Hyaluronan in joint cavitation

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Introduction

The view that the functional characteristics of connective tissues develop and are subsequently maintained, at least partly, by mechanisms that match their structure to their prevailing mechanical milieu is supported by many studies (for a review, see [1]). This relationship is clearly evident in the musculoskeletal system, where the morphology of different types of joint is closely related to the degree of movement that they facilitate. Diarthrodial joints provide extensive, essentially friction-free, movement and they can be distinguished from fibrous joints and synarthroses, which accommodate only small degrees of movement, on the basis that they contain cavities between opposed cartilaginous elements that are lined by synovium.

Diarthrodial joints permit mobility in some places while providing stability in others. This must involve functional adaptation of bone, cartilage, collagenous ligaments and tendons that cross the joint, but it must also involve, initially, the production and, later, the maintenance of a cavity separating opposed elements. This cavity must provide a non-adherent milieu in which these and associated synovial structures can move without damage or failure.

Many of the chemical and physical properties of hyaluronan (HA) make it an excellent candidate molecule in the facilitation of a joint's normal range of functions. Indeed, soon after its isolation [2] and chemical analysis [3], it was found that synovial fluid of normal joints contains high-Mr HA at particularly high concentrations [4]. Recent studies show that HA levels are decreased in rheumatoid joints, and that they are partly restored by intra-articular corticosteroid treatment, which concomitantly improves joint function [5,6]. This is consistent with the notion that high levels of synovial fluid HA are essential for functionally appropriate joint articulation.

HA differs from other glycosaminoglycans (GAGs) in that it lacks covalently linked polypeptide and is unsulphated. It is also well established that tissues in which HA is present at high concentrations are relatively soft [7]. In comparison with tissues with high tensile strength, which contain higher proportions of sulphated GAGs and collagen fibrils, synovial fluid is completely 'liquefied' and its functional properties are largely independent of collagen. It is clear that by determining the mechanisms that regulate the initial elaboration and, later, the maintenance of a synovial joint's high local concentrations of HA, it may be possible to formulate novel strategies for conserving, or indeed restoring, its contribution to a joint's functional competence where it is compromised by disease.

Joint cavities form by a process (cavitation), which involves elaboration of a fluid-filled, non-adherent 'space' between the opposed elements. Sites where such spaces initially develop during morphogenesis provide an ideal environment in which the cellular characteristics associated with the establishment and maintenance of high local HA levels can be determined. Furthermore, using chick embryos (readily accessible and easily manipulated) as a 'model' system also permits the mechanical environment to be modified during development, allowing the functional relevance of these cellular characteristics, as well as their controlling mechanisms, to be defined.

This review describes several key characteristics of cells at sites of joint cavitation, which suggest that differential increases in HA synthesis and its interaction with hyaluronan-binding proteins (HABPs) are essential for joint cavitation. It will also describe phenomenological evidence from in ovo immobilization experiments, which suggests that these characteristics are indeed related to the facilitation and development of a joint's functional competence. Finally, evidence from in vitro experiments will be presented, which suggests that these mechanomodulatory influences may be mediated by specific and direct alterations in HA synthesis, release and binding.
Joint morphogenesis

Most studies of limb development have investigated those mechanisms which control skeletal element patterning (addressing the question: where do joints form?), and few have addressed joint morphogenesis (how do joints form?) directly. These 'patterning' studies show that the initial stages of development involve dynamic reciprocal relationships between a thickened region of the ectoderm (apical ectodermal ridge; AER) and mesenchyme in the most distal regions of the developing limb. This maintains the mesenchyme in a proliferative, undifferentiated state (progress zone), which facilitates limb outgrowth.

With continued progress zone outgrowth, cells depart from the AER's influence and become 'committed' to particular fates. Many elegant studies have identified key regulatory genes, which seem to contribute to such skeletal 'commitment' [8,9] and, although these have provided an understanding of the organizational mechanisms that determine the specific location of discrete structures, few have addressed the morphogenetic changes associated with joint cavity formation.

Synovial joint cavity formation involves the separation of predetermined opposed cartilaginous elements, producing non-adherent articular surfaces and associated synovial structures. This morphogenic process is fundamentally similar in all species examined and is, for convenience, divided into two continuous phases: the initial formation and configuration of cartilage anlagen, and the subsequent formation of a cavity in an apparently continuous extracellular matrix (ECM).

The origins of a synovial joint become defined within an area of early blastemal mesenchyme found between developing avascular cartilaginous condensations, which are themselves enveloped by perichondrium. These condensations expand and the intervening presumptive joint becomes defined as a transverse region of persisting, densely packed, flattened cells (primitive blastemal remains), which become increasingly attenuated (early interzone). Later, the peripheral regions of the presumptive joint capsule/synovium become vascularized and subsequently tissue separation occurs within the avascular central region of the interzone by an, as yet, undetermined mechanism.

Thus, with time and despite continued expansion of the opposing pair of cartilaginous elements, the ever-decreasing proportion of blastemally associated mesenchymal cells within the limb will contribute directly to initial tissue separation at the joint line, as well as the later establishment, of the synovial lining. Consequently, the precise cellular differentiation associated with this joint tissue separation, or 'synovialization', can be addressed readily. Furthermore, using embryonic chick limb development as a model system, interventional studies enable the relationship between these characteristics, as well as their potential contribution to joint formation, to be investigated. With specific reference to the possible role of HA, this article focuses on local changes occurring at the site of this initial separation, as well as addressing the regulatory role of movement in creating, and later maintaining, this HA-rich fluid-filled space.

