Elastin biosynthesis in chick-embryo arteries

Studies on the intracellular site of synthesis of tropoelastin

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(Received 23 December 1983/Accepted 2 April 1984)

Electrophoretic analyses of the products of cell-free translation of elastin mRNA isolated from 17-day chick-embryo thoracic arteries have demonstrated that the elastin mRNA codes for polypeptides that are slightly larger than the cellular tropoelastin polypeptides synthesized and secreted by matrix-free artery cells. Pulse-chase experiments with cells labelled with [^3H]proline established that newly synthesized tropoelastin polypeptides were associated solely with membrane-bound particulate fractions. Cell-free translation of membrane-bound and free polyribosomes isolated from artery cells revealed that the tropoelastin mRNA was associated predominantly with the membrane-bound fraction. When rough-microsomal fractions, isolated from cells labelled with [^3H]proline for 10 min, were treated with proteinases in the presence and in the absence of detergent, the nascent tropoelastin polypeptides were shown to be susceptible to proteolysis only when the integrity of the membranes was destroyed by detergent treatment. In similar experiments tropoelastin polypeptides synthesized by membrane-bound polyribosomes in the nuclease-treated reticulocyte lysate were also resistant to the proteolytic-enzyme treatment. The results suggest that tropoelastin polypeptides are synthesized on membrane-bound polyribosomes and discharged into the lumen of the endoplasmic reticulum with co-translational removal of a signal peptide.

It is generally accepted that tropoelastin represents the soluble precursor of insoluble elastin, and cell-free translation studies with elastin mRNA have demonstrated that the primary translation products are two polypeptides designated tropoelastin a and tropoelastin b, of M_r approx. 73000 and 70000 respectively (for review see Foster *et al.*, 1983). Much is known about the structure of tropoelastin and the extracellular modifications leading to the formation of the very hydrophobic, highly insoluble, cross-linked extracellular elastic fibre. However, surprisingly little biochemical information is available on the intracellular synthesis and secretion of elastin, and other approaches, including both electron-microscopic and cytochemical studies, have failed to give a definitive view of the secretory pathway of elastin (Ross & Klebanoff, 1971; Gerrity *et al.*, 1975; Thyberg *et al.*, 1979; Damiano *et al.*, 1981).

The present paper describes investigations on the site of elastin synthesis in chick-embryo artery cells released from their surrounding matrix by enzymic digestion. These cells are particularly active in elastin synthesis (Uitto *et al.*, 1976; Rosenbloom & Cywinski, 1976; Cheah *et al.*, 1981), and the absence of any connective-tissue matrix permits the successful subcellular fractionation of such cells (Grant & Jackson, 1979), thereby permitting an analysis of some of the steps in the intracellular synthesis and secretion of tropoelastin.

Experimental

Materials

Fertile eggs obtained from Northern Biological Supplies, St. Annes, Lancs., U.K., were incubated for 17 days at 37°C in a Westernette egg incubator (Western Incubators, Chelmsford, Essex, U.K.).

L-[5- ^3H]Proline (26 Ci/mmol) was purchased

Abbreviation used: SDS, sodium dodecyl sulphate.

from Amersham International, Amersham, Bucks., U.K., and L-[3,4-³H]valine (60 Ci/mmol) was from New England Nuclear Chemicals, Dreieich, W. Germany. Sheep anti-(chick soluble elastin) serum was generously given by Dr. B. Sykes, University of Oxford, Oxford, U.K. Rabbit anti-sheep immunoglobulin G was purchased from Miles Laboratories, Stoke Poges, Slough, Bucks., U.K. Ribonuclease-free sucrose was obtained from B.R.L. Ltd., Cambridge, U.K. Chymotrypsin was supplied by Armour Pharmaceuticals Co., Eastbourne, Sussex, U.K. Dulbecco's 'A' phosphate-buffered saline was purchased from Oxoid, Basingstoke, Hants., U.K. Oligo(dT)-cellulose, heparin, sodium deoxycholate, Triton X-100 and Triton X-405 were purchased from Sigma Chemical Co., Poole, Dorset, U.K. Proteinase K, trypsin, acrylamide, *NN'*-methylenebisacrylamide, SDS and all other reagents (of AnalaR grade) were supplied by BDH Chemicals, Poole, Dorset, U.K.

