Proc. Natl. Acad. Sci. USA
Vol. 76, No. 12, pp. 6211–6215, December 1979
Biochemistry

Discontinuous translation of silk fibroin in a reticulocyte cell-free system and in intact silk gland cells

(mRNA/tRNA/gel electrophoresis/protease fingerprints/polyribosomes)

PAUL M. LIZARDI*, VIJAK MAHDAVI*, DENNIS SHIELDS†, AND GRACIELA CANDELAS‡

*The Rockefeller University, New York, New York 10021; †Albert Einstein College of Medicine of Yeshiva University, Bronx, New York 10461; and ‡Department of Biology, University of Puerto Rico, Rio Piedras, Puerto Rico

Communicated by Philip Siekevitz, September 14, 1979

ABSTRACT **Silk fibroin mRNA was translated in a rabbit reticulocyte cell-free system. Addition of tRNA from silk glands was essential for complete translation of the fibroin polypeptide. (M_r ≈400,000). Synthesis of full-sized product took at least 85 min. In addition to full-size product, a large number of smaller polypeptides were observed upon analysis by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Evidence is presented that these smaller polypeptides are growing fibroin chains that transiently accumulate as discrete size classes due to discontinuities in the translation process. These discontinuities, or pauses, occur at specific sites in the fibroin mRNA template. The relative duration of the pauses can be experimentally modulated by changing the source of the supplementary tRNA added to the *in vitro* system. Silk glands were incubated in organ culture under conditions where essentially exclusive labeling of newly synthesized fibroins was attained. Analysis in sodium dodecyl sulfate gels showed that the labeling pattern of nascent silk fibroins is similar to the pattern observed in the reticulocyte cell-free system. This result suggests that discontinuities or pauses in polypeptide chain elongation also occur *in vivo* under conditions of organ culture.**

Silk fibroin is one of the largest polypeptides in nature. Recent studies (1, 2) have shown that allelic variants of silk fibroin exist whose M_r is in the range of 350,000–415,000. The genetic machinery that directs the synthesis of this protein has been the subject of extensive study at the level of both DNA and mRNA (3–5). However, the translation of fibroin mRNA in an *in vitro* system has proven to be technically difficult, due in part to the large size and consequent lability of this messenger. Greene *et al.* (6) have demonstrated the synthesis of incomplete fibroin polypeptides in an *in vitro* system from Krebs ascites cells. In this paper we demonstrate the *in vitro* translation of full-length fibroin in a heterologous cell-free system from rabbit reticulocytes. An interesting aspect of these translation experiments is the absolute requirement for supplementation with insect tRNA. More striking is the finding that translation occurs discontinuously in the *in vitro* cell-free system as well as in silk gland cells in organ culture.

METHODS

Preparation of Labeled Fibroin. *Bombyx mori* larvae were raised as described (2, 7). Methods for labeling silkworms with [^{14}C]alanine and for the preparation of fibroin solutions by using lithium dodecyl sulfate ($LiDodSO_4$) have been described (2). The labeled fibroin used in these experiments was further purified by centrifugation in 5–20% sucrose gradients in 50 mM Tris·HCl, pH 7.4/1% $LiDodSO_4$/0.5 mM EDTA and collection of the fibroin peak, which sediments at about 10 S.

Preparation of mRNA and tRNA. RNA was extracted from posterior silk glands by a procedure based on proteinase K digestion and ethanol precipitation in the presence of saturated sodium perchlorate (8). Fibroin mRNA was prepared by two consecutive rounds of sucrose gradient centrifugation as described elsewhere (2). The mRNA was about 95% pure as judged by gel electrophoresis.

For tRNA isolation, RNA was extracted from either silk glands or dog brain by a phenol/chloroform extraction procedure (8). The RNA was fractionated by chromatography in a Bio-Gel A-1.5 m column (2.5 × 60 cm) in 20 mM Tris·HCl/0.5% sodium dodecyl sulfate ($NaDodSO_4$)/0.15 M NaCl/1 mM EDTA, pH 7.4. The 4S RNA peak was collected and precipitated twice with ethanol.

