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Biochemistry

Translation and characterization of messenger RNAs in differentiating chicken cartilage

(type II collagen/type I collagen/core protein of chondroitin sulfate proteoglycan)

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ABSTRACT **Total RNA, prepared from chicken limb bud cultures undergoing differentiation to cartilage, has been translated in a wheat germ cell-free protein-synthesizing system. Antibodies against chondroitin sulfate proteoglycan core protein immunoprecipitate a single component which migrates as a protein of 340,000 daltons in sodium dodecyl sulfate/polyacrylamide gels. The messenger RNA for this protein sediments at approximately 27 S in 70% formamide or aqueous sucrose gradients. The 340,000-dalton protein is present in cell-free translation products directed by RNA prepared from limb bud cultures and sternae and is absent in cell-free translation directed by RNA prepared from embryonic calvaria or liver. The level of synthesis of this protein is greatly reduced when RNA prepared from limb bud cultures inhibited from differentiation by BrdUrd is used. (Pre)proα1(I), -α2(I), and -α1(II) collagen bands have been identified on gels by electrophoresis of collagenase-digested or immunoprecipitated cell-free translation products directed by RNA from differentiating limb bud cultures, embryonic sternae, and embryonic calvaria.**

The differentiation of cultured chicken embryonic mesenchyme into cartilage provides a system for the study of the molecular mechanisms that control the coordinated expression of genes characteristic of this process. Stage 24 embryonic limb bud mesenchyme undergoes this differentiation into cartilage under the appropriate conditions in culture, paralleling its normal *in vivo* development (1). Such chondrogenesis is characterized by the accumulation of chondroitin sulfate proteoglycan (1, 2), type II collagen (2), and link protein(s) (3) in extracellular matrix. Prior to differentiation, these mesenchymal cells synthesize large amounts of type I collagen (4). In order to study this process at the molecular level, it is necessary to identify the messenger RNAs and nascent translation products for those genes specifically controlled during chondrogenesis. This communication is concerned with the cell-free translation of RNA from differentiating cartilage into putative chondroitin sulfate proteoglycan core protein and types I and II (pre)procollagens.

Variable amounts of information are available concerning the genes, mRNAs, and initial translation products of the proteins we have chosen to examine. Chondroitin sulfate proteoglycan subunit from chicken sternal or epiphyseal cartilage has been isolated as a very large [2–3 × 10^6 daltons (Dal)] (5, 6), polydisperse (7) molecule which contains 7–11% protein (8, 9). The presence of large amounts of carbohydrate attached to the subunit has prevented definitive characterization of the core protein, although extensive studies have suggested that it is of high molecular weight and contains regions differing in chemical composition and function (10). The identification and analysis of core protein translated from mRNA may provide an alternative approach to the study of this molecule. Preliminary reports of cell-free synthesis of core protein have appeared (11, 12).

Type II procollagen is somewhat better characterized, but information regarding its mRNA and the initial translation product is limited to a report demonstrating the cell-free translation of cartilage RNA into one major high molecular weight band of a collagenase-sensitive protein on sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gels (13). The mRNAs and nascent translation products for the α1 and α2 chains of type I collagen have received considerable attention (14–20). Two groups have reported the cloning of segments of cDNAs prepared from mRNA (18, 19), and the α1(I) chain has recently been shown to be synthesized in a prepro form (14).

MATERIALS AND METHODS

Fertile white leghorn chicken eggs were obtained from Sharpe Farms (Glen Ellyn, IL). High density limb bud cultures were prepared and, in some cases, treated with BrdUrd as described (21). Wheat germ was the generous gift of General Mills (Vallejo, CA), and rabbit reticulocyte lysate translation kits were obtained from New England Nuclear. Dynein was generously provided by F. D. Warner and D. Mitchell. All glassware was heated overnight at 190°C and plasticware was washed with 0.1% diethyl pyrocarbonate. Solutions were made RNase-free either by use of deionized water previously treated with diethyl pyrocarbonate or by direct addition of 1 part per 1000 diethyl pyrocarbonate 24 hr before use.

Total RNA Purification. RNA was purified from 8-day limb bud cultures and 16-day embryonic liver by using slight modifications of the guanidine extraction procedure (22) described by Adams *et al.* (23) with initial homogenization in a Dounce homogenizer followed by a 10-min centrifugation at 12,000 × *g* to remove nonsolubilized material prior to ethanol precipitation of RNA. RNA was prepared from 16-day embryonic calvaria either by the procedure of Monson and Goodman (17) or by the guanidine extraction procedure (22, 23) with initial homogenization in a Polytron. Total RNA from 17-day embryonic sternae was prepared by this latter procedure.