Preparation and incubation of cells

Matrix-free cells were isolated from the major thoracic arteries of 17-day chick embryos by the procedures of Schofield *et al.* (1977). The cells were incubated for 20 min (approx. 5×10^7 cells/ml) at 37°C in modified Krebs medium (Dehm & Prockop, 1971) before a labelling period of 10 min with [³H]proline (25 μ Ci/ml). In pulse-chase experiments the labelling period was terminated by the addition of unlabelled proline (2 mg/ml), and the incubation was terminated by cooling on ice.

Isolation of poly(A)-rich RNA

Artery cells isolated as described above were used as a source of tropoelastin mRNA. Cells (10^9) were homogenized with a glass/Teflon homogenizer (three strokes at 500 rev./min) in 10 mM-Tris/HCl buffer, pH 7.5, containing 1% SDS, 5 mM-EDTA and 200 μ g of proteinase K/ml, at 45°C. The homogenate (20 ml) was incubated for 1 h at 45°C before extraction with an equal volume of phenol/chloroform/3-methylbutan-1-ol (50:50:1, by vol.). The phases were separated by centrifugation at 1800 *g* for 10 min at 20–25°C. After re-extraction of the organic phase, the combined aqueous fraction was extracted with the phenol mixture until no further material collected at the interphase. The final aqueous phase was made 0.24 M with respect to ammonium acetate, and nucleic acids were precipitated overnight by addition of 2 vol. of cold ethanol (–20°C).

The precipitated nucleic acids were collected by centrifugation at 2500 *g* for 1 h at 0°C and washed with cold ethanol to remove residual phenol. DNA was removed by washing with 3 M-sodium acetate buffer, pH 6.0. The RNA pellet was washed with

0.1 M-sodium acetate, pH 6.0, in 70% (v/v) ethanol, dried under a stream of N₂ and finally dissolved in 10 mM-Tris/HCl buffer, pH 7.6, containing 0.5% SDS and 0.4 M-NaCl (5 ml). Poly(A)-rich RNA was bound to an oligo(dT)-cellulose column (4 cm $\times$ 1 cm), and rRNA was eluted with 10 mM-Tris/HCl buffer, pH 7.6, containing 0.4 M-NaCl. Poly(A)-rich RNA was finally eluted with 10 mM-Tris/HCl buffer, pH 7.6, then made 0.24 M with respect to ammonium acetate, and precipitated with 2 vol. of cold ethanol.

Separation of free and membrane-bound polyribosomes

Free and membrane-bound cytoplasmic polyribosomes were isolated by two separate procedures, and the association of tropoelastin mRNA with these fractions was assessed by cell-free translation in a reticulocyte-lysate system. Free and membrane-bound polyribosomes were separated by sucrose-density-gradient centrifugation (Craig *et al.*, 1979). Cells (usually 3×10^8 cells from approx. 100 embryos) were homogenized (7×10^7 cells/ml) in buffer A, comprising 50 mM-Tris/HCl, pH 7.6, 25 mM-KCl, 5 mM-magnesium acetate, 0.15 M-sucrose, 250 μ g of heparin/ml, and 0.2 vol. of rat liver high-speed supernatant prepared as described by Craig *et al.* (1979). A post-mitochondrial supernatant was obtained by centrifugation (10000 *g*_{max}, 10 min) and layered over a discontinuous sucrose gradient prepared from 2.3 M-sucrose (4 ml) and 1.0 M-sucrose (2 ml) in buffer A. Gradients were centrifuged at 95000 *g*_{av} for 19 h, and membrane-bound polyribosomes were recovered at the 2.3 M-/1.0 M-sucrose interphase, whereas the free polyribosomes pelleted. Both fractions were suspended in buffer A from which sucrose was omitted, and the membrane-bound polyribosomes were freed of membranes by treatment with 0.2 vol. of 50 mM-Tris/HCl buffer, pH 7.6, containing 25 mM-KCl, 5 mM-magnesium acetate and 10% (v/v) Triton X-100. Free and released polyribosomes were recovered by sedimentation at 250000 *g*_{av} for 3 h through a cushion (3 ml) of 40% (w/v) sucrose in buffer B, comprising 50 mM-Tris/HCl, pH 7.6, 25 mM-KCl, 5 mM-magnesium acetate and 0.2 vol. of liver high-speed supernatant. Intact membrane-bound polyribosomes were recovered by sedimentation through a cushion of 1.0 M-sucrose in buffer B under the same conditions.