***In Vitro* Translation.** The mRNA-dependent reticulocyte cell-free system described by Pelham and Jackson (9) was used. Unless otherwise specified, the incubation mixture contained 20 mM Hepes-KOH (pH 7.6), 140 mM potassium acetate, 2.5 mM $MgCl_2$, and 2 mM dithiothreitol. The final concentration of "energy mix" components was ATP, 1 mM; creatine phosphate, 10 mM; GTP, 0.2 mM; and creatine phosphokinase, 50 μg/ml. Nonradioactive amino acids (except alanine) were present at the following levels: glycine, 750 μM; serine, 280 μM; and the other 17 amino acids, 40 μM. In addition, each 50-μl incubation mixture contained 2 μCi (1 Ci = 3.7 × 10^{10} becquerels) of [^{14}C]alanine (168 Ci/mol). Unless otherwise specified, fibroin mRNA was added at a final concentration of 80 μg/ml and tRNA at 80 μg/ml ("standard" level of tRNA supplementation). The incubation temperature was 29°C. Reactions were stopped by addition of 4 vol of $NaDodSO_4$ gel loading buffer (3.7% $NaDodSO_4$).

Gel Electrophoresis. $NaDodSO_4$ gels were run with the buffers described by Neville (10). Gel loading buffer contained 75 mM Tris sulfate, 1% $NaDodSO_4$, 0.7 mM EDTA, 15% sucrose, 0.004% bromphenol blue, and 25 mM dithiothreitol (pH 8.7). For dissolution of reticulocyte lysate incubation mixtures, the $NaDodSO_4$ concentration was raised to 3.7%. Methods for two-dimensional electrophoresis with *Staphylococcus aureus* V8 protease were modified from those described by Cleveland *et al.* (11) and have been described in detail elsewhere (2). Fluorographic exposures of $NaDodSO_4$ gels were done as described by Bonner and Laskey (12). The radioactivity of dried gels was sometimes measured with a position-sensitive proportional counter developed by K. Goulianos, K. K. Smith, and S. White at The Rockefeller University.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; $LiDodSO_4$, lithium dodecyl sulfate.

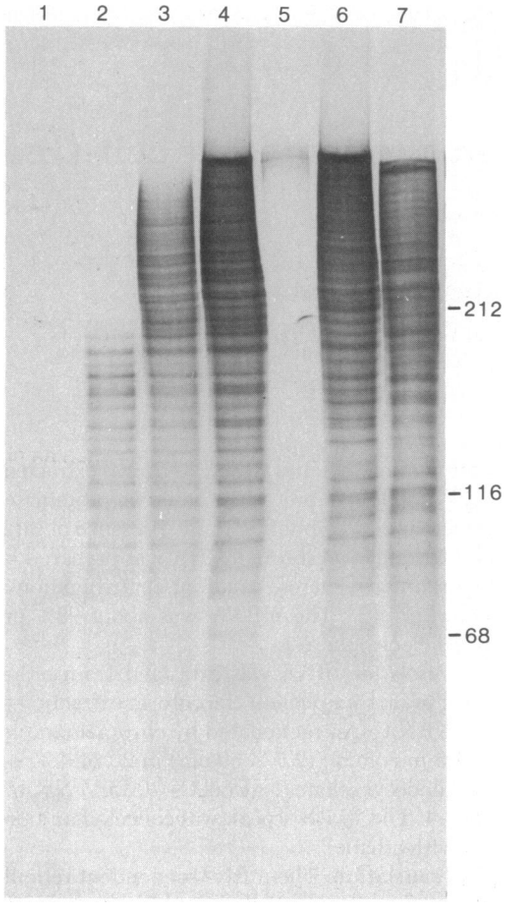

FIG. 1. Cell-free translation products of fibroin mRNA. mRNAs were incubated in a reticulocyte system and radioactive products were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis followed by fluorography. Scale on the right denotes the position of size markers (calibrated in $M_r \times 10^{-3}$). Lane 1, P50 mRNA, 120-min incubation without tRNA supplementation. Lanes 2, 3, and 4, P50 mRNA with silk gland tRNA supplementation, incubated for 30, 60, and 120 min, respectively. Lane 5, marker P50 fibroin (labeled *in vivo* with [^{14}C]alanine) that had been incubated for 100 min in nonradioactive reticulocyte lysate mixture. Lane 6, P22 mRNA, incubation as in lane 4. Lane 7, G1 mRNA, incubation as in lane 4. Each lane (except lane 5) contains equivalent amounts of lysate protein.

