PRESUMPTIVE mRNA FOR PROCOLLAGEN: OCCURRENCE IN MEMBRANE BOUND RIBOSOMES OF EMBRYONIC CHICK TENDON FIBROBLASTS

Richard HARWOOD, Ann D. CONNOLLY, Michael E. GRANT and David S. JACKSON
Department of Medical Biochemistry,
University of Manchester, Manchester M13 9PT, UK

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1. Introduction

Recent evidence has established that the collagen monomer is synthesised in a precursor form (procollagen) which is most fibrillar collagens comprises two pro-α_1 chains and one pro-α_2 chain (for review see ref. [1]) and that these polypeptides are assembled simultaneously [2] under the direction of separate monocistronic mRNAs [3]. Matrix-free cells isolated from embryonic chick tendons [4] provide an ideal system with which to commence studies on the procollagen mRNAs since in these cells protein synthesis is devoted predominantly to collagen synthesis and the absence of any matrix avoids the possibility of non-specific RNA–protein aggregation which can occur when intact tissue is employed. In this study we report that in tendon cells the major mRNA species synthesised is of a size appropriate to the presumptive procollagen mRNAs and is associated with collagen-synthesising membrane-bound polysomes.

2. Experimental

Matrix-free cells, isolated from 17-day-old embryonic chick tendons [4], were incubated in modified Krebs medium [4] with appropriate radioisotopes. RNA synthesis was studied by incubation of cells with [5-^{3}H]uridine, and the identification of collagen-synthesising polysomes was determined by incubating cells with [U-^{14}C]proline and subsequently assaying fractions for [^{14}C]hydroxyproline [5] after hydrolysis in 6N HCl at 105°C for 24 hr.

For the isolation of subcellular fractions, cells were homogenised (6 to 8 × 10^7 cells/ml) in a glass–teflon motor driven homogeniser in 0.25 M sucrose in 0.05 M Tris–HCl buffer (pH 7.5) containing 0.025 M KCl and 0.5 mM $MgCl_2$. Free and membrane-bound ribosomes were separated by the method of Blobel and Potter [6]. RNA was extracted from subcellular fractions as described by Schutz et al. [7] and the isolation of poly(A) containing RNA species was achieved by absorption on a cellulose (Sigmacell type 38) column followed by elution with water [7]. The RNA species were analysed by poly-acrylamide gel electrophoresis [8] and detected either by scanning at 265 nm using a Chromoscan Mark II (Joyce, Loebl and Co. Ltd., Gateshead, UK) or by slicing and assaying for radioactivity [8]. The molecular weight of the labelled RNA species was calculated using a standard curve of log mol wt. versus mobility obtained with standards of cytoplasmic RNA prepared from mouse Ehrlich ascites tumour cells [9]. In order to determine the sedimentation coefficient of labelled RNA, the Ehrlich ascites RNA standards were employed as internal markers in the sucrose gradient centrifugation procedure [9].

3. Results and Discussion

Tendon cells were incubated with [^{3}H]uridine (2 $\mu C_i/10^7$ cells/ml) for 1 hr and then cytoplasmic RNA was extracted and subjected to polyacrylamide gel electrophoresis. Radioactivity was associated with the major cytoplasmic RNA species (28S rRNA, 18S rRNA and 4–5S RNA) but the 18S RNA peak was

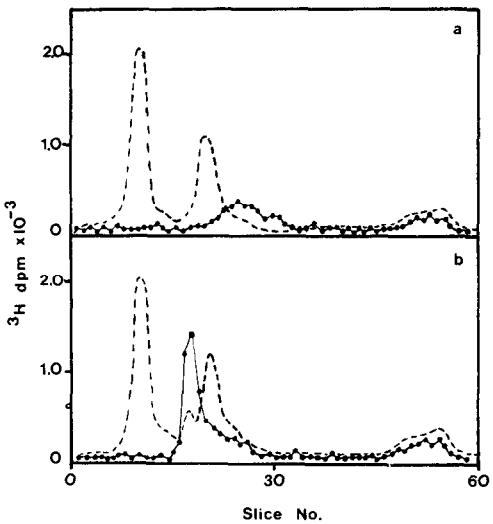
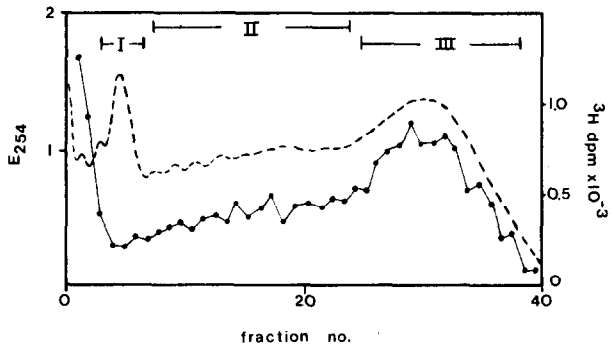


