# The isolation of a messenger RNA coding for the small subunit of fibroin from the posterior silkgland of the silkworm, *Bombyx mori*

Tetsuo Ohmachi+, Hideo Nagayama and Kensuke Shimura*

*Laboratory of Biochemistry, Department of Agricultural Chemistry, Tohoku University, Sendai 980, Japan*

Received 13 July 1982

*Messenger RNA, poly (A)-containing* *Fibroin small subunit, independent biosynthesis* (Bombyx mori)
*(Posterior silkgland)* *Fibroin biosynthesis, translation*

## 1. INTRODUCTION

Fibroin is a secretory protein synthesized in the posterior silkgland of the silkworm. The fibroin molecule consists of 2 polypeptides of different $M_r$ linked by a disulfide bond [1]. The larger peptide showed a typical amino acid composition of the so-called 'fibroin' and the smaller one differs markedly from the larger one in amino acid composition.

The whole translation process of fibroin biosynthesis, however, has not yet been elucidated. To investigate this process, we isolated and fractionated the nascent fibroin peptides from the posterior silkgland ribosomes and found that the small-molecule nascent peptides obtained have an amino acid composition similar to that of the small subunit of fibroin [2]. Accordingly, two possible mechanisms for fibroin biosynthesis has been postulated:

(1) A large precursor fibroin molecule is synthesized by translation depending on one (large) mRNA and cleaved into 2 peptides of different sizes;
(2) The 2 peptides are synthesized independently by the translation of 2 separate mRNAs.

Here, we describe the isolation of a new poly(A)-containing RNA from the posterior silkgland of the silkworm of $5.0 \times 10^5$ $M_r$ exhibiting a template activity to code for the small subunit of fibroin in a wheat germ cell-free system of protein synthesis. This result supports mechanism (2) for fibroin biosynthesis.

+Present address: Research Institute for Tuberculosis and Cancer, Tohoku University, Sendai 980, Japan

*To whom correspondence should be addressed

## 2. MATERIALS AND METHODS

### 2.1. *Preparation of messenger RNA*

The posterior silkglands of the silkworm on day 4 of the fifth instar were digested with proteinase K (300 μg/ml) at 37°C for 10 min and treated with SDS–phenol as in [4]. The total RNA extracted was subjected to 2 cycles of oligo(dT)-cellulose column chromatography. The poly(A)-containing RNA obtained was dissolved in buffer A consisting of 10 mM Tris–HCl (pH 7.6), 100 mM NaCl, 1 mM EDTA, 0.5% SDS and 20 μg polyvinyl sulfate/ml and applied to a Bio-Gel A-50m column (1.2 × 35 cm) pre-equilibrated with the same buffer.

### 2.2. *Translation of mRNA in a wheat germ cell-free system*

Wheat germ was obtained from Nissin Mills Corp. (Tokyo). An S-30 fraction was prepared as in [5]. To exhaust the endogenous mRNA, the S-30 fraction was digested with a staphylococcal nuclease as in [6]. The incubation mixture contained 10 mM Tris–HCl (pH 7.6), 135 mM KCl, 2.5 mM $Mg(CH_3COO)_2$, 1 mM DTT, 2 mM ATP, 0.2 mM GTP, 10 mM creatine phosphate, 25 μg/ml creatine kinase, 0.8 mM spermidine, 100 μCi/ml $^{14}C$-labeled amino acid mixture or [$^{35}S$]methionine, 400 μg/ml posterior silkgland tRNA and 30 μg/ml mRNA. Incubations were carried out at 30°C for 30 min, and then at 37°C for 30 min in the presence of RNase A.

*Published by Elsevier Biomedical Press*
00145793/82/0000–0000/$2.75 

2.3. *Preparation of antibody*

The small subunit of fibroin was prepared from the fibroin isolated from the posterior silkgland and purified as in [1]. Antibody against the small subunit of fibroin was prepared in the rabbit. The specificity of the antibody was tested by Ouchterlony double immunodiffusion analysis [7].

