Characterization of the tissue-specific proteoglycans synthesized by chondrocytes from nanomelic chick embryos

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Cartilage from the avian mutant nanomelia has been reported to synthesize cartilage-specific proteoglycans, PGS(SC)-I, at 1–2% of normal values [McKeown & Goetinck (1979) *Dev. Biol.* **71**, 203–215]. Proteoglycans were endogenously labelled with [^{35}S]sulphate and extracted from cartilage in 4M-guanidine hydrochloride and chromatographed on controlled-pore glass 1400. PGS(SC)-I was obtained from the void volume of these columns. Dissociative sucrose-density-gradient analysis revealed a greater than normal polydispersity in the nanomelic PGS(SC)-I. Fractions from both the controlled-pore glass 1400 void volume and sucrose gradients were tested for their ability to bind specific antibody against cartilage proteoglycan monomer. In all instances, binding of normal fractions was greater than 90%, whereas binding to nanomelic fractions ranged from 20 to 65%. Chromatography of PGS(SC)-I on controlled-pore glass 2500 resulted in 70% of the normal and 25% of the mutant proteoglycans eluting as aggregates. Chondroitin sulphate chains from mutant PGS(SC)-I appeared slightly larger than normal when chromatographed on controlled-pore glass 500. In addition, PGS(SC)-I from nanomelic cartilage is more susceptible to proteolysis *in vitro* than the PGS(SC)-I from normal cartilage. This evidence suggests that the small amount of cartilage-specific proteoglycan synthesized by nanomelic cartilage is not normal.

Avian chondrogenesis studied by either mesenchymal cell culture or limb organ culture is marked by definitive changes in the synthesis of sulphated proteoglycans. The sulphated proteoglycans synthesized during differentiation have been characterized by molecular-sieve chromatography (Goetinck *et al.*, 1974; DeLuca *et al.*, 1977; Vasan & Lash, 1979), dissociative sucrose-density-gradient analysis (Okayama *et al.*, 1976; Kitamura & Yamagata, 1976; Sawyer & Goetinck, 1981) and immunological specificity (Ho *et al.*, 1977; Royal *et al.*, 1980). Undifferentiated limb mesenchyme synthesizes a species of proteoglycan that is immunologically and physically distinct from the proteoglycans synthesized by cartilaginous tissues. Mesenchymal proteoglycans similar to cartilage proteoglycans have recently been shown to interact with exogenous hyaluronic acid to form aggregates (Royal *et al.*, 1980).

Molecular sieve chromatography of proteoglycans from limb mesenchyme, limb cartilage or sternal cartilage on controlled-pore glass 1400 separates two populations of proteoglycans, PGS-I, which elutes in the column void volume, and PGS-II,

which is included on the column. The PGS-I material from limb mesenchyme, PGS(LM)-I (the nomenclature is from McKeown & Goetinck, 1979), can be distinguished from the PGS(LC)-I by sedimentation rates in dissociative sucrose gradients and by the ability to bind specific antibody against cartilage proteoglycan (Royal *et al.*, 1980).

Chick embryos homozygous for the recessive lethal gene, nanomelia, exhibit a severe form of micromelia and die on about the 17th day of incubation (Landauer, 1965). Nanomelic embryos exhibit a wide range of skeletal deformities including a limb and axial skeleton that is both reduced in size and abnormal in shape. The skeletal defects result from malformations in the cartilaginous rudiments of these skeletal structures (Mathews, 1967). Nanomelic chondrocytes synthesize sulphated proteoglycans at only 10% of normal values, with the reduction in proteoglycan synthesis coming exclusively from the cartilage-specific proteoglycan species (PGS-I) (Mathews, 1967; Fraser & Goetinck, 1971; Palmoski & Goetinck, 1972; Pennypacker & Goetinck, 1976; McKeown & Goetinck, 1979). Preliminary results (McKeown &