Tightly membrane-bound, loosely membrane-bound and free polyribosomes were separated by the method of Ramsey & Steele (1977) after homogenization of cells in buffer A. The homogenate was centrifuged at 740 *g*_{max} for 2 min immediately, and then at 135000 *g*_{max} for 12 min to yield a supernatant containing free polyribosomes. The pellet

was resuspended in buffer A in which the KCl concentration was increased to 0.25M-KCl, and loosely bound polyribosomes were recovered in the supernatant after centrifugation as above. The pellet was resuspended in buffer A containing 0.25M-KCl and 1% (v/v) Triton X-100, centrifuged at 1500 g_{max} for 5min to remove nuclei, and the supernatant, containing tightly bound polyribosomes, was made 1.3% (w/v) with respect to sodium deoxycholate and clarified by centrifugation at 27000 g_{max} for 10min. The resulting supernatant, and the supernatants containing free and loosely bound polyribosomes, which had been made 1.3% with respect to sodium deoxycholate, were each layered over cushions (3ml) of 1.38M-sucrose in 50mM-Tris/HCl buffer, pH7.6, containing 0.25M-KCl, 5mM-magnesium acetate and 0.2vol. of liver high-speed supernatant. Centrifugation at 226000 g_{max} for 4h yielded pellets of tightly bound, loosely bound and free polyribosomes.

All polyribosomal pellets were suspended in 0.5mM-magnesium acetate and either used directly for cell-free translation studies or stored under liquid N₂.

Cell-free protein synthesis

Polyribosomal RNA was translated in the nuclease-treated rabbit reticulocyte-lysate cell-free system of Pelham & Jackson (1976). Translation was carried out in final volumes of 20 μ l containing 10 μ l of lysate, 5 μ l of polyribosomes (containing 5 μ g of RNA), 1 μ l of [³H]valine (10 μ Ci) and 4 μ l of a master mixture, containing ATP, GTP, phosphocreatine, creatine kinase and the other 19 naturally occurring amino acids, to give final concentrations identical with those described by Hunt & Jackson (1974). Optimization of the cell-free system indicated that 100mM-potassium acetate and 1mM-magnesium acetate should also be present in the incubations. Reactions were terminated by addition of 10mM-valine containing 5% (v/v) sodium deoxycholate and 5% (v/v) Triton X-100 (7 μ l), and elastin-related translation products were precipitated by addition of 2 μ l of sheep anti-(chick soluble elastin) antiserum. The samples were incubated overnight at 4°C, then anti-sheep immunoglobulin G was added and incubation continued for a further 16h at 4°C. Immunoprecipitates were collected by centrifugation (2500 g for 5min), washed three times with Dulbecco's 'A' phosphate-buffered saline containing 1% Triton X-405 and 10mM-valine, and finally dissolved in electrophoresis-sample-preparation buffer (Laemmli, 1970). Electrophoresis and fluorography were performed as described previously (Cheah *et al.*, 1979b), except that acetic

acid was substituted for dimethyl sulphoxide in the preparation of the fluorograms.

