Expression of cell-surface proteoglycan mRNA by human articular chondrocytes

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The expression of six cell-surface proteoglycans (syndecan, fibroglycan, amphiglycan, glypican, betaglycan and CD44) was studied at the mRNA level. Analysis was performed by Northern blotting using total RNA preparations from freshly isolated articular chondrocytes obtained from both juveniles and adults. Similar results were obtained for both age groups. By far the most abundant message was that for amphiglycan, CD44 message was next in relative abundance, and the messages for fibroglycan, glypican and betaglycan were all expressed at low levels. Syndecan message could not be detected by this technique. This pattern of expression was different to that observed in cultured skin fibroblasts, where the messages for amphiglycan, CD44,

fibroglycan and glypican were all expressed at a similar level. In contrast with the fibroblasts, where the amphiglycan message exhibits no size polymorphism, the chondrocyte amphiglycan message is present in three polymorphic forms, due to the use of alternative polyadenylation signals. When the newly isolated chondrocytes are maintained in monolayer culture for several passages, the amphiglycan message heterogeneity reverts to that characteristic of the fibroblasts. Thus human articular chondrocytes are characterized by both their high level of amphiglycan message expression and their use of alternative polyadenylation signals.

INTRODUCTION

Proteoglycans are often classified according to their tissue location as being extracellular, cell-associated or intracellular. The molecules in these sites differ both in their core protein structure and in the type of glycosaminoglycan chain that they possess. The cell-associated proteoglycans are characterized by the presence of heparan sulphate, though some may also bear chondroitin sulphate, and are intimately associated with the plasma membrane of the cell. The proteoglycans at the plasma membrane may be further divided according to their mode of association. The majority have a core protein that spans the plasma membrane, but some are linked to the outer surface of the membrane via a glycosyl-phosphatidylinositol bond [1]. Many functions have been ascribed to these proteoglycans, including the promotion of cell–cell and cell–matrix interactions and the facilitation of growth-factor responses [2].

The membrane-spanning proteoglycans are typified by the four members of the syndecan family: syndecan, syndecan-2 (also known as fibroglycan), syndecan-3 (also known as N-syndecan) and syndecan-4 (also known as ryudocan or amphiglycan). These molecules exhibit different patterns of cell, tissue and developmental expression [3], and their genes are located on different chromosomes [4]. Human syndecan cDNA encodes a protein of 310 amino acids [5], which includes a 34-residue cytoplasmic domain, a 25-residue membrane-spanning domain, and a 251-residue extracellular domain of which the terminal 17 residues represent a putative signal peptide. The extracellular domain contains the sites for glycosaminoglycan attachment, which are present in two clusters. The N-terminal cluster contains the heparan sulphate chains, and the C-terminal cluster near the plasma membrane possesses only chondroitin sulphate [6]. Human fibroglycan cDNA encodes a protein of 201 amino acids

[7,8], and has a shorter extracellular domain than syndecan. Human amphiglycan cDNA encodes a protein of 198 amino acids [9], having a similar structure to fibroglycan. A human cDNA sequence has yet to be reported for N-syndecan, but, based on the rat sequence [10], the primary translation product for this proteoglycan will be the largest of the syndecan family.

Membrane spanning proteoglycans unrelated to syndecan are also known, and include the transforming growth factor (TGF- β) type III receptor (also known as betaglycan) and the lymphocyte homing receptor (also known as CD44 or epican). These molecules are often termed ‘part-time’ proteoglycans, as they may exist in a form that does not possess a glycosaminoglycan chain. Human betaglycan cDNA encodes a protein of 849 amino acids (GenBank Accession Number L07594), and is of similar size to the published sequence for the rat cDNA [11,12]. It has a much larger extracellular domain than any member of the syndecan family. This extracellular domain has two sites for the attachment of glycosaminoglycan chains [13]. CD44 is perhaps the most complex of the cell-surface proteoglycans, in that its gene can undergo extensive alternative splicing to yield proteins with variable extracellular domains [14]. Splicing varies with cell type, but does not affect the acceptor sites for glycosaminoglycan attachment. Epican represents a form of CD44 expressed by keratinocytes, where the cDNA encodes a protein of 699 amino acids [15]. This is 339 amino acids larger than the smallest form of CD44 encoded by leucocytes.