Goetinck, 1979) indicate that the small amount of PGS-I synthesized by nanomelic chondrocytes is of the cartilage type. This conclusion is supported by the more recent finding that PGS(LM)-I synthesis by high-density mesenchymal cultures of cells from normal and nanomelic limb buds is identical, and that only at the onset of chondrogenesis when the normal cultures are starting to synthesize PGS(LC)-I can differences in proteoglycan synthesis be detected (Sawyer & Goetinck, 1981). Since nanomelic chondrocytes possess all the enzymic machinery for proper post-translational modification of the proteoglycan core protein (Stearns & Goetinck, 1979; Goetinck *et al.*, 1981), it is believed that the nanomelic phenotype results from the reduced availability of the cartilage proteoglycan core protein. This could result from either the decreased synthesis of a normal core protein or the synthesis of an abnormal core protein that is rapidly degraded. To distinguish between these two possibilities, the present study focuses on some of the properties of PGS(SC)-I synthesized by nanomelic chondrocytes. The present results indicate that the small amounts of tissue-specific proteoglycans synthesized by nanomelic chondrocytes are abnormal. A preliminary report on this work has been presented previously (McKeown *et al.*, 1980).

Experimental

Materials

Foetal-calf serum and Ham's F-12 (sulphate-free) medium were from Grand Island Biological Co., Grand Island, NY, U.S.A. Guanidine hydrochloride (grade 1) and bovine serum albumin and insoluble trypsin (100–200 units of enzyme activity/g of solid) were from Sigma Chemical Co., St. Louis, MO, U.S.A. Benzamidine hydrochloride and 6-aminocaproic acid were from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Controlled-pore glass beads were from Electronucleonics Inc., Fairfield, NJ, U.S.A. [³⁵S]Sulphate as the sodium salt (916 Ci/mol) was from New England Nuclear, Boston, MA, U.S.A. Cellulose nitrate centrifuge tubes were from Beckman Instruments Inc., Palo Alto, CA, U.S.A. Fertile eggs were obtained from flocks maintained by the Department of Animal Genetics at the University of Connecticut.

Methods

Organ culture and extraction of radiolabelled proteoglycans. Fertile eggs resulting from the matings between birds known to be heterozygous for the gene nanomelia were incubated for 14 days. Embryos were removed and identified phenotypically as either normal or nanomelic. Sterna were dissected, cleaned of perichondrium and incubated overnight in Ham's F-12 medium (sulphate-free). The medium

was supplemented with 10% foetal-calf serum and contained [³⁵S]sulphate at 40 μ Ci/ml. Cultures customarily contained three organs in 2.5 ml of medium and were maintained at 37°C in an atmosphere of CO₂/air (1:19). After an 18 h labelling period, the sterna were rinsed three times in cold phosphate-buffered saline and extracted for 48 h in 4 M-guanidine hydrochloride in 0.05 M-sodium acetate, pH 5.8, at 4°C. The extraction, subsequent dialysis, column chromatography and Farr assays were done at 4°C in the presence of proteinase inhibitors as described by Oegema *et al.* (1975) (0.01 M-sodium EDTA, 0.1 M-6-aminocaproic acid and 0.005 M-benzamidine hydrochloride). The radio-labelled extracts were clarified by centrifugation and dialysed against 0.05 M-sodium acetate, pH 5.8, containing 0.5 M-NaCl for removal of free isotope and guanidine hydrochloride.

Molecular-sieve chromatography of sulphated proteoglycans and glycosaminoglycans. All columns for chromatography had the dimensions 0.8 cm $\times$ 88 cm. Flow rates of approx. 15–20 ml/h were obtained with a peristaltic pump. Sample sizes applied to the columns were from 1 to 2 ml and were eluted using 0.05 M-sodium acetate, pH 5.8, containing 0.5 M-NaCl. Fractions (1 ml) were collected and radioactivity in 25 μ l portions was determined by liquid-scintillation spectrometry. Chromatography of proteoglycans on controlled-pore glass 1400 [actual pore size, 1250 Å (125 nm)] and controlled-pore glass 2500 [actual pore size, 2574 Å (257.4 nm)] has been described previously (Lever & Goetinck, 1976). Molecular-sieve chromatography of glycosaminoglycans on glyceryl-controlled-pore glass 500 [actual pore size, 544 Å (54.4 nm)] has also been described previously (McKeown & Goetinck, 1979).