Cell-Free Translation. Wheat germ extracts were prepared by a modification of the method of Roberts and Paterson (24). Wheat germ was selected by flotation on a mixture of CCl_4 and cyclohexane (25) of density sufficient to float approximately 80% of the wheat germ. No preincubation was used. The extract was spun twice at 30,000 × *g*, once before and once after passage through a Sephadex G-25 column. Dithiothreitol (2 mM) was used instead of 2-mercaptoethanol. The cell-free system (10–50 μl) was adjusted to contain 20 mM Hepes (pH 7.35), 120 mM KOAc, 1.2 mM $Mg(OAc)_2$, 2 mM dithiothreitol, 1 mM ATP, 0.2 mM GTP, 500 μM spermidine, 7.8 mM creatine phosphate, 40 μg of creatine phosphokinase (Boehringer Mannheim) per ml, 30 μM of each amino acid except methionine, 0.7–2.3 μM [^{35}S]methionine (580–1350 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) (New England Nuclear or Amersham),

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Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; Dal, dalton.

approximately 3 mg of wheat germ S30 protein per ml, and 70–160 μg of total RNA per ml. After a 3-hr incubation at 23°C, unlabeled methionine was added to 16 mM for an additional 10-min incubation. Total protein synthesis was determined by trichloroacetic acid precipitation according to the method of Boedtker *et al.* (20). Protein in the aliquots was precipitated by trichloroacetic acid for analysis by gel electrophoresis.

Immunoprecipitation of Cell-Free Translation Products. Antisera have been described (26). The antigen used for production of core protein antibodies was the chondroitin sulfate proteoglycan subunit digested with testicular hyaluronidase (EC 3.2.1.35). Cell-free products were diluted 1:42.5 with 120 mM KOAc/1.2 mM $Mg(OAc)_2$/1 mM phenylmethylsulfonyl fluoride, and 0.5-ml aliquots were centrifuged at 63,000 × *g* for 25 min in an SW 50.1 rotor at 0°C. The supernatant solution was further diluted 1:3 to a final concentration of 150 mM monovalent cation ($K^+ + Na^+$), 10 mM sodium phosphate (pH 7.4), 0.5% Triton X-100, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, and 0.02% sodium azide. The diluted reaction mixture was incubated for 1 hr with 11.2 μl of antiserum per ml. Sufficient goat anti-rabbit IgG or goat anti-guinea pig IgG (Miles; determined for each lot of second antibody) was added for 1 hr at room temperature followed by overnight incubation at 4°C. Immunoprecipitates were processed according to the procedure of Shapiro *et al.* (27).

Collagenase Digestion of Cell-Free Translation Products. Cell-free products were diluted 1:6.8 for digestion with varying amounts of purified bacterial collagenase (form III, Advance Biofactures, Lynbrook, NY) in 1 mM phenylmethylsulfonyl fluoride/20 mM Hepes, pH 7.4/2.6 mM *N*-ethylmaleimide/0.5 mM $CaCl_2$ for 10 min at 37°C. Reactions were stopped by the addition of 0.2 vol of cold 250 mM EDTA. Protein was precipitated with trichloroacetic acid for analysis by gel electrophoresis.

Polyacrylamide Gel Electrophoresis. Samples were solubilized and reduced by heating at 100°C for 3 min in 0.1 M dithiothreitol/2% $NaDodSO_4$/80 mM Tris·HCl, pH 6.8/20% glycerol. Electrophoresis was performed in 0.1% $NaDodSO_4$/polyacrylamide slab gels. A discontinuous buffer system with a 3.1% polyacrylamide stacking gel (62.5 mM Tris·HCl, pH 6.7) and a 5% polyacrylamide resolving gel (375 mM Tris·HCl, pH 8.9) was used. Reservoir buffer was 5.0 mM Tris/38.4 mM glycine/0.1% $NaDodSO_4$ at pH 8.4. Fluorographic analysis was performed by the procedure of Laskey and Mills (28).

