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Review

Hyaluronan synthesis and degradation in cartilage and bone

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Abstract. Hyaluronan (HA) is a large but simple glycosaminoglycan composed of repeating D-glucuronic acid, β1 – 3 linked to *N*-acetyl-D-glucosamine β1 – 4, found in body fluids and tissues, in both intra- and extracellular compartments. Despite its structural simplicity, HA has diverse functions in skeletal biology. In development, HA-rich matrices facilitate migration and condensation of mesenchymal cells, and HA participates in joint cavity formation and longitudinal bone growth. In adult cartilage, HA binding to aggrecan immobilises aggrecan, retaining it at the high concentrations required for compressive resilience. HA also appears to regulate bone remodelling by controlling osteoclast, osteoblast and osteocyte behaviour. The functions of HA depend on its intrinsic properties, which in turn rely on the degree of polymerisation by HA synthases, depolymerisation by hyaluronidases, and interactions with HA-binding proteins. HA synthesis and degradation are closely regulated in skeletal tissues and aberrant synthetic or degradative activity causes disease. The role and regulation of HA synthesis and degradation in cartilage, bone and skeletal development is discussed.

Keywords. Hyaluronan, hyaluronan synthase, hyaluronidase, cartilage, bone, growth plate, skeletal development, limb morphogenesis.

The biology of hyaluronan

Structure and properties of hyaluronan

Hyaluronan (HA) is the simplest glycosaminoglycan. It is unsulphated, unbranched, immunologically inert, and unlike other glycosaminoglycans, it is synthesised as a free polysaccharide rather than substituted on a core protein. Newly synthesised HA polymers of 2500 – 25 000 repeating disaccharides have a molecular mass of 10^6 – 10^7 Da.

HA has biophysical properties that distinguish it from other components of the extracellular matrix. In solution as a long-chain biopolymer it behaves as a stiffened random coil with a large hydrated volume, such that each molecule interacts with its neighbours to create viscoelastic solutions. It shows excluded volume effects, as it restricts the access of other macromolecules to its domain and as HA is polydisperse its properties are the aggregate properties of a population of molecules of varying chain length, rather than those of a unique species.

Molecular diffusion studies have shown that HA properties in aqueous solution at high concentration

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(> 1.0 mg/ml) reflect molecular entanglement with no evidence of chain-chain association [1, 2]. Molecular dynamics have been very helpful in explaining these biophysical properties by showing that the HA chain is very mobile and that the stiffening of the chain is due to a range of dynamic hydrogen bonds, which restrict glycosidic bond rotation between adjacent saccharides [3, 4]. This analysis is supported by the diffusion studies which showed that the major contribution to stiffening was from the hydrogen bonds between adjacent saccharides [2]. On its own, HA thus forms a dynamic network, which restricts the movement of other macromolecules, but is freely permeable to all components of low molecular weight. Although electron micrographs of HA have visualised apparent ropes and possible anti-parallel helices [5] and NMR studies have been interpreted to suggest that HA forms specific stable tertiary structures [5], there is no evidence that such structures exist in solution [6]. Under physiological conditions the properties of HA solutions can be predicted directly from the behaviour in dilute solution and even at very high concentrations, when the solutions are highly viscoelastic, individual chains remain mobile and they undergo no transition to a gel-like state. As discussed below, where HA is organised into higher order structures, it does so through interactions with a range of proteins, belonging to several families that have evolved to bind to it.

HA-rich pericellular matrices

Many cell types elaborate HA-rich pericellular matrices that confer important properties such as shielding against potentially harmful extracellular molecules or pathogens [7–13]. For example, chondrocytes that express abundant pericellular HA are difficult to transfect with plasmid DNA and transfection efficiency can be improved by hyaluronidase (HYAL) treatment [14, 15].

The assembly of HA-rich matrices can occur *via* HA binding to cell surface receptors. Pericellular HA is detected using an assay in which the surrounding matrix excludes erythrocytes (added in suspension) *via* steric exclusion, producing erythrocyte-free 'coats' [16]. The coats depend upon HA for their integrity and are lost after treatment with HYALs or HA oligosaccharides [17, 18]. Pericellular HA provides a direct link between the cell and its extracellular environment.

HA and CD44

It would be impractical to review the functions, synthesis and degradation of HA without considering the role of the widely expressed, archetypal HA receptor, CD44. The CD44 gene contains 21 exons, many of which are alternatively spliced to generate multiple isoforms [19, 20]. Chondrocytes express the standard isoform of CD44, CD44 s [21]. CD44-HA interactions link chondrocytes with their matrix and these interactions are essential for maintaining normal cartilage homeostasis [22]. Treatment of cartilage explants with antisense oligonucleotides that inhibit CD44 expression causes substantial loss of aggrecan [23], and treatment with HA oligosaccharides that compete with endogenous HA for binding to CD44 have both anabolic and catabolic effects [24]. These studies suggest that chondrocyte CD44-HA interactions modulate cartilage metabolism, and that disrupting these interactions promotes matrix remodelling [22]. Other studies have shown that CD44-HA interactions also modify chondrocyte survival and apoptosis [25, 26].

In bone, CD44 is expressed in haematopoietic marrow cells, osteoclasts and osteocytes, and less so in mature osteoblasts and osteoprogenitor cells [27]. CD44 is present at the osteocyte plasma and basolateral membrane, and the cytoplasmic processes of active osteoblasts, but not in osteoclast ruffled borders or clear zones in direct contact with bone [28]. HA-CD44 interactions restrict osteoblast-mediated osteoclastogenesis [29] and may play roles in inter-osteocyte and osteocyte-osteoclast communication [30]. The interactions might also provide 'stop signals' for bone-resorbing osteoclasts [31] and osteoclastogenesis [27–33].

HA biosynthesis by HA synthases

Early pioneering work on the identification and characterisation of the bacterial HA synthase (HAS), and its mechanism of action, provided the basis for identifying the HASs in eukaryotes [34–36]. The bacterial HAS (*has*) operon consists of the *hasA*, *hasB*, and *hasC* genes, which correspond with the HAS (*hasA*) responsible for polymerising HA, UDP-glucose dehydrogenase (*hasB,* UGDH) that converts UDP-glucose to UDP-glucuronic acid, and UDP-glucose pyrophosphorylase (*hasC*, UDPG-Ppase) that forms UDP-glucose from UTP and Glc-1-phosphate. Ablating any of these bacterial *has* genes disrupts HA synthesis and impairs assembly of their protective HA-rich glycocalyx.

Eukaryotic cells possess a similar set of functionally related enzymes for HA biosynthesis [37–41]. The vertebrate genome has three HAS genes, HAS1, HAS2 and HAS3 (Table 1), and separate genes encoding UGDH and UDPG-Ppase. HA synthesis is essential for embryo development and ablation of the *has2* gene in mice is embryo lethal at E9.5 [42], as is UGDH deletion in zebrafish [43]. In contrast, lacking one or both of the *has1* or *has3* genes is not lethal,

Table 1. Hyaluronan synthases (HASs) in humans and mice.

Enzyme	Human gene	Human chromosome	Mouse gene	Mouse chromosome
HAS1	*HAS1*	19q13.4	*has1*	17 A3.2
HAS2	*HAS2*	8g24.12	*has2*	15 D1
HAS3	*HAS3*	16q22.1	*has3*	8 D3

suggesting that *has2* is the critical HAS required for HA biosynthesis in development [44].

HAS enzymes polymerise HA by alternate transfer of UDP-*N*-acetyl-D-glucosamine, and UDP-glucuronic acid, with loss of the UDP-moiety after each sequential addition to the growing HA chain. Unlike other glycosaminoglycans, HA synthesis does not require attachment to a core protein to initiate polymerisation and does not occur within the Golgi. HA is synthesised at the inner face of the plasma membrane and either retained inside the cell [45] or exported to the extracellular matrix, possibly *via* the action of ABC transporters [46]. Nascent HA extruded into the extracellular space can be released, or retained at the cell surface to form the pericellular matrix. Although the mechanism for terminating HA polymerisation is unknown, it is likely to be influenced by substrate availability, intracellular environment, accessory proteins or HAS stability. These same factors could account for the finding that HAS mRNA levels do not always correlate with the levels of HA activity [47]; post-translational phosphorylation of HAS enzymes, as reported for HAS3 [48], could also be important. The third feature that distinguishes HA from other glycosaminoglycans is that HA chains are not modified by *N*-acetyl transferases, sulphotransferases or fucosyltransferases.

The three HAS enzymes share a high degree of sequence and structural homology. They each contain two N-terminal and five C-terminal membrane-spanning domains, and a central cytoplasmic region with approximately 80 % sequence identity. Despite this, HAS enzymes differ in their stability, the rate at which they elongate HA and the length of the HA they produce [49]. The molecular mass of HA produced *in vitro* by cells expressing HAS3 is $10^5 - 10^6$ Da, whereas cells expressing HAS1 and HAS2 can produce HA that is twice that size, and in the case of HAS2, even larger than 2×10^6 Da [49]. The pericellular coats formed by HAS1 transfectants *in vitro* are smaller than those formed by HAS2 and HAS3, which could be due to a reduced stability of the HAS1 enzyme, or it could be a consequence of *in vitro* expression, since the HA polymers produced by HAS3 *in vivo* and *in vitro* are significantly different in molecular mass [49]. The *in vivo* products of HAS activity can be as large as 1×10^7 Da.

HA degradation by HYALs

In vivo, HA is degraded by the action of oxygen free radicals [50] and HYALs [51, 52]. Only the HYALs are reviewed here. Most HA (80–90 %) is cleared from the circulation by endocytosis in the lymph nodes and liver, *via* the HA receptor for endocytosis, HARE [53]. Other HA receptors including CD44, LYVE-1, RHAMM and layilin [54–58] have local, tissue-specific roles in HA endocytosis. This is particularly important in cartilage and bone where HA is immobile and cannot reach the lymphatics. Once internalised, HA is degraded in lysosomes by HYALs and exoglycosidases.

Mammalian HYALs are endo-beta-*N*-acetylhexosaminidases that catalyse the hydrolysis of HA; some also degrade the related glycosaminoglycans, chondroitin sulphate (CS) and dermatan sulphate. Despite their presumed importance for degrading HA in development, morphogenesis, wound healing, inflammation and tumour metastasis, there are large gaps in what is known about these enzymes. For example, *hyal1, hyal2 and hyal3* null mice have been produced [51, 52] but their phenotypes are not published; the modified targeting of HA and CS by HYALs has not been confirmed, and it is unclear whether the putative GPI-linkage in the predicted *HYAL2* cDNA sequence is translated, universally, or only in selected cell types under certain conditions [59, 60].

The family of human HYAL genes include *HYAL1, HYAL2, HYAL3 HYAL4, HYALP1* and *PH-20* (*SPAM-1*, sperm adhesion molecule1). These six genes are clustered on two chromosomes [51] (Table 2). HYAL1 and HYAL2 are ubiquitously expressed and are considered the major HA-degrading enzymes in somatic tissues [52, 59]. HYAL1, together with β-D-glucuronidase and β-hexosaminidase, degrades HA to its constituent monosaccharides [51, 61]. This is the final step in HA degradation and occurs in lysosomes, even though HYAL1 is also found in plasma, synovial fluid and urine. Prior to that, HYAL2 is thought to cleave high molecular mass HA to intermediate-sized fragments of ~20 kDa (50 disaccharides) [59]. Studies in native chondrocytes and transfected cells including HeLa, COS-7 and C6 glioma cells [59, 60] show that HYAL2 expression is restricted to lysosomes, where it has a pH optimum of ~4.0. However, other studies in *Xenopus* [52, 62],

Table 2. Hyaluronidases (HYALs) in humans and mice.

Enzyme	Human gene	Human chromosome	Mouse gene	Mouse chromosome
HYAL1	*HYAL1*	3p21.2 cluster	*hyal1*	9 F1–F2 cluster
HYAL2	*HYAL2*	3p21.2 cluster	*hyal2*	9 F1–F2 cluster
HYAL3	*HYAL3*	3p21.2 cluster	*hyal3*	9 F1–F2 cluster
HYAL4	*HYAL4*	7q31.3 cluster	*hyal4*	6 A2 cluster
PH-20	*SPAM1*	7q31.3 cluster	*Ph-20*	6 A2 cluster
HYALP1	*HYALP1*	7q31.3 cluster	*hyalp1*	6 A2 cluster
			hyal5	6 A2 cluster

transfected HEK293 cells [63–66], and a breast tumour cell line [65] show that HYAL2 is GPI-anchored at the plasma membrane where it exhibits a broader pH spectrum and extracellular HA-degrading activity.

Less is known about the role of HYAL3, HYAL4 and HYALP1 in humans. HYAL3 is expressed in many tissues and there is some evidence from *in vitro* transcription/translation experiments that it has HA-degrading activity [67]. There is no evidence that human HYAL4 can degrade HA. *HYALP1* is an expressed pseudogene with a premature stop codon that blocks translation of the mRNA [68].

Mice have an additional hyaluronidase gene, *hyal5,* which is expressed in the testis [69]. Mouse HYAL1, HYAL2 and HYAL3 are expressed in most tissues [70] and again, HYAL1 and HYAL2 are thought to be the major HA-degrading enzymes. Mouse HYAL3 and HYAL4 have high homology (>75%) with their human orthologues, but there is no evidence that they are active. The mutation that prevents translation of the human *HYALP1* gene is absent in mice, so the rodent gene is translated; however, it is not clear whether the enzyme is active [71, 72]. Finally, transcription of mouse HYALs is complex; *hyal1* is co-transcribed with the downstream genes *Fus2* and *Hyal3* [73] and the *Fus2* gene, which codes for an acetyl transferase, is partially embedded within the *Hyal3* gene [74].

Products of HYAL activity

There is a vast literature describing the effects of HA fragments on numerous cell types and disease processes, including those of the skeletal system. However, there is scant evidence that such fragments exist *in vivo.* Stern and colleagues [75] have proposed elegant pathways detailing how HA fragments with size-specific activities, might be produced by HYALs. The pathways and activities of the fragments are compiled from data showing that HA oligosaccharides of low relative molecular mass (M_r) are inflammatory, angiogenic and immunomodulatory, whereas high M_r HA fragments are non-inflammatory, non-angiogenic and immunomodulatory. The same authors duly report that, to date, evidence for the presence of HA fragments *in vivo* is minimal, and they provide important caveats to bear in mind when reviewing the many published studies on HA fragments [76].