Results

Characterization of the product of cell-free translation of elastin mRNA

When poly(A)-rich mRNA isolated from chick-embryo arteries was translated in the nuclease-treated reticulocyte-lysate cell-free system, the major high- M_r [³H]valine-labelled polypeptide synthesized migrated on electrophoresis on SDS/polyacrylamide gels with M_r approx. 73000 (Fig. 1). This species could be precipitated by sheep anti-elastin serum (results not shown) and was observed to have a similar electrophoretic mobility to tropoelastin polypeptides secreted by matrix-free artery cells. The secreted proteins are known to contain hydroxyproline residues, and the presence of hydroxy groups was found to influence the electrophoretic behaviour. Thus non-hydroxylated tropoelastin isolated from cells labelled with [³H]proline in the presence of $\alpha\alpha'$ -bipyridine has a greater electrophoretic mobility than the fully hydroxylated secreted polypeptides (Fig. 1). An analogous effect of inhibition of hydroxylation is seen for the pro- α -chains of procollagen (Fig. 1), an observation reported previously by others (Harwood *et al.*, 1976; Cheah *et al.*, 1979a). When the cell-free product of tropoelastin mRNA was compared with the non-hydroxylated polypeptides isolated from artery cells, the cell-free product migrated more slowly, suggesting that it was of slightly higher M_r than the cellular protein.

Intracellular localization of tropoelastin

In initial experiments designed to assess the intracellular localization of newly synthesized tropoelastin polypeptides, the labelled cells were separated into particulate and soluble fractions by centrifugation at 105000 g_{av} for 1h after homogenization in 50mM-Tris/HCl buffer, pH7.5, containing 0.25M-sucrose, 25mM-KCl and 0.5mM-MgCl₂. The radiolabelled polypeptides present in the fractions were analysed by SDS/polyacrylamide-gel electrophoresis and fluorography (Cheah *et al.*, 1979b). The particulate fractions recovered from cells pulse-labelled for 10min with [³H]proline, and from cells pulse-labelled and chased for 10min with unlabelled proline, were found to contain a spectrum of polypeptides, but the major band present migrated in the position of tropoelastin polypeptides (Fig. 2). The identity of this band was confirmed by immunoprecipitation with anti-elastin serum (results not shown). No [³H]proline-labelled tropoelastin polypeptides were detectable in the cytosolic fractions, suggest-

Fig. 1. Comparison of hydroxylated and non-hydroxylated tropoelastin polypeptides with the products of elastin-mRNA cell-free translation

Matrix-free artery cells (2.5×10^7) were preincubated for 1 h in modified Krebs medium (1 ml) in the absence and in the presence of 1 mM- $\alpha\alpha'$ -bipyridine before a labelling period of 1 h with $20 \mu\text{Ci}$ of [^3H]-proline. Cells were collected by centrifugation at $1200g$ for 10 min; the pellets were solubilized in electrophoresis sample buffer ($500 \mu\text{l}$), and a portion ($20 \mu\text{l}$) was analysed by SDS/polyacrylamide-gel electrophoresis on 10%-acrylamide gels, followed by fluorography. Track 1, products labelled in the absence of $\alpha\alpha'$ -bipyridine. The major tropoelastin polypeptides are designated TE, and the other two major high- M_r polypeptides correspond to pro- $\alpha 1$ and pro- $\alpha 2$ chains of type I collagen. Track 2, products labelled in the presence of $\alpha\alpha'$ -bipyridine. Track 3, second experiment in which cellular proteins were labelled in the presence of $\alpha\alpha'$ -bipyridine. Track 4, products of cell-free translation when poly(A)-rich mRNA ($0.2 \mu\text{g}$) extracted from chick arteries was translated in the reticulocyte-lysate cell-free system ($20 \mu\text{l}$) for 1 h at 30°C in the presence of [^3H]valine.

ing that, during their intracellular translocation, the tropoelastin polypeptides remain within membrane-bound compartments.