The prototype for the phosphatidylinositol-anchored proteoglycans is glypican. Human glypican cDNA encodes a protein of 558 amino acids [16]. Attachment to the plasma membrane occurs near the C-terminus of the protein and involves proteolytic cleavage of the C-terminal tail. The molecule has four potential sites for glycosaminoglycan attachment, and only heparan sulphate appears to be present. Although the expression of

Abbreviations used: TGF, transforming growth factor; MMuLV-RT, Moloney murine leukaemia virus reverse transcriptase; FCS, fetal-calf serum; DMEM, Dulbecco's Modified Eagle Medium; MEM, Minimum Essential Medium; DEPC, diethyl pyrocarbonate; SSC, saline sodium citrate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; bFGF, basic fibroblast growth factor.

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glypican and the membrane-spanning proteoglycans has been studied in a variety of cell types and in a variety of species, there is currently no report on their expression by chondrocytes from any species. This work is aimed at addressing this deficit by studying the expression of the mRNA encoding six of the cell-surface proteoglycans described above in human articular chondrocytes.

METHODS

Materials

Guanidine isothiocyanate, antibiotics, trypsin, tissue-culture media and Moloney murine leukaemia virus reverse transcriptase (MMuLV-RT) were from Gibco-BRL (Burlington, Ontario, Canada). Fetal-calf serum (FCS) was from Bockneck (Rexdale, Ontario, Canada). Bacterial collagenase, hyaluronidase and Mops were from Sigma (St. Louis, MO, U.S.A.). Stratilinker UV cross-linker was from Stratagene (La Jolla, CA, U.S.A.). Taq DNA polymerase was from Perkin-Elmer Cetus (Montreal, Quebec, Canada). Deoxynucleotides and random hexanucleotide primers were from Pharmacia (Oakville, Ontario, Canada). Multiprime DNA labelling systems, Rapid-hyb buffer, X-ray film (Hyperfilm β Max) and [32 P]dCTP were from Amersham (Oakville, Ontario, Canada). TA cloning kits were from Invitrogen (San Diego, CA, U.S.A.). RNasin and Multi-Pol DNA sequencing systems were from Promega (through Fisher Scientific, Nepean, Ontario, Canada). Positively charged nylon membrane was from Boehringer-Mannheim (Montreal, Quebec, Canada). The Gel-Print 2000 photographic system and the Gel Print Tool Box version 2.0 software for densitometric analysis were from Biophotonics (through Bio-Can, Mississauga, Ontario, Canada).

Source of cartilage and chondrocyte isolation

Articular cartilage was obtained from two infants (aged 12 days and 3 months), two juveniles (aged 6 and 9 years) and two adults (aged 58 and 66 years). Tissue was collected from the distal femur

at the time of autopsy (within 20 h after death), from individuals who showed no signs of joint disease. The tissue was transported from the autopsy room to the laboratory in Dulbecco's Modified Eagle Medium (DMEM) containing antibiotics (25 units/ml penicillin, 25 μ g/ml gentamicin sulphate and 2.5 μ g/ml fungizone), where it was cleaned of any extraneous connective tissue and cut into small pieces for enzymic release of chondrocytes [17]. In some experiments isolated chondrocytes were resuspended in DMEM in the presence of 10% FCS and antibiotics (25 units/ml penicillin and 25 μ g/ml streptomycin), seeded at 5×10^6 cells per 150 cm² flask, and allowed to grow in monolayer culture. When the cells reached 80% confluence, they were either passaged by trypsin treatment or used for total RNA preparation.

Source of skin and fibroblast isolation

Full-thickness skin biopsies were obtained from the forearm of one infant (1 day), one juvenile (10 years) and two adults (24 and 37 years). Biopsies were placed immediately in explant culture in Minimum Essential Medium (MEM) containing 10% FCS and antibiotics (50 units/ml penicillin and 25 μ g/ml gentamicin sulphate). Fibroblasts were obtained by outgrowth from the skin explants. Growing cells were released by trypsin treatment with 0.25% trypsin in Puck's medium, and seeded in monolayer culture in MEM containing 10% FCS but no antibiotics. Fibroblasts were passaged another 4–6 times before being used for total RNA preparation.