Nevertheless, the sheer volume of data, coupled with the reproducible effects of specific fragments on critical biological processes such as angiogenesis, provide compelling reasons to believe that endogenous fragments exist. A dilemma for researchers at present is knowing how to interpret studies showing increased HYAL expression. One interpretation is that bioactive HA fragments (created by HYALs) are important for the system under study. Another interpretation is that removing HA is important or necessary for the system. The latter interpretation is more compelling when increased HYAL activity is coordinated with decreased HAS activity, as in precartilage condensations of the skeletal elements [77, 78]. Both interpretations could be equally important and there is no reason why they should be mutually exclusive. HA fragments may be short-lived and therefore difficult to detect. However, the few studies that have successfully identified HYAL-mediated HA fragmentation *in vivo* [79] give hope that more evidence will be forthcoming.

Cartilage

HA in cartilage

Adult articular cartilage is avascular, aneural and has very few cells (chondrocytes). Cartilage matrix contains a structural scaffold of type II collagen, and aggrecan densely substituted with CS and keratan sulphate chains that confer a high charge density, creating large hydrodynamic domains and osmotic swelling pressures. The swelling pressure of aggrecan is balanced by the tension in the collagen network, and the balance is essential for the compressive resilience of cartilage. Accordingly, the biomechanical proper-

ties of cartilage rely on an intact collagen network and a high concentration of aggrecan. Aggrecan monomers bind to HA and a small protein, called link protein, forming non-dissociable aggregates ($K_d < 1 \times 10^{-11}$ M) that can contain up to 100 aggrecan monomers ($M_r \sim 2 \times 10^6$ Da per monomer). The molecular mass of the aggregates is therefore vast and limits the opportunity for aggrecan loss from cartilage. Immobilising aggrecan in cartilage is one of most important functions of HA. Aggrecan structure and function has been reviewed elsewhere [80–83].

Newly synthesised HA has a very high molecular mass and although the concentration of HA in human articular cartilage increases with age, the molecular mass decreases ($M_r \sim 3 \times 10^5 - 6 \times 10^5$ Da) [84, 85]. These results suggest that HA in normal cartilage is degraded in a regulated manner during homeostasis and ageing. HA extracted from diseased cartilage is also decreased in size [86].

HASs in cartilage

HAS2 is the major HAS isoform expressed in human and animal articular cartilage and by articular chondrocytes in culture [47, 87, 88]. Human chondrocytes treated with antisense oligonucleotides that block HAS2 function respond by decreasing HA production, pericellular matrix formation and retention of newly synthesised aggrecan [89]. Conversely, overexpression of HAS2 *in vitro* produces a large molecular mass HA that supports pericellular coat formation [49]. Therefore, HAS2 appears to play a key role in producing HA necessary for the organisation and retention of normal cartilage matrix.

HAS3 is also expressed by articular chondrocytes [87, 88, 90] and hypertrophic growth plate chondrocytes [91], although at lower levels than HAS2, and cultured chondrocytes are variably reported to express HAS1 [47, 87–89, 91].

The decreasing levels of HA seen in joints of osteoarthritis (OA) patients can be supplemented by intra-articular injection of HA to provide therapeutic benefits [92, 93]. The benefits of intra-articular HA are consistent with observations of HAS2 down-regulation in an inflammatory model of arthritis [94] and up-regulation of HASs during repair of cartilage defects with autologous chondrocyte transplants [95].

HYALs in cartilage

Human and bovine chondrocytes express *HYAL1, HYAL2* and *HYAL3* [60, 64, 96] and *PH-20* mRNA has been detected in human chondrocytes [97]. We have found that 6-day-old mouse chondrocytes also express high levels of *hyal1, hyal2*, and *hyal3*, and low levels of *PH-20* mRNA (Fig. 1), but that mRNA for *hyal4, hyal5* and *hyalp1* was undetectable.

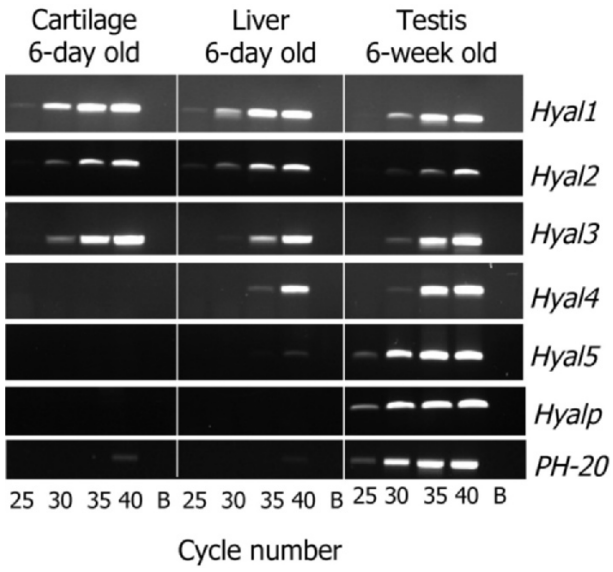

Figure 1. Expression of mouse hyaluronidases (HYALs). Messenger RNA for mouse HYALs was amplified by RT-PCR from extracts of 6-day-old epiphyseal cartilage, 6-day-old liver and 6-week-old testis; B: blank control. Mouse cartilage does not express *hyal4*, *hyal5* or *hyalp*, and *PH-20* was only weakly expressed.

It appears that cartilage HA is degraded by HYALs intracellularly or extracellularly, depending partly on its association with aggrecan. HA free of aggrecan, or HA complexed to sufficiently small aggrecan fragments, is internalised *via* CD44 and degraded within lysosomes [23, 98, 99]. In contrast, HA complexed to intact, or high molecular mass aggrecan is catabolised outside the cell, because it cannot be internalised [100]; the complex is simply too large.

The delivery of HA to lysosomes by CD44-mediated endocytosis is well documented for cartilage, but it is possible that only pericellular HA is endocytosed in this way and that inter-territorial HA, remote from the chondrocyte and without a CD44 attachment, might escape endocytosis. Aggrecan fragments have a long tissue half-life, and whereas large fragments have a half-life of approximately 3 years, smaller fragments, comprising only the G1 domain, survive in cartilage with an extraordinarily long half-life of 20–25 years [101, 102]. These G1 fragments might survive in cartilage because their distance from cell surfaces precludes CD44-medited endocytosis of the HA to which they are bound, or because CD44 receptors occupied by large aggrecan-HA complexes are not available to endocytose smaller complexes. HA is also likely to have a long half-life in cartilage, given that HA-aggrecan-link protein complexes do not dissociate under physiological conditions, and that HA and aggrecan are catabolised with identical kinetics in cartilage explants [103].

HA in cartilage extracts is substantially smaller than newly synthesised HA, and a number of studies suggest that HA might be degraded extracellularly [104–112]. Studies with adult articular cartilage explants show that catabolic stimuli induce concomitant release of HA, link protein and aggrecan G1 fragments into media [104, 105, 112–115], and that the released HA and G1 domains are small. Other *in vitro* studies in growth cartilages have also found evidence of extracellular HA degradation [106–109]. For example, intact aggrecan monomers are released from foetal cartilage without cleavage in the proteinase-sensitive region between G1 and G2 [108], and HA released from IL-1-stimulated foetal cartilage explants is smaller than HA released from unstimulated cultures [109]. Furthermore, electron microscopy studies show that HA-aggrecan aggregates in the hypertrophic zone are smaller than aggregates in other zones of the growth plate, due to a decrease in HA polymer length and a consequent decrease in the number of monomers attached [106, 107]. These studies provide convincing evidence for extracellular degradation of HA, particularly in growth cartilage.

HYALs and HASs in the synovial joint

The major HAS enzymes in human synovium are HAS1 and HAS2 [47]. Substantially more total *HAS* mRNA is expressed by human synovium than by human articular cartilage [47], and HA synthesis by synovium is further increased during inflammatory synovitis. Because of its rheological properties, HA in diarthrodial joints is a lubricant important for minimising friction between adjacent surfaces during articulation.

HA is secreted into the synovial fluid by synovial fibroblasts [116]. In inflammatory joint disease, both the molecular mass and the concentration of HA in synovial fluid is decreased [117–121]. The changes in concentration may be the result of dilution as synovial fluid volume increases [122], HA degradation, altered HA synthesis or a combination. *HYAL2* mRNA is increased in OA and rheumatoid arthritis (RA) synovium, whereas *HAS1/HAS2* mRNA is reduced [111]. *HAS3* mRNA expression is increased in RA synovium. This latter finding is particularly interesting and may explain the significantly lower size and concentration of HA in RA synovium, compared with either OA or normal synovium, as synthesis switches from a *HAS1*- to a *HAS3*-mediated process.

The reduced molecular mass of HA in synovial fluids of patients with arthropathy might exacerbate their disease in several ways. Along with the increase in synovial fluid volume, the reduction in HA molecular mass will decrease synovial fluid viscosity, leading to decreased friction-free articulation and increased cartilage damage. The importance of maintaining synovial fluid HA size and concentration is seen in viscosupplementation studies where intra-articular injection of high M_r HA reduces pain in inflamed joints and promotes wound healing [123–125]. This effect is dependent entirely on the elastoviscous properties of the HA solutions used. However, decreases in HA molecular mass can have other direct effects on chondrocytes and synovial tissues. For example, cartilage explants treated with HA fragments lose aggrecan *via* aggrecanase activity [24] and increase their expression of MMP-13 *via* an NF-κB-dependent signalling pathway [126]. These pro-catabolic effects of HA contrast with their anti-catabolic effects of failing to increase release of IL-1β into culture medium, induce AP-1 activation [127] or induce release of nitric oxide in the presence of IRAP, the IL-1 receptor antagonist [128]. Instead, HA oligosaccharides *in vitro* increase the expression of type II collagen, HAS2 and aggrecan. These apparent anomalies highlight the need for further studies on the role of HA fragments in cartilage biology.

Bone

HA in bone

Although we have known for many years that bone organ cultures produce HA, the precise function of HA in bone is unknown. One reason for this is that, unlike cartilage, HA has no clear structural role in bone. Bone has separate cell types for matrix synthesis (osteoblasts), matrix degradation (osteoclasts) and mechanosensory functions (osteocytes). The formation and resorption of bone is co-ordinated within remodelling, or basic multicellular units [129, 130] and an imbalance in bone formation and resorption underpins many diseases like osteoporosis, where targets for therapy are anti-resorptive and pro-osteogenic. Bone research has therefore focussed on the behaviour of osteoclasts and osteoblasts and most studies have addressed the effects of exogenous HA. The roles of endogenous HA synthesis and degradation are slowly being defined [131]; however, it is important to take care when extrapolating from the effects of exogenous HA.

Effects of HA on osteoclasts

HA is widely thought to have a role in regulating bone turnover, but it is unclear whether the HA that regulates osteoclasts is endogenously produced or derived from other cell types. Links between bone resorption and osteoclast HAS induction have, nevertheless, suggested that HA might have a role in osteoclast binding to bone surfaces and as a diffusion

barrier for the construction of 'sealing zones', both essential processes for bone resorption [131]. Intriguingly, HA taken orally reduces urinary markers of bone resorption and ovariectomy-induced bone loss [132], indicating that HA suppresses bone resorption. If HA is to be exploited therapeutically, the role of HA and the role, if any, of osteoclasts in HA synthesis must be determined [133].

There are some data to suggest that the effect of HA on osteoclasts might depend on the molecular mass of the HA. HA fragments of <8000 Da, but not high M_r HA, induce osteoclastic bone resorption *via* interactions between osteoclast RANK (receptor activator of NF-κB) and osteoblast RANKL (RANK ligand) [134]. In addition, HA synthesis and bone resorption, measured by [^{3}H]glucosamine-labelling and calcium release, respectively, are well correlated [135]. In contrast, studies showing that HA secretion is increased by treatment with resorption inhibitors such as dcAMP or colchicine, suggest that HA might also suppress osteoclast function [136–138]. Exogenous high M_r HA has an anti-osteoclastogenic effect that suppresses M-CSF-dependent pathways controlling RANK signalling *via* Toll-like receptor-4 (TLR-4); this could be an important effect since M-CSF/RANKL governs the differentiation of osteoclasts [139]. This high M_r HA/TLR-4 regulation of osteoclastogenesis is independent of CD44 in contrast to the pro-inflammatory activity of low M_r HA, which is also mediated through TLR-4 and is dependent in part upon CD44 [140]. *In vivo*, exogenous HA reduces RANKL-induced resorption and suggests that HA can restrict osteoclast function [139]. The likelihood that intact HA restricts, but fragmented HA promotes resorption is intriguing and warrants further investigation (Fig. 2).

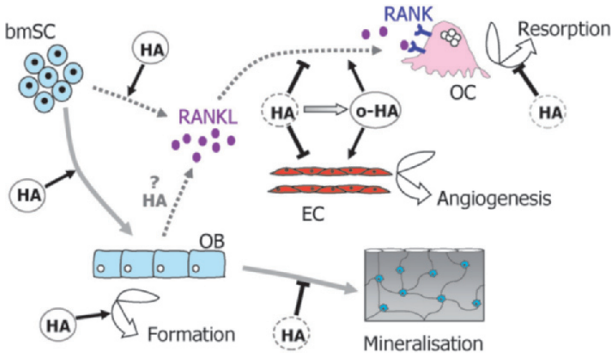

Figure 2. Proposed roles for hyaluronan (HA) and HA fragments in bone. Schematic model of proposed roles for HA in osteoblast (OB) recruitment from bone marrow stromal cell (bmSC) progenitors, bone formation and mineralisation. Roles for intact, high M_r HA (HA) and low M_r HA oligosaccharides (o-HA) in RANKL-related, osteoclast (OC) RANK-mediated promotion of bone resorption and in endothelial cell (EC) angiogenesis are also proposed.

Effects of HA on osteoblasts

In vitro studies measuring colony number, osteoblast proliferation, osteocalcin mRNA levels and alkaline phosphatase have examined the effect of HA on osteogenesis [141–143]. Although they are preliminary, these studies suggest that HA promotes osteogenesis in a manner dependent upon HA mass and concentration. High M_r HA is reportedly osteoinductive *in vivo* [144, 145]; however, this contrasts with another study showing that HA fails to promote bone formation in distraction osteogenesis [146]. Since other glycosaminoglycans might also affect bone, the osteoinductive effects of HA need further investigation and care taken when describing their specificity [147, 148]. Despite the many unknowns, the capacity for HA to modify osteoblast behaviour strongly suggests that HA can influence osteogenesis.