Sucrose-density-gradient centrifugation. Dissociative sucrose-density-gradient centrifugation was performed by the method of Kimata *et al.* (1974). Samples from organ culture extracts of the controlled-pore glass 1400 void volume were layered on a linear sucrose gradient (5–20%) containing 4 M-guanidine hydrochloride in 0.05 M-sodium acetate (pH 5.8). The gradients (12.4 ml) were centrifuged in an SW 40 rotor at 36 000 rev./min for 20 h at 4°C in a Beckman L5-65 ultracentrifuge. Gradients were fractionated by an ISCO gradient fractionator and 0.3 ml fractions were collected. In the instance where the gradient fractions were to be analysed further, radioactivity was determined in 50 μ l portions, and designated fractions were pooled, dialysed against 0.05 M-sodium acetate, pH 5.8, containing 0.5 M-NaCl.

Preparation of glycosaminoglycans. Glycosaminoglycans were obtained from proteoglycan aggregates and monomers [PGS(SC)-I] by β -elimination in 0.2 M-NaOH. Samples were left in

alkaline conditions for 72h at 20°C. Before chromatography, samples were neutralized with HCl.

Radioimmunoassay of proteoglycans. A radioimmunoassay of proteoglycans using antiserum prepared against avian cartilage-specific proteoglycan monomer has been described previously (Sparks *et al.*, 1980). This is a Farr (1958)-type assay in which antigen-antibody complexes are precipitated in 40%-saturated $(\text{NH}_4)_2\text{SO}_4$. Briefly, samples of [^{35}S]sulphate-labelled proteoglycan (25 μl) were mixed with either an equal volume of immune rabbit serum or normal rabbit serum and incubated at 4°C overnight. Antigen-antibody complexes were precipitated by the addition of an equal volume (50 μl) of 80%-saturated $(\text{NH}_4)_2\text{SO}_4$ and pellets were obtained by centrifugation. Supernatants were discarded, pellets were redissolved in 23 μl of 0.5 M-NaCl and radioactivity was determined by liquid-scintillation spectrometry. Samples to be tested for antibody binding contained from 1000–30000 d.p.m. and were done in duplicate. This range of antigen was well below antigen excess for this assay. Corrected specific binding was determined by the following formula:

$$\text{Bound (\%)} = \frac{\text{immune serum binding} - \text{normal serum binding}}{\text{total antigen added} - \text{normal serum binding}} \times 100$$

Limited proteolysis of cartilage proteoglycans. Normal and nanomelic samples of PGS(SC)-I (60 μg of protein/ml) were first dialysed against proteolysis buffer (50 mM-sodium phosphate, pH 7.5, containing 0.15 M-NaCl). Samples were warmed to 37°C, trypsin was added to give a final concentration of 0.25–0.50 units/ml and samples were kept at 37°C with constant shaking. At the indicated time points, 0.6 ml portions were removed and the enzyme was pelleted by centrifugation in a Beckman centrifuge. The supernatant (0.55 ml) was immediately frozen in solid CO_2 and acetone. All digested samples were then re-chromatographed on controlled-pore glass 1400 and proteolysis was monitored as loss of counts from controlled-pore-glass 1400 void volume. This assay is based on that of Bradley *et al.* (1975).

Analytical methods. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Radioactivity was determined with a Tracor Analytical Mark III scintillation spectrometer equipped with an external standard. The counting fluid was prepared by mixing 2 vol. of a toluene solution of 2,5-diphenyloxazole (4 g/litre) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.1 g/litre) and 1 vol. of Triton X-100.

Results

Characterization of [^{35}S]sulphate-labelled proteoglycans

[^{35}S]Sulphate-labelled cartilage extracts were

characterized by both molecular-sieve chromatography and dissociative sucrose-density-gradient analysis. Chromatography on controlled-pore glass 1400 separates the tissue-specific proteoglycans, PGS(SC)-I, from PGS(SC)-II, a species of proteoglycan found in many tissues as well as cartilage (Fig. 1*a*). The normal profile shows 90% of the incorporated sulphate eluting in the column void volume as PGS(SC)-I, whereas only 35% of the nanomelic proteoglycans elute in the controlled-pore glass 1400 void volume.

When sedimented in dissociative sucrose gradients, extracts from normal cartilage contain a large proportion of ^{35}S -labelled proteoglycans that sediment to the denser region of the gradient (Fig. 2). This proteoglycan species is designated as PGS(SC)-I because it is the same population that elutes in the controlled-pore glass 1400 void volume (McKeown & Goetinck, 1979; Fig. 3). Fig. 2 also shows that the nanomelic extracts contain small amounts of this faster sedimenting species of proteoglycan.