RESULTS

Translation of Total RNA from Limb Bud Cultures and Sternae. Total RNA was prepared from embryonic chicken sternae and limb bud cultures that had differentiated to form cartilage. The guanidine extraction procedure was used because $NaDodSO_4$/phenol extraction procedures did not provide sufficiently intact messenger RNA for cell-free translation into high molecular weight proteins, presumably due to the presence of ribonuclease in the cell homogenates. RNA was translated in a wheat germ cell-free system by using [^{35}S]methionine as the labeled amino acid. The system was optimized with respect to temperature, length of incubation, and K^+, Mg^{2+}, and spermidine concentrations for the synthesis of both high molecular weight products and the proteins of interest.

$NaDodSO_4$/polyacrylamide gel electrophoretic analysis of the translation products directed by RNAs from limb bud cultures (lane 1) and embryonic sternae (lane 2) is shown in Fig. 1. Characteristic translation products of these RNAs include a very large protein with a molecular weight above 300,000, several bands between 200,000 and 300,000 Dal, and a group of major products between 150,000 and 200,000 Dal in size. The band pattern in the 150,000- to 200,000-Dal region of translation products of RNA prepared from limb bud cultures is dependent to some extent on the particular RNA preparation and the K^+ concentration in the cell-free reaction (compare Figs. 1, 4, and 5). Antibodies against the core protein of cartilage chondroitin sulfate proteoglycan immunoprecipitated only the largest polypeptide from the cell-free translation products directed by RNA from cultured limb bud (Fig. 1, lane 3). No such product was observed with normal rabbit serum (Fig. 1, lane 4). Sufficient dilution of the labeled products and centrifugation to remove ribosomes were essential to prevent nonspecific precipitation of other proteins.

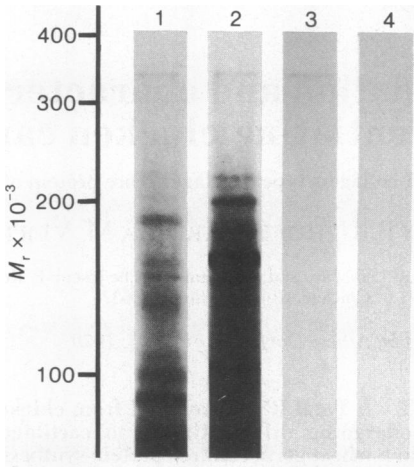

FIG. 1. Electrophoresis of cell-free translation products directed by RNA isolated from differentiating limb bud cultures and sternae. Samples were electrophoresed on 0.1% $NaDodSO_4$/5% polyacrylamide gels. Lanes: 1, translation products of total differentiating limb bud RNA; 2, translation products of total sternal RNA; 3, immunoprecipitate of limb bud product with rabbit antibodies against core protein; 4, immunoprecipitate of limb bud product with normal rabbit serum.

Fig. 2 compares the mobility of the immunoprecipitated translation product with a standard curve of electrophoretic mobility versus size for a number of standard proteins. There are few well-characterized polypeptides greater than 200,000 Dal in size; the largest standard commonly used is myosin at 200,000 Dal. In order to validate extrapolation above 300,000 Dal, we have included dynein, a ciliary protein with an estimated size of 330,000–360,000 Dal. The size of this protein has been determined by comparison with either crosslinked myosin (29) or crosslinked albumin (30). Core protein migrates slightly faster than dynein and we estimate its molecular weight to be

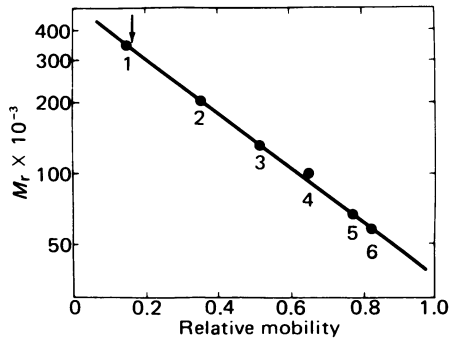

FIG. 2. Plot of molecular weight versus relative mobility on $NaDodSO_4$/polyacrylamide slab gels. Molecular weight standards are: 1, dynein (340,000); 2, myosin heavy chain (200,000); 3, α1(II) collagen chain (migrates as 130,000); 4, phosphorylase A (100,000); 5, bovine serum albumin (67,000); 6, pyruvate kinase (57,000). The position of the core protein synthesized in the cell-free system is indicated by the arrow.

approximately 340,000. Caution must be used in interpreting the apparent molecular weight because the validity of NaDodSO$_4$/polyacrylamide gel electrophoresis for molecular weight determination above 250,000 has not been well documented (31) and some proteins, most notably collagen, are known to behave anomalously (32, 33).