Effects of HA on bone remodelling

Bone remodelling involves activation of resorption, followed by new bone formation, which engages critical, local interactions between osteoblasts and osteoclasts. Non-contact co-cultures of osteoclasts with osteoblasts, or with osteoblast-conditioned medium, show that HA facilitates interactions between osteoblasts and osteoclasts to regulate osteoclast responses to parathyroid hormone (PTH). This is important as PTH stimulates remodelling by promoting formation and resorption, and intermittent PTH treatment increases bone mass, and points to indirect actions of HA on osteoclasts to control remodelling [149]. In addition, HA influences osteoclast progenitor recruitment. HA increases RANKL, but not osteoprotegerin (decoy receptor for RANK) levels in marrow stromal cells, suggesting that it drives osteoclast differentiation. It is possible that HA acts *via* CD44 to increase RANKL in bone marrow stromal cells, and hence remodelling, because anti-CD44 antibodies block these effects, the effects are absent in CD44-null mice, and the CD44-null mice have thicker cortices and smaller medullary areas [150]. Exogenous HA also promotes stromal cell proliferation and osteogenic gene expression [151], indicating a likely role in pre-osteoblast recruitment, proliferation and differentiation and osteoclast crosstalk (Fig. 2).

A cDNA microarray study analysing ligament cells from patients with an ectopic bone formation disease further suggests a role for HA in bone remodelling. This study showed that the HA binding protein, TNFip6 (TNFα-induced protein 6), was down-regulated during osteoblast differentiation and that TNFip6 over-expression could restrict mesenchymal

stem cell differentiation. This effect of TNFip6 was lost in the presence of exogenous HA or TNFip6 lacking the N-terminal HA-binding domain, but only partial loss was seen with TNFip6 lacking the C-terminal domain involved in protein interactions. This interesting study suggests not only that HA-associated TNFip6 controls osteoblast differentiation, but also that HA-binding to TNFip6 is a target for therapeutic intervention in ectopic ossification [152].

Many studies have shown that changes in HA content, synthesis, retention and release, in several bone cell types, disease states and dietary conditions are intimately associated with the control of bone remodelling [153–158]. For example, bone from patients with the brittle bone disease, osteogenesis imperfecta (OI), has three times more HA than normal bone. In addition to suggesting that HA contributes to the increased fragility characteristic of OI bone [159], such studies may provide clues about the function of HA.

Because local diffusion is limited in bone, an extensive vasculature is vital for the delivery of oxygen and nutrients. Vascular invasion is also critical for repair and development and insufficient blood supply is clearly linked with some bone pathologies [160]. Many studies have shown that high M_r HA is a potent anti-angiogenic factor [161–163] and that, since HA can inhibit blood vessel sprouting, HA gradients might provide directional cues for endothelial cells [164]. Conversely, HA oligosaccharides are angiogenic [165]; they increase endothelial cell proliferation, motility and tubule formation [166–169], and activate MAPK signalling in endothelial cells to promote proliferation [170]. High M_r HA does not have the same proliferative effects as HA oligosaccharides, suggesting a possible modulatory link between angiogenesis and osteogenesis in bone diseases such as osteoporosis and Paget's disease.

In hypoxic conditions such as fracture callus, bone cells requiring extra blood supply produce large amounts of angiogenic factors. Hypoxia also promotes HYAL activity, suggesting that HA processing into small, angiogenic fragments might help coordinate bone's response to hypoxia [171]. Indeed, HA as a supplement in bone grafts promotes bone healing [172].

HA synthesis by bone cells

HA synthesis was first identified in bone organ cultures [136, 137]. Both osteoblasts and osteoclasts synthesise HA, and in cultured bone explants treated with PTH, HA accumulates at periosteal surfaces and around osteocytes [173]. HA synthesis by osteoclasts is modulated by pro-resorptive stimuli, including 1,25-(OH)2D3 [174], PTH and glucocorticoids [175].

Isolated osteoblasts synthesise and release HA and intriguingly, the level of release is characteristic of their origin within bone and the method of isolation. For example, cells isolated from layers closer to, or within the bone matrix show the highest rates of HA synthesis [176, 177]; these cells are likely to be osteocytes. Other studies with human osteoblasts show that HA synthesis is also dependent on cell density [178, 179]. Clonal populations of isolated foetal osteoblasts can be distinguished on the basis of population-doubling times, morphology and responsiveness to PTH, as well as on the basis of a distinctive ratio of HA to other glycosaminoglycans that they synthesise [180, 181]. We have found that chick embryo osteoblasts release HA constitutively and express UGDH [182], an enzyme that we and others have found is also capable of regulating HA production [182, 183]. Foetal osteoblasts synthesise HA and increase the size of HA they produce in response to TGF-β [184, 185]. Thus, osteoblast HA synthesis *in vitro* is related to cell origin and conditions during isolation, and it can be regulated by osteogenic stimuli.

One potential function of HA in bone is as a regulator of mineralisation. HA binds hydroxyapatite in calcified cartilage and bone, and although it does not modify mineral growth, it might have a regulatory role in mineralisation [186]. High M_r HA increases osteoblast proliferation and mineralisation, whereas low M_r HA (60 kDa) increases proliferation but not mineralisation [142]. HA synthesis by osteoblasts is mediated by HAS2 and HAS3 [187, 188]. *HAS2* knockdown *in vitro* disrupts HA accumulation, coat formation, cell motility and cell invasiveness [188]. *HAS2* expression is not altered during mineralisation in the MG-63 cell line and the observed decrease in HA production corresponds to a decrease in *HAS3* expression [187]. Thus, endogenous HA synthesis may restrict mineralisation, and its regulation at the level of the HAS is important for the modulation of mineral deposition during osteogenesis.

The most crucial function of bone is to bear load and accordingly, much research is focussed on signalling mechanisms that ensure bone adapts to applied loads [189]. Studies showing that the MLO-Y4 osteocyte line elaborates an HA-rich matrix, essential for flow-induced increases in PGE_2 release, suggest a crucial role for pericellular HA in transducing mechanical signals [190]. Our recent studies have found that primary human osteoblasts also show magnitude-dependent modulation of HA release in response to mechanical strain *in vitro* (Clarkin et al., unpublished data), and we have previously found in other cell types that the supply of monosaccharide, up-stream of HAS (UGDH), can control HA release and pericellular HA coat formation [182]. Thus, HA synthesis by osteoblasts might also be regulated up-stream of HAS

[191]. Since strain induces anti-resorptive osteogenic behaviour, strain-induced HA release and the mechanosensory function of HA-rich osteocyte matrices, might indicate an indirect signalling role for HA in the control of bone remodelling.

Finally, HA acts as a survival factor in the bone marrow of multiple myeloma patients and contributes to pathophysiology [192, 193]. This is important because it is known that multiple myeloma bone disease develops almost exclusively in the marrow and leads to bone destruction by closely apposed osteoclasts. These findings suggests that the significant increases in *HAS1* mRNA and HA secretion seen in the bone marrow mesenchymal progenitor cells of these patients [193] may drive myeloma marrow cell survival or osteoclast bone destruction. This extends the seminal work showing that adherent stromal cells in haematopoietically active marrow produce HA [194].

HYAL in bone

Early studies in immature rat bone demonstrated that all three enzyme activities; HYAL, β-glucuronidase and β-*N*-acetylglucosaminidase required for the degradation of HA in bone were present in lysosomal-like intracellular compartments [195, 196]. At the time, the different mammalian HYALs were unknown, but it is now clear that osteoblasts express *HYAL2*, *HYAL3* and *HYAL4* [187, 197] and are distinct from most other cells for their lack of *HYAL1* expression. HYAL activity in osteoclasts has not been characterised. HYAL gene expression is increased during mineralisation: *HYAL2* increases by 62-fold, *HYAL3* by 13-fold and *HYAL4* expression increasing by 3-fold [187]. Since HYAL2 is thought to degrade HA to ~20-kDa intermediates, increased HYAL2 expression might produce intermediate-sized HA fragments with potential roles in intracellular signalling during mineralisation to promote bone formation [141, 198]. Coupled with the observation that HASs are down-regulated during mineralisation (see above: *Hyaluronan synthesis by bone cells*) the up-regulation of HYALs might have a direct effect of regulating bone matrix HA content. HYALs are also implicated in the disruption of matrix vesicles required for bone mineralisation. The liberation of aggrecan G1 domain fragments and link protein from vesicles is dependent upon HYAL treatment [167]. These results are consistent with the hypothesis that aggrecan is resorbed prior to bone mineralisation, with the decrease in aggrecan content corresponding to the mineralising front [199]. Osteoblasts might therefore play an active role in removing matrix components in preparation for matrix mineralisation during bone formation.

Limb development and morphogenesis

HA-rich pericellular matrices have key roles throughout skeletal embryogenesis, from the initial outgrowth of developing limb mesenchyme to chondrogenesis, joint cavitation and longitudinal bone growth. Dynamic changes in these HA-rich matrices rely on both HA synthesis and degradation.

HYALs and HASs in limb development

Limbs develop when embryonic mesenchyme emerges from the limb bud in a proximo-distal fashion to form the basic limb primordia. The emerging mesenchymal cells proliferate and migrate under the strict control of signals from the apical ectodermal ridge (AER), a zone of polarising activity localised to the distal periphery of the limb bud. HAS2 is abundantly expressed by the AER and distal subridge mesodermal cells [77] which secrete HA-rich matrices to provide highly hydrated paths for mesenchymal migration. The HA-rich matrix also helps separate mesenchymal cells from each other to prevent the cell-cell and/or cell-matrix interactions needed to trigger chondrogenesis [200, 201].

Just before the onset of cartilage formation, precisely at the time of condensation, mesenchymal cells express CD44 [202] and gain the capacity to bind HA [10, 203, 204], concomitant with decreases in intercellular HA levels, down-regulation of HAS2 expression [77], and increased HYAL activity [78]. This suggests that HA must first be removed from precartilage condensations in order for later chondrogenesis to proceed. Over-expression of *has2* in chick limb bud mesoderm interferes with this highly regulated process and results in limbs that are shortened, malformed and lacking in one or more elements [77], confirming that excess HA is detrimental to precartilage condensation. HYALs in prechondrogenic limb mesenchyme are maximally active at pH 2.5–4.5, suggesting they are lysosomal [205]. Resorption of the HA matrix of limb mesenchymal cells is thought to occur *via* receptor-mediated endocytosis and translocation of HA to lysosomes [205, 206]. CD44 is the primary receptor for HA-mediated endocytosis in many systems [79, 207, 208]; however, it is possible that HA endocytosis in prechondrogenic limb mesenchyme is achieved by a receptor other than CD44 given that $CD44^{-/-}$ mice lack a developmental phenotype [209]. Alternatively, transient, acidic microenvironments which exist within extracellular matrices might facilitate extracellular HA catabolism by acid-active HYALs [205]. Evidence for this comes from a study in breast cancer cells, which showed that HA binding to CD44 activates a specific Na^+-H^+ exchanger (ROK/NHE1) that is co-localised with

HYAL2 and CD44 in lipid rafts and is linked to changes in lysosomal pH and extracellular acidification [65]. While local pH gradients might provide favourable environments for low pH enzymes, there is also recent evidence that HYAL2 is active at pH 6.0–7.0 [63].

These findings do not address the role of the HASs in ensuing chondrogenesis. The regulation of HA synthesis at stages after the initiation of chondrogenesis has not been resolved, nor has the relationship between HA synthesis and the synthesis of sulphated glycosaminoglycans that are required for cartilage elaboration.

HYALs and HASs in joint cavity formation

Cells within the developing limb destined, by prepatterning, to form a future synovial joint are known to separate individual chondrogenic regions by 'interzones'. An interzone defines a region that consists of three cell layers comprising two outer intermediate layers either side of an inner central cell layer. Joint cavity formation (cavitation) begins from within the central cell layer of the interzone as a cell-free space is established to separate the adjacent cartilage elements. The cells that form the interzone remain prechondrogenic and produce many factors that actively restrict chondrogenesis prior to cavitation and can be distinguished from adjacent cartilaginous elements by their failure to secrete an aggrecan-rich matrix. Instead, HA is secreted by cells of the interzone prior to and during overt cavitation. This newly synthesised HA is readily detected using a biotinylated HA-binding protein as a probe for free binding sites on the HA polymer [210–212].

The precise mechanisms regulating joint cavity formation are not known and apoptosis may [213, 214], or may not [215, 216], have a role in cavitation. Movement is essential for establishing the joint cavity in developing chicks (reviewed in [217]). Paralysis induced in the developing chick embryo abrogates joint cavity formation and induces joint fusion across the interzone region, associated with increased sulphated proteoglycan, but decreased local HA content [218]. The application of mechanical strain can also induce secretion of large amounts of HA by cells derived from embryonic chick articular surfaces *in vitro*. It is therefore thought that embryo movement promotes local HA secretion into the interzone matrix to facilitate cavitation. HA accumulated in the interzone is thought to reach its full swelling potential by absorbing water, thereby creating a cell-free extracellular space between adjacent cartilage elements. Temporospatial patterns of *has* mRNA expression and *in vitro* studies using cells derived from chick embryonic articular surfaces suggest that *has2* is the major HAS involved in this process [44, 219, 220]. Once formed, fibroblasts at the intimal surface of synovial membranes, which express predominantly HAS1 and HAS2 [47], continue to synthesise high M_r HA.

HYALs and HASs in longitudinal bone growth

After secondary centres of ossification are established in the epiphyses of growing bones, longitudinal growth depends on the rate at which chondrocytes proliferate and mature into hypertrophic cells in the growth plate, which comprises zones of morphologically and biochemically distinct cells. Cells in the resting zone are round and sparse, relatively quiescent and surrounded by a voluminous matrix. Chondrocytes in the proliferative zone are flattened and stacked in ordered columns, separated by longitudinal septae. Cells in the prehypertrophic zone retain this columnar organisation, and as they hypertrophy (hypertrophic zone) begin to increase their metabolic activity and enlarge by as much as tenfold [221] to occupy up to 60 % of the tissue volume [222]. The terminally differentiated hypertrophic chondrocytes are eventually removed in the erosion zone at the chondro-osseous border. The hypertrophic zone contains higher HA concentrations than resting or proliferative zones [223, 224], and this is associated with increasing levels of calcium and phosphate, presumably in preparation for mineralisation [225].