Cartilage-specific proteoglycans, PGS(SC)-I, were further characterized by molecular-sieve chromatography on controlled-pore glass 2500. This

column separates proteoglycan aggregate from monomer (Lever & Goetinck, 1976; Royal *et al.*, 1980). According to the profiles in Fig. 1*b*), 75% of normal cartilage PGS(SC)-I chromatographs as aggregates [PGS(SC)-Ia], and 25% elutes as a monomer [PGS(SC)-Ib]. Chromatography of the nanomelic PGS(SC)-I results in only 20% of the radioactivity eluting in the aggregate position.

Antibody binding to PGS(SC)-I fractions from normal and nanomelic cartilage extracts

When PGS(SC)-I from normal and nanomelic cartilage extracts are sedimented in dissociative sucrose density gradients, the profiles shown in Fig. 3*a*) are obtained. The normal proteoglycans sediment in the denser regions of the gradient. However, the nanomelic profile is broader and peaks in the less dense regions of the gradient. The designated fractions from these gradients were pooled, dialysed and tested for their ability to bind antiserum directed against the cartilage monomer (Fig. 3*b*). Antibody binding to the normal proteoglycans reaches almost 100%. The nanomelic proteoglycans in the less-dense regions of the gradient (fractions 20–30) exhibit very little specific binding with the antiserum. The more-dense regions of the gradient (fractions 35–45) contain a significant proportion of molecules that can be bound by antibody. Antibody binding in these fractions is from 35 to 65%.

Antibody binding was also tested by using proteo-

Fig. 1. Molecular-sieve chromatography and antibody binding of proteoglycans

(a) A 2 ml sample of cartilage extracts from either normal or nanomelic sterna was chromatographed on controlled-pore glass 1400. (b) A 1 ml sample of PGS(SC)-I from either the normal or nanomelic controlled-pore glass 1400 void volume was chromatographed on controlled-pore glass 2500. (c) Corrected specific binding of controlled-pore glass 1400 fractions to antibodies against cartilage-specific proteoglycan monomer. (d) Corrected specific binding of proteoglycan aggregates (controlled-pore glass 2500 void volume) and monomers (controlled-pore glass 2500 included fractions) to antibody against cartilage-specific proteoglycan monomer.

Fig. 2. Dissociative sucrose-density-gradient analysis of $[^{35}\text{S}]$ sulphate-labelled proteoglycans

A 0.8 ml sample of cartilage extracts from either normal or nanomelic sterna was layered on the gradient. Sedimentation is from left to right. Abbreviations used: T, top; B, bottom.

Fig. 3. Dissociative sucrose-density-gradient analysis of PGS(SC)-I fractions

(a) A 1 ml sample from controlled-pore glass 1400 void volume of either normal or nanomelic fractions was layered on the gradient. Sedimentation is from left to right. (b) Corrected specific binding of designated fractions from the gradients to antibodies against cartilage-specific proteoglycans.

glycans from fractions obtained from molecular-sieve chromatography. Fig. 1(a) shows the profiles obtained when normal and nanomelic ^{35}S -labelled extracts are chromatographed on controlled-pore glass 1400. The void-volume fractions containing

PGS(SC)-I were tested for their ability to bind antibody (Fig. 1c). Binding in all void-volume fractions of the normal was greater than 95%, whereas binding to the nanomelic fractions was only 50%. The

PGS(SC)-I fractions were chromatographed on controlled-pore glass 2500 to separate aggregate [PGS(SC)-Ia] from monomer [PGS(SC)-Ib] (Fig. 1b) and tested for their ability to bind antibody (Fig. 1d). Greater than 90% of all normal proteoglycan fractions can bind antibody. Nanomelic proteoglycans that chromatograph as aggregates exhibit greater binding (60%) than those that chromatograph in the monomer position (20%).

Molecular-sieve chromatography of glycosaminoglycans from normal and nanomelic PGS(SC)-I

The glycosaminoglycan moieties from the cartilage-specific proteoglycans [PGS(SC)-I] synthesized by normal and nanomelic chondrocytes were characterized by molecular-sieve chromatography. The sulphated glycosaminoglycans were removed by β -elimination and chromatographed on controlled-pore glass 500 (Fig. 4). Both normal and nanomelic glycosaminoglycans elute in an included position on the column. The nanomelic material elutes slightly ahead of and in a broader peak than normal, suggesting that these molecules may represent a population of glycosaminoglycans with a wider range, although higher average molecular weight, than normal.

