Identification of messenger RNA for human type II collagen

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Total RNA was purified from human fetal calvaria and articular cartilage. Messenger RNAs for type I and II collagens were identified by hybridization using cDNA clones for chicken proα1(I)-, proα2(I)- and proα1(II)collagen mRNAs and by analysis of cell-free translation products of these RNAs by polyacrylamide gel electrophoresis. The size of human proα1(II)collagen mRNA is approx. 5100 bases. Translatability of cartilage specific type II collagen mRNA was found to be concentration dependent: with increasing total RNA concentrations the relative translation of type II collagen mRNA was reduced with respect to type I mRNAs.

Collagen *Human type II collagen* *Cartilage* *Hybridization* *mRNA* *Cell-free translation*

1. INTRODUCTION

Type II collagen forms about one half of the organic matrix of articular cartilage. In addition to maintaining tissue strength and flexibility, cartilage specific type II collagen plays an important role during embryonic development of cartilage and bone [1]. At least 9 different collagen types are now known. Types I and II are the major collagens expressed during cartilage differentiation. Type I collagen consists of two identical α1(I)-chains and one α2(I)-chain and type II collagen of 3 α1(II)-chains. The different chains are products of different genes: the corresponding mRNAs are translated into (pre)proα-chains which undergo extensive post-translational modification including association of three proα-chains into triple helix before secretion into extracellular matrix [2].

Messenger RNA for chicken type II collagen has been identified [3] and used for the construction of the corresponding cDNA clones [4,5] with subsequent identification of the chicken [6] and human [7] genes for type II collagen. This study was undertaken to characterize the mRNA for human type II collagen. Alterations in the structure and expression of this collagen type are of special interest in studies on human anticular diseases.

Abbreviation: cDNA, complementary DNA

2. MATERIALS AND METHODS

Total RNA was purified from calvaria and articular cartilage of human fetuses obtained at therapeutic abortions and from calvaria and sterna of 17-day-old chick embryos as in [8].

For hybridizations total RNAs were denatured with glyoxal and dimethyl sulfoxide and electrophoresed in 0.75% agarose gels [9]. After the fractionation one part of the gel was stained with ethidium bromide; RNA from the other part was transferred by blotting to nitrocellulose [10]. The filters were hybridized with plasmid DNAs nick-translated using [^{32}P]dCTP (>3000 Ci/mmol, Amersham International, England) and a commercial reagent kit (BRL, Gaithersburg, MD). The following plasmids were used as probes: pCAL1 and pCAL2 containing sequences complementary to chicken proα1(I)- and proα2(I)collagen mRNAs [11], and pCAR1 containing cDNA for chicken

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proα1(II)collagen mRNA [4,6]. The filters were washed at 50°C as [10] and exposed with Kodak X-omat film using intensifying screens.

Messenger RNAs in the total RNA preparations were translated in a rabbit reticulocyte lysate system with [^{35}S]methionine (approx. 1400 Ci/mmol) as the radioactive precursor (both from Amersham International). The incubations were performed as suggested by the supplier using different RNA concentrations. Electrophoretic fractionation of the translation products was performed in 5–11% polyacrylamide gradient gels [12] followed by fluorographic analysis [13]. Where indicated, aliquots of the translation reactions were incubated prior to electrophoresis with purified bacterial collagenase (form III, Advance Biofactures, Lynbrook, NY) as in [3].

3. RESULTS AND DISCUSSION

The presence of type II collagen mRNA in total RNA preparations from human fetal cartilage was shown by nucleic acid hybridization using cloned cDNAs for chicken type I and II collagen mRNAs as probes. RNA from calvaria was chosen for reference as this tissue actively produces type I collagen but does not contain any type II collagen mRNA [3]. Nick-translated plasmid pCAR1 hybridized strongly to mRNA bands in chicken and human cartilage (fig.1). We showed previously that the major mRNA for chicken proα1(II)collagen was 5300 bases long (with variable amounts of a larger mRNA species of 7000 bases) [4]. The larger band is not detectable in fig.1 (lane B1). The probe also hybridized strongly to an mRNA of about 5100 bases in human cartilage RNA (lane B2) and slightly to two mRNAs in human calvaria (lane B3). As these two bands were also recognized by pCAL1 (lane C3) and cell-free translation of this RNA did not produce any preproα1(II)-chains, the hybridization is most likely due to cross-hybridization between pCAR1 and human proα1(I)collagen mRNAs and not to the presence of type II collagen mRNA in human calvaria. The