RESULTS

When the mRNA-dependent reticulocyte cell-free system was optimized for fibroin mRNA translation, the addition of tRNA obtained from posterior silk glands gave a 3-fold stimulation of [^{14}C]alanine incorporation whereas little or no stimulation was obtained with tRNA from *Escherichia coli*. Analysis of the cell-free translation products by $NaDodSO_4$/polyacrylamide gel electrophoresis (35-cm slab, 3.2–12% acrylamide) is shown in Fig. 1. In the absence of exogenous tRNA (lane 1) no high molecular weight products were observed, whereas in the presence of silk gland tRNA (lanes 2–4) a time-dependent increase in molecular weight was observed, resulting in the eventual synthesis and accumulation of full-size fibroin (compare lanes 4 and 5). Other experiments have shown that the full-length product appears after 85 min of incubation (fibroin contains approximately 5200 amino acid residues).

A striking feature of the fluorograph shown in Fig. 1 is the ladder of radioactive bands smaller than full-size fibroin. Several observations suggest that proteolytic activities do not play a role in the generation of these bands: (*i*) The pattern of small bands observed at short translation times (15–30 min) remains unchanged as translation proceeds to completion of full-length fibroin. (*ii*) If protein synthesis is stopped at any time by addition of cycloheximide and incubation is continued for an additional hour in the absence of protein synthesis, the original band pattern is not altered. (*iii*) When *in vivo*-labeled fibroin is incubated for 100 min in the reticulocyte translation system, no proteolytic degradation is observed (Fig. 1, lane 5).

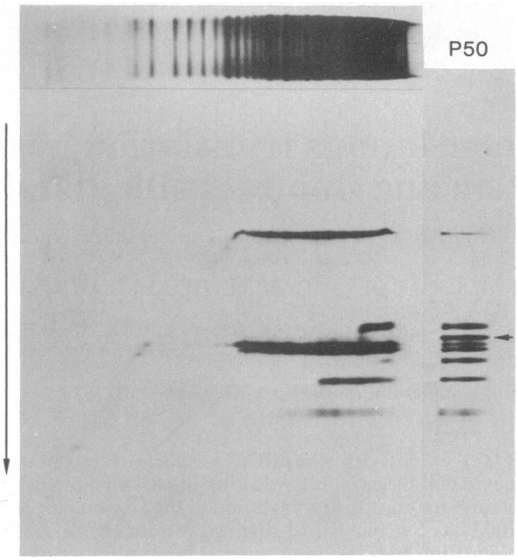

FIG. 2. Two-dimensional $NaDodSO_4$/polyacrylamide gel electrophoresis fingerprint of protease-digested translation products of fibroin mRNA. mRNA from strain P50 was incubated in the reticulocyte system with tRNA supplementation. A 6-mm-wide strip from the first-dimension slab gel (3.2–10% acrylamide) was loaded horizontally over a second-dimension slab gel (4–12% acrylamide) so that the largest products of translation were towards the right side. A sample of P50 fibroin (labeled *in vivo* with [^{14}C]alanine) was loaded on a slot on the right side of the gel strip. V8 protease *in situ* was used for digestion during the stacking phase of the second dimension gel as described (2, 11). (*Left*) Fluorographs of a first-dimension gel strip (chains growing from left to right) (*Upper*) and a second-dimension slab gel fingerprint (*Lower*). Large arrow on the left denotes the direction of electrophoresis in the second dimension. (*Right*) Digestion products of *in vivo*-labeled P50 fibroin. Small arrow on the right side marks a digestion product of P50 fibroin that seems to be absent in the fingerprint of translation products.

To determine unequivocally whether the multiple bands observed in Fig. 1 were incomplete fibroin polypeptides, we took advantage of the fact that fibroin can be digested with *S. aureus* V8 protease to yield a specific pattern of subfragments with molecular weights in the range of 15,000–100,000 (2). Fig. 2 *left* shows the results of an experiment in which a gel strip containing translation products of P50 mRNA was subjected to a second dimension of electrophoresis after *in situ* digestion with V8 protease. Fig. 2 *right* shows a reference marker of [^{14}C]alanine-labeled P50 fibroin that was subjected to digestion and electrophoresis in the same gel. The two-dimensional fingerprint shows that all the radioactive products of translation are fibroin polypeptides in various stages of completion. Furthermore, it shows the order of the products of proteolysis as they appear in sequence during translational elongation (left to right).