As hypertrophic chondrocytes enlarge [221] to occupy an increased tissue volume [222], the HA content also increases. Pavasant et al. [223] showed that this HA accumulates in the pericellular space surrounding the hypertrophic chondrocyte in its lacunae, leading the authors to postulate that HA swelling pressures in this space contribute directly to hypertrophic expansion and impact directly on bone growth, and hence overall body stature. Conversely, aberrant HA production could lead to abnormal or insufficient hypertrophic expansion and might contribute to the phenotype in some skeletal dysplasias [223]. It is not clear which synthases produce HA in human hypertrophic chondrocytes; however, *has2* and *has3* mRNAs are maximally expressed in prehypertrophic rabbit growth plate chondrocytes [226] and *has1–3* are detected by *in situ* hybridisation in hypertrophic chondrocytes of mouse growth plate [91]. The growth plate is highly dynamic; within each zone there is extensive remodelling and it appears that in the hypertrophic zone, HA production and degradation proceed simultaneously. Electron microscopy studies have shown that aggrecan aggregates in the hypertrophic zone are smaller than in other zones, due to a decrease in the length of HA to which aggrecan is bound [106, 107]. Other studies show that HA released from rabbit growth plate chondrocytes during *in vitro* differentiation has a broad ranging

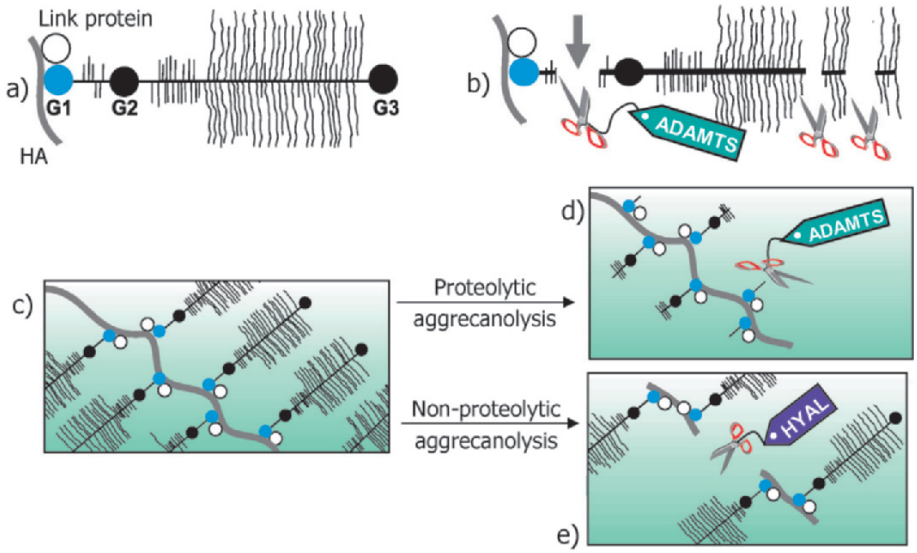

Figure 3. Proteolytic and non-proteolytic mechanisms of aggrecanolysis. (*a*) Intact aggrecan monomer showing the G1, G2 and G3 globular domains, with the N-terminal G1 domain bound to HA and link protein. Keratan sulphate chains are represented as short straight lines attached to the core protein; chondroitin sulphate chains are represented as longer wavy lines. (*b*) Proteolysis of an aggrecan monomer by ADAMTS enzymes. Cleavage in the region between the G1 and G2 domains is most detrimental for cartilage function. (*c*) Aggrecan aggregate showing aggrecan monomers and link protein assembled on a single HA polymer. (*d*) Fragments of degraded aggrecan monomers bound to intact HA. (*e*) Intact aggrecan monomers bound to short (degraded) HA.

molecular mass [226]. Since *hyal1* and *hyal2* are expressed in the proliferative and hypertrophic zones of rabbit growth plate [227], these results suggest that HYALs, and synthases producing high M_r HA, are active concurrently.

Depolymerisation of HA by HYALs in growth plate cartilage will simultaneously depolymerise the HA-aggrecan-link protein aggregates. HYALs therefore provide a non-proteolytic mechanism for degrading aggrecan aggregates and facilitating their loss from cartilage (Fig. 3). The early literature records some controversy over whether aggrecan is [228–231] or is not [232–235] lost from the hypertrophic zone just before cartilage calcifies. However, it is now clear that aggrecan is lost from the hypertrophic zone [236], and clear that, although the concentration of aggrecan increases [110, 236], there is a net loss of aggrecan before calcification [236]. A number of recent studies suggest that aggrecan loss from hypertrophic cartilage might not involve the aggrecanases or MMPs traditionally associated with aggrecanolysis. Analyses of mice with knockin [237, 238] or knockout [239–241] mutations show that ablation of aggrecanase activity [238, 239, 241, 242], or MMP activity [237] does not disrupt growth plate organisation or bone formation. Some strains of mice showed differences in their susceptibility to experimental arthritis [238, 239, 241], but surprisingly, all the strains developed normally without skeletal deformity. These results challenge the prevailing dogma about aggrecan degradation in growth cartilage, and suggest that aggrecan loss from the growth plate might not be driven by the same proteolytic mechanisms that drive aggrecan loss from articular cartilage in joint disease. Instead, these data are consistent with emerging evidence that there might be "non-proteolytic" mechanisms for aggrecanolysis in epiphyseal cartilage [109] that involve hyaluronidases [108, 110] (Fig. 3).

Extensive growth plate remodelling occurs in the lower hypertrophic zone, where blood vessels from the metaphysis break through the transverse septa into the hypertrophic zone lacunae. This is followed by the appearance of osteoblasts that deposit osteoid that will mineralise to form bone. Immunohistochemical studies show that osteoprogenitor cells at the metaphyseal border endocytose HA *via* CD44 for degradation in lysosomes [33]. Hypertrophic chondrocytes, osteoclasts and septoclasts adjacent to invading capillaries [243] might also degrade HA. Septoclasts in particular appear to secrete lysosomal enzymes including cathepsin-B [244]. Indeed, their abundant lysosomal content makes it tempting to speculate that septoclasts, as well as cells in the lower hypertrophic zone, are responsible for local HYAL release and degradation of HA-aggrecan aggregates by lysosomal exocytosis. Lysosomal exocytosis occurs when conventional lysosomes (distinct from the specialised secretory lysosomes found in haematopoietic cells and osteoclasts [245]) fuse with the plasma membrane in response to cell wounding in a calcium-dependent process thought to be a repair mechanism for patching holes in cell membranes [246–251]. During hyper-

trophy, chondrocytes expand their volume substantially over a short period of 3–4 days, experiencing rapid increases in stretch-induced load. This mechanical challenge might induce lysosomal exocytosis and release of HYALs in hypertrophic chondrocytes [252]. The invasion of blood vessels into the growth plate is critical for initiating bone formation. Vessel ingrowth delivers new mesenchymal cells that differentiate into osteoblasts that secrete bone matrix. The presence of low molecular mass HA fragments in the medium of differentiating chondrocytes *in vitro* is intriguing considering that (exogenous) fragments of HA are angiogenic [165, 166, 253–255]. It is possible that, in addition to secreting angiogenic factors such as VEGF (vascular endothelial growth factor) [256], cells at the bottom of the growth plate might stimulate capillary invasion into the hypertrophic zone by inducing HYAL activity that generates low M_r HA fragments. This is consistent with the synergistic effects of VEGF and exogenous HA oligosaccharides on the angiogenic response in bovine microvascular endothelial cells [257] and it is tempting to hypothesise that hypertrophic chondrocytes might also respond synergistically to endogenous HA fragments and VEGF. Although deficiencies in HYALs are not widely reported, there are two patients (siblings) with mucopolysaccharidosis type IX, a lysosomal storage diseases caused by a mutation in the *HYAL1* gene [258, 259]. The phenotype in these patients is mild, and includes periarticular soft-tissue masses, erosions of the acetabulum and short stature [258]. The short stature is consistent with an impaired ability to resorb HA-aggrecan aggregates in the growth plate, leading to abnormal or delayed cartilage resorption and defective ossification and growth of long bones.

Summary

For such a simple molecule, HA is remarkable for its diversity of functions, its distribution across species and its non-Newtonian physical properties. HA links cells with their extracellular environment by binding to CD44 and when the linkages are disrupted, cells respond by modulating gene expression and matrix remodelling. HA is produced as large polymers by HASs located at the cell membrane, and it is degraded by HYALs. Selected HYALs can, in collaboration with lysosomal exoglycosidases, degrade HA to its monosaccharide constituents; others depolymerise large HA polymers to fragments of variable sizes. HA fragments *in vitro* have bioactivities that are distinct from the parent molecule, and efforts to identify such fragments *in vivo*, and to confirm their bioactivities, are in progress. The biochemical properties of the HYALs vary and might depend on cell, tissue, age, disease or development; these variable properties include their pH optima, substrate specificity, and their site of expression within the cell.

HA production by HASs is essential for the development, and the function, of synovial joints. In development, HAS2 expression at the AER generates HA that provides hydrated paths for the migration of mesenchymal cells, and separates cells to prevent cell-cell interactions that trigger chondrogenesis. Indeed chondrogenesis requires removal of HA, and the down-regulation of HAS activity, up-regulation of HYAL activity and increased expression of CD44 are coordinated to achieve this. Failure to remove excess HA prior to chondrogenesis *in vivo* results in shortened and malformed limbs. Skeletal growth occurs in the growth plate where dynamic synthesis and degradation of HA occur simultaneously to continuously remodel growing bones. The angiogenic properties of exogenous HA oligosaccharides seen *in vitro* could be important for directing vascular invasion into the growth plate, and also for bone healing.

In the adult, high M_r HA facilitates lubrication and articulation in the joint space; it immobilises and concentrates aggrecan in articular cartilage to achieve high compressive resilience and in bone, it is thought to suppress resorption and limit mineralisation. Promoting HA synthesis as an adjunct therapy for managing joint diseases might therefore have efficacy in stabilising cartilage and bone matrices and increasing friction-free movement.

Although much is known about where and when HA is produced and degraded, much remains to be learned about the activities of the HASs and HYALs and how they are regulated. Future studies with targeted mutations in the HAS and HYAL genes in mice will provide clearer insights into the functions of these important enzymes.

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1 Gribbon, P., Heng, B. C. and Hardingham, T. E. (1999) The molecular basis of the solution properties of hyaluronan investigated by confocal fluorescence recovery after photobleaching. Biophys. J. 77, 2210–2216.
2 Gribbon, P., Heng, B. C. and Hardingham, T. E. (2000) The analysis of intermolecular interactions in concentrated hyaluronan solutions suggest no evidence for chain-chain association. Biochem. J. 350, 329–335.

