other factors such as Bmsage, which is a silk gland–specific basic helix-loop-helix transcription factor interacting with Fkh, may be necessary [102]. The coordination of these transcription factors will be interpreted by the context (i.e., structure) of each promoter. The expressions of fibH and ser1 were not induced in the ASG under the misexpression of Awh and Antp, respectively, and the expression level of fibH in the MSG-P induced by the misexpression of Awh was very low [47,48]. POU-M1, en and in, which are efficiently expressed in these portions, may exert a suppressive function on these genes [46,55,74,76,98,103]. A typical POU-M1-binding element was detected in the promoter of fibH and ser1 (Table 2), but not around the promoter of fibL and fibx. In Drosophila, En has the ability to recruit the corepressor Groucho and repress target genes [104–106]. For example, En suppresses the expression of fkh under coordination with Abd-A in Drosophila.

7. Perspective

We have demonstrated that the homeodomain proteins Antp and Awh are involved in regulating the region-specific expression of silk genes in the silk gland of Bombyx mori by direct binding to the regulatory elements of the silk genes [47–51]. The region-specific regulatory functions of Antp and Awh on the silk genes are achieved through region-specific expression of Antp and Awh. Therefore, it is important to analyze the upstream regulatory mechanisms responsible for the region-specific expression of Antp and Awh. In the silk gland, other Hox and homeobox genes are also expressed with unique regional specificities along the AP axis, and the homeodomain-containing transcriptional factors encoded by these genes may be involved in the cascades regulating the regional specific expression of silk genes. A large number of ATTA core AT-rich elements consist of the consensus sequences recognized by homeodomain proteins in and around the silk genes’ promoters [50,51,78,107,108]. Several AT-rich stretches have also been found around the promoter of Awh (Takiya, unpublished results). Therefore, the silk gland of Bombyx mori is an appropriate system for analyzing how Hox and other homeodomain proteins select and regulate their specific target genes in vivo, in spite of the relative lack of DNA-binding specificity in vitro. This will provide an insight into the mechanisms by which Hox genes determine regional identities along the AP axis of animal bodies.

The expression of Antp mRNA was detectable in the entire MSG, whereas the Antp protein was only found in the MSG-M and MSG-P and was scarcely detectable in the MSG-A with Western blotting [47]. These findings suggest that post-transcriptional regulatory mechanisms also restrict the function of Antp in the silk gland. Antp mRNA of Drosophila has an internal ribosomal entry site (IRES), and the activity of Antp is regulated not only at the step of transcription, but also at the step of translation. [109]. The translational regulation of some Hox mRNAs is necessary for the normal development of vertebrates [110,111]. Therefore, it is possible that other Hox genes expressed in the silk gland are regulated at translational and/or post-translational steps, as is Antp.

The expression pattern of Hox genes along the AP axis during embryogenesis corresponds to the tandem array on the chromosome and is called “colinearity” [12,112]. Although colinearity is conserved in many animals, the biological significance of maintaining the colinearity during animal evolution remains unclear. Expression levels of Hox genes appear to be affected by the distance
from one enhancer, and the distance from the remote enhancer determines the chromatin structure affecting the expression level of each Hox gene [112–114]. When Hox genes are expressed in overlapped regions, the more posterior Hox genes tend to govern specificity of the regions. The phenomenon is termed “posterior prevalence”. It is not clear at present whether the mechanisms underlying posterior prevalence play a role in the silk gland. However, Hox genes are expressed in regionally specific, but partly overlapping patterns that roughly correspond to those during embryogenesis. The mechanisms of posterior prevalence may be involved in the differentiation of each part of the silk gland and also in the region-specific expression of silk protein genes.

The silk gland of Bombyx mori is a large and single-cell layered organ, and it is easy to obtain many materials for the preparation of proteins, RNAs and other cell components produced in a region-specific manner. We have the ability to analyze the promoter activities of various constructs transiently in the silk gland and other tissues using a gene gun system [50,77,101,115,116]. Technologies to prepare transgenic silkworms and manipulate the genome have been established and are being improved [117–121]. Therefore, the silk gland is an appropriate system to analyze the issues described above both biochemically and genetically. The silkworm Bombyx mori in which Mendel’s law was first confirmed in the animal kingdom [122] is promising to provide novel insight into the molecular basis underlying the regulation of animal body plans by Hox genes.

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