2.4. *Immunoprecipitation*

Reaction mixture (10 μl) was suspended in 50 μl 10 mM Tris–HCl buffer (pH 7.6) containing 150 mM NaCl and 2 M urea. To this mixture, 100 μl of the anti-small subunit sera was added and incubated at 37°C for 60 min. Then, 50 μg purified small subunit was added as a carrier and incubated for 60 min. The precipitates were collected by centrifugation and washed with the same buffer as above and then with the buffer containing 0.5% Triton X-100.

2.5. *Gel electrophoresis*

Electrophoresis of RNA was performed in 1.6% polyacrylamide–0.6% agarose gel under fully denaturing conditions [8]. The gels were stained with methylene blue. SDS–polyacrylamide gel electrophoresis of the cell-free translation products was performed in 15% slab gels using the buffer system in [9]. The gels were subjected to a fluorography according to [10].

## 3. RESULTS

The total RNA fraction extracted from the posterior silkgland by the proteinase K–phenol method (section 2) was subjected to oligo(dT)–cellulose column chromatography (not shown). The poly(A)-containing RNA obtained was further fractionated by a Bio-Gel A-50m column. Two peaks (I and II) were observed (fig.1). The $M_r$ of peak I RNA was estimated at $\sim 6.5 \times 10^6$ by gel electrophoresis (fig.2,3), suggesting that this large RNA may be identical to the fibroin mRNA isolated [3]. Peak II RNA was of a small molecular size, est. $M_r \sim 5.0 \times 10^5$.

The peak II RNA was then translated in a wheat germ cell-free system. The incorporation of [$^{35}$S]-methionine into trichloroacetic acid-insoluble materials was stimulated ~20-fold by the addition of this RNA (table 1). Fig.4 shows a fluorogram of the translation products of this small-$M_r$ RNA ob-

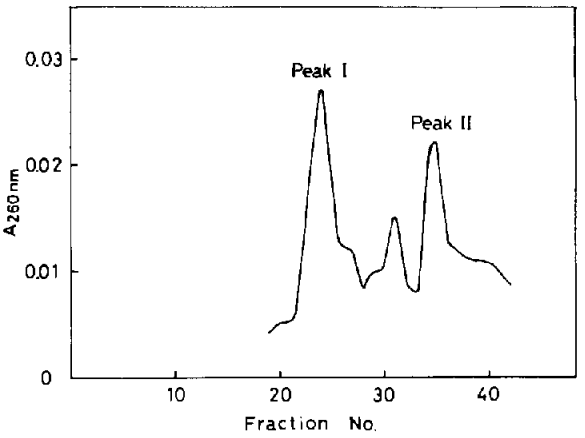


Fig.1. Bio-Gel A-50m column chromatography of poly(A)-containing RNA. Poly(A)-containing RNA obtained from an oligo(dT)–cellulose column was subjected to a Bio-Gel A-50m column (1.2 × 35 cm). The elution buffer contained 10 mM Tris–HCl (pH 7.6), 100 mM NaCl, 1 mM EDTA, 0.5% SDS and 20 μg polyvinyl sulfate/ml. The peak between peak I and peak II is 18 S rRNA.

tained by SDS–polyacrylamide gel electrophoresis. In the absence of the RNA, no translation products were observed (lane 1). In the presence of this RNA, the main translation product was found to migrate at almost the same position as that of the small subunit of fibroin (lane 2). The translation products of the peak II RNA was further sub-

Table 1

Immunoprecipitation of the translation products of the peak II RNA with the antisera of the small subunit

| Addition of RNA | [$^{35}$S]Met incorporated | (cpm) |
|---|---|---|
| – | Trichloroacetic acid precipitate | 2563 |
| – | Immunoprecipitate | 1506 |
| + | Trichloroacetic acid precipitate | 45 346 |
| + | Immunoprecipitate | 33 403 |

The translation of peak II RNA was done as in section 2. After 30 min incubation and the subsequent RNase A treatment, the trichloroacetic acid insoluble radioactivity and the antiserum-precipitable radioactivity were determined