3 Almond, A., Brass, A. and and Sheehan, J. K. (1998) Deducing polymeric structure from aqueous molecular dynamics simulations of oligosaccharides: Predictions from simulations of hyaluronan tetrasaccharides compared with hydrodynamic and X-ray fibre diffraction data. J. Mol. Biol. 284, 1425–1437.
4 Almond, A., Brass, A. and Sheehan, J. K. (1998) Dynamic exchange between stabilized conformations predicted for hyaluronan tetrasaccharides: Comparison of molecular dynamics simulations with available NMR data. Glycobiology 8, 973–980.
5 Scott, J. E. and Heatley, F. (1999) Hyaluronan forms specific stable tertiary structures in aqueous solution: A 13C NMR study. Proc. Natl. Acad. Sci. U. S.A 96, 4850–4855.
6 Cooper, N. J., Mugford, M., Symmons, D. P., Barrett, E. M. and Scott, D. G. (2002) Total costs and predictors of costs in individuals with early inflammatory polyarthritis: A community-based prospective study. Rheumatology (Oxford) 41, 767–774.
7 Clarris, B. J. and Fraser, J. R. (1968) On the pericellular zone of some mammalian cells *in vitro.* Exp. Cell Res. 49, 181–193.
8 Underhill, C. B. and Toole, B. P. (1982) Transformation-dependent loss of the hyaluronate-containing coats of cultured cells. J. Cell Physiol. 110, 123–128.
9 Goldberg, R. L. and Toole, B. P. (1984) Pericellular coat of chick embryo chondrocytes: Structural role of hyaluronate. J. Cell Biol. 99, 2114–2122.
10 Knudson, C. B. and Toole, B. P. (1985) Changes in the pericellular matrix during differentiation of limb bud mesoderm. Dev. Biol. 112, 308–318.
11 McGuire, P. G., Castellot, J. J. Jr. and Orkin, R. W. (1987) Size-dependent hyaluronate degradation by cultured cells. J. Cell Physiol. 133, 267–276.
12 Knudson, W. and Knudson, C. B. (1991) Assembly of a chondrocyte-like pericellular matrix on non-chondrogenic cells. Role of the cell surface hyaluronan receptors in the assembly of a pericellular matrix. J. Cell Sci. 99, 227–235.
13 Heldin, P. and Pertoft, H. (1993) Synthesis and assembly of the hyaluronan-containing coats around normal human mesothelial cells. Exp. Cell Res. 208, 422–429.
14 Lu Valle, P., Iwamoto, M., Fanning, P., Pacifici, M. and Olsen, B. R. (1993) Multiple negative elements in a gene that codes for an extracellular matrix protein, collagen X, restrict expression to hypertrophic chondrocytes. J. Cell Biol. 121, 1173–1179.
15 Madry, H. and Trippel, S. B. (2000) Efficient lipid-mediated gene transfer to articular chondrocytes. Gene Ther. 7, 286–291.
16 Clarris, B. J. and Fraser, J. R. (1968) On the pericellular zone of some mammalian cells *in vitro.* Exp. Cell Res. 49, 181–193.
17 Knudson, W., Aguiar, D. J., Hua, Q. and Knudson, C. B. (1996) CD44-anchored hyaluronan-rich pericellular matrices: An ultrastructural and biochemical analysis. Exp. Cell Res. 228, 216–228.
18 Knudson, C. B. (1993) Hyaluronan receptor-directed assembly of chondrocyte pericellular matrix. J. Cell Biol. 120, 825–834.
19 Screaton, G. R., Bell, M. V., Jackson, D. G., Cornelius, F. B., Gerth, U. and Bell, J. I. (1992) Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. Proc. Natl. Acad. Sci. USA 89, 12160–12164.
20 Yu, Q. and Toole, B. P. (1996) A new alternatively spliced exon between v9 and v10 provides a molecular basis for synthesis of soluble CD44. J. Biol Chem. 271, 20603–20607.
21 Salter, D. M., Godolphin, J. L., Gourlay, M. S., Lawson, M. F., Hughes, D. E. and Dunne, E. (1996) Analysis of human articular chondrocyte CD44 isoform expression and function in health and disease. J. Pathol. 179, 396–402.
22 Knudson, C. B. and Knudson, W. (2004) Hyaluronan and CD44: Modulators of chondrocyte metabolism. Clin. Orthop. Relat. Res. S152–162.
23 Chow, G., Knudson, C. B., Homandberg, G. and Knudson, W. (1995) Increased expression of CD44 in bovine articular chondrocytes by catabolic cellular mediators. J. Biol. Chem. 270, 27734–27741.
24 Knudson, W., Casey, B., Nishida, Y., Eger, W., Kuettner, K. E. and Knudson, C. B. (2000) Hyaluronan oligosaccharides perturb cartilage matrix homeostasis and induce chondrocytic chondrolysis. Arthritis Rheum. 43, 1165–1174.
25 Takahashi, K., Hashimoto, S., Kubo, T., Hirasawa, Y., Lotz, M. and Amiel, D. (2000) Effect of hyaluronan on chondrocyte apoptosis and nitric oxide production in experimentally induced osteoarthritis. J. Rheumatol. 27, 1713–1720.
26 Lisignoli, G., Grassi, F., Zini, N., Toneguzzi, S., Piacentini, A., Guidolin, D., Bevilacqua, C. and Facchini, A. (2001) Anti-Fas-induced apoptosis in chondrocytes reduced by hyaluronan: Evidence for CD44 and CD54 (intercellular adhesion molecule 1) involvement. Arthritis Rheum. 44, 1800–1807.
27 Noonan, K. J., Stevens, J. W., Tammi, R., Tammi, M., Hernandez, J. A. and Midura, R. J. (1996) Spatial distribution of CD44 and hyaluronan in the proximal tibia of the growing rat. J. Orthop. Res. 14, 573–581.
28 Nakamura, H., Kenmotsu, S., Sakai, H. and Ozawa, H. (1995) Localization of CD44, the hyaluronate receptor, on the plasma membrane of osteocytes and osteoclasts in rat tibiae. Cell Tissue Res. 280, 225–233.
29 Fujii, Y., Fujii, K., Nakano, K. and Tanaka, Y. (2003) Crosslinking of CD44 on human osteoblastic cells upregulates ICAM-1 and VCAM-1. F. E.B. S. Lett. 539, 45–50.
30 Yamazaki, M., Nakajima, F., Ogasawara, A., Moriya, H., Majeska, R. J. and Einhorn, T. A. (1999) Spatial and temporal distribution of CD44 and osteopontin in fracture callus. J. Bone Joint Surg. Br. 81, 508–515.
31 Spessotto, P., Rossi, F. M., Degan, M., Di Francia, R., Perris, R., Colombatti, A. and Gattei, V. (2002) Hyaluronan-CD44 interaction hampers migration of osteoclast-like cells by down-regulating MMP-9. J. Cell Biol. 158, 1133–1144.
32 Sterling, H., Saginario, C. and Vignery, A. (1998) CD44 occupancy prevents macrophage multinucleation. J. Cell Biol. 143, 837–847.
33 Pavasant, P., Shizari, T. M. and Underhill, C. B. (1994) Distribution of hyaluronan in the epiphysial growth plate: Turnover by CD44-expressing osteoprogenitor cells. J. Cell Sci. 107, 2669–2677.
34 DeAngelis, P. L., Papaconstantinou, J. and Weigel, P. H. (1993) Molecular cloning, identification, and sequence of the hyaluronan synthase gene from group A *Streptococcus pyogenes.* J. Biol Chem. 268, 19181–19184.
35 Dougherty, B. A. and van de Rijn, I. (1994) Molecular characterization of hasA from an operon required for hyaluronic acid synthesis in group A steptococci. J. Biol. Chem. 269, 169–175.
36 Weigel, P. H., Hascall, V. C. and Tammi, M. (1997) Hyaluronan synthases. J. Biol. Chem. 272, 13997–14000.
37 Watanabe, K. and Yamaguchi, Y (1996) Molecular identification of a putative human hyaluronan synthase. J. Biol. Chem. 271, 22945–22948.
38 Spicer, A. P., Augustine, M. L. and McDonald, J. A. (1996) Molecular cloning and characterization of a putative mouse hyaluronan synthase. J. Biol. Chem. 271, 23400–23406.
39 Shyjan, A. M., Heldin, P., Butcher, E. C., Yoshino, T. and Briskin, M. J. (1996) Functional cloning of the cDNA for a human hyaluronan synthase. J. Biol. Chem. 271, 23395–23399.
40 Itano, N. and Kimata, K. (1996) Expression cloning and molecular characterization of HAS protein, a eukaryotic hyaluronan synthase. J. Biol. Chem. 271, 9875–9878.
41 Itano, N. and Kimata, K. (1996) Molecular cloning of human hyaluronan synthase. Biochem. Biophys. Res. Commun. 222, 816–820.
42 Camenisch, T. D., Spicer, A. P., Brehm-Gibson, T., Biesterfeldt, J., Augustine, M. L., Calabro, A., Jr., Kubalak, S., Klewer, S. E. and McDonald, J. A. (2000) Disruption of

hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. J. Clin. Invest. 106, 349–360.
43 Walsh, E. C. and Stainier, D. Y. (2001) UDP-glucose dehydrogenase required for cardiac valve formation in zebrafish. Science 293, 1670–1673.
44 Tien, J. Y. and Spicer, A. P. (2005) Three vertebrate hyaluronan synthases are expressed during mouse development in distinct spatial and temporal patterns. Dev. Dyn. 233, 130–141.
45 Hascall, V. C., Majors, A. K., De La Motte, C. A., Evanko, S. P., Wang, A., Drazba, J. A., Strong, S. A. and Wight, T. N. (2004) Intracellular hyaluronan: A new frontier for inflammation? Biochim. Biophys. Acta 1673, 3–12.
46 Schulz, T., Schumacher, U. and Prehm, P. (2007) Hyaluronan export by the A. B.C transporter M. R.P5 and its modulation by intracellular cGMP. J. Biol. Chem. 282, 20999–21004.
47 Recklies, A. D., White, C., Melching, L. and Roughley, P. J. (2001) Differential regulation and expression of hyaluronan synthases in human articular chondrocytes, synovial cells and osteosarcoma cells. Biochem. J. 354, 17–24.
48 Goentzel, B. J., Weigel, P. H. and Steinberg, R. A. (2006) Recombinant human hyaluronan synthase 3 is phosphorylated in mammalian cells. Biochem. J. 396, 347–354.
49 Itano, N., Sawai, T., Yoshida, M., Lenas, P., Yamada, Y., Imagawa, M., Shinomura, T., Hamaguchi, M., Yoshida, Y., Ohnuki, Y., Miyauchi, S., Spicer, A. P., McDonald, J. A. and Kimata, K. (1999) Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties. J. Biol. Chem. 274, 25085–25092.
50 Schiller, J., Fuchs, B., Arnhold, J. and Arnold, K. (2003) Contribution of reactive oxygen species to cartilage degradation in rheumatic diseases: Molecular pathways, diagnosis and potential therapeutic strategies. Curr. Med. Chem. 10, 2123–2145.
51 Csoka, A. B., Frost, G. I. and Stern, R. (2001) The six hyaluronidase-like genes in the human and mouse genomes. Matrix Biol. 20, 499–508.
52 Lepperdinger, G., Mullegger, J. and Kreil, G. (2001) Hyal2 – less active, but more versatile? Matrix Biol. 20, 509–514.
53 Weigel, J. A. and Weigel, P. H. (2003) Characterization of the recombinant rat 175-kDa hyaluronan receptor for endocytosis (HARE). J. Biol. Chem. 278, 42802–42811.
54 Lesley, J., Hyman, R. and Kincade, P. W. (1993) CD44 and its interaction with extracellular matrix. Adv. Immunol. 54, 271–335.
55 Hayflick, J. S., Kilgannon, P. and Gallatin, W. M. (1998) The intercellular adhesion molecule (ICAM) family of proteins. New members and novel functions. Immunol. Res. 17, 313–327.
56 Turley, E. A., Belch, A. J., Poppema, S. and Pilarski, L. M. (1993) Expression and function of a receptor for hyaluronan-mediated motility on normal and malignant B lymphocytes. Blood 81, 446–453.
57 Banerji, S., Ni, J., Wang, S. X., Clasper, S., Su, J., Tammi, R., Jones, M. and Jackson, D. G. (1999) L.Y.V.E.-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. J. Cell Biol. 144, 789–801.
58 Bono, P., Rubin, K., Higgins, J. M. and Hynes, R. O. (2001) Layilin, a novel integral membrane protein, is a hyaluronan receptor. Mol. Biol. Cell 12, 891–900.
59 Lepperdinger, G., Strobl, B. and Kreil, G. (1998) HYAL2, a human gene expressed in many cells, encodes a lysosomal hyaluronidase with a novel type of specificity. J. Biol. Chem. 273, 22466–22470.
60 Chow, G., Knudson, C. B. and Knudson, W. (2006) Expression and cellular localization of human hyaluronidase-2 in articular chondrocytes and cultured cell lines. Osteoarthritis Cartilage 14, 849–858.
61 Stern, R (2003) Devising a pathway for hyaluronan catabolism: Are we there yet? Glycobiology 13, 105R-115R.
62 Mullegger, J. and Lepperdinger, G. (2002) Degradation of hyaluronan by a Hyal2-type hyaluronidase affects pattern formation of vitelline vessels during embryogenesis of *Xenopus laevis*. Mech. Dev. 111, 25–35.
63 Harada, H. and Takahashi, M. (2007) CD44-dependent intracellular and extracellular catabolism of hyaluronic acid by hyaluronidase-1 and -2. J. Biol. Chem. 282, 5597–5607.
64 Chow, G. and Knudson, W. (2005) Characterization of promoter elements of the human HYAL-2 gene. J. Biol. Chem. 280, 26904–26912.
65 Bourguignon, L. Y., Singleton, P. A., Diedrich, F., Stern, R. and Gilad, E. (2004) CD44 interaction with Na^+-H^+ exchanger (NHE1) creates acidic microenvironments leading to hyaluronidase-2 and cathepsin B activation and breast tumor cell invasion. J. Biol. Chem. 279, 26991–27007.
66 Rai, S. K., Duh, F. M., Vigdorovich, V., Danilkovitch-Miagkova, A., Lerman, M. I. and Miller, A. D. (2001) Candidate tumor suppressor HYAL2 is a glycosylphosphatidylinositol (GPI)-anchored cell-surface receptor for jaagsiekte sheep retrovirus, the envelope protein of which mediates oncogenic transformation. Proc. Natl. Acad. Sci. USA 98, 4443–4448.
67 Lokeshwar, V. B., Schroeder, G. L., Carey, R. I., Soloway, M. S. and Iida, N. (2002) Regulation of hyaluronidase activity by alternative mRNA splicing. J. Biol. Chem. 277, 33654–33663.
68 Csoka, A. B., Scherer, S. W. and Stern, R. (1999) Expression analysis of six paralogous human hyaluronidase genes clustered on chromosomes 3p21 and 7q31. Genomics 60, 356–361.
69 Kim, E., Baba, D., Kimura, M., Yamashita, M., Kashiwabara, S. and Baba, T. (2005) Identification of a hyaluronidase, Hyal5, involved in penetration of mouse sperm through cumulus mass. Proc. Natl. Acad. Sci. USA 102, 18028–18033.
70 Kim, E., Baba, D., Kimura, M., Yamashita, M., Kashiwabara, S. and Baba, T. (2005) Identification of a hyaluronidase, Hyal5, involved in penetration of mouse sperm through cumulus mass. Proc. Natl. Acad. Sci. USA 102, 18028–18033.
71 Miller, K. A., Shao, M. and Martin-DeLeon, P. A. (2007) Hyalp1 in murine sperm function: Evidence for unique and overlapping functions with other reproductive hyaluronidases. J. Androl. 28, 67–76.
72 Reitinger, S., Laschober, G. T., Fehrer, C., Greiderer, B. and Lepperdinger, G. (2007) Mouse testicular hyaluronidase-like proteins SPAM1 and HYAL5 but not HYALP1 degrade hyaluronan. Biochem. J. 401, 79–85.
73 Baba, D., Kashiwabara, S., Honda, A., Yamagata, K., Wu, Q., Ikawa, M., Okabe, M. and Baba, T. (2002) Mouse sperm lacking cell surface hyaluronidase PH-20 can pass through the layer of cumulus cells and fertilize the egg. J. Biol. Chem. 277, 30310–30314.
74 Shuttleworth, T. L., Wilson, M. D., Wicklow, B. A., Wilkins, J. A. and Triggs-Raine, B. L. (2002) Characterization of the murine hyaluronidase gene region reveals complex organization and cotranscription of Hyal1 with downstream genes, Fus2 and Hyal3. J. Biol. Chem. 277, 23008–23018.
75 Stern, R. (2004) Hyaluronan catabolism: A new metabolic pathway. Eur. J. Cell Biol. 83, 317–325.
76 Stern, R., Asari, A. A. and Sugahara, K. N. (2006) Hyaluronan fragments: An information-rich system. Eur. J. Cell Biol. 85, 699–715.
77 Li, Y., Toole, B. P., Dealy, C. N. and Kosher, R. A. (2007) Hyaluronan in limb morphogenesis. Dev. Biol. 305, 411–420.
78 Oster, G. F., Murray, J. D. and Maini, P. K. (1985) A model for chondrogenic condensations in the developing limb: The role of extracellular matrix and cell tractions. J. Embryol. Exp. Morphol. 89, 93–112.
79 Teder, P., Vandivier, R. W., Jiang, D., Liang, J., Cohn, L., Pure, E., Henson, P. M. and Noble, P. W. (2002) Resolution of lung inflammation by CD44. Science 296, 155–158.
80 Dudhia, J. (2005) Aggrecan, aging and assembly in articular cartilage. Cell. Mol. Life Sci. 62, 2241–2256.

