the first h following injection of [^{3}H]-C-16 JH, the total radioactivity recovered from the insect was similar to the amount injected in transectioned as in control females (table). JH catabolism, as determined by the ratio hexane-soluble JH remaining to the total recovered radioactivity, was similar in experimental and control females. Radiochromatograms of homogenized locusts or hemolymph extracts indicated that the only labeled compound in the hexane phase (see legend to the table) had the same retention time as C-16 JH. The half-life of [^{3}H]-C-16 JH was 23 min in operated and sham-operated locusts, in agreement with a previous determination[9]. The half-life of the natural enantiomer of C-16 JH is probably longer than the half-life of the [^{3}H]-C-16 JH[10]. The discrepancy between JH biosynthesis and JH titer in NCA-I-transectioned females therefore does not seem to result from a reduced catabolism.

Only 1 min after injection of [^{3}H]-C-16 JH into the hemocoele, the percentage of labeled hormone recovered in the hemolymph of control animals was low (< 10%). However, the labeled JH was not catabolized because at this time point the recovered radioactivity in the total animal was about 100%, and 80% of this radioactivity was [^{3}H]-C-16 JH (table). This result suggested the retention of a large amount of JH by the tissues and/or the uptake of the JH by the gut. After severance of NCA-I, the percentage of labeled hormone recovered in the hemolymph was 20%, which suggested a reduction of the JH tissue binding and/or the gut uptake. Those factors might be determining agents in the regulation of hemolymph JH titer and could contribute to maintain a high hemolymph JH titer in NCA-I-transectioned females even though JH biosynthesis by the CA was very low.

In conclusion, in *Locusta,* as in *Leptinotarsa*[11] and *Diploptera*[3], JH biosynthesis reflects JH titer in the hemolymph in normal females. Our experiments with allatectomy confirm that in *Locusta* females, the CA seem to be the exclusive source of JH. Moreover, in the locust the retention of JH by the tissues and/or the uptake by the gut were significant and could also be involved in the regulation of the JH titer. Evidence for these processes was found after nervous disconnection of the CA, which reduced JH biosynthesis and uptake of the JH from the hemolymph. The reduced uptake by the tissues and/or the gut promotes JH conservation in the hemolymph and could partially counterbalance the deficiency in JH biosynthesis, and explain the high JH titer and the normal accomplishment of all JH-mediated physiological events.

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Molecular cloning of the fibroin light chain complementary DNA and its use in the study of the expression of the light chain gene in the posterior silk gland of *Bombyx mori*[1]

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Summary. Fibroin light chain (L-chain) mRNA (mol. wt 4.0×10^5 daltons) was purified from the posterior silk gland of the silkworm, *Bombyx mori* (J-131 strain). Double-stranded complementary DNA was synthesized and inserted into the PstI site of pBR322 employing the oligo(dC)-oligo(dG) tailing method. Several recombinant plasmids containing the inserts of about 800 base pairs were isolated. Hybridization-translation assay demonstrated that these clones hybridized specifically with the fibroin L-chain mRNA. One of these clones (pLA23) was used as a probe to investigate relative concentrations of the fibroin L-chain gene and mRNA in the posterior silk glands at different stages of late larval development.
Key words. Silkworm; *Bombyx mori;* silk gland; mRNA; complementary DNA; fibroin light chain; molecular cloning.

A large amount of fibroin is synthesized and secreted in the posterior silk gland of the silkworm at the 5th instar and this system has been considered to be a suitable model for investigating control mechanisms underlying the expression of a specific gene in a specific eukaryotic tissue. The fibroin molecule consists of two polypeptides of different mol. wts linked by disulfide bonds[4-6]. The large polypeptide, termed a heavy chain (H-chain), has a mol. wt of approximately 350,000 and an amino acid composition of the so-called 'fibroin-type', that is, particularly rich in glycine, alanine and serine. On the other hand the

small polypeptide, termed a light chain (L-chain), has a mol. wt of approximately 25,000 and is relatively rich in acidic and hydrophobic amino acids. Couble et al.[7,8] called a small polypeptide in the silk fibroin P25 protein, and we think the L-chain and the P25 are the same protein species. Messenger RNAs for the H-chain[9] and the L-chain[10] have been isolated and their genes located on different chromosomes, i.e. the H-chain gene on the 25th chromosome[11] and the L-chain gene on the 14th chromosome[12]. The structure of the H-chain gene has been studied extensively at the DNA sequence level[13,14], but the L-chain gene has not been analyzed. Couble et al.[8] showed using an 80% pure P25 complementary DNA(cDNA) preparation as a probe that level of the P25 mRNA in the posterior silk gland changed in parallel with the level of the H-chain mRNA.