Fig. 2. Fluorogram of the [^3H]proline-labelled polypeptides of the soluble and particulate cell fractions

Cells ($5 \times 10^7/\text{ml}$) were labelled with [^3H]proline ($20 \mu\text{Ci}/\text{ml}$) for 10 min and chased with unlabelled proline ($2 \text{mg}/\text{ml}$) for a further 10 min. Particulate and soluble fractions were prepared as described in the text after the pulse and after the chase periods. Samples were dissolved in electrophoresis sample buffer and analysed by SDS/polyacrylamide-gel electrophoresis on 10%-acrylamide gels, followed by fluorography. Samples in tracks 1–4 are as follows: 1, soluble fraction after chase; 2, soluble fraction after pulse; 3, particulate fraction after chase; 4, particulate fraction after pulse. Tropoelastin polypeptides of interest are labelled TE.

Cell-free translation of polyribosomal RNA

In order to determine the site of synthesis of tropoelastin polypeptides, free and membrane-bound polyribosomes were isolated from artery cells and used to direct cell-free protein synthesis. Analysis of the [^3H]valine-labelled products of translation by SDS/polyacrylamide-gel electro-

Fig. 3. Fluorogram of [^3H]valine-labelled polypeptides when free and membrane-bound polyribosomes were translated in the reticulocyte-lysate cell-free system

Free and membrane-bound polyribosomes were prepared from matrix-free chick artery cells, and samples ($5\mu\text{g}$ of RNA) were translated in the reticulocyte-lysate system ($20\mu\text{l}$) for 1 h at 30°C . Total cell-free products and tropoelastin polypeptides precipitated with sheep anti-elastin serum (see the text) were analysed by SDS/polyacrylamide-gel electrophoresis on 10%-acrylamide gels, followed by fluorography. Samples in tracks 1–4 are as follows: 1, translation products of free polyribosomes; 2, translation products of free polyribosomes after immunoprecipitation; 3, translation products of membrane-bound polyribosomes; 4, translation products of membrane-bound polyribosomes after immunoprecipitation.

phoresis and fluorography demonstrated the synthesis of tropoelastin polypeptides in approximately similar proportions when the system was

programmed with equal amounts of both free and membrane-bound polyribosomal RNA (Fig. 3). However, the yield of free polyribosomes from these cells was consistently found to represent only 8–12% of the yield of membrane-bound polyribosomes as judged by RNA content in the fractions. Accordingly, it appears that approx. 90% of the tropoelastin mRNA is associated with membrane-bound polyribosomes prepared by the method of Craig *et al.* (1979), in which contamination of free polyribosomes with membrane-bound polyribosomes is minimized by sedimentation of the free polyribosomes through 2.3M-sucrose. It is noteworthy that the other products of cell-free translation showed considerable differences in their distribution, in that the free polyribosomes directed the synthesis of a large number of polypeptides over a wide M_r range, whereas the membrane-bound polyribosomal mRNA generated a very limited number of polypeptides.

An alternative fractionation procedure was employed to obtain a preparation of highly purified tightly membrane-bound polyribosomes devoid of contaminating free polyribosomes (Ramsey & Steele, 1977). This membrane-bound fraction derived from high-salt-washed membranes contained most of the tropoelastin mRNA, but the presence of tropoelastin polypeptides in the cell-free products obtained by translation of the free and loosely bound polyribosomes prepared by this procedure suggested that a small proportion of the elastin mRNA was associated with free polyribosomes (results not shown).

Directional discharge of tropoelastin polypeptides synthesized on membrane-bound polyribosomes

When rough-microsomal fractions were isolated by the method of Harwood *et al.* (1974) from matrix-free artery cells labelled with [^3H]proline for 10 min, it was possible to demonstrate that the ^3H -labelled tropoelastin polypeptides were protected within the lumen of the microsomal vesicles. Digestion of the microsomal preparation with a chymotrypsin/trypsin mixture ($50\mu\text{g}$ of each proteinase/ml at 4°C for 1 h) resulted in only limited digestion of the tropoelastin polypeptides, which are known to be particularly trypsin-sensitive (Rosenbloom & Cywinski, 1976). However, incubation of the microsomal preparation with proteinases in the presence of detergent (1% sodium deoxycholate plus 1% Triton X-100) resulted in complete digestion of the ^3H -labelled polypeptides (Fig. 4).