81 Roughley, P. J. (2006) The structure and function of cartilage proteoglycans. Eur. Cell Mater. 12, 92–101.
82 Mörgelin, M., Heinegård, D., Engel, J. and Paulsson, M. (1994) The cartilage proteoglycan aggregate: Assembly through combined protein-carbohydrate and protein-protein interactions. Biophys. Chem. 50, 113–128.
83 Hardingham, T. E. and Fosang, A. J. (1992) Proteoglycans: Many forms, many functions. FASEB J. 6, 861–870.
84 Holmes, M. W. A., Bayliss, M. T. and Muir, H. (1988) Hyaluronic acid in human articular cartilage. Biochem. J. 250, 435–441.
85 Bayliss, M. T. and Dudhia, J. (2002) Hyaluronan synthesis in human articular cartilage. In: Hyaluronan, vol. 2, pp. 297–302, Kennedy, J., Phillips, G., Williams, P. and Hascall, V. (eds.), MFK Group Ltd, Cambridge.
86 Pita, J. C., Muller, F. J., Manicourt, D. H., Buckwalter, J. A. and Ratcliffe, A. (1992) Early matrix changes in experimental osteoarthritis and joint disuse atrophy. In: Articular Cartilage and Osteoarthritis, pp. 455–470, Kuettner, K. E., Schleyerbach, R., Peyron, J. G. and Hascall, V. C. (eds.), Raven press, New York.
87 Hiscock, D. R., Caterson, B. and Flannery, C. R. (2000) Expression of hyaluronan synthases in articular cartilage. Osteoarthritis Cartilage 8, 120–126.
88 Ohno, S., Tanimoto, K., Fujimoto, K., Ijuin, C., Honda, K., Tanaka, N., Doi, T., Nakahara, M. and Tanne, K. (2001) Molecular cloning of rabbit hyaluronic acid synthases and their expression patterns in synovial membrane and articular cartilage. Biochim. Biophys. Acta 1520, 71–78.
89 Nishida, Y., Knudson, C. B., Nietfeld, J. J., Margulis, A. and Knudson, W. (1999) Antisense inhibition of hyaluronan synthase-2 in human articular chondrocytes inhibits proteoglycan retention and matrix assembly. J. Biol. Chem. 274, 21893–21899.
90 Schagemann, J. C., Mrosek, E. H., Landers, R., Kurz, H. and Erggelet, C. (2006) Morphology and function of ovine articular cartilage chondrocytes in 3-D hydrogel culture. Cells Tissues Organs 182, 89–97.
91 Takada, Y., Sakiyama, H., Kuriiwa, K., Masuda, R., Inoue, N., Nakagawa, K., Itano, N., Saito, T., Yamada, T. and Kimata, K. (1999) Metabolic activities of partially degenerated hypertrophic chondrocytes: Gene expression of hyaluronan synthases. Cell Tissue Res. 298, 317–325.
92 Lohmander, L. S., Dalen, N., Englund, G., Hamalainen, M., Jensen, E. M., Karlsson, K., Odensten, M., Ryd, L., Sernbo, I., Suomalainen, O. and Tegnander, A. (1996) Intra-articular hyaluronan injections in the treatment of osteoarthritis of the knee: A randomised, double blind, placebo controlled multicentre trial. Hyaluronan Multicentre Trial Group. Ann. Rheum. Dis. 55, 424–431.
93 Tamir, E., Robinson, D., Koren, R., Agar, G. and Halperin, N. (2001) Intra-articular hyaluronan injections for the treatment of osteoarthritis of the knee: A randomized, double blind, placebo controlled study. Clin. Exp. Rheumatol. 19, 265–270.
94 Kydd, A. S., Reno, C. R., Tsao, H. W. and Hart, D. A. (2007) Early inflammatory arthritis in the rabbit: The influence of intraarticular and systemic corticosteroids on mRNA levels in connective tissues of the knee. J. Rheumatol. 34, 130–139.
95 Mrosek, E., Erggelet, C., McDonald, J. A. and Kurz, H. (2003) Hyaluronan synthases in normal and regenerating joint cartilage. Cells Tissues Organs 173, 93–104.
96 Flannery, C. R., Little, C. B., Hughes, C. E. and Caterson, B. (1998) Expression and activity of articular cartilage hyaluronidases. Biochem. Biophys. Res. Commun. 251, 824–829.
97 El Hajjaji, H., Cole, A. A. and Manicourt, D. H. (2005) Chondrocytes, synoviocytes and dermal fibroblasts all express P. H.-20, a hyaluronidase active at neutral pH. Arthritis Res. Ther. 7, R756–768.
98 Hua, Q., Knudson, C. B. and Knudson, W. (1993) Internalization of hyaluronan by chondrocytes occurs *via* receptor-mediated endocytosis. J. Cell Sci. 106, 365–375.
99 Aguiar, D. J., Knudson, W. and Knudson, C. B. (1999) Internalization of the hyaluronan receptor CD44 by chondrocytes. Exp. Cell Res. 252, 292–302.
100 Embry, J. J. and Knudson, W. (2003) G1 domain of aggrecan cointernalizes with hyaluronan *via* a CD44-mediated mechanism in bovine articular chondrocytes. Arthritis Rheum. 48, 3431–3441.
101 Maroudas, A., Bayliss, M. T., Uchitel-Kaushansky, N., Schneiderman, R. and Gilav, E. (1998) Aggrecan turnover in human articular cartilage: Use of aspartic acid racemization as a marker of molecular age. Arch. Biochem. Biophys. 350, 61–71.
102 Verzijl, N., DeGroot, J., Bank, R. A., Bayliss, M. T., Bijlsma, J. W., Lafeber, F. P., Maroudas, A. and TeKoppele, J. M. (2001) Age-related accumulation of the advanced glycation endproduct pentosidine in human articular cartilage aggrecan: The use of pentosidine levels as a quantitative measure of protein turnover. Matrix Biol. 20, 409–417.
103 Morales, T. I. and Hascall, V. C. (1988) Correlated metabolism of proteoglycans and hyaluronic acid in bovine cartilage organ cultures. J. Biol. Chem. 263, 3632–3638.
104 Fosang, A. J., Tyler, J. A. and Hardingham, T. E. (1991) Effect of interleukin-1 and insulin-like growth factor-1 on the degradation and turnover of proteoglycan and hyaluronate in pig articular cartilage in explant culture. Matrix 11, 17–24.
105 Chockalingam, P. S., Zeng, W., Morris, E. A. and Flannery, C. R. (2004) Release of hyaluronan and hyaladherins (aggrecan G1 domain and link proteins) from articular cartilage exposed to ADAMTS-4 (aggrecanase 1) or ADAMTS-5 (aggrecanase 2). Arthritis Rheum. 50, 2839–2848.
106 Campo, R. D. and Romano, J. E. (1986) Changes in cartilage proteoglycans associated with calcification. Calcif. Tissue Int. 39, 175–184.
107 Buckwalter, J. A., Rosenberg, L. C. and Ungar, R. (1987) Changes in proteoglycan aggregates during cartilage mineralization. Calcif. Tissue Int. 41, 228–236.
108 Sztrolovics, R., White, R. J., Roughley, P. J. and Mort, J. S. (2002) The mechanism of aggrecan release from cartilage differs with tissue origin and the agent used to stimulate catabolism. Biochem. J. 362, 465–472.
109 Sztrolovics, R., Recklies, A. D., Roughley, P. J. and Mort, J. S. (2002) Hyaluronate degradation as an alternative mechanism for proteoglycan release from cartilage during interleukin-1[beta]-stimulated catabolism. Biochem. J. 362, 473–479.
110 Plaas, A. H.K. and Sandy, J. D. (1993) A cartilage explant system for studies on aggrecan structure, biosynthesis and catabolism in discrete zones of the mammalian growth plate. Matrix 13, 135–147.
111 Yoshida, M., Sai, S., Marumo, K., Tanaka, T., Itano, N., Kimata, K. and Fujii, K. (2004) Expression analysis of three isoforms of hyaluronan synthase and hyaluronidase in the synovium of knees in osteoarthritis and rheumatoid arthritis by quantitative real-time reverse transcriptase polymerase chain reaction. Arthritis Res. Ther. 6, 514–520.
112 Durigova, M., Roughley, P. J. and Mort, J. S. (2007) Mechanism of proteoglycan aggregate degradation in cartilage stimulated with oncostatin M. Osteoarthritis Cartilage (in press).
113 Sugimoto, K., Iizawa, T., Harada, H., Yamada, K., Katsumata, M. and Takahashi, M. (2004) Cartilage degradation independent of MMP/aggrecanases. Osteoarthritis Cartilage 12, 1006–1014.
114 Cheng, F., Heinegård, D., Fransson, L., Bayliss, M., Bielicki, J., Hopwood, J. and Yoshida, K. (1996) Variations in the chondroitin sulfate-protein linkage region of aggrecans from bovine nasal and human articular cartilages. J. Biol. Chem. 271, 28572–28580.
115 Yasumoto, T., Bird, J. L., Sugimoto, K., Mason, R. M. and Bayliss, M. T. (2003) The G1 domain of aggrecan released from porcine articular cartilage forms stable complexes with hyaluronan/link protein. Rheumatology (Oxford) 42, 336–342.

116 Edwards, J. C., Wilkinson, L. S., Jones, H. M., Soothill, P., Henderson, K. J., Worrall, J. G. and Pitsillides, A. A. (1994) The formation of human synovial joint cavities: A possible role for hyaluronan and CD44 in altered interzone cohesion. J. Anat. 185, 355–367.
117 Balazs, E. A., Watson, D., Duff, I. F. and Roseman, S. (1967) Hyaluronic acid in synovial fluid. I. Molecular parameters of hyaluronic acid in normal and arthritis human fluids. Arthritis Rheum. 10, 357–376.
118 Pitsillides, A. A., Worrall, J. G., Wilkinson, L. S., Bayliss, M. T. and Edwards, J. C. (1994) Hyaluronan concentration in non-inflamed and rheumatoid synovium. Br. J. Rheumatol. 33, 5–10.
119 Pitsillides, A. A., Will, R. K., Bayliss, M. T. and Edwards, J. C. (1994) Circulating and synovial fluid hyaluronan levels. Effects of intraarticular corticosteroid on the concentration and the rate of turnover. Arthritis Rheum. 37, 1030–1038.
120 Dahl, L. B., Dahl, I. M., Engstrom-Laurent, A. and Granath, K. (1985) Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. Ann. Rheum. Dis. 44, 817–822.
121 Vuorio, E., Einola, S., Hakkarainen, S. and Penttinen, R. (1982) Synthesis of underpolymerized hyaluronic acid by fibroblasts cultured from rheumatoid and non-rheumatoid synovitis. Rheumatol. Int. 2, 97–102.
122 Wallis, W. J., Simkin, P. A., Nelp, W. B. and Foster, D. M. (1985) Intraarticular volume and clearance in human synovial effusions. Arthritis Rheum. 28, 441–449.
123 Balazs, E. A. and Denlinger, J. L. (1993) Viscosupplementation: A new concept in the treatment of osteoarthritis. J. Rheumatol. 20 Suppl. 39, 3–9.
124 Balazs, E. A. (2004) Viscosupplementation for treatment of osteoarthritis: From initial discovery to current status and results. Surg. Technol. Int. 12, 278–289.
125 Gomis, A., Pawlak, M., Balazs, E. A., Schmidt, R. F. and Belmonte, C. (2004) Effects of different molecular weight elastoviscous hyaluronan solutions on articular nociceptive afferents. Arthritis Rheum. 50, 314–326.
126 Ohno, S., Im, H. J., Knudson, C. B. and Knudson, W. (2006) Hyaluronan oligosaccharides induce matrix metalloproteinase 13 *via* transcriptional activation of NFkappaB. and p38 MAP kinase in articular chondrocytes. J. Biol. Chem. 281, 17952–17960.
127 Ohno, S., Im, H. J., Knudson, C. B. and Knudson, W. (2005) Hyaluronan oligosaccharide-induced activation of transcription factors in bovine articular chondrocytes. Arthritis Rheum. 52, 800–809.
128 Iacob, S. and Knudson, C. B. (2006) Hyaluronan fragments activate nitric oxide synthase and the production of nitric oxide by articular chondrocytes. Int. J. Biochem. Cell Biol. 38, 123–133.
129 Parfitt, A. M., Drezner, M. K., Glorieux, F. H., Kanis, J. A., Malluche, H., Meunier, P. J., Ott, S. M. and Recker, R. R. (1987) Bone histomorphometry: Standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J. Bone Miner. Res. 2, 595–610.
130 Sims, N. and Baron, R. (2000) Bone cells and their function. In: Skeletal Growth Factors, pp. 1–16, Canalis, E. (ed.), Lippincott Williams & Wilkins, Philadelphia.
131 Prince, C. W. (2004) Roles of hyaluronan in bone resorption. BMC Musculoskelet. Disord. 5, 12.
132 Stancikova, M., Svik, K., Istok, R., Rovensky, J. and, Velebny, V. (2004) The effects of hyaluronan on bone resorption and bone mineral density in a rat model of estrogen deficiency-induced osteopenia. Int. J. Tissue React. 26, 9–16.
133 Raisz, L. G. (1979) Physiological and pharmacologic regulation of bone resorption – A 1978 update. J. Periodontol. 50, 3–6.
134 Ariyoshi, W., Takahashi, T., Kanno, T., Ichimiya, H., Takano, H., Koseki, T. and Nishihara, T. (2005) Mechanisms involved in enhancement of osteoclast formation and function by low molecular weight hyaluronic acid. J. Biol. Chem. 280, 18967–18972.
135 Larjava, H., Mielityinen, H., Tenovuo, J., Jalkanen, M. and Paunio, K. (1982) The effect of human dental plaque on bone resorption and hyaluronic acid synthesis in *in-vitro* cultures of fetal rat calvaria. Arch. Oral. Biol. 27, 147–150.
136 Severson, A. R. (1978) The effect of cyclic nucleotides on the incorporation of ^{3}H-glucosamine into hyaluronate in bone organ culture. Horm. Metab. Res. 10, 256–260.
137 Severson, A. R. (1979) Colchicine stimulation of hyaluronate synthesis and secretion in bone organ culture. J. Cell Physiol. 101, 341–348.
138 Severson, A. R. (1983) Inhibition of bone resorption and increased incorporation of ^{3}H-glucosamine into hyaluronate in bone organ cultures treated with dibutyryl cyclic AMP and colchicine. Exp. Cell Biol. 51, 70–76.
139 Chang, E. J., Kim, H. J., Ha, J., Ryu, J., Park, K. H., Kim, U. H., Lee, Z. H., Kim, H. M., Fisher, D. E. and Kim, H. H. (2007) Hyaluronan inhibits osteoclast differentiation *via* Toll-like receptor 4. J. Cell Sci. 120, 166–176.
140 Taylor, K. R., Yamasaki, K., Radek, K. A., Di Nardo, A., Goodarzi, H., Golenbock, D., Beutler, B. and Gallo, R. L. (2007) Recognition of hyaluronan released in sterile injury involves a unique receptor complex dependent on Toll-like receptor 4, CD44, and MD-2. J. Biol. Chem. 282, 18265–18275.
141 Pilloni, A. and Bernard, G. W. (1998) The effect of hyaluronan on mouse intramembranous osteogenesis *in vitro*. Cell Tissue Res. 294, 323–333.
142 Huang, L., Cheng, Y. Y., Koo, P. L., Lee, K. M., Qin, L., Cheng, J. C. and Kumta, S. M. (2003) The effect of hyaluronan on osteoblast proliferation and differentiation in rat calvarial-derived cell cultures. J. Biomed. Mater. Res. A 66, 880–884.
143 Nagahata, M., Tsuchiya, T., Ishiguro, T., Matsuda, N., Nakatsuchi, Y., Teramoto, A., Hachimori, A. and Abe, K. (2004) A novel function of *N*-cadherin and Connexin43: Marked enhancement of alkaline phosphatase activity in rat calvarial osteoblast exposed to sulfated hyaluronan. Biochem. Biophys. Res. Commun. 315, 603–611.
144 Itoh, S., Matubara, M., Kawauchi, T., Nakamura, H., Yukitake, S., Ichinose, S. and Shinomiya, K. (2001) Enhancement of bone ingrowth in a titanium fiber mesh implant by rhBMP-2 and hyaluronic acid. J. Mater. Sci. Mater. Med. 12, 575–581.
145 Sasaki, T. and Watanabe, C. (1995) Stimulation of osteoinduction in bone wound healing by high-molecular hyaluronic acid. Bone 16, 9–15.
146 Cho, B. C., Park, J. W., Baik, B. S., Kwon, I. C. and Kim, I. S. (2002) The role of hyaluronic acid, chitosan, and calcium sulfate and their combined effect on early bony consolidation in distraction osteogenesis of a canine model. J. Craniofac. Surg. 13, 783–793.
147 Cochran, D. L., Wisner, L. A., Richards, M. F. and Rouse, C. A. (1990) The induction of specific metabolic alterations in mouse calvarial organ cultures by glycosaminoglycans. Arch. Oral. Biol. 35, 515–522.
148 Hurley, M. M., Kream, B. E. and Raisz, L. G. (1990) Structural determinants of the capacity of heparin to inhibit collagen synthesis in 21-day fetal rat calvariae. J. Bone Miner. Res. 5, 1127–1133.
149 Wong, G. L. (1984) Paracrine interactions in bone-secreted products of osteoblasts permit osteoclasts to respond to parathyroid hormone. J. Biol. Chem. 259, 4019–4022.
150 Cao, J. J., Singleton, P. A., Majumdar, S., Boudignon, B., Burghardt, A., Kurimoto, P., Wronski, T. J., Bourguignon, L. Y. and Halloran, B. P. (2005) Hyaluronan increases RANKL expression in bone marrow stromal cells through CD44. J. Bone Miner. Res. 20, 30–40.
151 Zou, L. J., Zou, X. N., Li, H., Mygind, T., Kassem, M. and Binger, C. (2007) The effect of hyaluronan on osteogenic

