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Type X collagen, a product of hypertrophic chondrocytes

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The synthesis of collagen types IX and X by explants of chick-embryo cartilages was investigated. When sternal cartilage labelled for 24h with [^{3}H]proline was extracted with 4M-guanidinium chloride, up to 20% of the ^{3}H-labelled collagen laid down in the tissue could be accounted for by the low-M_r collagenous polypeptides (H and J chains) of type IX collagen; but no type X collagen could be detected. Explants of tibiotarsal and femoral cartilages were found to synthesize type IX collagen mainly in zones 1 and 2 of chondrocyte proliferation and elongation, whereas type X collagen was shown to be a product of the hypertrophic chondrocytes in zone 3. Pulse–chase experiments with tibiotarsal (zone-3) explants demonstrated a time-dependent conversion of type X procollagen into a smaller species whose polypeptides were of M_r 49000. The processed chains [α1(X) chains] were shown by peptide mapping techniques to share a common identity with the proα1(X) chains of M_r 59000. No evidence for processing of type IX collagen was obtained in analogous pulse–chase experiments with sternal tissue. When chondrocytes from tibiotarsal cartilage (zone 3) were cultured on plastic under standard conditions for 4–10 weeks they released large amounts of type X procollagen into the medium. However, 2M-$MgCl_2$ extracts of the cell layer were found to contain mainly the processed collagen comprising α1(X) chains. The native type X procollagen purified from culture medium was shown by rotary shadowing to occur as a short rod-like molecule 148nm in length with a terminal globular extension, whereas the processed species comprising α1(X) chains of M_r 49000 was detected by electron microscopy as the linear 148nm segment.

Although the major collagenous protein of cartilage is type II collagen (Miller, 1976), it is now known that several minor collagenous species also occur in mammalian and avian cartilages (for review see Mayne & von der Mark, 1983). The first evidence for collagen heterogeneity in cartilage was provided by the identification of the three α-sized cartilage-specific chains designated 1α, 2α and 3α (Burgeson & Hollister, 1979). More recently, several distinct low-M_r collagenous molecules have been demonstrated in biosynthetic studies and in pepsin digests of cartilaginous tissues.

The non-disulphide-bonded G collagen (Gibson *et al.*, 1981, 1982, 1983) or short-chain (SC) collagen (Schmid & Conrad, 1982*a*,*b*; Capasso *et al.*, 1982; Mayne *et al.*, 1983) has been detected in chick chondrocyte cultures, and its pepsin-resistant domain (M_r 45000) has been extracted from chick tibiotarsal cartilage (Kielty *et al.*, 1984; Capasso *et al.*, 1984). It is recognized that this short-chain collagen represents a unique gene product, which we propose should be designated collagen type X. Accordingly the polypeptides of G collagen are referred to below in the present paper as proα1(X) collagen chains.

Chick sternal chondrocytes also synthesize the interchain-disulphide-bonded type IX procollagen (Mayne, 1985; van der Rest *et al.*, 1985), comprising polypeptides referred to as J and H chains (Gibson *et al.*, 1983), pHMW (Bruckner *et al.*, 1983) or pMa and pMb (von der Mark *et al.*, 1984). A mammalian species with similar characteristics has been isolated from rat chondrosarcoma and designated ptype M by Duance *et al.* (1984). Pepsin-resistant fragments of type IX collagen

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have also been isolated from sternal cartilage of young chickens, and are described as HMW and LMW (Reese & Mayne, 1981) or M1 and M2 (von der Mark *et al.*, 1982). The homologous mammalian fragments were initially described by Shimokomaki *et al.* (1980, 1981) and Ayad *et al.* (1981, 1982).

In the present paper we describe further studies on the characterization of type X collagen and its synthesis by hypertrophic chondrocytes of chick-embryo epiphysial cartilages. In addition, we provide evidence for the processing of type X procollagen in tibiotarsal cartilages in organ culture.

Experimental

Materials

Culture medium, foetal-calf serum and antibiotics were obtained from Gibco Bio-Cult, Paisley, Scotland, U.K. Pepsin (EC 3.4.23.1) (1:10000, from pig stomach mucosa), phenylmethanesulphonyl fluoride, *N*-ethylmaleimide and cycloheximide were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Pepstatin A was supplied by Scientific Marketing, London N.1, U.K. *Staphylococcus aureus* V8 proteinase (batch no. 0015) was obtained from Miles Laboratories, Slough, Berks., U.K., and highly purified bacterial collagenase (EC 3.4.24.3) was purchased from Advanced Biofactures, New York, NY, U.S.A. L-[5-^{3}H]Proline (23 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation and maintenance of chondrocyte cultures