The majority of the incomplete fibroin polypeptides resided in active polysomes. A sample of translation mixture was incubated for 100 min, followed by addition of cycloheximide (50 μg/ml) and Triton X-100 (0.1%). Polysomes were pelleted by centrifugation for 30 min at 160,000 $\times$ g_{max} in a Beckman Air-fuge. The polysome pellet contained 62% of the acid-precipitable radioactivity. $NaDodSO_4$/polyacrylamide gel electrophoresis showed that the polysome-bound radioactivity

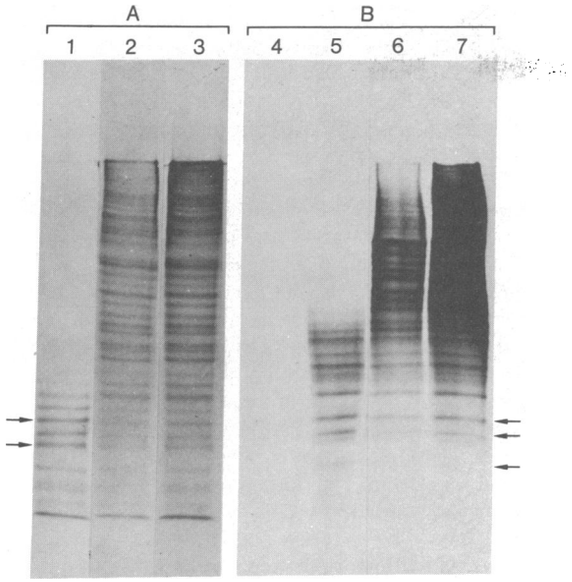

FIG. 3 Translation products obtained after "chase" with inhibitors of chain initiation. (*A*) *In vitro* translation of fibroin mRNA (strain P22) followed by NaDodSO$_4$/polyacrylamide gel electrophoresis in 3.4–5.0% gradient gel. All incubations proceeded for a total of 120 min. Lane 1, cycloheximide (50 μg/ml) added after 20 min of incubation. Lane 2, m^{7}Gp (0.7 mM) added after 20 min. Lane 3, control without inhibitors. (*B*) *In vitro* translation of fibroin mRNA (strain R1) followed by NaDodSO$_4$/polyacrylamide gel electrophoresis in 3.2–12.0% gradient gel. All incubations were for 120 min. Lane 4, edeine (12 μM) added before starting the incubation. Lane 5, edeine (12 μM) added after 5 min of incubation, followed by cycloheximide (50 μg/ml) 15 min later. Lane 6, edeine added after 5 min and incubation continued an additional 115 min. Lane 7, control without inhibitors. Arrows denote bands in the fluorograph that exhibit reduced intensity after chase with inhibitors of initiation.

consisted of the typical discrete bands of incomplete fibroin polypeptides (not shown). This material could be released from the polysomes by incubation for 5 min in the presence of 1 mM puromycin prior to addition of cycloheximide. This result suggests that the pattern of incomplete fibroin polypeptides reflects the distribution of growing polypeptide chains. This idea was supported further by experiments in which the elongation of incomplete fibroin polypeptides was followed as a function of time. Fig. 3 (lane 2) shows the results of an experiment in which translation was allowed to proceed for 20 min, at which time m^{7}Gp was added to inhibit polypeptide chain initiation. Elongation was then allowed to continue for an additional 100 min. Lane 1 contains material from a parallel incubation that received cycloheximide at 20 min to stop all protein synthesis. The change in intensity of the radioactive bands in the lower part of the gel (compare bands marked with arrows in lanes 1 and 2) suggests that a portion of the incomplete fibroin polypeptides participated in continued chain elongation. Fig. 3*B* shows a similar experiment in which a different inhibitor of initiation, edeine, was used to stop initiation after 5 min of incubation. Comparison of the amount of radioactivity in small polypeptides in lanes 5 and 6 (15 and 115 min after addition of edeine) shows that these bands became fainter with longer incubation times. This reduction was quantitated by using a device that measures radioactive disintegrations directly from a dried gel (see *Methods*). It was found that the amount of radioactivity in the bands marked with arrows drops to about one-third in lane 6 relative to lane 5. This change is necessarily an underestimate of the actual extent of participation of incomplete polypeptides in chain elongation because the bands in lane 6 can gain radioactivity from the continuing elongation of smaller polypeptides. Unfortunately, the large size of the pool of charged [^{14}C]alanyl-tRNA made it impractical to carry out a true chase experiment in which nonradioactive alanine would stop incorporation of radioactivity after the initiation block.