By using material prepared from limb bud cultures, a protein with mobility identical to the 340,000-Dal protein has been obtained from the following: (*i*) immunoprecipitates of completed nascent chains labeled by incubation of isolated polysomes in the wheat germ cell-free system in the presence of [^{35}S]methionine, (*ii*) RNA-directed wheat germ cell-free reactions using [^{3}H]serine or [^{3}H]proline, and (*iii*) RNA-directed rabbit reticulocyte cell-free translation using [^{35}S]methionine (data not shown).

Total RNA from sternae was sedimented on 70% formamide sucrose gradients to further characterize the mRNAs present (Fig. 3). Only those fractions sedimenting at approximately the same rate as the large rRNA (27 S) were active in the synthesis of the putative core protein. Similar results have been obtained on aqueous gradients with RNA prepared from limb bud cultures. Indirect immunoprecipitation of cell-free products directed by gradient-fractionated limb bud RNA using antibodies against core protein shows specific immunoprecipitation of only the 340,000-Dal protein.

The mRNA for a protein of this size and with the amino acid composition reported for core protein (8, 9) would be expected to have at least 8.2 kilobases and would sediment more rapidly than the large rRNA on denaturing gradients. Because 70% formamide gradients are not fully denaturing and RNAs larger than 27S rRNA can sediment more slowly than the 27S rRNA on these gradients (34), no precise estimate of the molecular weight of the core protein mRNA can be made from these data. However, the relatively rapid rate of sedimentation of this mRNA is consistent with the large size of the protein.

Tissue Specificity of the 340,000-Dal Product. Differentiating limb bud cultures are known to contain cell types other than chondrocytes (35, 36). In order to determine whether the immunoprecipitated protein is specific for the cartilage phenotype, cell-free translation products directed by RNA from limb bud cultures, sternae, and several noncartilage tissues were compared. These include the following: (*i*) 17-day embryonic sternae which are primarily composed of chondrocytes and synthesize large amounts of type II collagen and proteoglycan, (*ii*) 16-day embryonic calvaria, a noncartilagenous connective tissue in which type I collagen is reported to be 60% of the total protein synthesized (37), (*iii*) embryonic liver, and (*iv*) limb bud cultures inhibited from differentiating to cartilage by BrdUrd (21). Cell-free translation products of these RNAs are displayed by NaDodSO$_4$/polyacrylamide slab gel electrophoresis in Fig. 4. The 340,000-Dal protein was found only in the translation products of RNA from cultured limb bud and sternae. Sternal products also included a major protein with an apparent molecular weight of approximately 160,000, which we believe to be type II (pre)procollagen (see below).

Some mRNAs have been reported to compete poorly for initiation of translation in cell-free systems in the presence of other messenger RNAs (38, 39). In order to lessen such problems and to increase the sensitivity for detection of core protein mRNA in tissues other than cartilage, RNAs from calvaria, liver, and BrdUrd-treated limb bud cultures were fractionated by sedimentation on aqueous sucrose gradients. Fractions corresponding to those enriched in core protein mRNA in gradients of limb bud or sternal RNA (Fig. 3) were translated in the cell-free system and the products were immunoprecipitated with antibodies against core protein (data not shown). No immunoprecipitable core protein was detected in translation products from fractionated liver or calvaria RNA. Translation products of RNA fractions from BrdUrd-treated cultures contained low levels of core protein, consistent with other findings that indicate incomplete inhibition of differentiation by BrdUrd (2).

Identification of (Pre)Procollagen Chains in Translation Products Directed by RNA from Differentiating Cartilage. During the differentiation of mesenchymal cells to cartilage, a switch from synthesis of type I to type II collagen occurs (2, 4). In order to identify messenger RNAs and nascent translation products for the two collagen types, we investigated the sensitivity of cell-free translation products to collagenase and the reactivity of products with antibodies against types I and II collagen. Collagenase-digestions of the total translation products of RNAs prepared from differentiating limb bud cultures, sternae, and calvaria are shown in Fig. 5*A*. Calvaria RNA directs the synthesis of two major proteins sensitive to collagenase with apparent molecular weights of approximately 190,000 and 150,000. These bands appear to correspond to α1(I) and α2(I) (pre)procollagen chains, respectively (15–17). Other minor