Preparation of total cellular RNA

Total cellular RNA was extracted from chondrocytes or fibroblasts by the acid guanidinium isothiocyanate/phenol/chloroform method of Chomczynski and Sacchi [18]. The cell layer in each 150 cm² flask was extracted with 3 ml of denaturing solution on ice for 15 min. An equal volume of chloroform/3-methylbutan-1-ol solution (49:1, v/v) was then added to the extract, and the mixture was well vortex-mixed. RNA was then precipitated from the aqueous phase by addition of an equal

Table 1 Characteristics of cDNA probes

Primers are presented in a 5' to 3' orientation, with that for the coding strand being above that for the non-coding strand. The region covered by the amplified probe is delineated by the bases at either terminus. The numbering refers to the cited reference. The C content of the probe relates to the number of cytosine residues in the non-coding strand and is given relative to the total length of the probe.

Proteoglycan	Primers	Region	C content	Reference
Amphiglycan-5'	CTCCTAGAAGGCCGATACTTCT GGACCTCCGTTCTCTCAAAGAT	120–465	101/346	9
Amphiglycan-3'	ACTGGCTTAACGAGTCACAG CAGACAACAGTGTGAGAGGT	2191–2548	116/358	9
Betaglycan	CCAAATGGAGGCCCTTCCGTTT CTCACCTGCTGAAGGCTGCAAT	1803–2348	159/546	–†
CD44	GGTATGGGTTTCATAGAAGGGCA ACAGCTCCATTGCCACTGTTG	362–2072	106/483*	15
Fibroglycan	GGGAGCTGATGAGGATGTAG CACTGGATGGTTTGCGTTCT	759–1153	93/395	8
Glypican	ATCACCGACAAGTTCTGGGGTA CATCTTCTCACTGCACAGTGTC	1119–1436	116/318	16
Syndecan	CCCTGAAGATCAAGATGGCTCT CCCGAGGTTTCAAAGTGAACT	295–858	148/560	5

* The probe is shorter than that predicted from the published sequence, due to exon deletion of bp797–2024 in the chondrocyte form of CD44 message.

† Genebank accession number L07594.

volume of propan-2-ol, and resuspended in 50 μ l of diethyl pyrocarbonate (DEPC)-treated water containing 20 units/ml RNasin. RNA solutions were stored at -80°C .

Northern blotting

Total RNA (10 μ g) was subjected to electrophoresis on a 1%-agarose gel containing 1.11% formaldehyde in a Mops buffer [19]. Fractionated RNA was blotted on to positively charged nylon membranes in $20\times$ SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0), and cross-linked to the membrane using Stratolinker UV cross-linker adjusted to the auto-cross-link setting (120000 μ J). Hybridization was performed with a variety of ^{32}P -labelled cDNA probes by using the Rapid-hyb buffer according to the manufacturer's instructions. Blots were then washed with $2\times$ SSC/0.1% SDS for 15 min at room temperature, followed by $0.2\times$ SSC/0.1% SDS for $2\times$ 15 min at 65°C . The hybridized probe was detected by exposure of the blot to X-ray film. Between each different probe the blot was stripped by washing with 10 mM phosphate buffer (pH 6.8)/50% formamide at 70°C for 2 h. At the end of each series of probes, the original probe was re-utilized to determine whether RNA had been lost from the membrane by the consecutive stripping procedures. This final probing revealed message levels at about 80% of those observed with the initial probing after the use of seven intervening stripping procedures. The time of exposure to the X-ray film varied from overnight to 1 week, depending on the message under study. In some cases the message abundance reflected in the X-ray films was quantified by using a Gel-Print 2000 system and related software. The message abundance in each integrated band was normalized with respect to the number of radiolabelled cytosine residues in each probe.

Preparation of cDNA probes

cDNA probes for Northern blotting were prepared by PCR after reverse transcription of mRNA using MMuLV reverse transcriptase. The PCR amplification was carried out for 40 cycles (94°C for 45 s, 60°C for 30 s, and 72°C for 45 s) using Taq DNA polymerase. Primers being used for the PCR were synthesized with an Applied Biosystems 392 DNA synthesizer, based on published sequence data for the corresponding human cDNAs (Table 1). The source of mRNA varied, depending on the particular proteoglycan under study. For amphiglycan, betaglycan and CD44, RNA from human chondrocytes was used for reverse transcription. For fibroglycan and glypican, RNA from human fibroblasts was used, whereas for syndecan, RNA from human fetal kidney was used. All PCR products were TA-cloned according to the manufacturer's instructions, and the identity of the cloned DNA was confirmed by sequence analysis. Sequencing was carried out by the dideoxy chain-termination method [20], with a Multi-Pol DNA-sequencing system kit according to the manufacturer's instructions. Each probe was labelled with [^{32}P]dCTP (3000 Ci/mmol) by the random primer technique. The only probe not prepared in the above manner was that for human glyceraldehyde-3-phosphate dehydrogenase (GAPD) [21]. This probe was a 901 bp fragment excised from the 1.2 kb cDNA insert in the plasmid pHcGAPNR obtained from the Repository of Human DNA Probes and Libraries (American Type Culture Collection, Rockville, MD, U.S.A.).