Proteolysis of tissue-specific proteoglycans from normal and nanomelic cartilage

Tissue-specific proteoglycans from the void volume of controlled-pore glass 1400 columns were subjected to limited proteolysis by trypsin. The susceptibility of the normal and nanomelic proteoglycans to proteolysis was tested by treating samples with trypsin for increasing lengths of time and then re-chromatographing the samples on controlled-pore glass 1400. Fig. 5 shows some of the profiles

Fig. 4. *Molecular-sieve chromatography of glycosaminoglycans from PGS(SC)-I*

Polysaccharide chains were removed from normal and nanomelic controlled-pore glass 1400 void volume fractions by β -elimination and chromatographed on controlled-pore glass 500.

obtained when trypsin-treated samples are chromatographed. Three of the six time points are represented here. The fractions designated in Figs 5(a) and 5(b) were chosen to represent the void volume. The percentage of material that still eluted from the column void volume was then plotted against the length of time of enzyme treatment (Fig. 6). This indicates that the nanomelic proteoglycans are more susceptible to proteolysis than are normal molecules.

Discussion

Tissue-specific proteoglycans [PGS(SC)-I] extracted from normal and nanomelic cartilage can be isolated from the void volume of a controlled-pore glass 1400 column. Other connective tissues besides cartilage synthesizes proteoglycans that elute in the void volume of controlled-pore glass 1400 (i.e. skin

Fig. 5. *Molecular-sieve chromatography of trypsin-treated PGS(SC)-I*

Normal (a) and nanomelic (b) controlled-pore glass 1400 void-volume fractions were treated with trypsin for various lengths of time and then re-chromatographed on controlled-pore glass 1400. The fractions chosen as void volume are designated in the Figure.

Fig. 6. Time course of proteolysis of normal and nanomelic PGS(SC)-I

After digestion with trypsin for the designated time periods, proteoglycans were re-chromatographed on controlled-pore glass 1400 and the percentage that still chromatographed in the void volume was determined.

fibroblasts and limb mesenchyme). These proteoglycans can be distinguished from cartilage-type proteoglycans by their sedimentation rate in dissociative sucrose gradients (McKeown & Goetinck, 1979). Cartilage-type proteoglycans will sediment to the dense regions of a sucrose gradient, whereas skin- and mesenchymal-type proteoglycans sediment to regions of intermediate density. The profiles obtained when normal and nanomelic ^{35}S -labelled proteoglycans are sedimented in dissociative sucrose gradients (Figs. 2 and 3) indicate that the nanomelic tissue-specific proteoglycans sediment as cartilage-type proteoglycans, although they exhibit more polydispersity and appear smaller in size than normal proteoglycans. When the nanomelic proteoglycan fractions from such a gradient are tested with specific antibody to cartilage proteoglycan monomer, only the more dense regions of the gradient show significant antibody binding. Those molecules in the less-dense regions of the gradient show little reactivity with the antiserum. This antiserum is specific for cartilage proteoglycan and contains no detectable antibodies against chondroitin sulphate (Sparks *et al.*, 1980) or hyaluronic acid (P. McKeown-Longo & P. F. Goetinck, unpublished work). Therefore, the recognized antigenic determinants are believed to lie on or near the proteoglycan core protein. Those nanomelic molecules that are not bound by antibody may represent cartilage-type proteoglycans that are smaller than normal and are

therefore missing antigenic determinants. Alternatively, they could represent the synthesis of some residual mesenchymal-type proteoglycan or of a distinct population of proteoglycans that cannot be detected in normal chicken embryos because of the presence of large amounts of cartilage proteoglycans. It is, however, evident that the material from the denser region of the gradient represents cartilage-specific proteoglycans. This is in agreement with previous data that indicated that nanomelic chondrocytes do make the transition from the synthesis of mesenchymal- to cartilage-type proteoglycans (McKeown & Goetinck, 1979; Sawyer & Goetinck, 1981). However, these proteoglycans do not sediment quite as fast in sucrose gradients, nor do they bind cartilage proteoglycan antibody as well as molecules from normal.