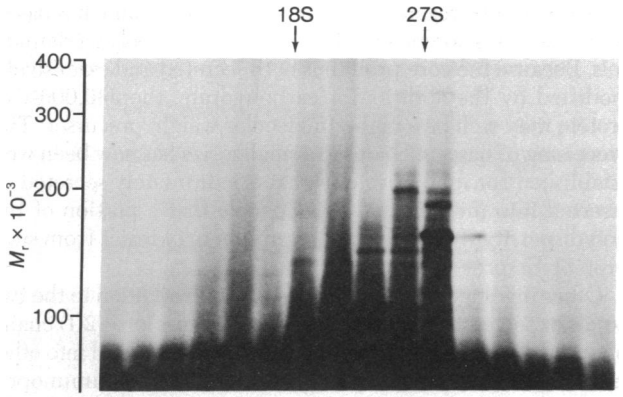

FIG. 3. Cell-free translation of sternal RNA fractionated by sucrose gradient centrifugation. Total sternal RNA (25 μg) was sedimented through a 5–20% sucrose gradient containing 70% formamide and 10 mM Tris·HCl, pH 7.5, at 38,000 rpm for 17.5 hr at 25°C in an SW 50.1 rotor. The RNA was heated at 65°C for 2 min in 70% formamide and cooled in ice water prior to sedimentation to eliminate aggregation of RNA. Fractions of 0.3 ml were collected and absorbance was monitored at 280 nm by using an ISCO gradient fractionator. Calf liver tRNA (5 μg), 10.3 μl of 3 M NaOAc, and 2.5 vol of absolute EtOH were added per fraction for overnight precipitation. Precipitates were successively washed with 3 M NaOAc, 70% EtOH, and 100% EtOH, dried, and translated in 10-μl reactions directly in the vials used for gradient fractionation. Sedimentation was from left to right. Arrows mark the positions of the large (27 S) and small (18 S) rRNAs in the gradient.

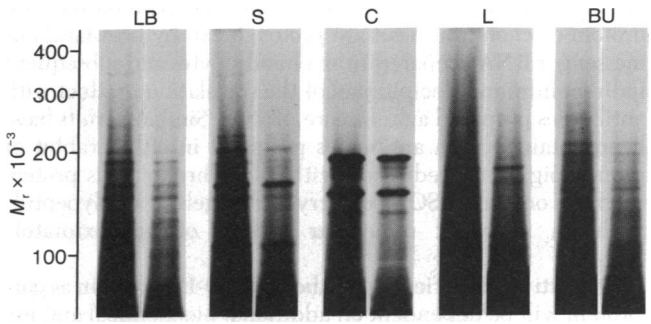

FIG. 4. Comparison of total cell-free translation products directed by RNAs prepared from different tissues. Each sample is shown at two different exposures so that both faint high molecular weight bands and more intense lower molecular weight bands may be visualized. LB (differentiating limb bud cultures), S (sternae), C (calvaria), L (liver), and BU (BrdUrd) inhibited limb bud cultures.