Fig. 2. SDS polyacrylamide gel electrophoresis of [^{3}H] uridine-labelled RNA from free and membrane-bound ribosomes of tendon cells incubated in the presence of actinomycin D. (a) RNA from ribosomes, (b) RNA from membrane-bound ribosomes. (•–•–•) radioactivity; (– – –) E_{265} nm.

examine whether this RNA species was associated with polysomes of the same size as those synthesising collagen. Tendon cells were incubated either for 1 hr with 200 μC$_i$ [^{3}H] uridine in the presence of actinomycin D or for 4 min with 50 μC$_i$ [^{14}C)proline. Membrane-bound ribosomes were isolated from the [^{3}H] uridine-labelled and [^{14}C] proline-labelled cells and polyribosomal distribution profiles were obtained by sucrose density gradient centrifugation (fig. 3). The isolated ribosomes were pooled to yield Fraction I containing single ribosomes, Fraction II containing light and medium polysomes (< 250S) and Fraction III containing heavy polysomes (> 250S). Hydroxy [^{14}C] proline assays indicated that collagen synthesis was occurring on the heavy polysomes (fig. 3). When RNA was extracted from identical regions of the [^{3}H] uridine-labelled polysome profile and analysed by gel electrophoresis, the 22S RNA species was found to be present only in Fraction III containing the heavy polysomes (fig. 4).

Thus, it can be concluded that in tendon cells procollagen synthesis occurs on large membrane-bound polysomes of similar size to those observed to synthesise collagen in embryonic chick limbs [3] and guinea-pig wound healing tissue [13]. Using



Fraction	I	II	III
total ^{14}C dpm	8189	21723	34796
^{14}C-hydroxy proline dpm	164	918	3526

Fig. 3. Distribution of [^{3}H] uridine-labelled RNA and location of collagen synthesising polysomes in sucrose density gradient profiles of membrane-bound ribosomes from tendon cells. Membrane-bound ribosomes from [^{3}H]-uridine and [^{14}C] proline labelled cells were analysed on 15–40% sucrose gradients. (•–•–•) [^{3}H] RNA; (– – –) E_{254} nm. Regions of the [^{14}C] proline labelled gradient were pooled to yield Fractions I, II and III which were assayed for hydroxy [^{14}C] proline content [5].

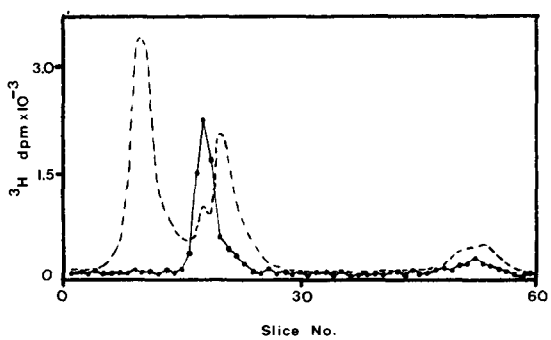


Fig. 4. SDS polyacrylamide gel electrophoresis of [^{3}H] uridine-labelled RNA from heavy membrane-bound polysomes (Fraction III). (•–•–•) radioactivity; (– – –) E_{265} nm.

isotopic labelling techniques it has been possible to identify an RNA species which has the characteristics of presumptive mRNA for procollagen and is associated solely with large collagen-synthesising polysomes.