Profiles of normal PGS(SC)-I on controlled-pore glass 2500 shows that greater than 75% of these molecules elute in the aggregate position (void volume). Only 20% of the nanomelic PGS(SC)-I appears aggregated, with the remainder being slightly included on the column. This profile differs from that of PGS(LM)-I, in which the material is separated into two major peaks, one eluting in the void volume and one in the total volume (Royal *et al.*, 1980). This is therefore consistent with the contention that nanomelic PGS(SC)-I represents the cartilaginous species of proteoglycans. The fact that proteoglycan aggregates are formed via the ionic interaction between a specialized region of the proteoglycan core protein and hyaluronic acid (Hardingham *et al.*, 1976) suggests that those proteoglycans that are included on the controlled-pore glass 2500 do not have a functional hyaluronic acid-binding region. The nanomelic material eluting in the controlled-pore glass 2500 void volume is assumed to have a functional hyaluronic acid-binding region based solely on its chromatographic behaviour. Because of the limited amount of ^{35}S -labelled proteoglycan from nanomelic cartilage, isolated proteoglycan monomers were not tested for their ability to bind exogenous hyaluronic acid. Such experiments have been done using normal proteoglycans (Lever & Goetinck, 1976; McKeown & Goetinck, 1979; Royal *et al.*, 1980). Antibody binding to the column fractions is similar to that obtained using fractions from the sucrose gradients. Only 50% of the nanomelic molecules in the controlled-pore glass 1400 void volume can be bound by antibody, in contrast with 95% binding by proteoglycans from normal cartilage. When the nanomelic controlled-pore glass 1400 void volume is chromatographed on controlled-pore glass 2500, the molecules that elute as aggregates show a greater specificity of binding than those that are included on the column. The results of antibody binding suggest that those molecules from the controlled-pore glass

1400 void volume that sediment to the denser regions of the sucrose gradient (Fig. 3a, fractions 35–45) are probably the same molecules that elute in the controlled-pore glass 2500 void volume. The lighter population of proteoglycans (Fig. 3b, fractions 20–30) are most likely the molecules that are included on the controlled-pore glass 2500.

One reason for the slower sedimentation rate in sucrose density gradients of the nanomelic PGS(SC)-I could be that these molecules have less polysaccharide on them than normal. Kato *et al.* (1978) have shown that decreased length of polysaccharide molecules on proteoglycans results in a slower sedimentation rate in dissociative sucrose gradients. This does not appear to be the case for nanomelic PGS(SC)-I (Fig. 4). The nanomelic polysaccharide moieties are equal in length if not longer than those obtained from normal PGS(SC)-I. Another possibility is that the nanomelic PGS(SC)-I has fewer glycosaminoglycan chains per unit of protein than does the normal form. Protein and uronic acid assays done on one set of normal and nanomelic PGS(SC)-I fractions support this theory. In addition, all nanomelic fractions tested with antibody exhibited larger amounts of non-specific binding than normal fractions. High protein/carbohydrate ratios are believed to increase non-specific binding of proteoglycans in the Farr assay used in the present study (Sparks *et al.*, 1980; McKeown-Longo *et al.*, 1981).

The nanomelic PGS(SC)-I has also been shown to be more susceptible to proteolysis than normal PGS(SC)-I. As mentioned above, one reason for the reduced availability of proteoglycan core protein within nanomelic chondrocytes may result from the increased turnover of an abnormal core protein. Abnormal proteins have been shown to have shorter half lives *in vivo*, which can be correlated to an increased susceptibility *in vitro* to proteolysis (Bond, 1971; Goldberg, 1972). If the core protein of the nanomelic PGS(SC)-I were abnormal, this would be expected to result in the synthesis of proteoglycans, which were more susceptible to proteolysis. This has been shown to be the case, but whether this increased susceptibility to proteolysis is a function of the core protein itself or a reflection of fewer carbohydrate chains on an otherwise normal core cannot be determined. However, recent data from this laboratory have shown that nanomelic chondrocytes do not contain a 246 000-mol.wt. intracellular protein, which has been identified as the proteoglycan core protein in normal chondrocytes (Argaves *et al.*, 1981). In the light of this and of the evidence presented here, i.e., the reduced ability of the nanomelic PGS(SC)-I to form aggregates and to bind antibody, it is concluded that the small amount of cartilage-specific proteoglycans synthesized by nanomelic chondrocytes is abnormal, and that this

abnormality is a result of an abnormal proteoglycan core protein.

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