The observation that discrete size classes of incomplete fibroin are intermediates of translation can be most simply explained by the occurrence of discontinuities in the polypeptide elongation process. One might expect that such discontinuities reflect specific features of the mRNA sequence being translated. This possibility can be tested by translation of fibroin mRNAs from different strains of *B. mori*. These strains synthesize fibroins of different length (2) and, therefore, the mRNAs are expected to differ in sequence. The translation products of three different mRNA genotypes are shown in Fig. 1, lanes 4, 6, and 7. Close inspection of the fluorograph shows that the patterns of radioactive products in these three lanes are different, especially for bands with M_r larger than 68,000.

Several additional experiments were carried out in an effort to understand the mechanism responsible for the apparent elongation discontinuities or "pauses." The results of an experiment that tested the effect of various heterologous tRNAs on the translational pause pattern are shown in Fig. 4. Lane 2 shows the previously observed pattern of products obtained with silk gland tRNA supplementation. This pattern is the same whether or not the silk gland tRNA has been deacylated by means of a preliminary incubation at alkaline pH (not shown). The effect of changes in the ratio of mRNA to silk gland tRNA can be seen in lanes 3 and 4; lane 6 shows that a mammalian tRNA fraction (dog brain) is much less efficient in stimulating the synthesis of large fibroin polypeptides. When an equimolar

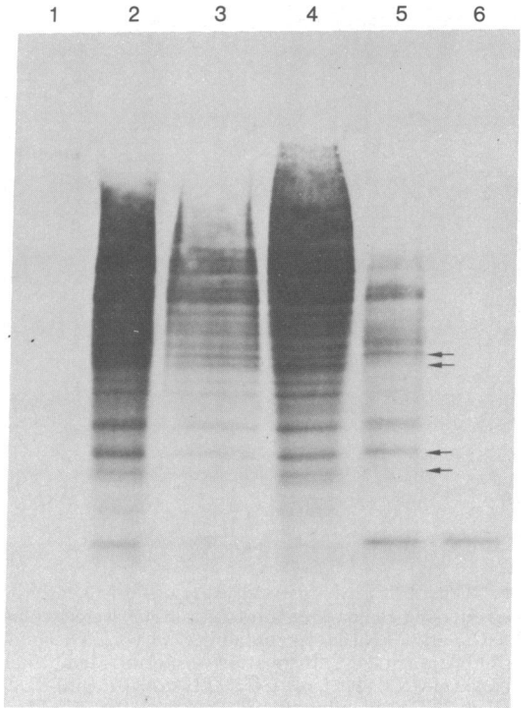

FIG. 4. Translation products of fibroin mRNA in a reticulocyte cell-free system with different tRNA supplements. The mRNA was from *B. mori* strain R1; NaDodSO$_4$/polyacrylamide gel electrophoresis was in 3.2–12.0% gradient gel. Lane 1, *in vivo* labeled fibroin standard. Lane 2, "standard" silk gland tRNA supplementation (80 μg/ml). Lane 3, same as lane 2, but one-fifth of standard mRNA concentration. Lane 4, 3× silk gland tRNA (240 μg/ml), standard mRNA concentration. Lane 5, silk gland tRNA (40 μg/ml) plus dog brain tRNA (40 μg/ml). Lane 6, dog brain tRNA (80 μg/ml).

mixture of silk gland and dog brain tRNA was added (lane 5), the stimulation of translation was intermediate between the levels obtained when a similar amount of either individual tRNA was added (see lanes 2 and 6). Of particular significance is the fact that the relative intensities of some bands change dramatically depending on the tRNA supplement being used. For example, the arrows in lane 5 point to two pairs of bands where this change in relative intensity is especially striking. The smaller band of each pair becomes more intense in the more efficient translation conditions (compare lanes 4 and 5). This result suggests that changing tRNA populations can differentially affect the rate of polypeptide chain elongation at specific pause sites in the mRNA template.