RESULTS

The mRNA levels for six cell-surface proteoglycans were analysed by Northern blotting of total RNA preparations from freshly isolated human articular chondrocytes. The message levels in

Figure 1 Northern-blot analysis of total cellular RNA hybridized with cDNA probes for (a) the 5'-region of human amphiglycan, (b) the 3'-region of human amphiglycan and (c) human GAPD

Total RNA was extracted from human skin fibroblasts (F), neonatal human articular chondrocytes (C_N) or adult human articular chondrocytes (C_A). The positions of the 18S and 28S rRNA are indicated. The autoradiographs were exposed overnight.

RNA preparations from neonatal and adult chondrocytes were compared with each other and with message levels from total RNA preparations isolated from cultured human skin fibroblasts. Message was detected for five of the proteoglycans (amphiglycan, fibroglycan, glypican, betaglycan and CD44) in both chondrocytes and fibroblasts, though the relative abundance of the different messages varied between the two cell types. In contrast, there was little variation between the relative abundance of these five messages for chondrocyte RNA preparations obtained from different individuals within the two age groups being studied. Northern blotting failed to detect any message for the proteoglycan syndecan in RNA preparations from either chondrocytes or fibroblasts.

By far the most abundant message under study in the chondrocyte RNA preparations was that for the proteoglycan amphiglycan. When 10 μ g of total RNA was used for analysis, the amphiglycan message was readily visible on autoradiographs after an overnight exposure following hybridization (Figure 1a). All other proteoglycan messages exhibited lower-intensity signals even when autoradiography was allowed to proceed for 1 week. The high amphiglycan message level in both adult and neonatal chondrocytes was in marked contrast with the low message level observed for this proteoglycan in fibroblasts, even though equivalent RNA loading was confirmed by analysis of GAPD message levels (Figure 1c). The difference between chondrocytes and fibroblasts was related not only to the amphiglycan message level, but also to its size heterogeneity. Messages of three distinct sizes were observed for the chondrocytes, with their relative abundance increasing with their size. Only the largest of these three messages was present within the fibroblast RNA preparations (Figure 1a). The message heterogeneity within the chondrocyte RNA preparations appeared to be due to the use of alternative polyadenylation signals, rather than the use of alternative promoters or alternative splicing, as hybridization using a probe hybridizing to the extreme 3'-end of the published

Figure 2 Northern-blot analysis of total cellular RNA hybridized with cDNA probes for (a) human betaglycan and (b) human CD44

Total RNA was extracted from human skin fibroblasts (F), neonatal human articular chondrocytes (C_N) or adult human articular chondrocytes (C_A). The positions of the 18S and 28S rRNA are indicated. The autoradiographs were exposed for 1 week.

amphiglycan cDNA 3'-untranslated region revealed only the message of largest size (Figure 1b).

The second most abundant message among the proteoglycans under study in the chondrocytes was that for CD44, which was also present at a higher abundance in chondrocytes than in fibroblasts (Figure 2b). As with amphiglycan, the chondrocyte CD44 message exhibited size heterogeneity, with three species being detected. Two of these isoforms were of similar size to the 18S rRNA marker, whereas the third was larger, being of similar size to the 28S rRNA marker. Similar size heterogeneity was also observed for the fibroblast CD44 message. However, although the two smaller RNA species were more abundant in the chondrocytes, the larger species was more abundant in the fibroblasts. The relative abundance of the different message isoforms did not appear to vary with age in the chondrocytes.

The messages for betaglycan, fibroglycan and glypican were all detected in low abundance in the chondrocytes. Those for betaglycan (Figure 2a) and glypican (Figure 3b) were present as single species in both chondrocytes and fibroblasts. Although the betaglycan message appeared more abundant in the chondrocyte RNA preparations, the glypican message was more abundant in the fibroblasts. In fact, of the molecules under study, glypican was the only one which had a higher abundance of message in fibroblasts than in chondrocytes. The message for fibroglycan was present at a similar level in both chondrocytes and fibroblasts, and exhibited two isoforms of similar size and relative abundance in both cell types (Figure 3a).