In the present study, we constructed recombinant plasmids containing the double-stranded fibroin L-chain cDNA and analyzed relative concentrations of the L-chain gene and L-chain mRNA during late larval development, using one of the L-chain cDNA clones as a specific probe.

Materials and methods. Total RNA was extracted from the fresh posterior silk gland of the silkworm on the 5th day of the 5th instar according to the method of Greene et al.[15], and was subjected to two cycles of oligo(dT)-cellulose column chromatography[16]. The poly(A)$^+$RNA was subjected to 5–20% linear sucrose density gradient centrifugation[9], and the size of the RNA in each fraction was determined by agarose-gel electrophoresis under denaturing conditions according to Lizardi and Engelberg[17], and McMaster and Carmichael[18]. In vitro translation of RNA (0.5 µg) was carried out in 10 µl of rabbit reticulocyte lysate (Amersham) containing 0.5 µCi of ^{14}C-protein hydrolysate (Amersham) at 30 °C for 75 min, and products were subjected to sodium dodecyl sulfate (SDS)–12.5% polyacrylamide slab gel electrophoresis according to Laemmli[19] and to fluorography according to Chamberlain[20]. The translation products were treated with an anti-L-chain serum[21] followed with a goat antirabbit Ig G (Cappel) to precipitate the L-chain, if any. Single-stranded cDNA synthesis was carried out at 37 °C for 90 min in a total volume of 0.125 ml. The reaction mixture contained 6.25 µg of oligo $(dT)_{10}$, 8 µg of the 1.2 kb poly(A)$^+$RNA, designated as fibroin L-chain mRNA, 100 units of RAV-2 reverse transcriptase (Takara Shuzo) and 25 µCi of ^{32}P-dCTP(3000 Ci/mmole, Amersham) in 50 mM Tris-HCl (pH 8.3)–10 mM $MgCl_2$–2 mM dithiothreitol–50 mM KCl and 1 mM each of the four deoxynucleotide triphosphates. After the reaction, the mixture was treated in 0.17 M NaOH at 70 °C for 20 min, neutralized with HCl, phenol extracted, and passed through a Sephadex G-100 column. Double-stranded cDNA synthesis was carried out at 15 °C for 12 h in 0.125 ml containing 21.5 units of DNA polymerase I-large fragment (NEB) in 68 mM KH_2PO_4(pH 7.5)–6.8 mM $MgCl_2$–5 mM dithiothreitol and 1 mM each of the four deoxynucleotide triphosphates. The reaction mixture was phenol-extracted and passed through a Sephadex G-100 column.

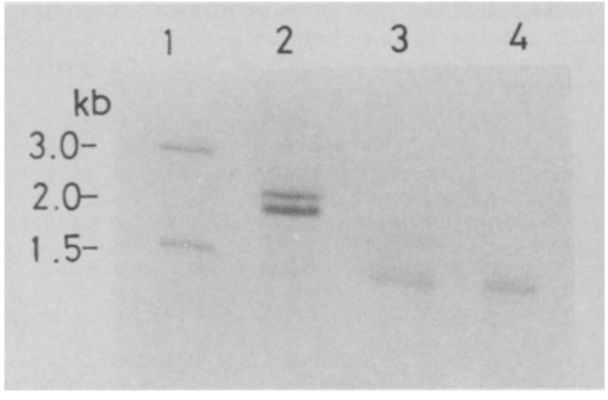

Figure 1. Denaturing agarose gel electrophoresis of poly(A)$^+$RNA in the sucrose gradient fractions. Lane 1: *E.coli* rRNA, lane 2: silkworm 18SrRNA (upper band), lane 3: sucrose gradient fraction No. 9 (0.8 µg RNA), lane 4: sucrose gradient fraction No. 10 (0.8 µg RNA). Stained with methylene blue.