Experiments have been done to test whether tRNA supplementation has any effect on mRNA breakdown. Analysis of labeled RNA in formamide/sucrose gradients (not shown) demonstrates that the presence or absence of exogenous tRNA during translation does not change the mRNA size profile after 90 min of incubation. The sedimentation profiles suggest that mRNA chains suffer an average of two breaks per molecule whether or not tRNA is added. Gel electrophoresis of the same mRNA samples shows a smear with no evidence of discrete degradation products. This result suggests that "early quitter" polypeptides produced by truncated mRNA molecules are not a major factor in generating the discrete patterns of incomplete fibroins that we have shown.

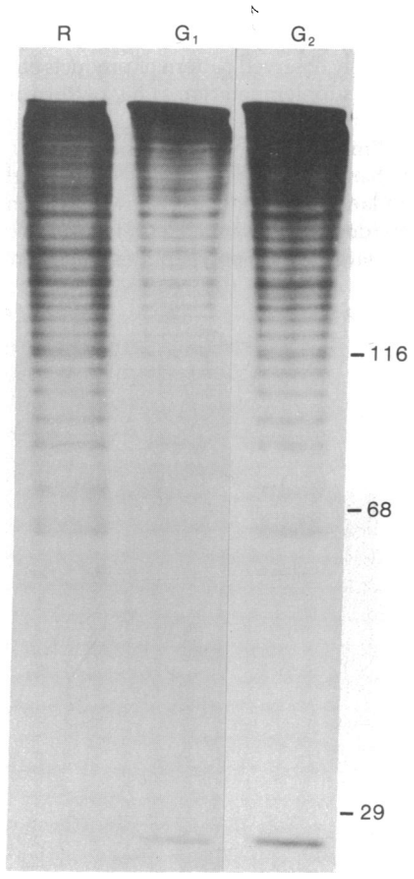

FIG. 5. Comparison of organ culture and fibroin synthesized *in vitro*. Posterior silk glands from inbred strain P50 were incubated in 0.15 M NaCl/0.015 M Na citrate containing 7 μCi of [^{14}C]alanine (168 Ci/mol) for 60 min at 24°C. Incubation was terminated by homogenization in 50 mM Tris·HCl, pH 7.4/5% $LiDodSO_4$/5 mM EDTA/0.5 mM $CdCl_2$/0.7 mM phenylmethylsulfonyl fluoride/0.5 mM *p*-chloromercuribenzoic acid/2 mM *N*-ethylmaleimide. The total $LiDodSO_4$-solubilized material was incubated for 2 min at 100°C, clarified by centrifugation (3 min at 12,000 × g_{max}), and processed for $NaDodSO_4$/polyacrylamide gel electrophoresis in a 4–12% gradient gel. Lanes G_1 and G_2, material labeled in gland culture (two different fluorographic exposures of the same gel lane). Lane R, products of *in vitro* translation of P50 mRNA in a reticulocyte system with standard silk gland tRNA supplementation, analyzed in the same slab gel as the gland culture samples.

The fact that fibroin is by far the major polypeptide product synthesized by posterior silk glands during the late stages of the fifth larval instar (13) offers an opportunity to test whether discontinuous translation may take place in living cells. Labeling of posterior silk gland cells with a mixture of radioactive amino acids results in virtually exclusive labeling of fibroin, with only about 10% of the label present in other proteins. If labeling is done with [^{14}C]alanine, which is about 4 times more abundant in fibroin than in most other proteins, the amount of nonfibroin incorporation becomes negligible. Fig. 5 shows the results of such an experiment, in which labeling in gland culture was carried out for 60 min. The fluorograph (lanes G_1 and G_2) shows a ladder of labeled bands which extend from about 50,000 to 400,000 daltons. The pattern is nearly identical to that obtained by *in vitro* translation of purified fibroin mRNA (lane R). The major difference is in the relatively greater accumulation of full-size fibroin in the cultured glands, where polypeptide elongation proceeds faster than in the reticulocyte system. Radioactivity in the ladder of labeled bands can be chased into full-length fibroin in the presence of unlabeled alanine (not shown); therefore, the ladder does not represent early quitter polypeptides. We interpret the ladder of incomplete polypeptides as representative of the distribution of growing fibroin molecules in polysomes, implying the existence of discontinuities or pauses in the process of chain elongation in intact silk gland cells. Comparison of several patterns of ladders from gland cultured cells and from *in vitro* translation shows two interesting differences: relative band intensity (i.e., relative pause duration) is markedly different at a number of loci, and individual fibroin bands of M_r 30,000–68,000 synthesized in gland culture display slightly lower electrophoretic mobilities when compared to their counterparts synthesized *in vitro*. This could be due to glycosylation of the nascent fibroin molecules, which would be expected to occur in gland cells (14) but not in the reticulocyte cell-free system. Other minor modifications of nascent fibroin, such as the proteolytic removal of a signal sequence (15), would not be expected to produce detectable differences in the mobility of large polypeptides such as those shown in Fig. 5.