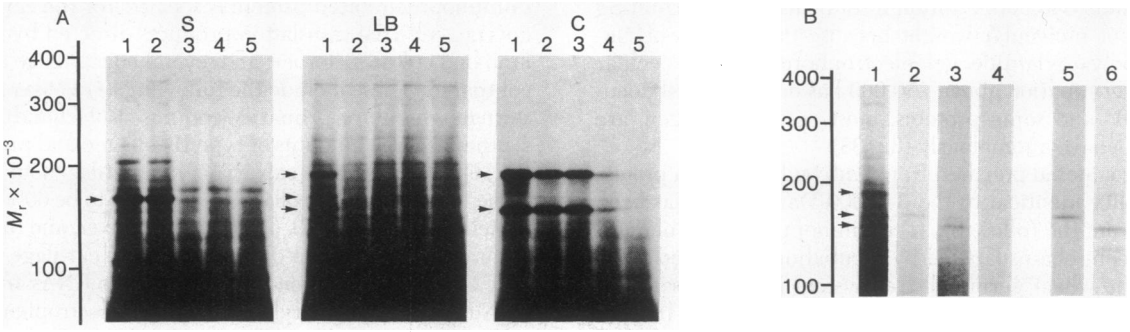

FIG. 5. Identification of (pre)procollagens in total translation products from differentiating limb bud (LB), sternae (S), and calvaria (C).(*A*) Collagenase sensitivity of cell-free translation products, the synthesis of which was directed by total RNA from sternae, differentiating limb bud cultures, and calvaria. Lanes 1–5: 0, 0.37, 2.0, 10, and 100 units of collagenase per ml, respectively. (*B*) Immunoprecipitation with guinea pig antibodies against type I and type II collagen. Lanes: 1, total products directed by differentiating limb bud RNA; 2, immunoprecipitate obtained from total limb bud product with antibodies against type II collagen; 3, immunoprecipitate obtained from total limb bud product with antibodies against type I collagen; 4, immunoprecipitate obtained from total limb bud product with normal guinea pig serum; 5, immunoprecipitate from total sternal product with antibodies against type II collagen; 6, immunoprecipitate from total calvaria product with antibodies against type I collagen. Arrows indicate the positions of presumptive (pre)proα1(I), -α2(I), and -α1(II) chains.

collagenase-sensitive bands were also present in calvaria mRNA translation products. Sternal RNA was translated into one major collagenase-sensitive product that migrated as a protein of 160,000 Dal as well as a number of minor collagenase-sensitive bands of higher and lower molecular weights. Included among these are bands that migrated at the same rate as the two type I (pre)procollagen bands. Translation products of limb bud RNA include three major bands sensitive to collagenase which correspond to the primary collagenase-sensitive translation products of RNA from calvaria and sternae. The 340,000-Dal polypeptide immunoprecipitated by antibodies against proteoglycan core protein is insensitive to collagenase in both limb bud and sternal cell-free products.

Immunoprecipitation of products, the cell-free synthesis of which was directed by limb bud RNA, with antibodies against type I or type II collagen is shown in Fig. 5*B*. Antibodies against type II collagen precipitated one predominant band which migrated at the same rate as the major band present in the sternal translation products (Fig. 5*B*, lane 2). Antibodies against type I collagen precipitated the band presumably corresponding to the α2(I) chain and not the band corresponding to the α1(I) chain (Fig. 5*B*, lane 3). Normal serum does not precipitate either of these bands (Fig. 5*B*, lane 4). Immunoprecipitation of translation products directed by RNA from sternae and calvaria with anti-type II and anti-type I collagen antisera, respectively, is shown in lanes 5 and 6.

DISCUSSION

We have identified the presumptive nascent chain for the core protein of chondroitin sulfate proteoglycan by translation of messenger RNA prepared from chondrocytes and subsequent indirect immunoprecipitation of the translation product with antibodies prepared against core protein. Similar results have been obtained with antibodies prepared in either rabbit or guinea pig. After reduction with dithiothreitol, this protein migrates on $NaDodSO_4$/polyacrylamide gels as a polypeptide with an apparent molecular weight of approximately 340,000.

Definitive identification of the 340,000-Dal protein as core protein will be dependent on additional biochemical and immunological characterization of core protein isolated from tissue and the nascent protein synthesized in RNA-dependent cell-free translation reactions. The antisera used in these studies have been characterized (26). They react strongly with hyaluronidase-digested [$^{35}SO_4$]chondroitin sulfate proteoglycan subunit and do not react with type I procollagen, type II procollagen, or link protein(s). Immunohistochemical studies demonstrate specific reactivity with chondrocytes and not fibroblast-like cells in culture. If the 340,000-Dal protein is not core protein, currently available antisera against core protein must not react with nascent unprocessed core protein (or core protein is not being synthesized) and must contain antibodies against a 340,000-Dal protein which is synthesized in cartilage tissue, not in liver and calvaria, and in greatly reduced amounts in limb bud cultures inhibited from differentiation by BrdUrd.

The size of core protein in cartilage has been difficult to ascertain previously due to the lack of availability of fully intact pure protein. On the basis of buoyant density and keratan sulfate content, a molecular weight of 200,000 was suggested by Hascall and Riolo (40) for bovine nasal cartilage core protein. Most studies of proteoglycan have suggested polydispersity (7, 41). Evidence presented here suggests that the apparent molecular weight of the nascent core protein is about 340,000. This estimate must be regarded with some caution because it is based only on electrophoretic mobility in $NaDodSO_4$/polyacrylamide gels. Because the core protein is both secreted and extensively modified by the addition of carbohydrate, the 340,000-Dal protein may well be a higher molecular weight precursor. The processing of nascent chains to a smaller size has now been well established for many proteins that are ultimately secreted or inserted into membranes. It is possible that a portion of the polydispersity reported for core protein may result from such proteolytic processing.