Chondrocytes were isolated from 17-day-chick-embryo sterna, and zone 3a/b from the distal end of the tibiotarsus (Stocum *et al.*, 1979) by trypsin/bacterial-collagenase digestion, after careful removal of surrounding perichondrium (Gibson *et al.*, 1982). Cells were plated on plastic at a density of 4×10^3 cells/cm^2. The culture medium used was Dulbecco's modified Eagle's medium supplemented with glutamine (2mM), ascorbate (25μg/ml), penicillin (100 units/ml) and streptomycin (0.1mg/ml) plus either 2% (v/v) or 10% (v/v) foetal-calf serum. When the primary monolayers reached confluency, they were subcultured by trypsinization and replated at a cell density of 5×10^2 cells/cm^2. The secondary cultures were fed every 48h on medium containing 2% and 10% foetal-calf serum alternately (Schmid & Linsenmeyer, 1983). Once confluency was reached cells were maintained under the above regime for up to 10 weeks, and spent medium was collected and stored at −20°C in the presence of the following proteinase inhibitors: phenylmethanesulphonyl fluoride (2mM), *N*-ethylmaleimide (10mM), 6-aminohexanoic acid (25mM) and EDTA (25mM). A floating cell population of sternal chondrocytes, isolated after the initial plating-out, was also maintained on long-term culture for up to 6 weeks.

Organ-culture experiments

Sterna and cartilage from the distal end of the tibiotarsus and femur of 17-day chick embryos were dissected and carefully cleaned of perichondrial membrane. The tibiotarsal cartilage was further dissected into zones 1 and 2, and zone 3a/b (Stocum *et al.*, 1979). The tissue was finely diced, and equilibrated at 37°C with shaking for 15min in Dulbecco's modified Eagle's medium containing penicillin (100 units/ml) and streptomycin (0.1 mg/ml) in an atmosphere of CO_2/air (1:19). The cartilage was then incubated in medium containing L-[5-^{3}H]proline (20μCi/ml), ascorbate (25μg/ml), 0.5% foetal-calf serum and β-aminopropionitrile fumarate (64μg/ml), for up to 2h. The medium was removed, and proteinase inhibitors were added as described above, with the addition of cycloheximide to 5mM, before storage at −20°C. In pulse–chase studies the above protocol was followed, but after the labelling period the tissues were extensively washed and then incubated for various times from 1 to 48h in medium containing ascorbate (25μg/ml) and L-proline (10mM), 0.5% foetal calf serum and β-aminopropionitrile (64μg/ml). Tissue from 12 embryos was used for each time point. At the end of each incubation the medium was removed and frozen at −20°C after addition of proteinase inhibitors.

Isolation of newly synthesized labelled polypeptides from medium and cartilage

Cartilage slices were extracted over 48h at 4°C in either 2M-$MgCl_2$ or 4M-guanidinium chloride containing proteinase inhibitors. Extracts were extensively dialysed in 0.1M-acetic acid containing pepstatin (0.5μg/ml) and then freeze-dried. Under these conditions over 95% of the non-diffusible [^{3}H]proline radioactivity was extracted provided that β-aminopropionitrile was present throughout the incubation.

Labelled proteins in the medium were precipitated by addition of $(NH_4)_2SO_4$ to 30% saturation, and recovered by centrifugation. The precipitates were resuspended in 0.1M-acetic acid containing pepstatin, and dialysed extensively against this solution. This fraction of newly synthesized proteins was stored at −20°C.

Electrophoretic analysis

Labelled polypeptides synthesized in organ and cell culture were examined by discontinuous sodium dodecyl sulphate/polyacrylamide-gel elec-

trophoresis (Laemmli, 1970) and fluorography (Bonner & Laskey, 1974; Laskey & Mills, 1975), with conditions as described by Gibson *et al.* (1983).

Purification of type X collagen from chondrocyte culture medium

Type X collagen was isolated from the medium of long-term tibiotarsal chondrocyte cultures and purified as described by Schmid & Linsenmeyer (1983). Briefly, the pooled spent medium was clarified by centrifugation, then twice precipitated by $(NH_4)_2SO_4$ at 30% saturation. The final precipitate was dissolved in 0.5M-acetic acid, and type II collagen was then precipitated by addition of NaCl to 0.9M. After centrifugation, the supernatant was adjusted to 2M-NaCl, which precipitated type X collagen. This species was collected by centrifugation, and further purified by CM-cellulose chromatography (Kielty *et al.*, 1984).

Electron microscopy

Freeze-dried collagen samples were dissolved in 0.1M-acetic acid. Subsequent dilution was with 0.1M-acetic acid or 0.2M-ammonium acetate. Samples of about 2μg/ml were spread in a thin layer on freshly cleaved mica, frozen in liquid N_2 and dried at approx. −80°C. The samples were then rotary-shadowed at 5° by evaporation of platinum from a tungsten filament, carbon-coated and floated on to 400-mesh grids. An AEI EM6B electron microscope was used, operated at 80kV. Length measurements were made on ×20000 micrographs by using a Joyce–Loebl Magiscan image analysis system. Otherwise micrographs were taken at ×60000. A cross-grating replica of 2160 lines/mm was used as a magnification standard.