DISCUSSION

Mills *et al.* (16) have reported discontinuities in the transcriptional elongation of Qβ RNA. Their data suggest that the discontinuities are the result of specific secondary structure features of the polynucleotide. We have done experiments that suggest that if fibroin mRNA contains stranded domains, these probably do not exceed 15–18 base pairs (based on the complete resistance of fibroin mRNA to digestion by high concentrations of RNase III in 0.1 M salt). The existence of relatively short double-stranded loops in the mRNA remains a possibility. Several observations provide indirect evidence against a major role of mRNA secondary structure in discontinuous translation: (*i*) Fibroin elongation in the reticulocyte system proceeds much faster in the presence of 180 mM potassium acetate than in 35 mM potassium acetate; the pauses appear to be longer at the lower salt concentration. (*ii*) Different tRNA supplements alter the relative amounts of nascent chain accumulation at adjacent elongation pause loci. (*iii*) Relative chain accumulation at adjacent loci is often different in the reticulocyte system as compared to cultured gland cells. It seems unlikely that mRNA

secondary structure would be basically different in either case.

An alternative hypothesis would be that discontinuous elongation reflects different times spent by the ribosome in the recognition-binding reactions at each codon. Very little is known about the intrinsic rate constants for recognition and binding of specific tRNAs (17). If these rate constants were very different for different tRNAs, one should observe discontinuous elongation even at optimal concentrations of charged tRNAs. On the other hand, the tRNA supplementation experiment shown in Fig. 4 suggests that the relative concentrations of specific charged tRNAs could be largely responsible for the discontinuities in the elongation rate. Silk fibroin has a very unusual sequence; glycine, alanine, and serine together account for about 85% of the total amino acids (18). During the late stages of the fifth larval instar, where fibroin synthesis becomes dominant, the intracellular pool of tRNA isoacceptors undergoes dramatic changes that optimize the availability of tRNAs required for fibroin synthesis (19–21). Alanine, glycine, and serine isoacceptors are rapidly synthesized and attain levels of about 70% of the total tRNA complement (22). One of these tRNAs is a new alanine isoacceptor that appears specifically at this time in the posterior silk gland and is not detectable in other silkworm tissues (23). In view of this, it is not surprising that the tRNA pool of the reticulocyte lysate is inadequate to support fibroin polymerization and that silk gland tRNA supplementation is highly stimulatory. Because a similar level of stimulation is obtained with either acylated or deacylated silk gland tRNA, it can be concluded that the activating enzymes in the reticulocyte lysate are able to charge insect tRNA. Of course, the enzymes may not charge all insect tRNAs with the same efficiency. The fact that chain elongation is discontinuous even at high levels of tRNA supplementation might be explained by the persistence of suboptimal concentration ratios for specific tRNAs. Recently we have undertaken similar translation experiments with the wheat germ (24) and the HeLa (25, 26) cell-free systems in the presence of placental RNase inhibitor (G. Scheele and P. Blackburn, personal communication). Preliminary experiments suggest that elongation discontinuities occur at exactly the same loci as in the reticulocyte system. However, the relative duration of each elongation pause (deduced from relative band intensities) is different in each cell-free system.

The observation that discontinuous translation occurs in intact silk gland cells is intriguing. We know that the processing of rRNA precursors becomes markedly slower under the gland culture conditions used in these experiments (unpublished observations). It is possible that tRNA metabolism or amino acid charging is also being affected in some way. Nevertheless, the fact that we can observe discontinuities in fibroin chain elongation in glands incubated for only 15 min after removal from the animal (not shown) suggests that we may simply be observing a slight accentuation of a normal cellular process. Protzel and Morris (27) have presented evidence that strongly suggests that the elongation of α and β globin may proceed discontinuously in intact rabbit reticulocytes.