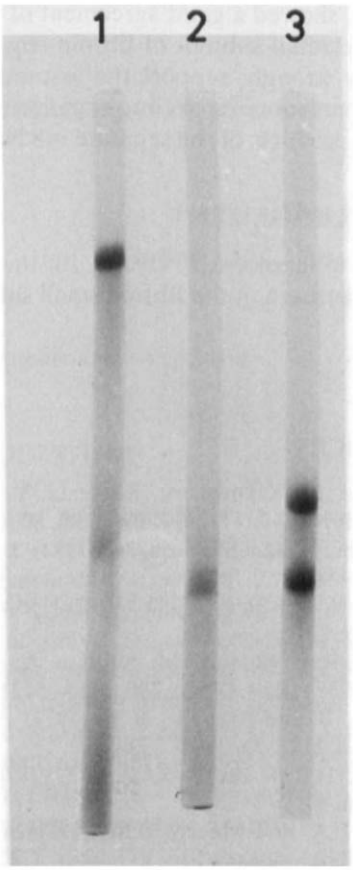


Fig.2. Gel electrophoresis of peak I and peak II RNAs. The peak I and peak II RNAs were subjected to the electrophoresis in 1.6% polyacrylamide–0.6% agarose gels under fully denaturing conditions [8]: (1) peak I in fig.1; (2) peak II in fig.2; (3) *E. coli* rRNA.

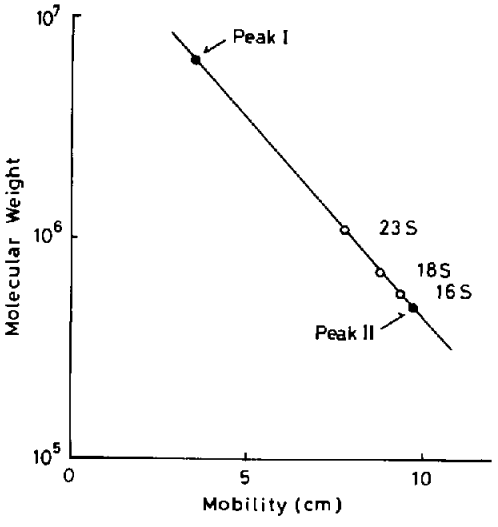


Fig.3. $M_r$-estimation by a semilogarithmic plot of electrophoretic mobility of RNA.

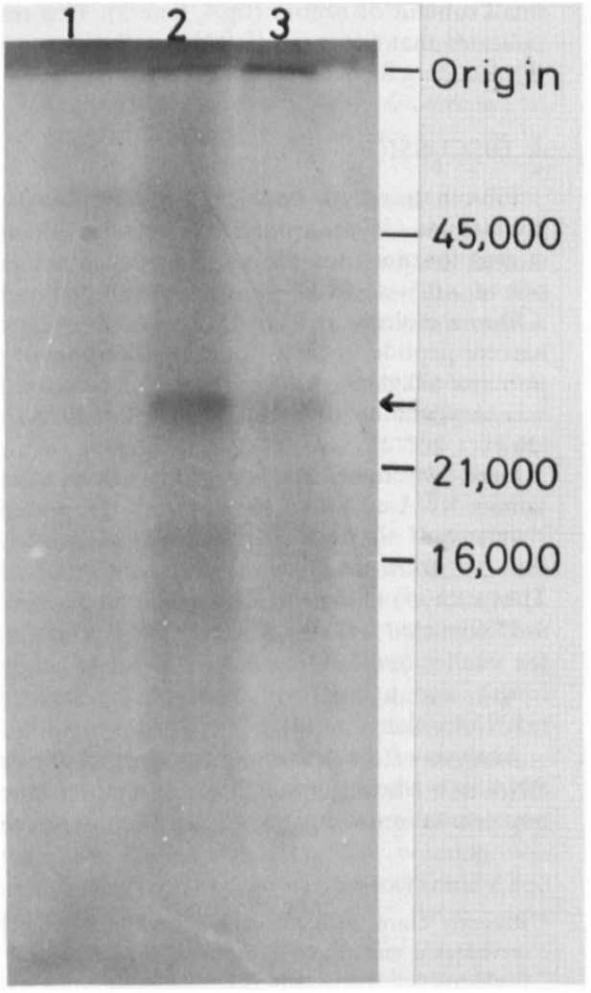


Fig.4. SDS–polyacrylamide gel electrophoresis of the translation products of the peak II RNA. The peak II RNA was incubated in a wheat germ cell-free system containing a $^{14}C$-labeled amino acids mixture and the translation products were subjected to the electrophoresis in 15% SDS–polyacrylamide slab gels: (1) no added RNA; (2) translation products of the peak II RNA; (3) immunoprecipitates of the translation products. The position of marker $M_r$ is shown on the right.