Results

Synthesis of collagen types IX and X by chick-embryo chondrocytes in organ culture

Chick-embryo cartilages from the sterna and from the distal ends of the tibiotarsus and of the femur were incubated for 2h in the presence of [^{3}H]proline, as described in the Experimental section. Radiolabelled polypeptides were recovered from the tissue after extraction with 4M-guanidinium chloride and analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and fluorography (Fig. 1). All the major

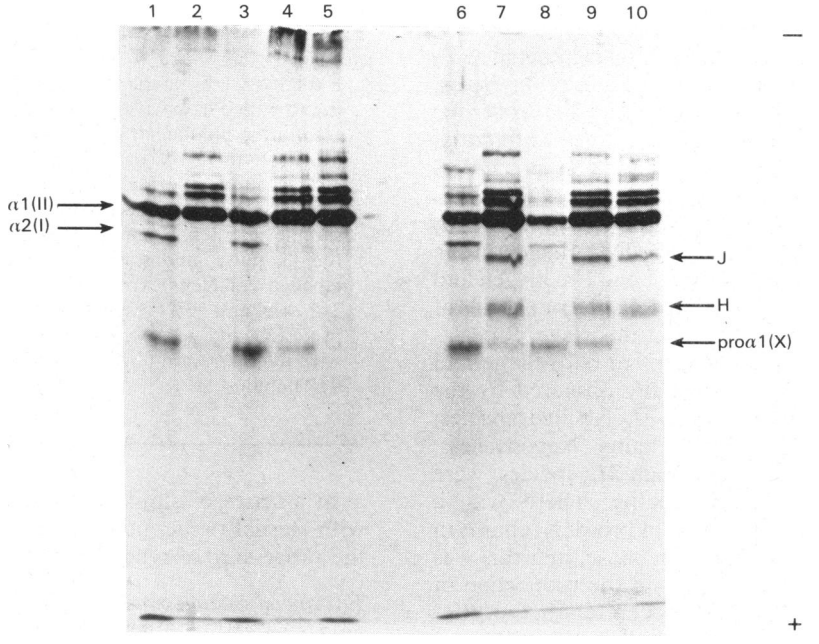

Fig. 1. *Fluorogram of* [^{3}*H*]*proline-labelled proteins synthesized by explants of epiphysial cartilage and sterna*
Organ cultures of tibiotarsal cartilage from zone 3 (tracks 3 and 8) and zones 1 and 2 combined (tracks 4 and 9), of femoral cartilage from zone 3 (tracks 1 and 6) and zones 1 and 2 combined (tracks 2 and 7), and of sternal cartilage (tracks 5 and 10), were labelled for 2h with [^{3}H]proline. Proteins deposited in the matrix were extracted with 4M-guanidinium chloride and analysed on an 8%-polyacrylamide gel under non-reduced (tracks 1–5) and reduced conditions (tracks 6–10). The positions of H and J chains of type IX collagen and of proα1(X) are identified, and the migration positions of standards of α1(II) chains and α2(I) chains are also indicated.

labelled polypeptides were found to be collagenous, as judged by their susceptibility to bacterial-collagenase digestion. High-M_r collagens migrating in positions corresponding to proα1(II), pα1(II) and α1(II) chains were synthesized by the three tissues (Fig. 1). Small amounts of type I collagen were also synthesized by the tibiotarsal and femoral zone-3 cartilages, as judged by the presence of α2(I) chains. In addition, sterna synthesized the low-M_r collagenous polypeptides comprising type IX collagen, and designated J and H chains (Fig. 1). These polypeptides together accounted for up to 20% of the labelled collagen laid down in the sterna, as determined by laser densitometric scanning of the fluorograms. There was no evidence for the synthesis of type X collagen by the sternal tissue. In contrast, tibiotarsal and femoral cartilages from zones 1/2 and from zone 3a/b synthesized type X procollagen, and this species comprised approx. 4% and 18% respectively of the labelled collagen species incorporated in these cartilage segments. Type IX procollagen was also synthesized in zones 1/2, and represented up to 26% of deposited collagens. In each type of cartilage, the proportions of the labelled species recovered from the incubation medium reflected those deposited in the extracellular matrix.