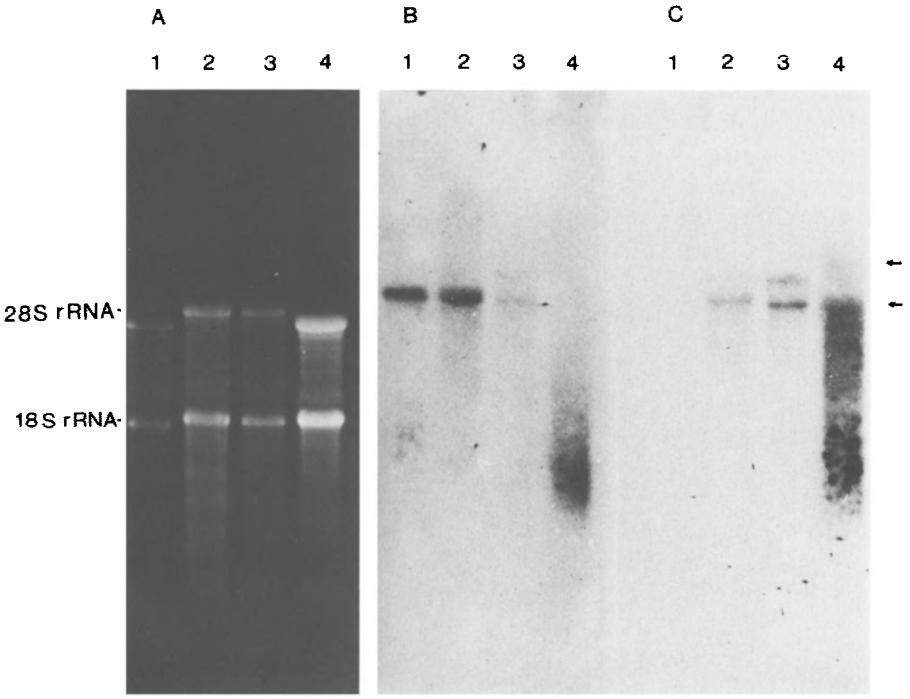

Fig.1. Analysis of RNAs from calvaria and cartilage by hybridization with cDNA probes. The RNA samples (10–20 μg each) were electrophoresed in 0.75% agarose gels: (1) chicken cartilage, (2) human cartilage, (3) human calvaria and (4) chicken calvaria. (A) Ethidium bromide staining of the gel with human rRNAs marked. (B) Hybridization pattern obtained with plasmid pCAR1. (C) Hybridization pattern with pCAL1. For clarity the two mRNAs for chicken proα1(I)collagen are marked to the right as the RNA is somewhat degraded.

analysis of the published cDNA sequences covered by probes pCAL1 and pCAR1 shows an 86.2% homology between chick and human α1(I)-mRNAs whereas chick α1(II)- and human α1(I)-mRNAs share a homology of 76.0%. The mRNA for human proα2(I)collagen shows considerably less homology with pCAL1 (65.6%) and pCAR1 (68.2%) [14–16]. No specific hybridization of pCAR1 was seen to chick calvaria RNA (lane B4).

Fig.1C shows hybridization of pCAL1 to an identical nitrocellulose filter. The probe hybridized to two mRNA bands in chicken (lane C4) and human (lane C3) calvaria as reported [11,17], but also slightly to two mRNA bands in human car-