The relative abundance of the different proteoglycan messages is not easy to assess with accuracy by direct observation of Northern blots, as the probes used for detection are never of the same specific activity. Visual quantification is further hampered when multiple message isoforms are present, and when variation in the level of different messages necessitates the use of different exposure times for detection within the linear range of the film used for autoradiography. All of these potential problems are applicable to the chondrocyte messages under study. To over-

Figure 3 Northern-blot analysis of total cellular RNA hybridized with cDNA probes for (a) human fibroglycan and (b) human glypican

Total RNA was extracted from human skin fibroblasts (F), neonatal human articular chondrocytes (C_N) or adult human articular chondrocytes (C_A). The positions of the 18S and 28S rRNA are indicated. The autoradiographs were exposed for 1 week.

come these problems, autoradiographs were subjected to densitometry, and message levels were assessed by integrating the area under all isoform peaks and correcting for the number of radiolabelled cytosine residues in each probe. This type of analysis indicates that for the chondrocytes the amphiglycan message represents about 83 % of the total message for the five proteoglycans detected upon Northern blotting (Figure 4). CD44 message represents about 12 % of this total, and betaglycan, fibroglycan and glypican messages all represent less than 5 %. In the fibroblasts the relative abundance of the different messages does not show this extreme diversity. The amphiglycan message represents about 31 % of the total message for the five proteoglycans, whereas CD44, fibroglycan and glypican messages each represent 20–23 %, and the betaglycan message represents about 3 %. The vast abundance of the amphiglycan message in the chondrocytes is more apparent when one considers that the total message for the five proteoglycans under study is at least 4 times greater in the chondrocytes than the fibroblasts.

This method of analysis can also be used to provide quantification of the different message isoforms for those proteoglycans that show heterogeneity in message size. For amphiglycan the three message forms are present in proportions of about 5:4:1 in order of decreasing size in the chondrocytes (Figure 4). No such heterogeneity is present for the fibroblasts. For CD44, both chondrocytes and fibroblasts show three messages in proportions of about 2:5:3 and 5:3:2 respectively, in order of decreasing size. Finally, for fibroglycan the ratio of the two observed message sizes is about 7:3 for the chondrocytes and 8:2 for the fibroblasts, with the message of larger size being most abundant in both cases.

In addition to the variations in relative abundance, the chondrocytes differ from the fibroblasts in the message isoforms being expressed. This difference was not observed when the chondrocytes were maintained in monolayer culture for several passages. For the amphiglycan messages, the two smaller isoforms were decreased in their relative abundance, such that

Figure 4 Quantification of various proteoglycan messages in total cellular RNA from (a) human articular chondrocytes and (b) human skin fibroblasts

The message levels for each of five proteoglycans is depicted as a percentage of the total message for all five proteoglycans. Quantification was performed by densitometric analysis of autoradiographs of Northern blots, and bars shown indicate averages with S.D. for the different RNA preparations examined. For those proteoglycans that show heterogeneity in message size, the bars are divided according to the relative abundance of the different message isoforms. Isoform abundance is presented in order of decreasing message size from left to right in the bars.

Figure 5 Northern-blot analysis of total cellular RNA hybridized with cDNA probes for (a) the 5'-region of human amphiglycan or (b) human CD44

Total RNA was extracted from freshly isolated human articular chondrocytes (C), chondrocytes which were in primary monolayer culture (P_0) or had been passaged once (P_1) or twice (P_2), or cultured human skin fibroblasts (F). The positions of the 18S and 28S rRNA are indicated. The autoradiographs were exposed for 1 week.

after a second passage message heterogeneity resembled that in cultured skin fibroblast RNA preparations (Figure 5a). A similar situation was observed for the CD44 messages, where the relative abundance of the isoform of largest size increased with passage

to give a message pattern analogous to that obtained from the cultured skin fibroblast RNA preparations (Figure 5b).

DISCUSSION

Message levels for six cell-associated proteoglycans were studied in freshly isolated human chondrocytes. It is hoped that under such conditions, where no subsequent cell culture is performed, that the message levels, or at least their relative abundances reflect those present *in situ*. This being the case then human chondrocytes are characterized by their high expression of amphiglycan message, moderate expression of CD44 message, and low expression of fibroglycan, betaglycan and glypican messages. The syndecan message was never detected. Although there was some variation in message abundance between different individuals, this pattern of message expression was consistent at all ages. Thus high amphiglycan message expression would appear to be a characteristic of all human chondrocytes *in situ*.