Processing of type X collagen in prelabelled organ cultures

Pulse–chase experiments were conducted with tibiotarsa (zone 3) of 17-day chick embryos. Labelled collagens extracted from tibiotarsa (zone 3) after a 1 h chase contained the interstitial fibrillar collagens (I and II) and their precursors (Fig. 2). In addition, type X procollagen was present, together with an additional species of M_r 49000. The corresponding labelled collagen in the medium pool contained types I and II collagen and precursors, but no type X procollagen or species of M_r 49000 could be detected at this stage. Processing of the interstitial collagens in both tissue and medium fractions was partially obscured by the presence of additional high-M_r labelled species, possibly including 1α2α3α chains. Nevertheless, by 6 h, the predominant high-M_r species were α1(I), α1(II) and α2(I) chains. There was a diminution in the proportion of proα1(X) chains in the zone-3 extracts after a 4 h chase, and this was accompanied by an increase in the proportion of the species of M_r 49000. Indeed, after 6 h, the latter was a major component, with only traces of proα1(X) chains remaining (Fig. 2). On the basis of experiments described below, we have designated the species of M_r 49000 as the α1(X) chain. Pulse–chase experiments conducted with tibiotarsa from 12-day chick embryos showed identical processing (results not shown).

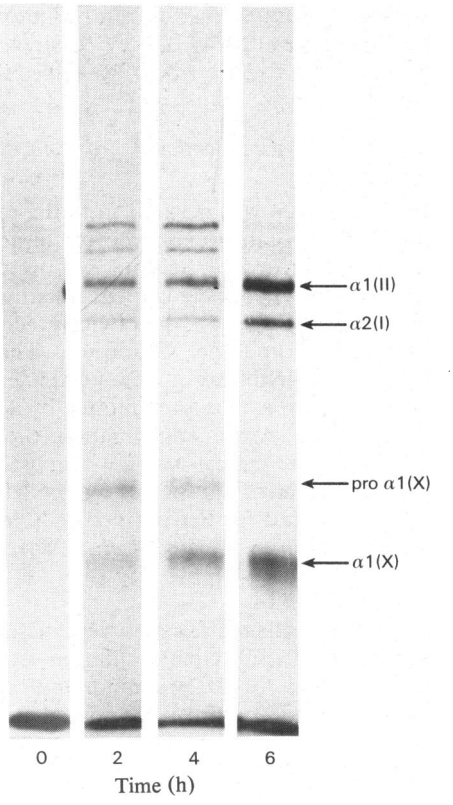

Fig. 2. *Fluorogram of [^{3}H]proline-labelled collagens extracted from tibiotarsal cartilage (zone 3) after pulse–chase*

Explants were incubated with [^{3}H]proline for 1 h, then washed extensively and incubated in medium containing unlabelled proline (100 μg/ml) for up to 6 h. Proteins in the tissue were extracted with 4M-guanidinium chloride and analysed on 8%-polyacrylamide gels under reducing conditions. The sample in track 1 contains species deposited after the 1 h pulse, and samples in tracks 2–4 contain labelled collagens extracted after chase periods of 2 h, 4 h and 6 h respectively. The position of proα1(X) and α1(X) chains are arrowed, together with the migration positions of standard α1(II) and α2(I) chains.

In a series of similar pulse–chase experiments with sternal tissue, no evidence was obtained for the processing of type IX collagen chains.

Enzyme digestion studies and peptide mapping of proα1(X) and its processed derivative

In order to identify the polypeptide of M_r 49000 as a product of type X procollagen, a labelled tissue extract of 17-day tibiotarsa (zone 3) containing both species was subjected to two-dimensional peptide mapping, after digestion with *S. aureus* V8 proteinase (Fig. 3*a*) and CNBr (Fig. 3*b*) respect-

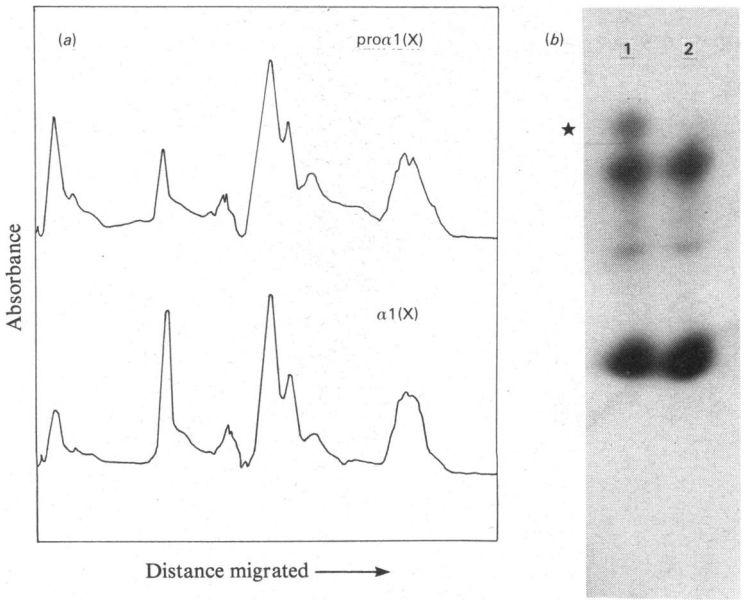

Fig. 3. *Peptide mapping of [^3H]proline-labelled proα1(X) chain and its processed derivative*
Labelled samples containing proα1(X) and α1(X) chains were separated by electrophoresis in 8%-polyacrylamide gels under reducing conditions. Disc gels containing electrophoresed proteins were embedded in 15%-polyacrylamide slab gels and subjected to digestion with either *S. aureus* V8 proteinase or CNBr as described in the text. The resulting peptides were separated by electrophoresis and detected by fluorography. In (*a*) the fluorogram of the V8-proteinase digestion products was scanned with an LKB laser densitometer at 632.8nm. The upper track shows the peptide profile of the proα1(X) chain and the lower track that of its processed derivative, α1(X). In (*b*) the fluorogram of CNBr-cleavage peptides shows the peptides of proα1(X) chains in track 1, and track 2 contains peptides of α1(X) chains.