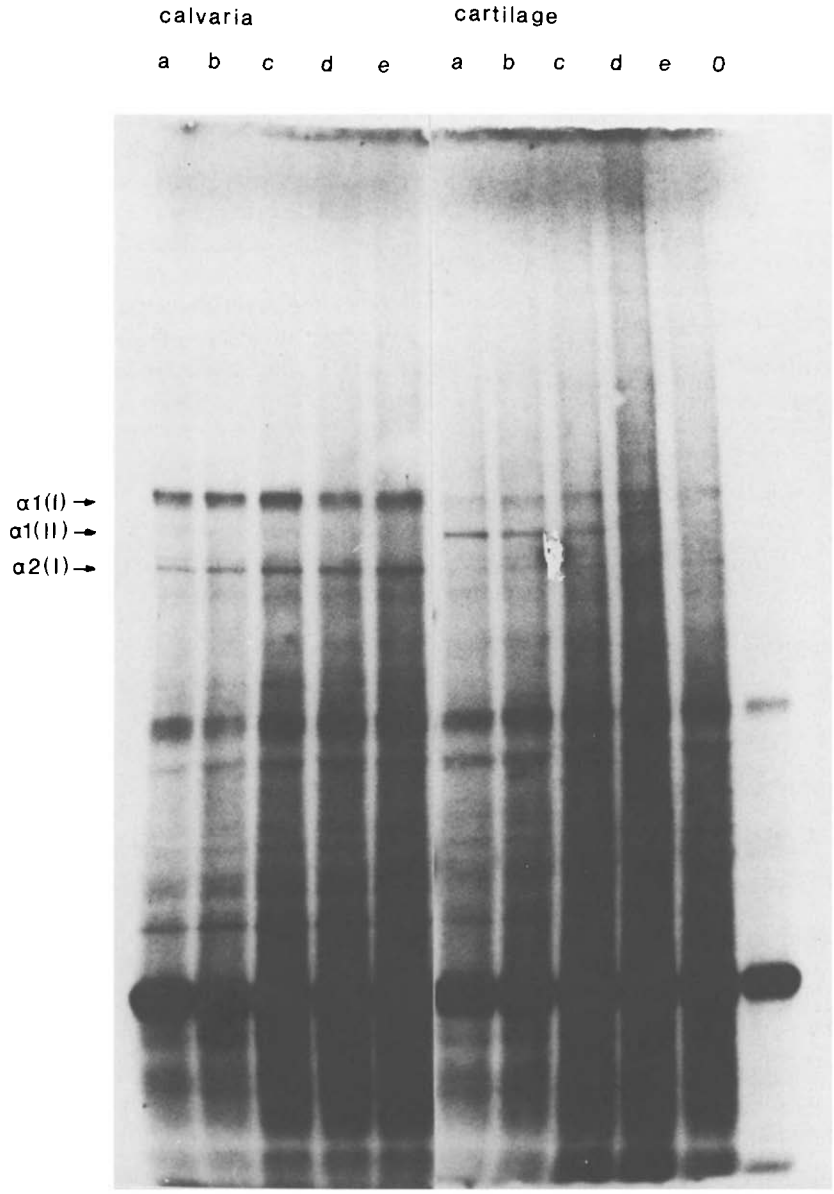

Fig.2. Cell-free translation of human cartilage and calvaria RNAs. The different RNA concentrations in the reactions were: (a) 10, (b) 25, (c) 50, (d) 100, (e) 150 ng/μl. (0) No RNA added. Marked in the figure are the preproα-chains of type I and II collagens.

tilage RNA (lane C2). Some type I collagen mRNA is present in our human cartilage RNA (fig.2), but part of the hybridization observed may be due to cross-hybridization of the type I and II sequences even under the stringent washing conditions used. Similarly, plasmid pCAL2 hybridized to two mRNAs in human calvaria RNAs and weakly to the corresponding mRNA in human calvaria (not shown). Recently the homology between chicken and human type II collagen mRNAs was utilized to isolate a genomic clone for human type II collagen using pCAR1 as the probe [7].

Electrophoretic fractionation of the cell-free translation products directed by human calvaria mRNAs revealed two major bands (fig.2) which were sensitive to bacterial collagenase (fig.3). These must correspond to the preproα1(I)- and preproα2(I)-chains as type I collagen is the major protein produced by calvaria [3]. The bands migrated very close to the chick preproα-chains (not shown). A similar analysis of cartilage mRNAs revealed 3 collagenase-sensitive translation products two of which comigrated with the chains of type I collagen (fig.2,3). This strongly suggests that the third band corresponds to the cartilage specific preproα1(II)-chain. Further evidence for this is provided by the fact that the band comigrated with chick preproα1(II)-chain (not shown). Cell-free translation of the RNAs from cartilage at different concentrations revealed that the translatability of the proα1(II)collagen mRNA was inhibited at high RNA concentrations with reference to type I collagen (fig.2). A similar inhibition has also been observed in cell-free translation of type III collagen at high RNA concentrations [18].

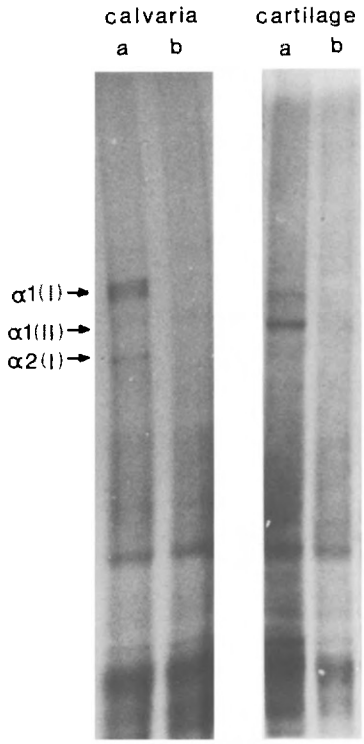

Fig.3. Identification of collagenous translation products by sensitivity to bacterial collagenase. Human cartilage and calvaria RNA (25 ng/μl) was translated in a reticulocyte lysate system and electrophoresed before (a) and after (b) digestion with bacterial collagenase. The preproα-chains of type I and II collagens are marked in the figure.

The mRNA for human proα1(II)collagen identified in RNA isolated from cartilage should prove to be useful in studies on identification and expression of human type II collagen genes.

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