differentiation of porcine bone marrow stromal cells *in vitro*. Int. J. Cancer 32, 500.
152 Tsukahara, S., Ikeda, R., Goto, S., Yoshida, K., Mitsumori, R., Sakamoto, Y., Tajima, A., Yokoyama, T., Toh, S., Furukawa, K. and Inoue, I. (2006) Tumour necrosis factor alpha-stimulated gene-6 inhibits osteoblastic differentiation of human mesenchymal stem cells induced by osteogenic differentiation medium and BMP-2. Biochem. J. 398, 595–603.
153 Waddington, R. J., Embery, G. and Last, K. S. (1989) Glycosaminoglycans of human alveolar bone. Arch Oral. Biol. 34, 587–589.
154 Prince, C. W. and Navia, J. M. (1983) Glycosaminoglycan alterations in rat bone due to growth and fluorosis. J. Nutr. 113, 1576–1582.
155 Vogel, H. G. (1979) Influence of maturation and aging on mechanical and biochemical parameters of rat bone. Gerontology 25, 16–23.
156 Dickson, I. R. and Roughley, P. J. (1993) The effects of vitamin D deficiency on proteoglycan and hyaluronate constituents of chick bone. Biochim. Biophys. Acta 1181, 15–22.
157 Kent, G. N., Walker, D. G. and Cohn, D. V. (1979) Decreased response to calcitonin in osteopetrotic microphthalmic mouse bone. Nature 280, 71–72.
158 Genever, P. G. and Dickson, I. R. (1996) Influence of vitamin D status on hyaluronan localization in bone. Bone 18, 429–435.
159 Oohira, A. and Nogami, H. (1989) Elevated accumulation of hyaluronate in the tubular bones of osteogenesis imperfecta. Bone 10, 409–413.
160 Burkhardt, R., Kettner, G., Bohm, W., Schmidmeier, M., Schlag, R., Frisch, B., Mallmann, B., Eisenmenger, W. and Gilg, T. (1987) Changes in trabecular bone, hematopoiesis and bone marrow vessels in aplastic anemia, primary osteoporosis, and old age: A comparative histomorphometric study. Bone 8, 157–164.
161 Toole, B. P., Biswas, C. and Gross, J. (1979) Hyaluronate and invasiveness of the rabbit V2 carcinoma. Proc. Natl. Acad. Sci. USA 76, 6299–6303.
162 Banerjee, S. D. and Toole, B. P. (1992) Hyaluronan-binding protein in endothelial cell morphogenesis. J. Cell Biol. 119, 643–652.
163 Toole, B. P., Turner, R. E. and Banerjee, S. D. (1993) Hyaluronan-binding protein in chondrogenesis and angiogenesis in the developing limb. Prog. Clin. Biol. Res. 383B, 437–444.
164 Borselli, C., Oliviero, O., Battista, S., Ambrosio, L. and Netti, P. A. (2007) Induction of directional sprouting angiogenesis by matrix gradients. J. Biomed. Mater. Res. A 80, 297–305.
165 West, D. C., Hampson, I. N., Arnold, F. and Kumar, S (1985) Angiogenesis induced by degradation products of hyaluronic acid. Science 228, 1324–1326.
166 West, D. C. and Kumar, S. (1989) The effect of hyaluronate and its oligosaccharides on endothelial cell proliferation and monolayer integrity. Exp. Cell Res. 183, 179–196.
167 Slevin, M., West, D., Kumar, P., Rooney, P. and Kumar, S. (2004) Hyaluronan, angiogenesis and malignant disease. Int. J. Cancer 109, 793–794; author reply 795–796.
168 Taylor, K. R., Trowbridge, J. M., Rudisill, J. A., Termeer, C. C., Simon, J. C. and Gallo, R. L. (2004) Hyaluronan fragments stimulate endothelial recognition of injury through TLR4. J. Biol. Chem. 279, 17079–17084.
169 Lokeshwar, V. B., Lokeshwar, B. L., Pham, H. T. and Block, N. L. (1996) Association of elevated levels of hyaluronidase, a matrix-degrading enzyme, with prostate cancer progression. Cancer Res. 56, 651–657.
170 Slevin, M., Krupinski, J., Kumar, S. and Gaffney, J. (1998) Angiogenic oligosaccharides of hyaluronan induce protein tyrosine kinase activity in endothelial cells and activate a cytoplasmic signal transduction pathway resulting in proliferation. Lab. Invest. 78, 987–1003.
171 Gao, F., Okunieff, P., Han, Z., Ding, I., Wang, L., Liu, W., Zhang, J., Yang, S., Chen, J., Underhill, C. B., Kim, S. and Zhang, L. (2005) Hypoxia-induced alterations in hyaluronan and hyaluronidase. Adv. Exp. Med. Biol. 566, 249–256.
172 Yee, A. J., Bae, H. W., Friess, D., Robbin, M., Johnstone, B. and Yoo, J. U. (2003) Augmentation of rabbit posterolateral spondylodesis using a novel demineralized bone matrix-hyaluronan putty. Spine 28, 2435–2440.
173 Midura, R. J., Su, X., Morcuende, J. A., Tammi, M. and Tammi, R. (2003) Parathyroid hormone rapidly stimulates hyaluronan synthesis by periosteal osteoblasts in the tibial diaphysis of the growing rat. J. Biol. Chem. 278, 51462–51468.
174 Wong, G. L., Luben, R. A. and Cohn, D. V. (1977) 1,25-Dihydroxycholecalciferol and parathormone: Effects on isolated osteoclast-like and osteoblast-like cells. Science 197, 663–665.
175 Wong, G. L. (1979) Basal activities and hormone responsiveness of osteoclast-like and osteoblast-like bone cells are regulated by glucocorticoids. J. Biol. Chem. 254, 6337–6340.
176 Chopra, R. K., Li, Z. M., Vickery, S. and Anastassiades, T. (1990) Newly synthesized proteoglycans secreted by sequentially derived populations of cells from new-born rat calvaria: Effects of transforming growth factor-beta and matrigenin activity. Cell Differ. Dev. 32, 47–59.
177 Luben, R. A., Wong, G. L. and Cohn, D. V. (1976) Biochemical characterization with parathormone and calcitonin of isolated bone cells: Provisional identification of osteoclasts and osteoblasts. Endocrinology 99, 526–534.
178 Fedarko, N. S., Termine, J. D., Young, M. F. and Robey, P. G. (1990) Temporal regulation of hyaluronan and proteoglycan metabolism by human bone cells *in vitro*. J. Biol. Chem. 265, 12200–12209.
179 Anastassiades, T. P. and Chan, C. (1993) Stimulatory effect of T. G.F-beta on anionic glycoconjugate synthesis by rat calvarial cells: Specificity, uncoupling of cell density dependence, and modulation by chondroitin sulfate. J. Bone Miner. Res. 8, 1301–1309.
180 Midura, R. J., Evanko, S. P. and Hascall, V. C. (1994) Parathyroid hormone stimulates hyaluronan synthesis in an osteoblast-like cell line. J. Biol. Chem. 269, 13200–13206.
181 Aubin, J. E., Heersche, J. N., Merrilees, M. J. and Sodek, J. (1982) Isolation of bone cell clones with differences in growth, hormone responses, and extracellular matrix production. J. Cell Biol. 92, 452–461.
182 Clarkin, C. E., Allen, S. J., Wheeler, B., Wheeler-Jones, C. P. D. and Pitsillides, A. A. (2007) UGDH. is a positive modulator of chondrogenesis. Int. J. Exp. Pathol. in press.
183 Vigetti, D., Ori, M., Viola, M., Genasetti, A., Karousou, E., Rizzi, M., Pallotti, F., Nardi, I., Hascall, V. C., De Luca, G. and Passi, A. (2006) Molecular cloning and characterization of UDP-glucose dehydrogenase from the amphibian *Xenopus laevis* and its involvement in hyaluronan synthesis. J. Biol. Chem. 281, 8254–8263.
184 Ogawa, Y., Sawamura, S. J., Ksander, G. A., Armstrong, R. M., Pratt, B. M. and McPherson, J. M. (1990) Transforming growth factors-beta1 and beta2 induce synthesis and accumulation of hyaluronate and chondroitin sulfate *in vivo*. Growth factors 3, 53–62.
185 Hunter, G. K., Heersche, J. N. and Aubin, J. E. (1983) Isolation of three species of proteoglycan synthesized by cloned bone cells. Biochemistry 22, 831–837.
186 Boskey, A. L. and Dick, B. L. (1991) Hyaluronan interactions with hydroxyapatite do not alter *in vitro* hydroxyapatite crystal proliferation and growth. Matrix 11, 442–446.
187 Adams, J. R., Sander, G. and Byers, S. (2006) Expression of hyaluronan synthases and hyaluronidases in the M. G.63 osteoblast cell line. Matrix Biol. 25, 40–46.
188 Nishida, Y., Knudson, W., Knudson, C. B. and Ishiguro, N. (2005) Antisense inhibition of hyaluronan synthase-2 in human osteosarcoma cells inhibits hyaluronan retention and tumorigenicity. Exp. Cell Res. 307, 194–203.
189 Pitsillides, A. A. and Lanyon, L. E. (1998) Mechanical strain-associated N. O. production by bone cells. In: Nitric oxide in