The human chondrocyte amphiglycan message is also characterized by its size polymorphism, in which three distinct message sizes are apparent. Previous studies using a variety of human cell lines have revealed only the presence of a single message of about 2.6 kb [9], which would correspond to the largest chondrocyte message. At present it is not clear whether this difference is a distinct feature of cartilage or an artefact of cell culture. Certainly, when the chondrocytes were maintained in monolayer cell culture for several passages, their amphiglycan messages reverted to a pattern characteristic of cultured skin fibroblasts and all other cell lines. This observation provides a warning concerning the extrapolation of data from cultured cells to the state *in situ*. A similar triplet pattern for amphiglycan messages has previously been reported in several embryonic-chick tissues [22], indicating that at least in this species this pattern of message polymorphism is not unique to cartilage. As in the human, the message of largest size predominated.

The origin of the human amphiglycan message polymorphism is likely to reside in the use of alternative polyadenylation signals within the 3'-untranslated region. Several potential polyadenylation signals reside downstream of that used in cultured fibroblasts to give a message of ~2.6 kb [9]. The other sites would be expected to yield messages of about 1.5 and 2.1 kb, compatible with the sizes observed in the present work. This contention is supported by the observation that a cDNA probe residing in the 3'-untranslated region between the last two polyadenylation signals reveals only the message of largest size. It is therefore unlikely that the observed polymorphism arises by alternative splicing of the multiple exons forming the coding region of the gene [22] or by the use of alternative promoters. However, one cannot categorically rule out the participation of such events if they should happen to generate products that show little size change and hence cannot be resolved on Northern blotting. The use of alternative polyadenylation signals has also been suggested to account for the heterogeneity of fibroglycan messages [8], though in this case cell culture does not appear to result in any difference in signal usage.

The pattern of CD44 expression in the human chondrocytes is compatible with that described for a variety of haematopoietic cells [23], with three messages of 1.6, 2.2 and 5.0 kb, where message heterogeneity is also thought to be due to the use of multiple polyadenylation signals [14]. At least 17 other CD44 transcripts have been described to date, due to the fact that 12 of the 19 exons encoding CD44 can undergo alternative splicing. The majority of such splicing occurs within the centre of the coding region encoded by exons 6–14. The absence of expression

of these exons by the human chondrocytes was demonstrated by sequencing the cDNA probe used for Northern blotting. The primers for this probe were designed from sequence data derived from human keratinocyte cDNA encoding the proteoglycan epican, an alternative form of CD44 [15]. The expected PCR product size for epican would be 1711 bp, whereas that obtained was only 483 bp (Table 1). The difference represents the absence of the six alternative exons expressed in epican, but not in leucocytes or chondrocytes.

It remains to be ascertained whether the actual cell-surface proteoglycans are expressed to the same relative degree as their corresponding messages, or whether a prevalence of amphiglycan or CD44 would have any specific effect on chondrocyte function. In the case of CD44 and betaglycan, the core proteins of the proteoglycans have been shown to interact with hyaluronate and TGF β , respectively [24,25], functions that are not duplicated by the other cell-surface proteoglycans and would depend on the specific expression of the macromolecules. However, all the cell-surface proteoglycans containing heparan sulphate have the potential to interact with basic fibroblast growth factor (bFGF) and modulate its interaction with its specific receptors [26,27]. The question remains whether this apparent redundancy of proteoglycans at the cell surface has any functional significance. Perhaps the different core proteins affect the precise structure of the heparan sulphate and so influence bFGF binding, or perhaps they influence intracellular signalling in a differential manner [1]. Then again it is possible that these different protein structures influence turnover within the plasma membrane by affecting the rate of proteolysis or internalization of the ectodomain [28]. It is certainly well recognized that different cell types exhibit different cell-surface proteoglycans [3,29] and that these differences may change with development. One may therefore speculate that a specific cell-surface proteoglycan complement endows the cell with a specific function. In human chondrocytes this function would appear to require significant amphiglycan expression at all ages, though it is possible that the structure of the heparan sulphate, and hence the functional properties of the amphiglycan, could vary with age.

We thank: Nadia Nikolajew for typing the manuscript and Jane Wishart for preparing the Figures; the pathology departments at l'Hôpital Sainte-Justine, the Royal Victoria Hospital and the Montreal General Hospital for the provision of autopsy specimens; and the Medical Research Council of Canada, the National Institutes of Health and the Shriners of North American for financial support.

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