Other investigators have reported that, in addition to the two bands expected for the (pre)proα1(I) and (pre)proα2(I) chains of collagen, RNA prepared from calvaria is translated into other minor bands which are collagenase-sensitive or immunoprecipitable with antibodies against collagen (15–17, 20). We also find several such bands (see Figs. 4 and 5). Some of these bands migrate between the (pre)proα1(I) and (pre)proα2(I) chains and might be either the result of premature termination of translation, proteolysis of (pre)proα1(I) chains, or translation of partially degraded mRNAs cleaved at specific sites. We find a relatively intense band migrating as a protein of 130,000 Dal which is both collagenase-sensitive and immunoprecipitated by antibodies against type I collagen. These antibodies were prepared against native type I collagen isolated from lathrytic chicken embryos and primarily react with the presumptive α2 chain and not the α1 chain (see above).

In translation products of sternal RNA, we find a single major

band which appears to be (pre)proα1(II) collagen migrating as a protein of 160,000 Dal. There are bands of intermediate intensity, both larger (205,000 Dal) and smaller (110,000 Dal) than the major product, as well as a number of fainter bands which are all collagenase-sensitive. Two of the minor bands correspond in mobility to those for the (pre)proα1(I) and (pre)proα2(I) chains. No bands other than the apparent 160,000-Dal band were detected in immunoprecipitates by using antibodies against type II collagen, but the procedure may not be sufficiently sensitive to detect small amounts of other components. The 205,000-Dal band might represent another collagenous protein. The 110,000-Dal protein is translated from an RNA smaller in size that those identified as type I and type II mRNAs (see Fig. 3) and thus could not be generated by premature termination of translation. It is possible that this RNA is a specific partial degradation product of the mRNA for (pre)proα1(II) chains. However, the product is not immunoprecipitated by antibodies against type II collagen. An alternative explanation for the apparent collagenase sensitivity of minor bands could be contamination of the collagenase by very specific proteases.

When the total translation products of RNA prepared from differentiating limb bud cultures and sternae are compared, a number of differences are observed, probably reflecting the multiple cell types present in limb bud cultures. The major difference is the translation of limb bud RNA into relatively large amounts of type I collagen and small amounts of type II collagen, compared with sternal RNA. Minor differences are difficult to interpret due to the fact that cell-free translation products do not necessarily reflect the true relative mRNA concentrations.

The translation products directed by RNA from BrdUrd-inhibited cultures are very similar to those directed by limb bud RNA with the exception that the cartilage-specific products, core protein and (pre)proα1(II) collagen, are lacking.

These studies demonstrate the translation of RNA into putative chondroitin sulfate proteoglycan core protein and type II (pre)procollagen, products characteristic of the cartilage phenotype.