bone and joint diseases, pp. 151–168, Hukkanen, M. V. J., Polak, J. M. and Hughes, S. P. F. (eds.), Cambridge University Press, Cambridge.
190 Reilly, G. C., Haut, T. R., Yellowley, C. E., Donahue, H. J. and Jacobs, C. R. (2003) Fluid flow induced PGE2 release by bone cells is reduced by glycocalyx degradation whereas calcium signals are not. Biorheology 40, 591–603.
191 Magee, C., Nurminskaya, M. and Linsenmayer, T. F. (2001) U. D.P-glucose pyrophosphorylase: Up-regulation in hypertrophic cartilage and role in hyaluronan synthesis. Biochem. J. 360, 667–674.
192 Vincent, T., Molina, L., Espert, L. and Mechti, N. (2003) Hyaluronan, a major non-protein glycosaminoglycan component of the extracellular matrix in human bone marrow, mediates dexamethasone resistance in multiple myeloma. Br. J. Haematol. 121, 259–269.
193 Calabro, A., Oken, M. M., Hascall, V. C. and Masellis, A. M. (2002) Characterization of hyaluronan synthase expression and hyaluronan synthesis in bone marrow mesenchymal progenitor cells: Predominant expression of H. A.S1 mRNA. and up-regulated hyaluronan synthesis in bone marrow cells derived from multiple myeloma patients. Blood 100, 2578–2585.
194 Gallagher, J. T., Spooncer, E. and Dexter, T. M. (1983) Role of the cellular matrix in haemopoiesis. I. Synthesis of glycosaminoglycans by mouse bone marrow cell cultures. J. Cell Sci. 63, 155–171.
195 Vaes, G. (1967) Hyaluronidase activity in lysosomes of bone tissue. Biochem. J. 103, 802–804.
196 Vaes, G. and Jacques, P. (1965) Studies on bone enzymes. The assay of acid hydrolases and other enzymes in bone tissue. Biochem. J. 97, 380–388.
197 Adams, J. R. J. and Byers, S. (2005) Hyaluronan metabolism in the M. G.63 osteoblast cell line. In: Hyaluronan: Its structure, metabolism, biological activities and therapeutic applications, vol. 2, pp. 459–463, Balazs, E. A. and Hascall, V. C. (eds.), Winmar Enterprises, Edgewater.
198 Deed, R., Rooney, P., Kumar, P., Norton, J. D., Smith, J., Freemont, A. J. and Kumar, S. (1997) Early-response gene signalling is induced by angiogenic oligosaccharides of hyaluronan in endothelial cells. Inhibition by non-angiogenic, high-molecular-weight hyaluronan. Int. J. Cancer 71, 251–256.
199 Baylink, D., Wergedal, J. and Thompson, E. (1972) Loss of protein polysaccharides at sites where bone mineralization is initiated. J. Histochem. Cytochem. 20, 279–292.
200 Toole, B. P., Jackson, G. and Gross, J. (1972) Hyaluronate in morphogenesis: Inhibition of chondrogenesis *in vitro.* Proc. Natl. Acad. Sci. U. S.A 69, 1384–1386.
201 Kosher, R. A., Savage, M. P. and Walker, K. H. (1981) A gradation of hyaluronate accumulation along the proximo-distal axis of the embryonic chick limb bud. J. Embryol. Exp. Morphol. 63, 85–98.
202 Rousche, K. T. and Knudson, C. B. (2002) Temporal expression of CD44 during embryonic chick limb development and modulation of its expression with retinoic acid. Matrix Biol. 21, 53–62.
203 Knudson, C. B. and Toole, B. P. (1987) Hyaluronate-cell interactions during differentiation of chick embryo limb mesoderm. Dev. Biol. 124, 82–90.
204 Maleski, M. P. and Knudson, C. B. (1996) Hyaluronan-mediated aggregation of limb bud mesenchyme and mesenchymal condensation during chondrogenesis. Exp. Cell Res. 225, 55–66.
205 Kulyk, W. M. and Kosher, R. A. (1987) Temporal and spatial analysis of hyaluronidase activity during development of the embryonic chick limb bud. Dev. Biol. 120, 535–541.
206 Nicoll, S. B., Barak, O., Csoka, A. B., Bhatnagar, R. S. and Stern, R. (2002) Hyaluronidases and CD44 undergo differential modulation during chondrogenesis. Biochem. Biophys. Res. Commun. 292, 819–825.
207 Kaya, G., Rodriguez, I., Jorcano, J. L., Vassalli, P. and Stamenkovic, I. (1997) Selective suppression of CD44 in keratinocytes of mice bearing an antisense CD44 transgene driven by a tissue-specific promoter disrupts hyaluronate metabolism in the skin and impairs keratinocyte proliferation. Genes Dev. 11, 996–1007.
208 Zoller, M., Herrmann, K., Buchner, S., Seiter, S., Claas, C., Underhill, C. B. and Moller, P. (1997) Transient absence of CD44 expression and delay in development by anti-CD44 treatment during ontogeny: A surrogate of an inducible knockout? Cell Growth Differ. 8, 1211–1223.
209 Protin, U., Schweighoffer, T., Jochum, W. and Hilberg, F. (1999) CD44-deficient mice develop normally with changes in subpopulations and recirculation of lymphocyte subsets. J. Immunol. 163, 4917–4923.
210 Archer, C. W., Morrison, H. and Pitsillides, A. A. (1994) Cellular aspects of the development of diarthrodial joints and articular cartilage. J. Anat. 184, 447–456.
211 Edwards, J. C., Wilkinson, L. S., Jones, H. M., Soothill, P., Henderson, K. J., Worrall, J. G. and Pitsillides, A. A. (1994) The formation of human synovial joint cavities: A possible role for hyaluronan and CD44 in altered interzone cohesion. J. Anat. 185, 355–367.
212 Pitsillides, A. A., Archer, C. W., Prehm, P., Bayliss, M. T. and Edwards, J. C. (1995) Alterations in hyaluronan synthesis during developing joint cavitation. J. Histochem. Cytochem. 43, 263–273.
213 Abu-Hijleh, G., Reid, O. and Scothorne, R. J. (1997) Cell death in the developing chick knee joint: I. Spatial and temporal patterns. Clin. Anat. 10, 183–200.
214 Mitrovic, D. (1978) Development of the diarthrodial joints in the rat embryo. Am. J. Anat. 151, 475–485.
215 Kavanagh, E., Abiri, M., Bland, Y. S. and Ashhurst, D. E. (2002) Division and death of cells in developing synovial joints and long bones. Cell Biol. Int. 26, 679–688.
216 Ito, M. M. and Kida, M. Y. (2000) Morphological and biochemical re-evaluation of the process of cavitation in the rat knee joint: Cellular and cell strata alterations in the interzone. J. Anat. 197, 659–679.
217 Pitsillides, A. A. (2006) Early effects of embryonic movement: 'A shot out of the dark'. J. Anat. 208, 417–431.
218 Osborne, A. C., Lamb, K. J., Lewthwaite, J. C., Dowthwaite, G. P. and Pitsillides, A. A. (2002) Short-term rigid and flaccid paralyses diminish growth of embryonic chick limbs and abrogate joint cavity formation but differentially preserve pre-cavitated joints. J. Musculoskelet. Neuronal. Interact. 2, 448–456.
219 Dowthwaite, G. P., Flannery, C. R., Flannelly, J., Lewthwaite, J. C., Archer, C. W. and Pitsillides, A. A. (2003) A mechanism underlying the movement requirement for synovial joint cavitation. Matrix Biol. 22, 311–322.
220 Dowthwaite, G. P., Ward, A. C., Flannely, J., Suswillo, R. F., Flannery, C. R., Archer, C. W. and Pitsillides, A. A. (1999) The effect of mechanical strain on hyaluronan metabolism in embryonic fibrocartilage cells. Matrix Biol. 18, 523–532.
221 Hunziker, E. B., Schenk, R. K. and Cruz-Orive, L. M. (1987) Quantitation of chondrocyte performance in growth-plate cartilage during longitudinal bone growth. J. Bone Joint Surg. Am. 69, 162–173.
222 Buckwalter, J. A. (1983) Proteoglycan structure in calcifying cartilage. Clin. Orthop. Relat. Res. 207–232.
223 Pavasant, P., Shizari, T. and Underhill, C. B. (1996) Hyaluronan contributes to the enlargement of hypertrophic lacunae in the growth plate. J. Cell Sci. 109, 327–334.
224 Alini, M., Matsui, Y., Dodge, G. R. and Poole, A. R. (1992) The extracellular matrix of cartilage in the growth plate before and during calcification: Changes in composition and degradation of type I. I. collagen. Calcif. Tissue Int. 50, 327–335.
225 Mwale, F., Tchetina, E., Wu, C. W. and Poole, A. R. (2002) The assembly and remodeling of the extracellular matrix in the growth plate in relationship to mineral deposition and

cellular hypertrophy: An in situ study of collagens II and IX and proteoglycan. J. Bone Miner. Res. 17, 275–283.
226 Suzuki, A., Tanimoto, K., Ohno, S., Nakatani, Y., Honda, K., Tanaka, N., Doi, T., Ohno-Nakahara, M., Yoneno, K., Ueki, M. and Tanne, K. (2005) The metabolism of hyaluronan in cultured rabbit growth plate chondrocytes during differentiation. Biochim. Biophys. Acta 1743, 57–63.
227 Tanimoto, K., Suzuki, A., Ohno, S., Honda, K., Tanaka, N., Doi, T., Nakahara-Ohno, M., Yoneno, K., Nakatani, Y., Ueki, M., Yanagida, T., Kitamura, R. and Tanne, K. (2004) Hyaluronidase expression in cultured growth plate chondrocytes during differentiation. Cell Tissue Res. 318, 335–342.
228 Lohmander, S. and Hjerpe, A. (1975) Proteoglycans of mineralizing rib and epiphyseal cartilage. Biochim. Biophys. Acta 404, 93–109.
229 Franzen, A., Heinegard, D., Reiland, S. and Olsson, S. E. (1982) Proteoglycans and calcification of cartilage in the femoral head epiphysis of the immature rat. J. Bone Joint Surg. Am. 64, 558–566.
230 Boyde, A. and Shapiro, I. M. (1980) Energy dispersive X-ray elemental analysis of isolated epiphyseal growth plate chondrocyte fragments. Histochemistry 69, 85–94.
231 Thyberg, J., Lohmander, S. and Friberg, U. (1973) Electron microscopic demonstration of proteoglycans in guinea pig epiphyseal cartilage. J. Ultrastruct. Res. 45, 407–427.
232 Howell, D. S. and Carlson, L (1968) Alterations in the composition of growth cartilage septa during calcification studied by microscopic x-ray elemental analysis. Exp. Cell Res. 51, 185–195.
233 Poole, A. R., Pidoux, I. and Rosenberg, L. (1982) Role of proteoglycans in endochondral ossification: Immunofluorescent localization of link protein and proteoglycan monomer in bovine fetal epiphyseal growth plate. J. Cell Biol. 92, 249–260.
234 Scherft, J. P. and Moskalewski, S. (1984) The amount of proteoglycans in cartilage matrix and the onset of mineralization. Metab. Bone Dis. Relat. Res. 5, 195–203.
235 Hargest, T. E., Gay, C. V., Schraer, H. and Wasserman, A. J. (1985) Vertical distribution of elements in cells and matrix of epiphyseal growth plate cartilage determined by quantitative electron probe analysis. J. Histochem. Cytochem. 33, 275–286.
236 Matsui, Y., Alini, M., Webber, C. and Poole, A. R. (1991) Characterization of aggregating proteoglycans from the proliferative, maturing, hypertrophic, and calcifying zones of the cartilaginous physis. J. Bone Joint Surg. Am. 73, 1064–1074.
237 Little, C. B., Meeker, C. T., Hembry, R. M., Sims, N. A., Lawlor, K. E., Golub, S. B., Last, K. and Fosang, A. J. (2005) Matrix metalloproteinases are not essential for aggrecan turnover during normal skeletal growth and development. Mol. Cell. Biol. 25, 3388–3399.
238 Little, C. B., Meeker, C. T., Golub, S. B., Lawlor, K. E., Farmer, P. J., Smith, S. M. and Fosang, A. J. (2007) Blocking aggrecanase cleavage in the aggrecan interglobular domain abrogates cartilage erosion and promotes cartilage repair. J. Clin. Invest. 117, 1627–1636.
239 Glasson, S. S., Askew, R., Sheppard, B., Carito, B., Blanchet, T., Ma, H. L., Flannery, C. R., Peluso, D., Kanki, K., Yang, Z., Majumdar, M. K. and Morris, E. A. (2005) Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature 434, 644–648.
240 Glasson, S. S., Askew, R., Sheppard, B., Carito, B. A., Blanchet, T., Ma, H. L., Flannery, C. R., Kanki, K., Wang, E., Peluso, D., Yang, Z., Majumdar, M. K. and Morris, E. A. (2004) Characterization of and osteoarthritis susceptibility in ADAMTS-4-knockout mice. Arthritis Rheum. 50, 2547–2558.
241 Stanton, H., Rogerson, F. M., East, C. J., Golub, S. B., Lawlor, K. E., Meeker, C. T., Little, C. B., Last, K., Farmer, P. J., Campbell, I. K., Fourie, A. M. and Fosang, A. J. (2005) ADAMTS5 is the major aggrecanase in mouse cartilage *in vivo* and *in vitro*. Nature 434, 648–652.
242 Rogerson, F. M., East, C. J., Golub, S. B., Stanton, H. and Fosang, A. J. (2007) Aggrecan catabolism in mice lacking both ADAMTS-4 and ADAMTS-5 activities: Evidence for another aggrecanase in cartilage. Trans. Orthop. Res. Soc. 32, 82.
243 Lee, E. R. (2006) Proteolytic enzymes in skeletal development: Histochemical methods adapted to the study of matrix lysis during the transformation of a "cartilage model" into bone. Front. Biosci. 11, 2538–2553.
244 Lee, E. R., Lamplugh, L., Shepard, N. L. and Mort, J. S. (1995) The septoclast, a cathepsin B-rich cell involved in the resorption of growth plate cartilage. J. Histochem. Cytochem. 43, 525–536.
245 Griffiths, G. (2002) What's special about secretory lysosomes? Semin. Cell Dev. Biol. 13, 279–284.
246 Jaiswal, J. K., Andrews, N. W. and Simon, S. M. (2002) Membrane proximal lysosomes are the major vesicles responsible for calcium-dependent exocytosis in nonsecretory cells. J. Cell Biol. 159, 625–635.
247 Reddy, A., Caler, E. V. and Andrews, N. W. (2001) Plasma membrane repair is mediated by Ca(2+)-regulated exocytosis of lysosomes. Cell 106, 157–169.
248 McNeil, P. L. (2002) Repairing a torn cell surface: Make way, lysosomes to the rescue. J. Cell Sci. 115, 873–879.
249 Cocucci, E., Lorusso, A., Ongania, G. N., Klajn, A. and Meldolesi, J. (2006) Non-secretory exocytoses in the brain. J. Physiol. Paris 99, 140–145.
250 Meldolesi, J (2003) Surface wound healing: A new, general function of eukaryotic cells. J. Cell. Mol. Med. 7, 197–203.
251 Wang, P., Chicka, M. C., Bhalla, A., Richards, D. A. and Chapman, E. R. (2005) Synaptotagmin VII is targeted to secretory organelles in PC12 cells, where it functions as a high-affinity calcium sensor. Mol. Cell. Biol. 25, 8693–8702.
252 Bastow, E. R., Wilson, R., Golub, S. B. and Fosang, A. J. (2007) Lysosomal exocytosis in hypertrophic chondrocytes. Trans. Orthop. Res. Soc. 32, 593.
253 Sattar, A., Rooney, P., Kumar, S., Pye, D., West, D. C., Scott, I. and Ledger, P. (1994) Application of angiogenic oligosaccharides of hyaluronan increases blood vessel numbers in rat skin. J. Invest. Dermatol. 103, 576–579.
254 Slevin, M., Kumar, S. and Gaffney, J. (2002) Angiogenic oligosaccharides of hyaluronan induce multiple signaling pathways affecting vascular endothelial cell mitogenic and wound healing responses. J. Biol. Chem. 277, 41046–41059.
255 Rooney, P., Kumar, S., Ponting, J. and Wang, M. (1995) The role of hyaluronan in tumour neovascularization. Int. J. Cancer 60, 632–636.
256 Horner, A., Bishop, N. J., Bord, S., Beeton, C., Kelsall, A. W., Coleman, N. and Compston, J. E. (1999) Immunolocalisation of vascular endothelial growth factor (VEGF) in human neonatal growth plate cartilage. J. Anat. 194, 519–524.
257 Montesano, R., Kumar, S., Orci, L. and Pepper, M. S. (1996) Synergistic effect of hyaluronan oligosaccharides and vascular endothelial growth factor on angiogenesis *in vitro*. Lab. Invest. 75, 249–262.
258 Natowicz, M. R., Short, M. P., Wang, Y., Dickersin, G. R., Gebhardt, M. C., Rosenthal, D. I., Sims, K. B. and Rosenberg, A. E. (1996) Clinical and biochemical manifestations of hyaluronidase deficiency. N. Engl. J. Med. 335, 1029–1033.
259 Triggs-Raine, B., Salo, T. J., Zhang, H., Wicklow, B. A. and Natowicz, M. R. (1999) Mutations in HYAL1, a member of a tandemly distributed multigene family encoding disparate hyaluronidase activities, cause a newly described lysosomal disorder, mucopolysaccharidosis IX. Proc. Natl. Acad. Sci. USA 96, 6296–6300.