The above experiment was repeated to assess the fate of the tropoelastin polypeptides synthesized in the reticulocyte cell-free system under the direction of membrane-bound polyribosomal mRNA. The accessibility of the newly synthesized polypeptides

Fig. 4. Susceptibility to digestion with proteinases of ^3H -labelled tropoelastin (TE) polypeptides in a rough-microsomal fraction prepared from artery cells

Rough-microsomal fractions were isolated by the procedures of Harwood *et al.* (1974) from matrix-free cells (1×10^8) that had been incubated for 10 min with [^3H]proline ($20 \mu\text{Ci/ml}$) at a cell density of 5×10^7 cells/ml. The microsomal pellet was suspended in 50 mM-Tris/HCl buffer, pH 7.5 (300 μl), containing 25 mM-KCl, 0.5 mM-MgCl₂ and 0.25 M-sucrose, and samples (100 μl) were treated with a mixture of trypsin and chymotrypsin in the absence and in the presence of detergents (see the text). Samples were analysed by SDS/polyacrylamide-gel electrophoresis followed by fluorography. Fluorograms were scanned with an LKB laser densitometer at 632.8 nm. —, Control microsomal fraction; ----, fraction treated with enzymes; -.-.-, fraction treated with enzymes in the presence of detergents.

to degradation by added proteinases was investigated. In the presence of detergents the tropoelastin polypeptides were totally degraded, whereas the intact membranes afforded some protection against degradation (Fig. 5a). In contrast, the cell-free products of membrane-bound polyribosomal mRNA from which membranes had been stripped before translation were totally degraded when incubated with the proteinase mixture in the absence as well as in the presence of detergents (Fig. 5b). Also of note was the difference in electrophoretic mobility of the tropoelastin polypeptides synthesized in the cell-free system in the presence and in the absence of membranes. The [^3H]-valine-labelled tropoelastin polypeptides, which were protected from proteinase treatment by the microsomal membranes (designated TE in Fig. 5a), were of slightly lower M_r than the cell-free product of the membrane-stripped polyribosomal mRNA (arrowed in Fig. 5b). Such observations and those reported in Fig. 1 are consistent with the removal of a signal peptide during the directional discharge of the tropoelastin polypeptides into the lumen of the endoplasmic reticulum.

Fig. 5. Susceptibility to digestion with proteinases of ^3H -labelled tropoelastin (TE) polypeptides synthesized on membrane-bound polyribosomes

Membrane-bound polyribosomes (6 μg of RNA) isolated from artery cells were translated in the reticulocyte-lysate cell-free system (20 μl) (a) with membranes intact and (b) after treatment with detergents to obtain 'stripped' polyribosomes. The translation products were treated with a mixture of trypsin and chymotrypsin in the absence and in the presence of detergents and analysed as indicated in Fig. 4 legend. —, Control incubation; ----, sample incubated with proteinases; -.-.-, sample incubated with proteinases in the presence of detergents.

Discussion

Studies conducted with secreted proteins such as serum proteins, pancreatic enzymes, immunoglobulins etc. have led to the general concept that proteins destined for export are synthesized on membrane-bound ribosomes and then transferred into the cisterna of the endoplasmic reticulum before secretion via the Golgi apparatus (for reviews see Palade, 1975; Sabatini *et al.*, 1982). A considerable amount of information is available on the synthesis and secretion of collagen (for reviews see Grant & Jackson, 1976; Prockop *et al.*, 1979), but details of the intracellular assembly and