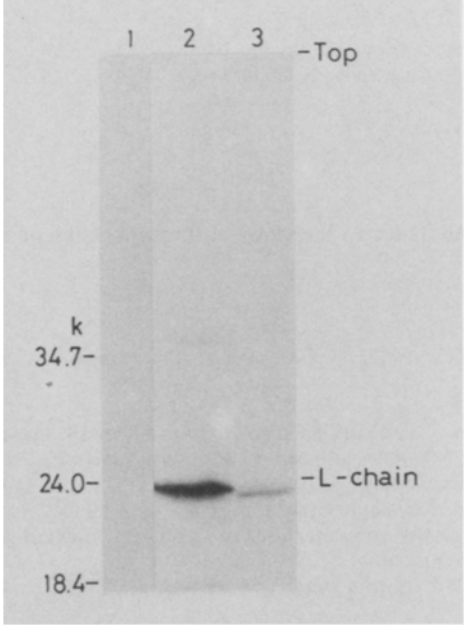

Figure 2. SDS-12.5% polyacrylamide gel electrophoretic profile of in vitro translation products of the 1.2 kb poly(A)$^+$RNA. Lane 1: reaction in the absence of exogenous RNA, lane 2: reaction with the 1.2 kb poly(A)$^+$RNA, lane 3: immunoprecipitate of the translation products of the 1.2 kb poly(A)$^+$RNA with the anti-L-chain serum.

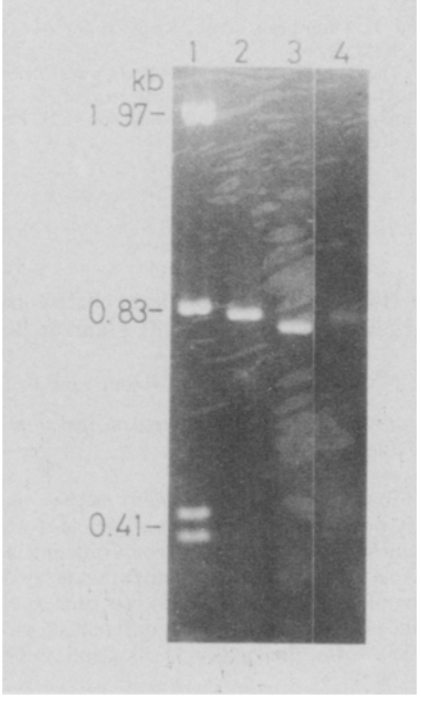

Figure 3. Determination of the sizes of the L-chain cDNA inserts. Recombinant plasmids were digested with PstI and subjected to electrophoresis on an agarose gel and stained with ethidium bromide. Lane 1: PM2/HindIII, size markers, lane 2: pLA23, lane 3: pLA75, lane 4: pLC52.