ively. Fluorographic analysis of the peptide maps revealed that proα1(X) and the species of M_r 49000 have a common identity. Thus the *S. aureus* V8-proteinase maps of the two collagenous polypeptides had virtually identical profiles (Fig. 3*a*). The CNBr fingerprints also showed common identities, but the proα1(X) digest contained one extra peptide (starred in Fig. 3*b*).

When samples containing type X procollagen or its processed derivative were digested with pepsin (Gibson *et al.*, 1982), only the previously characterized pepsin-resistant sequence (M_r 45000) of type X collagen remained in each case (results not shown).

Chondrocyte cultures

Long-term cultures of chondrocytes from chick-embryo tibiotarsal and femoral zones 3a/b provided a good source of type X procollagen for purification and subsequent analyses (Fig. 4). After 4 weeks in secondary culture, type X procollagen accounted for more than 80% of the collagen secreted into the medium by the zone-3 chondrocytes, but only approx. 10% of that secreted by cells cultured from zones 1 and 2. At 10 weeks in culture, the zone-3 chondrocytes were still secreting large amounts of type X procollagen, but this by now only accounted for approx. 50% of the labelled collagens in the medium. There was a concomitant increase in type I collagen synthesis. Type X procollagen could also be extracted from the cell layer with 2M-$MgCl_2$, but not 0.2M-acetic acid (Fig. 5). The type X collagen extracted from the cell layer comprised predominantly α1(X) chains, although some proα1(X) species were detected. Over the periods of culture employed there was no evidence for the synthesis of type IX collagen in either zone-1/2 or zone-3 tibiotarsal chondrocyte cultures. The nature of the high-M_r reducible bands was not investigated but these components are probably related in part to the precursors of collagen type II.

Sternal chondrocytes maintained on plastic in long-term culture exhibited dedifferentiation as characterized by a switch from synthesis of type II to type I collagen after 2 weeks. However, long-term culture of a population of floating sternal chondrocytes retained the capacity to synthesize not only type II collagen but also types IX and X procollagens (Fig. 5).

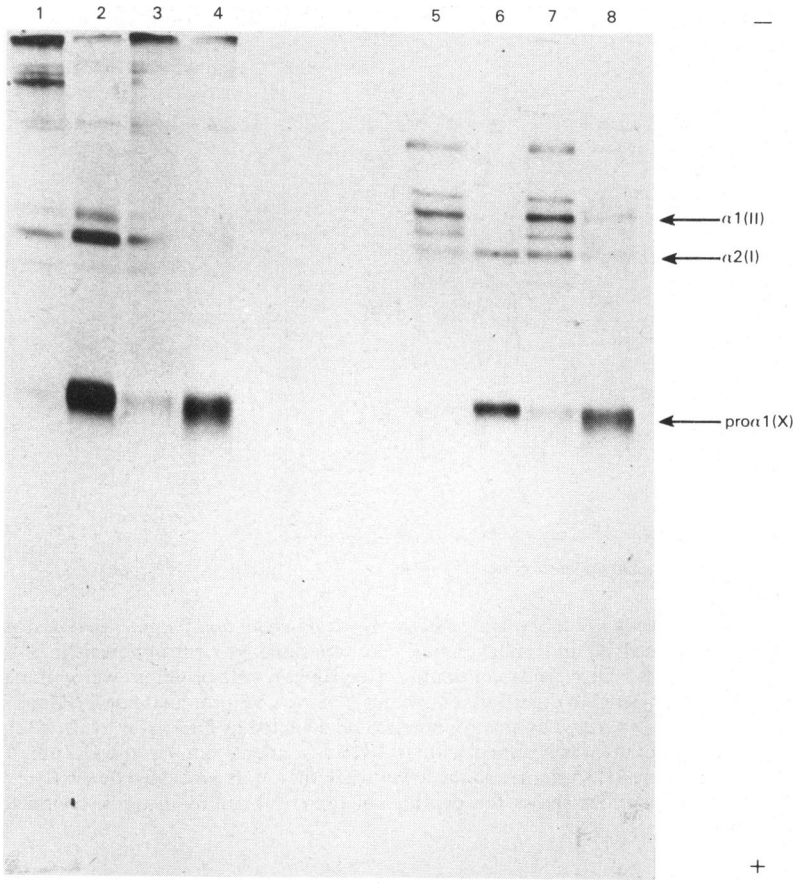

Fig. 4. *Fluorogram of [^3H]proline-labelled proteins in the medium synthesized by cells from zones of epiphysial cartilages after 4 weeks in culture*