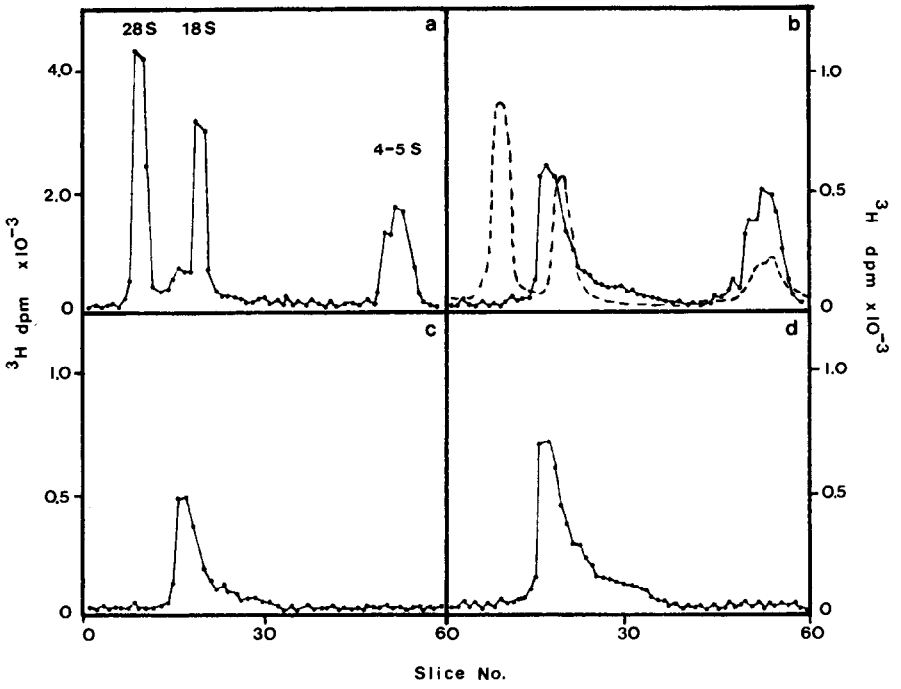


Fig. 1. SDS polyacrylamide gel electrophoresis of [^{3}H] uridine-labelled cytoplasmic RNA from tendon cells incubated ± actinomycin D. (a) RNA from control cells, (b) RNA from actinomycin D treated cells, (c) poly(A)-containing RNA from control cells, (d) poly(A)-containing RNA from actinomycin D treated cells. (•–•–•) radioactivity; (– – –) E_{265} nm.

heterogeneous and exhibited a high molecular weight shoulder on 2.4% gels (fig. 1a). When RNA synthesis was studied in cells incubated in the presence of actinomycin D (0.04 μg/ml), the major [^{3}H] uridine-labelled species migrated as a component of molecular weight 1.09 × 10^6 daltons (fig. 1b). On sucrose density gradient centrifugation this RNA species was found to have a sedimentation coefficient of 22S and since actinomycin D at 0.04 μg/ml suppresses ribosomal RNA synthesis [10] this labelled species was presumed to be messenger RNA.

Recent evidence has demonstrated that mammalian mRNAs contain a poly-adenylate sequence at the 3′-terminus of the molecule (for review see ref. [11]), the only exception established to date being histone mRNA. The presence of a poly(A) sequence in messenger RNAs affords a means for their isolation since such RNAs are retained by a column of cellulose and can be eluted subsequently with water [7]. When [^{3}H] uridine-labelled cytoplasmic RNA from cells incubated ± actinomycin D was chromatographed on a cellulose column, the 22S RNA species was bound to the column and could be recovered by elution with water (fig. 1c, d). The behaviour of this 22S RNA on the cellulose column demonstrates that it is a poly(A)-containing RNA and further supports the suggestion that this species is a messenger.

The molecular weights of the three polypeptides of procollagen are approx. 120 000 daltons [1] and hence it can be calculated that the molecular weight of their mRNAs must be approx. 1 × 10^6 daltons, i.e. of similar size to the 22S mRNA species detected in the tendon cells. Since procollagen, like other proteins for export, is synthesised on membrane-bound polyribosomes [12], the distribution of this mRNA species between free and bound ribosomes was determined (fig. 2). [^{3}H] uridine-labelled 22S mRNA could not be detected in the RNA extract of free ribosomes from tendon cells (fig. 2a) and was associated exclusively with membrane-bound polysomes (fig. 2b).

The above results are in accordance with the concept that this 22S RNA is the procollagen messenger, and further experiments were designed to

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