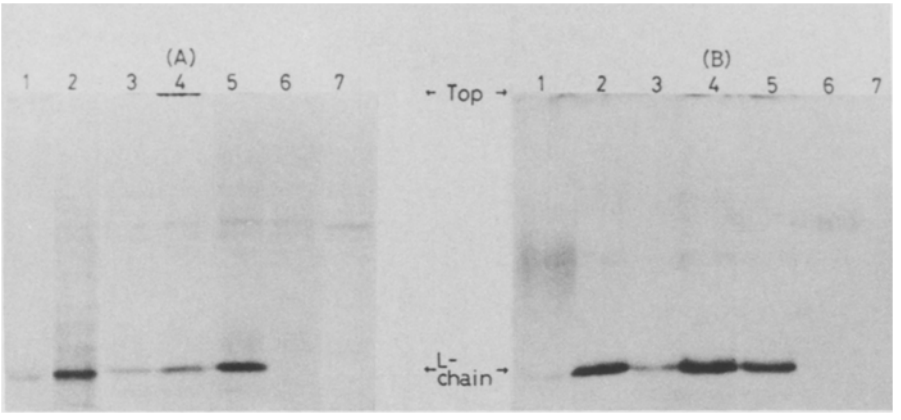

Figure 4. Hybridization-translation assay. In vitro translation products with total poly(A)$^+$ RNA of the posterior silk gland (lane 2), with RNA eluted from the pLA23-filter (lane 3), with RNA eluted from the pLA75-filter (lane 4), with RNA eluted from the pLC52-filter (lane 5), with the fraction eluted from the pBR322-filter (lane 6), or in the absence of exogenous RNA (lane 7). Lane 1, in vivo labeled ^{14}C-L-chain. Translation products (A) and precipitates with anti-L-chain serum (B) were subjected to SDS-polyacrylamide gel electrophoresis and fluorography.

The double-stranded cDNA was treated with Sl nuclease to obtain blunt ends. The digestion was carried out at 37 °C for 1 h in 0.125 ml containing 1 unit of S1 nuclease (Seikagaku Kogyo) in 50 mM CH_3COONa (pH 4.5)–200 mM NaCl–1 mM $ZnSO_4$. The 3'-ends of the double-stranded cDNA were extended with approximately 30 dCMP-residues using terminal deoxynucleotidyl transferase (Takara Shuzo) according to Norgard et al.[22]. The dC-tailed cDNA was annealed with an approximately equimolar amount of dG-tailed pBR322 (BRL) which had been cleaved with PstI. Transformation of *E. coli* LE392 with the recombinant plasmid was carried out by the $CaCl_2$ method[23].

Clones containing the fibroin L-chain cDNA sequence were first identified by colony hybridization[24] using either ^{32}-P-labeled L-chain mRNA or ^{32}P-labeled L-chain cDNA as a probe. For this purpose, the L-chain mRNA was degraded into an average size of about 100 nucleotides by alkali and end-labeled with γ-^{32}P-ATP and T4 polynucleotide kinase (Takara Shuzo). Recombinant plasmids were isolated by the method of Marko et al[25]. The size of the DNA insert was determined by digesting the recombinant plasmid with PstI and subjecting it to electrophoresis on a horizontal 1.5% agarose gel in 8 mM Tris-HCl(pH 7.5)–1 mM CH_3COONa–0.4 mM EDTA. Presence of the L-chain cDNA sequence was confirmed by the hybridization-translation assay as follows. About 20 µg of the EcoRI-digested plasmid DNA was heat denatured (100 °C, 10 min) and immobilized on a nitrocellulose filter. The filter was incubated with polyl(A)$^+$ RNA from the posterior silk gland for 16 h at 50 °C in 0.5 ml of 0.2 M PIPES (pH 6.4)–0.2% SDS–0.4 M NaCl–65% formamide including 50 µg of yeast tRNA and washed successively in the solutions as described by Parnes et al[26]. The hybridized RNA was dissociated and eluted in 150 µl of water by heating in boiling water bath for 3 min, and precipitated with ethanol. In vitro translation was performed in 20 µl of the reticulocyte lysate containing 8 µCi of ^{14}C-protein hydrolysate and products were subjected, either directly or after immunoprecipitation with the anti-L-chain serum, to electrophoresis and fluorography as described above.

Total RNA and DNA samples from the posterior silk gland were prepared according to the published methods[15,27]. Probe-excess dot-blot hybridization was carried out as follows. One to 10 µg of RNA or 10 µg DNA were spotted and fixed on a nitrocellulose filter according to Thomas[28] and hybridized with an excess of ^{32}P-labeled (by nick-translation) L-chain cDNA clone (pLA23) or H-chain genomic clone (pJ9HPE1; containing 4 kb of the H-chain structural gene[29]) at 65 °C for 36 h in 6 X SSC (SSC = 0.15 M NaCl–0.015 M Na_3–citrate, pH 7.0) containing 0.1% each of Ficoll, bovine serum albumin, polyvinylpyrrolidone–0.1% SDS-100 µg/ml of single stranded calf thymus DNA. Northern blot hybridization was performed according to the method of Thomas[28]. After the reaction, the nitrocellulose filter was washed successively in 2 X SSC–0.1% SDS and 1 X SSC–0.1% SDS at 65 °C and autoradiographed using a Kodak X-Omat S film at −80 °C. The autoradiogram was scanned by a densitometer (Shimadzu CS-900) and each peak area was cut out and weighed.