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1. Caplan, A. I., Zwilling, E. & Kaplan, N. O. (1968) *Science* **160,** 1009–1010.
2. Levitt, D. & Dorfman, A. (1974) *Curr. Top. Dev. Biol.* **8,** 103–149.
3. Vasan, N. S. & Lash, J. W. (1977) *Biochem. J.* **164,** 179–183.
4. von der Mark, K. & von der Mark, H. (1977) *J. Cell Biol.* **73,** 736–747.
5. Hascall, V. C. & Sajdera, S. W. (1970) *J. Biol. Chem.* **245,** 4920–4930.
6. Pasternack, S. G., Veis, A. & Breen, M. (1974) *J. Biol. Chem.* **249,** 2206–2211.
7. Kimura, J. H., Osdoby, P., Caplan, A. I. & Hascall, V. C. (1978) *J. Biol. Chem.* **253,** 4721–4729.
8. Nevo, Z., Horwitz, A. L. & Dorfman, A. (1972) *Dev. Biol.* **28,** 219–228.
9. Hascall, V. C., Oegema, T. R., Brown, M. & Caplan, A. I. (1976) *J. Biol. Chem.* **251,** 3511–3519.
10. Heinegard, D. & Axelsson, I. (1977) *J. Biol. Chem.* **252,** 1971–1979.
11. Upholt, W. B., Vertel, B. M., Ho, P.-L. & Dorfman, A. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36,** 650 (abstr.).
12. Treadwell, B. V., Mankin, D. & Mankin, H. J. (1979) *Transactions of the 25th Annual Meeting of the Orthopaedia Research Society* (Orthopaedic Research Society, Montreal), Vol. 4, p. 130.
13. Harwood, R., Grant, M. E. & Jackson, D. S. (1975) *FEBS Lett.* **57,** 47–50.
14. Palmiter, R. D., Davidson, J. M., Gagnon, J., Rowe, D. W. & Bornstein, P. (1979) *J. Biol. Chem.* **254,** 1433–1436.
15. Howard, B. H., Adams, S. L., Sobel, M. E., Pastan, I. & de Crombrugghe, B. (1978) *J. Biol. Chem.* **253,** 5869–5874.
16. Rowe, D. W., Moen, R. C., Davidson, J. M., Byers, P. H., Bornstein, P. & Palmiter, R. D. (1978) *Biochemistry* **17,** 1581–1590.
17. Monson, J. M. & Goodman, H. M. (1978) *Biochemistry* **17,** 5122–5128.
18. Lehrach, H., Frischauf, A. M., Hanahan, D., Wozney, J., Fuller, F., Crkvenjakov, R., Boedtker, H. & Doty, P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5417–5421.
19. Sobel, M. E., Yamamoto, T., Adams, S. L., DiLauro, R., Avvedimento, V. E., de Crombrugghe, B. & Pastan, I. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5846–5850.
20. Boedtker, H., Frischauf, A. M. & Lehrach, H. (1976) *Biochemistry* **15,** 4765–4770.
21. Levitt, D. & Dorfman, A. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1253–1257.
22. Cox, R. A. (1968) *Methods Enzymol.* **12,** 120–129.
23. Adams, S. L., Sobel, M. E., Howard, B. H., Olden, K., Yamada, K. M., de Crombrugghe, B. & Pastan, I. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3399–3403.
24. Roberts, B. E. & Paterson, B. M. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2330–2334.
25. Shih, D. S. & Kaesberg, P. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1799–1803.
26. Vertel, B. M. & Dorfman, A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1261–1264.
27. Shapiro, D. J., Taylor, J. M., McKnight, G. S., Palacios, R., Gonzalez, C., Kiely, M. L. & Schimke, R. T. (1974) *J. Biol. Chem.* **249,** 3665–3671.
28. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56,** 335–341.
29. Ogawa, K. & Gibbons, I. R. (1976) *J. Biol. Chem.* **251,** 5793–5801.
30. Warner, F. D., Mitchell, D. R. & Perkins, C. R. (1977) *J. Mol. Biol.* **114,** 367–384.
31. Quittner, S., Watts, L. A. & Roxby, R. (1978) *Anal. Biochem.* **89,** 187–195.
32. Furthmayr, H. & Timpl, R. (1971) *Anal. Biochem.* **41,** 510–516.
33. Weber, K. & Osborn, M. (1975) in *The Proteins,* ed. Neurath, H. (Academic, New York), 3rd Ed., Vol. 1, pp. 179–223.
34. Boedtker, H. & Lehrach, H. (1976) in *Progress in Nucleic Acid Research and Molecular Biology,* eds. Cohn, W. E. & Volkin, E. (Academic, New York), Vol. 19, pp. 253–260.
35. Vertel, B. M. (1976) in *Scanning Electron Microscopy/1976/II Part V,* Proceedings of the Workshop on Advances in Biomedical Applications of the SEM (Chicago Press Corp., Chicago), pp. 291–298.
36. Osdoby, P. & Caplan, A. I. (1976) *Dev. Biol.* **52,** 283–299.
37. Diegelmann, R. F. & Peterkofsky, B. (1972) *Dev. Biol.* **28,** 443–453.
38. Sonenshein, G. E. & Brawerman, G. (1976) *Biochemistry,* **15,** 5501–5505.
39. Kabat, D. & Chappell, M. R. (1977) *J. Biol. Chem.* **252,** 2684–2690.
40. Hascall, V. C. & Riolo, R. L.(1972) *J. Biol. Chem.* **247,** 4529–4538.
41. Rosenberg, L., Hellmann, W. & Kleinschmidt, A. K. (1975) *J. Biol. Chem.* **250,** 1877–1883.