mitted to an immunoprecipitation examination by using the antisera of fibroin small subunit. About 75% of incorporated [$^{35}$S]methionine counts in the translation system was precipitated with the antisera (table 1). The labeled precipitates were then subjected to the SDS–polyacrylamide gel electrophoresis. The main band of the polypeptides was observed at the same position as that of the marker small subunit of fibroin (fig.4, lane 3). This result indicates that the peak II RNA is the messenger RNA coding for the small subunit of fibroin.

## 4. DISCUSSION

Fibroin has long been regarded as one large polypeptide. However, recent studies demonstrated the presence of another smaller polypeptide of $\sim 2.5 \times 10^4$ $M_r$ joined by disulfide bond in a fibroin molecular [1,11–13]. We have isolated a nascent peptide fraction from the ribosomes of the posterior silkgland, whose amino acid composition was very similar to the smaller peptide of fibroin [2].

Here, we have isolated a new poly(A)-containing RNA of $5.0 \times 10^5$ $M_r$ from the posterior silkgland of silkworm (fig.1–3), which is clearly different from the fibroin mRNA isolated in [3]. The size of this new RNA (corresponding to $\sim 1500$ nucleotides) is sufficiently large to code for the small subunit of fibroin ($\sim 300$ amino acid residues), and agreed with that of the unknown mRNA* isolated in [14].

Analysis of the translation product of the new RNA in a wheat germ cell-free system by immunoprecipitation technique (table 1) and gel electrophoresis, showed a good agreement of the product with the small subunit of fibroin (fig.4). These results may strongly support the assumption that the 2 different subunits of fibroin molecule are the translation products of the separate mRNAs.

* Recently Garel et al. have reported that this RNA served as a messenger RNA for P25 protein in a reticulocyte cell-free system (Satellite Symposium of the FEBS Special Meeting in Athens, 22 April 1982)

## ACKNOWLEDGEMENT

We wish to thank Mr F. Oyama for the preparation of the antisera of the fibroin small subunit.

## REFERENCES

[1] Shimura, K., Kikuchi, A., Katagata, Y., Ohtomo, K. and Hyodo, A. (1976) J. Biochem. 80, 693–702.
[2] Ohmachi, T. and Shimura, K. (1981) J. Biochem. 89, 531–541.
[3] Suzuki, Y. and Brown, D.D. (1972) J. Mol. Biol. 63, 409–429.
[4] Greene, R.A., Morgan, M., Shatkin, A. and Gage, L.P. (1975) J. Biol. Chem. 250, 5114–5121.
[5] Roberts, B. and Paterson, B.M. (1972) Proc. Natl. Acad. Sci. USA 70, 2330–2334.
[6] Pelham, H.R.B. and Jackson, R.J. (1976) Eur. J. Biochem. 67, 247–256.
[7] Kabat, E.A. and Mayer, M.M. (1961) in: Experimental Immunochemistry (Thomas, C.C. ed) 2nd edn, pp. 1–99, Springfield IL.
[8] Lizardi, P.M. and Engelberg, A. (1979) Anal. Biochem. 98, 116–122.
[9] Laemmli, U.K. (1970) Nature 227, 680–685.
[10] Bonner, W.H. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83–88.
[11] Tashiro, Y., Otsuki, E. and Shimadzu, T. (1972) Biochim. Biophys. Acta 257, 198–209.
[12] Sasaki, T. and Noda, H. (1973) Biochim. Biophys. Acta 310, 91–103.
[13] Gamo, T., Inokuchi, T. and Laufer, H. (1977) Insect Biochem. 7, 285–295.
[14] Couble, P., Garel, A. and Prudhomme, J.-C. (1981) Dev. Biol. 82, 139–149.