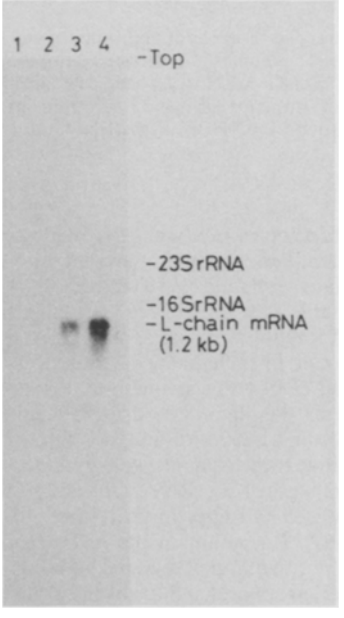

Figure 5. Northern blot hybridization of the total RNA from the middle or posterior silk gland with ^{32}P-pLA23. 2.5 µg (lane 1) and 5.0 µg (lane 2) of the RNA from the middle silk gland at the 2nd day of the 5th instar. 2.5 µg (lane 3) jand 5.0 µg (lane 4) of the RNA from the posterior silk gland at the 2nd day of the 5th instar.

Results and discussion. Poly(A)$^+$ RNA purified from the posterior silk gland was fractionated by sucrose density gradient centrifugation and fractions (No. 9 and 10) under an RNA peak slightly smaller than the 16S marker were collected. Denaturing gel electrophoresis demonstrated that these fractions consisted of a major RNA species of 4.0–4.2 × 10^5 daltons or 1.2 kb (fig. 1). When these RNA fractions were combined and subjected

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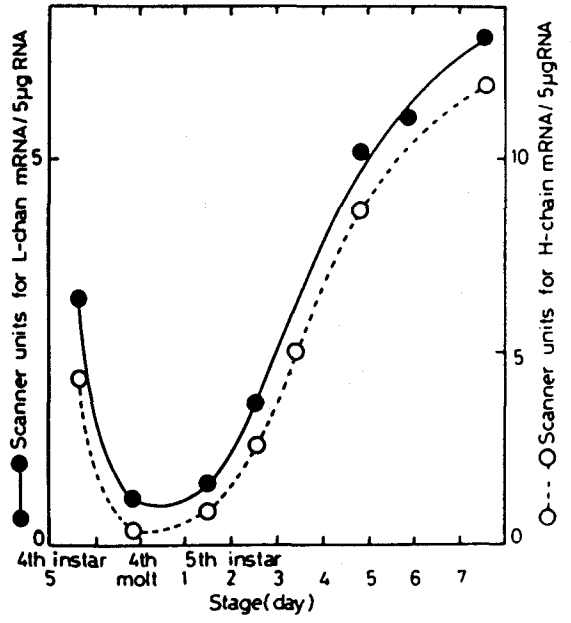

Figure 6. Changing concentrations of the fibroin H- and L-chain mRNAs during the late larval development. Relative concentrations of the L-chain mRNA (●) and H-chain mRNA (○) were determined by the dot blot hybridization of the posterior silk gland RNA from different stages with excess ^{32}P-pLA23 and ^{32}P-pJ9HPE1, respectively.