Cultures of femoral-cartilage chondrocytes from zones 1 and 2 combined (tracks 1 and 5) and zone 3 (tracks 2 and 6), and of tibiotarsal cartilage chondrocytes from zones 1 and 2 combined (tracks 3 and 7) and zone 3 (tracks 4 and 8), were labelled for 24h with [^{3}H]proline. Proteins in the medium precipitable with $(NH_4)_2SO_4$ at 30% saturation were analysed by electrophoresis on an 8%-polyacrylamide gel under non-reduced (tracks 1–4) and reduced conditions (tracks 5–8). The migration positions of α1(II), α2(I) and proα1(X) chains are indicated.

Purification and rotary shadowing of type X collagen

Type X collagen was purified from tibiotarsal zone 3 chondrocyte cultures as described by Schmid & Linsenmeyer (1983). An additional purification step involving the use of CM-cellulose chromatography was introduced (Kielty *et al.*, 1984). The purified material, which contained predominantly proα1(X) chains, but also some α1(X) chains (approx. 5%), was observed in the native form in the electron microscope by the rotary-shadowing technique (Fig. 6). The helical portion of the molecule had a mean total length of 148.5 ± 1 nm (Fig. 6), and the proα1(X) chains contained a large globular domain at one end. Assuming triple-helical conformation, in which each amino acid has a residue length of 0.286 nm along the axis of the molecule and an average M_r of 95 as determined from the amino acid analyses of type X collagen (Kielty *et al.*, 1984), one chain will contain approx. 519 residues, which corresponds to M_r 49000. This value correlates exactly with the molecular size as determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The rotary-shadowing data obtained with 0.2M-ammonium acetate or 0.1M-acetic acid were identical, although the latter solvent resulted in higher background and appeared to enhance the interactions of the globular terminal domains of the type X precursor molecules (Fig. 6).

Discussion

This project has made use of a chick-embryo organ-culture system to study the synthesis and

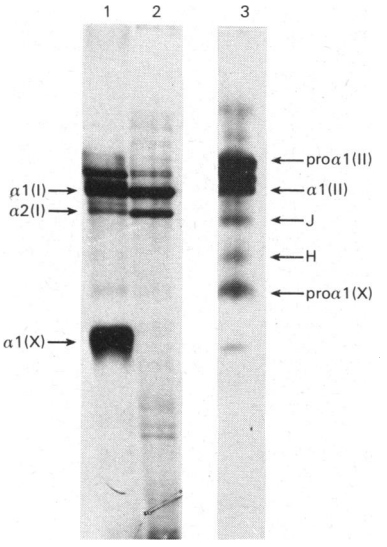

Fig. 5. *Fluorogram of [^{3}H]proline-labelled proteins synthesized by cultured chondrocytes from tibiotarsal (zone-3) cartilage and sternal cartilage*

Chondrocytes derived from zone 3 of tibiotarsal cartilage were maintained in culture for 4 weeks and then labelled with [^{3}H]proline for 24h. Proteins deposited in the cell layer/matrix were extracted with 0.2M-acetic acid (track 2) followed by 4M-guanidinium chloride (track 1), and analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis under reducing conditions. In a second experiment a floating population of sternal chondrocytes (see the Experimental section) was labelled with [^{3}H]proline for 24h, and the proteins in the medium precipitable with $(NH_4)_2SO_4$ at 30% saturation were similarly analysed (track 3). The positions of H and J chains of type IX collagen and of proα1(X) and α1(X) chains are identified, and the migration positions of standard proα1(II), α1(II), α1(I) and α2(I) chains are indicated.

potential processing of the low-M_r cartilage-specific type IX and X collagens. Earlier investigations with chondrocytes in culture contributed in large part to the identification and characterization of the precursors of these collagens released into the culture medium (Gibson *et al.*, 1982, 1983; Schmid & Conrad, 1982*a*,*b*). However, in organ culture the embryonic cartilage deposits all its collagenous components in the extracellular matrix and only a small proportion of newly synthesized macromolecules subsequently diffuse into the medium. Consequently, it has been possible to assess the distribution and the fate of [^{3}H]proline-labelled collagenous polypeptides synthesized by organ cultures of sternal and epiphysial cartilages.