transport of elastin remain ill-defined. Both fibrous proteins are known to be produced within the same chick artery cells (Kao *et al.*, 1980), but their synthesis and secretion appear to be independently regulated (Uitto *et al.*, 1976), and the possibility that the highly hydrophobic non-glycosylated elastin precursors might follow an alternate route of secretion has been considered (Grant *et al.*, 1971). Subsequent electron-microscopic, cytochemical and immunochemical studies (Ross & Klebanoff, 1971; Gerrity *et al.*, 1975; Thyberg *et al.*, 1979; Damiano *et al.*, 1981) have implicated Golgi-associated vesicles in elastin secretion, but the suggestion that elastic-fibre components may possibly be released via the cytoplasm has not been ruled out (Thyberg *et al.*, 1979).

There have been few biochemical studies of elastin synthesis, translocation and secretion, but the demonstration that the primary translation product of elastin mRNA has a short hydrophobic *N*-terminal leader sequence not found in the secreted protein (Karr & Foster, 1981; Davidson *et al.*, 1982; Krawetz *et al.*, 1983) is consistent with the signal hypothesis of Blobel and colleagues (for review see Blobel, 1977). Thus it is postulated that the hydrophobic signal is likely to play a role in protein-membrane recognition, resulting in co-translational vectorial discharge into the lumen of the endoplasmic reticulum (Sabatini *et al.*, 1982).

The studies reported in the present paper were undertaken with chick-embryo matrix-free artery cells to identify the intracellular site of tropoelastin synthesis and to establish the fate of nascent polypeptides. Initial studies on the products of cell-free translation of poly(A)-rich mRNA extracted from chick arteries confirmed that tropoelastin was the major [³H]proline- and [³H]valine-labelled product. Normally up to 30% of the proline residues in chick tropoelastin may be hydroxylated *in vivo* (Uitto *et al.*, 1976), but under the conditions of cell-free synthesis the polypeptides do not undergo post-translational modifications. Accordingly, the electrophoretic mobilities of the cell-free product and of cellular non-hydroxylated tropoelastin were compared (Fig. 1). Such experiments demonstrated that the cellular tropoelastin was of lower *M_r* than the tropoelastin polypeptides synthesized in the cell-free system, suggesting that tropoelastin mRNA codes for a polypeptide with a signal sequence that is removed during transfer of the nascent chains into the lumen of the endoplasmic reticulum.

That the tropoelastin polypeptides indeed undergo a vectorial discharge to become segregated within the cisterna of the endoplasmic reticulum was demonstrated in a series of subsequent experiments. In preliminary short-term pulse-chase studies it was found that newly synthesized

tropoelastin polypeptides were associated solely with the particulate membrane fractions (Fig. 2). Further studies of the distribution of tropoelastin mRNA between free and membrane-bound polyribosomes indicated that a small proportion of the message was on free polyribosomes, but that most was associated with membrane-bound polyribosomes. This small proportion of tropoelastin mRNA present in the free-polyribosome fraction probably represents those polyribosomes carrying only short nascent polypeptide chains before their association with the endoplasmic reticulum (Blobel, 1977). Evidence in favour of the directional discharge of the tropoelastin polypeptides synthesized on membrane-bound polyribosomes was obtained in experiments (Fig. 4) demonstrating that the newly synthesized polypeptides were protected within rough-microsomal vesicles. Confirmatory evidence for the transfer of nascent polypeptides across microsomal membranes was obtained by translating membrane-bound polyribosomal mRNA in the cell-free system in the presence of membranes. Under these circumstances the signal peptide appeared to be removed during the co-translational transfer of the tropoelastin polypeptides across the microsomal membrane (Fig. 5).

These studies provide direct biochemical evidence for the assembly of tropoelastin polypeptides on membrane-bound ribosomes and the co-translational processing of the nascent polypeptides as they pass into the lumen of the endoplasmic reticulum. Further work is required to establish which subcellular compartments are involved in the transport of the tropoelastin polypeptides to the cell surface.

We thank Ms. Jill Jeffrey for excellent technical assistance and acknowledge the financial support of the Science and Engineering Research Council.

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