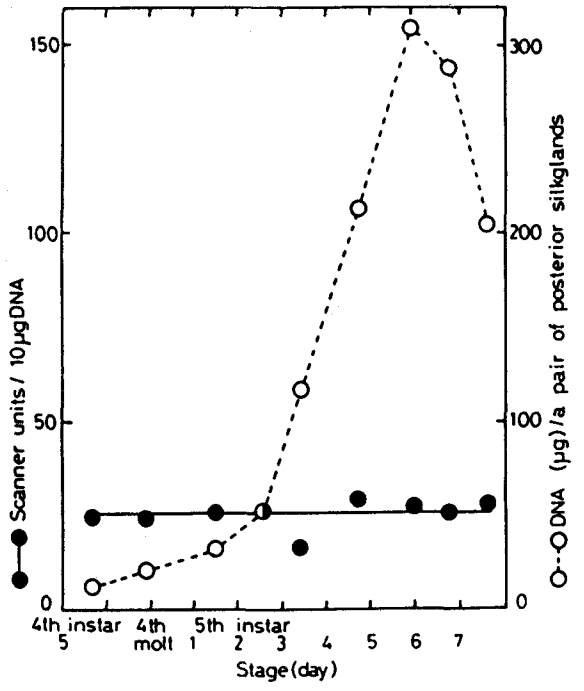

Figure 7. DNA contents and relative concentrations of the fibroin L-chain gene in the posterior silk gland during the late larval development. Amounts of DNA (○) extracted from the posterior silk glands at different stages were measured by A_{260}. Relative concentrations of the L-chain gene (●) were determined by dot blot hybridization of the posterior silk gland DNA with excess ^{32}P-pLA23.

to in vitro translation in a reticulocyte lysate system, a major product was identified which co-migrated with the fibroin L-chain on SDS-polyacrylamide gel electrophoresis, and the product was precipitable with anti-fibroin L-chain serum (fig. 2). The combined RNA fraction was used as an L-chain mRNA fraction for the construction of L-chain cDNA clones.

Double-stranded cDNA was synthesized, inserted into the PstI site of pBR322 by the oligo(dC)-oligo(dG) tailing method and transformed *E. coli* LE392. About 3000 tetracycline-resistant, ampicillin-sensitive transformants were obtained starting with 8 μg of the partially purified mRNA. Out of 230 colonies tested, 115 colonies showed hybridization with the ^{32}P-labeled L-chain mRNA or cDNA. Recombinant plasmids were isolated from several of these positive clones, digested with PstI, and subjected to agarose-gel electrophoresis. Sizes of the DNA inserts ranged from about 200 to 800 base pairs. Three clones (pLA23, pLA75, pLC52) which contained relatively long DNA inserts are shown in figure 3. These recombinant plasmids were subjected to the hybridization-translation assay as described in 'materials and methods'. As shown in figure 4A, all the three RNA preparations, which had been hybridized and eluted from the recombinant DNA-filters, directed the synthesis of a 25,000-dalton protein, which was absent when the eluate from the pBR322-filter or no exogenous RNA was added to the reticulocyte lysate system. When these translation products were immunoprecipitated with the anti-L-chain serum, only the above 25,000-dalton protein was recovered from the precipitate (fig. 4B). These results indicated that all the three recombinant plasmids contained complementary sequences to a part of the fibroin L-chain mRNA.

The pLA23 was ^{32}P-labeled by nick-translation and used as a specific probe for the L-chain mRNA. The ^{32}P-pLA23 formed a hybrid with a 1.2 kb RNA in the posterior silk gland RNA from the 2nd day of the 5th instar, but it did not form a hybrid with the middle silk gland RNA from the same larval stage (fig. 5). Next, changing levels of the concentration of the L-chain mRNA in the posterior silk gland during the late larval development were compared with those of the fibroin H-chain mRNA. RNA samples from the posterior silk glands were prepared daily from the 5th day of the 4th instar to the 7th day of the 5th instar. Different amounts of each RNA sample were spotted and immobilized on the nitrocellulose membrane filter and hybridized with the ^{32}P-pLA23 or ^{32}P-pJ9HPE1, a Charon 4A clone carrying a part of the H-chain structural gene sequence[29], under conditions of probe-excess. Figure 6 shows that the concentration of the L-chain mRNA on the last day of the 4th instar was almost equal to that on the 4th day of the 5th instar, whereas the concentration decreased to a very low level during the 4th molting. The concentration began to increase again from the 2nd day of the 5th instar, and this continued until the 7th day. This changing pattern of the L-chain mRNA concentration was paralleled by that of the H-chain mRNA concentration.