It is not known whether the rate-limiting step for fibroin translation *in vivo* is at the level of chain initiation or elongation. Early in the fifth instar, when the tRNA population is not yet optimal for fibroin mRNA decoding, chain elongation may be rate limiting for this specific mRNA. Later in the instar, when fibroin mRNA is synthesized at a very rapid rate while ribosome synthesis slows down (28), the rate-limiting step in translation is likely to be at the level of chain initiation. If this is the case, it might be inconsequential whether elongation proceeds continuously or discontinuously. Still, it may be advantageous for the cell to adjust the elongation rate in order to maintain certain levels of polysome loading or to optimize the length of time during which nascent chains are accessible to modifying activities such as proteases or glycosylating enzymes. If the cell were to accomplish this modulation by adjusting the concentrations of a few specific tRNAs, discontinuous chain elongation would necessarily ensue. More detailed studies on developing silk glands may clarify the role of tRNA-mediated modulation of translation in cell differentiation.

We thank Zofia Borowska for a gift of edeine and John Dunn for a gift of RNase III. We also thank A. Engelberg for his expert technical assistance and R. Zielinski for conscientious proofreading of the manuscript. This work was supported by National Institutes of Health Grants GM-22865 (to P.M.L.) and AM-21860 (to D.S.). P.M.L. is the recipient of a National Institutes of Health Research Career Development Award. V.M. is a fellow of the Swiss National Fund for Scientific Research.

1. Sprague, K. U., Roth, M. B., Manning, R. F. & Gage, L. P. (1979) *Cell* **17**, 407–413.
2. Lizardi, P. M. (1979) *Cell* **18**, 581–589.
3. Suzuki, Y. (1975) *Adv. Biophys.* **8**, 83–114.
4. Manning, R. F. & Gage, L. P. (1978) *J. Biol. Chem.* **253**, 2044–2052.
5. Tsujimoto, Y. & Suzuki, Y. (1979) *Cell* **16**, 425–436.
6. Greene, R. A., Morgan, M., Shatkin, A. J. & Gage, L. P. (1975) *J. Biol. Chem.* **250**, 5114–5121.
7. Suzuki, Y. & Brown, D. D. (1972) *J. Mol. Biol.* **63**, 409–429.
8. Lizardi, P. M. & Engelberg, A. (1979) *Anal. Biochem.* **98**, 116–122.
9. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256.
10. Neville, D. M. (1971) *J. Biol. Chem.* **246**, 6328–6334.
11. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U.K. (1977) *J. Biol. Chem.* **252**, 1102–1106.
12. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
13. Tashiro, Y., Morimoto, T., Matsuura, S. & Nagata, S. (1968) *J. Cell Biol.* **38**, 574–588.
14. Sinohara, H. (1977) *Insect Biochem.* **7**, 3–4.
15. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 835–851.
16. Mills, D. R., Dobkin, C. & Kramer, F. R. (1978) *Cell* **15**, 541–550.
17. Lodish, H. F. (1976) *Annu. Rev. Biochem.* **45**, 39–72.
18. Lucas, F. & Rudall, K. M. (1968) in *Comprehensive Biochemistry*, eds. Florkin, M. & Stotz, E. H. (Elsevier, New York), Vol. 26, pp. 475–558.
19. Garel, J. P., Mandel, P., Chavancy, G. & Daillie, J. (1971) *FEBS Lett.* **12**, 249–252.
20. Chavancy, G., Daillie, J. & Garel, J. P. (1971) *Biochimie* **53**, 1187–1194.
21. Delaney, P. & Siddiqui, M. A. Q. (1975) *Dev. Biol.* **44**, 54–62.
22. Garel, J. P., Garber, R. L. & Siddiqui, M. A. Q. (1977) *Biochemistry* **16**, 3618–3624.
23. Meza, L., Araya, A., Leon, G., Krauskopf, M., Siddiqui, M. A. Q. & Garel, J. P. (1977) *FEBS Lett.* **77**, 255–260.
24. Roberts, B. E. & Paterson, D. M. (1972) *Proc. Natl. Acad. Sci. USA* **70**, 2330–2334.
25. McDowell, M., Joklik, W. K., Villa-Komaroff, L. & Lodish, H. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2649–2653.
26. Villa-Komaroff, L., Guttman, N., Baltimore, D. & Lodish, H. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4157–4161.
27. Protzel, A. & Morris, A. J. (1974) *J. Biol. Chem.* **249**, 4594–4600.
28. Suzuki, Y. & Suzuki, E. (1974) *J. Mol. Biol.* **88**, 393–407.