The synthesis of type X collagen was found to occur almost exclusively in the cartilaginous zone 3 of hypertrophying chondrocytes within the developing long bones, whereas type IX collagen (H and J chains) was very prominent in the organ cultures of the sterna and of the zones of chondrocyte proliferation and elongation (zones 1/2) of the tibiotarsa and femurs (Fig. 1). It is noteworthy that synthesis of collagen type X was not detected in the sternal cultures (Fig. 1), and in our studies on the minor collagens released by pepsin digestion of sternal cartilages we also failed to detect any type-X-collagen-related polypeptides (Kielty *et al.*, 1984). Recently, Gibson *et al.* (1984) have reported that in culture the large hypertrophic-like chondrocytes derived from the presumptive calcification region of chick-embryo sterna synthesize G collagen (i.e. type X), unlike the smaller chondrocytes isolated from the permanent cartilaginous zone. This observation is consistent with our earlier studies, which demonstrated that sternal chondrocytes cultured in collagen gels assumed, with time, a rounded morphology similar to that of hypertrophic chondrocytes in growth cartilage and switched to the synthesis of large amounts of type X procollagen (Gibson *et al.*, 1981, 1982). It has now been shown that chondrocytes isolated from the hypertrophying zone 3 of chick-embryo epiphysial growth plate and maintained in long-term culture (up to 6–8 weeks) synthesize increasing amounts of collagen type X with time (Fig. 4). Large amounts of this species are released into the medium, which, as described also by Schmid & Linsenmeyer (1983), provides a rich source of intact type X procollagen for purification. In such cultures substantial proportions of type X polypeptides are also deposited in the cell layer, from which they can only be extracted by using strongly dissociative solutions or proteolysis.

The distinct distribution of these low-M_r collagens within chick-embryo cartilage presumably reflects their roles in contributing to extracellular matrices of distinct character and function. The likelihood that collagen types IX and X assume markedly different states of organization within the extracellular matrix is indicated by the studies reported in Fig. 2. In these experiments the type X collagen deposited in the cartilagenous matrix during organ culture is shown to undergo a rapid conversion into a smaller triple-helical species (whose polypeptides are of M_r 49000), which may represent the predominant form of type X collagen *in vivo*. Such a conversion would be analogous to the processing of cartilage type II procollagen to collagen molecules, which are then capable of forming fibrils. It was also observed that in cell cultures of zone-3 tibiotarsal chondrocytes some of the type X collagen polypeptides were also processed to a related species of M_r 49000 (Fig. 5). In contrast, no evidence was obtained for the process-