Lastly, the question whether the observed increase in the L-chain mRNA concentration during the 5th instar is caused by specific amplification of the L-chain gene was examined by subjecting DNAs isolated from the posterior silk glands at different stages to dot blot hybridization with excess ^{32}P-pLA23. Figure 7 shows that although the amount of DNA per pair of posterior silk glands increased more than tenfold by the 6th day of the 5th instar, the concentration of the L-chain gene was unchanged throughout these stages.

The present study, using a L-chain cDNA clone as a specific probe, demonstrates that the expression of the L-chain gene is probably limited in the posterior silk gland and is coordinated with the expression of the H-chain gene during the late larval development. These results confirm the observations made by Couble et al.[8] using an L-chain cDNA preparation as a probe. The large increase in the concentration of the L-chain mRNA during the 5th instar should indicate a large increase in the absolute amount of the L-chain mRNA, as the total amount of RNA per pair of posterior silk glands does increase during the same period[6]. The present finding that the relative concentration

of the L-chain gene is kept constant despite the massive synthesis of DNA during the 5th instar implies that the expression of the L-chain gene is activated transcriptionally during the 5th instar. It is of interest to elucidate how the two genes, located on different chromosomes, are expressed coordinately. It has been shown that the 5'-flanking region of the sericin gene, which is expressed in the middle silk gland, contains a homologous sequence to the one in the 5'-flanking region of the fibroin H-chain gene[30]. The L-chain cDNA clones constructed in this study should serve as useful probes in screening L-chain genomic clones from the library, which is a prerequisite for the determination of base sequences of the L-chain gene and its flanking regions.

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Differences in cardiac myosin light chain LC1 among human, monkey and sheep

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Summary. The atrial and ventricular myosin light chains of human, monkey and sheep hearts were compared by dodecylsulfate polyacrylamide gel electrophoresis. The atrial light chain 2 and ventricular light chain 2 are similar among these mammals. However, the atrial light chain 1 of monkey has different electrophoretic mobility from those of human and sheep. The monkey ventricular light chain 1 has same mobility as that of sheep but different from that of human.
Key words. Monkey cardiac myosin; human cardiac myosin; sheep cardiac myosin; atrial myosin light chains; ventricular myosin light chains.

Myosin is the major contractile protein of the myofibril. The myosin molecule is a large ($M_r \sim 500{,}000$), asymmetric protein consisting of a rod-like tail and two globular heads[2,3]. It is comprised of two heavy chains ($M_r \sim 200{,}000$) and two pairs of light chains. The heavy chains interact with each other to form both the rod-like tail and the globular heads. The light chains are located in close proximity at two different sites at the globular head regions. The light chain compositions of cardiac myosins are heterogenous. The atrial and ventricular myosin light chains of mammalian hearts can be separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) into four different polypeptides; atrial light chain 1 (ALC1), atrial light chain 2 (ALC2), ventricular light chain 1 (VLC1) and ventricular light chain 2 (VLC2)[4-10]. The order of migration in SDS polyacrylamide gel (with increasing electrophoretic mobility) for lower mammals are, in general: VLC1, ALC1, ALC2 and VLC2[5-7]. In the case of human cardiac myosin, ALC1 migrates slower than VLC1[7-10]. Because of this difference between humans and the lower mammals and our interest in studying human cardiac myosin light chains in relation to human heart diseases[11], we have compared the light chains of human and monkey. Monkey, being a primate, may have similar cardiac myosin light chain to that of the human. In this report, we present results on the differences in electrophoretic mobility of ALC1 and VLC1 among human, monkey and sheep.

Materials and methods. Mature sheep hearts were obtained from a local abattoir. Adult monkey (*Cercopithecus Aethiops*, African Green) hearts were purchased from Connaught Laboratory, Ltd, Toronto, Canada. Human heart tissues were obtained immediately after death from a 22-year-old male accident victim with unknown medical history. All tissues were stored in liquid nitrogen until used. Myosins were prepared as described by Wikman-Coffelt et al.[12]. Protein was determined by the procedure of Bradford[13] using rabbit γ-globulin as standard. SDS