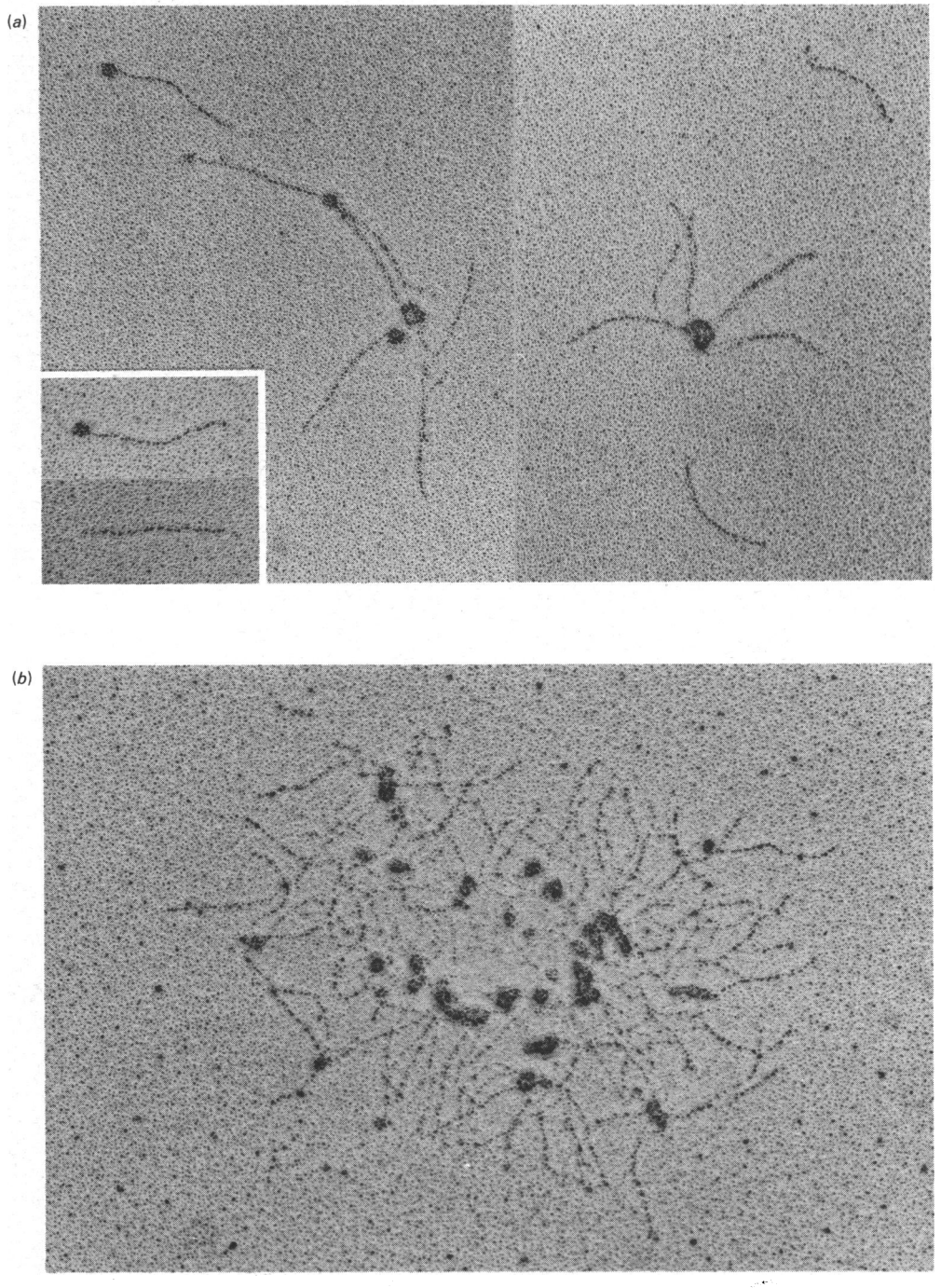

Fig. 6. *Electron micrographs of rotary-shadowed preparations of type X procollagen* (× *145000*)
The sample of type X procollagen secreted by tibiotarsal (zone-3) chondrocytes in culture was purified by salt fractionation and ion-exchange chromatography. The sample containing only type X procollagen with approx. 5% type X collagen was dissolved in 0.1 M-acetic acid (1 mg/ml) and diluted to 2 μg/ml with either (*a*) 0.2 M-ammonium acetate or (*b*) 0.1 M-acetic acid. The upper left-hand frame demonstrates the presence of procollagen type X molecules with a pronounced globular extension at one end, together with occasional molecules of the type X collagen lacking the extension (see bottom inset). The upper right-hand frame illustrates the tendency of the molecules to aggregate, and this aggregation is increased in (*b*) when 0.1 M-acetic acid is used as solvent.

ing of type IX collagen polypeptides in pulse–chase experiments conducted over 48h (results not shown). Moreover, we have been able to demonstrate the presence of the two characteristic chains of type IX collagen (M_r 69000 and 84000) in 6M-guanidinium chloride extracts of chick-embryo sterna (C. M. Kielty, J. T. Thomas & M. E. Grant, unpublished work). In the above context it is noteworthy that Gibson *et al.* (1984) have shown, by using scanning electron microscopy, that the extracellular matrices elaborated by sternal chondrocytes synthesizing collagen types IX and X respectively have very different ultrastructural appearances. There are also reports that antibodies raised to pepsin-derived fragments of minor cartilage collagens (now considered to be derived from type IX collagen) localize in immunofluorescence studies to the pericellular matrix of chondrocytes in articular cartilage (Duance *et al.*, 1982; Ricard-Blum *et al.*, 1982). Further studies will be required to identify the exact location and role of collagen type X in the cartilage growth plate and its potential involvement in the processes of endochondral calcification and bone formation.

The processing of the type X procollagen was shown by rotary-shadowing techniques to involve the scission of a large globular domain from one end of the molecule (Fig. 6). The native type X procollagen purified from cell-culture medium was seen to comprise a short helical segment 148nm in length with a terminal globular extension. The processed species comprising α1(X) chains of M_r 49000 were seen to be present as the linear 148nm segment. This overall structure and conformation of the type X procollagen is totally consistent with the structure predicted in our previous studies, but it still remains to be established whether the large non-collagenous extension peptide is *N*-terminal (Gibson *et al.*, 1982). When type X procollagen and its processed product were treated with pepsin, the M_r of the polypeptides was decreased to 45000, and these molecules will form SLS-crystallites of 130nm in length (Kielty *et al.*, 1984; Schmid *et al.*, 1984). On the basis of these decreases in M_r and in apparent length, it must be concluded that one or more pepsin-sensitive sites are present in the α1(X) chains and may occur as short telopeptides similar to those in the common interstitial fibrillar collagens (Heathcote & Grant, 1980). It may be noted that Capasso *et al.* (1984) have described the processing of a short-chain cartilage collagen of M_r 64000 (probably identical with type X collagen) deposited in culture matrix, with the consequent release into the medium of a non-collagenous component of M_r 30000. We have found no evidence for the existence of a similar-sized non-collagenous domain in the organ-culture experiments described here. Nor have we detected any higher-M_r precursors of the type X procollagen polypeptides (G chains, M_r 59000) as proposed by Remington *et al.* (1983) in their studies on rabbit growth-plate cultures. Thus further studies will be required to establish that the proα1(X) chains described above do represent the primary translation products of the type-X-collagen mRNA.

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