HA synthesis

HA biosynthesis differs fundamentally from that of other GAGs, which are synthesized in the Golgi complex. Despite much debate, it is now accepted that HA is synthesized at the plasma membrane and extruded directly into the ECM [10], and that the reducing end of nascent HA chains is elongated by alternate addition of sugar residues derived from UDP-glucuronate and UDP-N-acetylglucosamine, by the enzyme HA synthase [11-13]. However, the mechanisms regulating HA synthesis remain enigmatic and it is possible that monosaccharide supply constitutes a key point controlling the rate of its synthesis.

All GAG monosaccharides are synthesized by enzymic interconversion of nucleotide sugars [14]. UDP-glucose dehydrogenase (UDPGD, EC 1.1.1.22) is responsible for the conversion of UDP-glucose into UDP-glucuronate, and it has been shown that UDPGD activity is an irreversible, rate-limiting step in UDP-glucuronate production [15]. In addition to regulating the rate of UDP-glucuronate supply, it has been suggested that UDPGD has a pivotal role in controlling the type of monosaccharides produced by utilization of UDP-glucose [16]. Indeed, it has been shown that UDP-amino sugar (such as UDP-N-acetylglucosamine of HA) availability does not normally appear to limit the GAG synthesis rate [17]. Consistent with this, our findings using a quantitative cytochemical assay for UDPGD activity applied to sections of both human cartilage explants and human and rabbit synovium
support the notion that this appears to constitute a key regulatory point in GAG synthesis [18,19].

The role of HA in joint cavitation

Many processes, including necrotic cell death, vascular ingression, cellular migration, apoptosis, and increased breakdown of ECM components, have been implicated in the cavity formation process [20–22]. However, the mechanisms controlling joint cavity formation remain to be established. It is, nevertheless, evident that cavitation must involve both local and precisely defined loss of tensile properties within the presumptive joint’s interzonal matrix. This could be mediated by several types of event, such as: (i) mechanically or enzymically related degradation of previously coherent elements of the matrix; (ii) by changes in local synthesis and secretion of non-coherent ECM components with low tensile strength; or (iii) by a combination of such factors.

Alteration in local ECM components is supported by histochemical studies indicating joint line-selective differences [23] and by immunolabelling studies, which indicated a loss of type II collagen and keratan sulphate-containing proteoglycans from the joint interzone, as well as the appearance of type I collagen, concomitant with cavitation [24,25]. These findings were extended by in situ hybridization, which showed transient procollagen type IIA mRNA expression, as well as consistent type I collagen mRNA expression in interzonal cells before cavitation [21]. Such changes in collagen content may lead one to assume that such fibrous components would have to be removed for cavitation to occur. However, evidence for the localized degradation of such ECM components at the forming joint line is limited [26,27], and these studies fail to provide a basis for the localized loss in tensile strength required to facilitate tissue separation.

Using biotinylated HA-binding region link-protein complex, we and others demonstrated the appearance of staining for free HA at the joint line, concomitant with the first signs of overt cavitation [25,28]. On this basis it was postulated that locally produced HA was secreted into the presumptive joint space, and that it was instrumental in joint cavity formation. This proposal was supported by studies in which the use of radiolabelled precursors showed that HA constitutes a major component of the material extracted from newly formed spaces at another embryonic site, into which neural crest cells migrate during craniofacial development [29]. Since HA is considered to be an essential component of the cell-free, highly hydrated matrices responsible for such separation, this warranted further examination.

It is clear that these results are consistent with the elaboration of a non-coherent ECM component with low tensile strength (HA), yet they have several limitations: (i) such HA staining may not produce a meaningful measure of local HA concentration; (ii) staining may be absent from areas in which HA is otherwise occupied; and (iii) the site and dynamics of HA synthesis remain purely speculative. Thus such HA staining may indicate that local HA synthesis is involved in joint space genesis or, alternatively, that HA diffuses into a space formed by other mechanisms.

Using the novel application of an in situ biochemical method for UDPGD activity [30] to embryonic limbs, together with immunolabelling using a monoclonal antibody raised against a 52,000-M, streptococcal protein (which binds nascent HA and is affinity-labelled by both UDP-N-acetylgalcosamine and UDP-glucuronate [31]), recently described as a HA-synthase-associated protein (HAS-AP), as well as autoradiography for labelled sulphate incorporation, we evaluated the role of local cells in the synthesis of HA during joint cavitation. These studies showed that as joint cavities form, local cells at the developing articular surfaces exhibit increased UDPGD activity and HAS-AP immunolabelling, while local sulphate incorporation is reduced. In fully cavitated joints, these characteristics are maintained in all cells directly bordering the joint cavity, and detailed microdensitometric analysis established that the peak in UDPGD activity per cell was apparent at the exact site and occurred synchronously with the first signs of overt cavity formation [28]. Since cells in presumptive interzones also incorporate markedly reduced levels of labelled sulphate compared with neighbouring epiphyseal chondrocytes, this suggests that local UDP-glucuronate production is used preferentially in the synthesis of HA, rather than sulphated GAGs. Thus it is evident that the differentiation of cells bordering sites of active separation in developing joints appears to involve alterations associated specifically with differential synthesis of HA.

These findings are in agreement with the proposal that HA is involved in the generation of fluid-filled spaces, such as those found between
the external corneal epithelium and endothelium during development [32]. Our studies extend this notion by providing the first evidence that cells that border a site where such separation is apparent appear to be actively involved in the synthesis of HA. These findings do not, however, necessarily establish that HA is responsible for joint 'space' generation, and it remains possible that this involves some other mechanism and that, as a consequence, local HA synthesis is stimulated so as to fill this newly formed cavity.

The role of HABPs and their cytoskeletal linking elements in cavitation

HA's characteristic multiple disaccharide repeats provide potential binding sites for molecules collectively known as hyaladherins. Members of this group are divided on the basis that they mediate HA binding either within the matrix or at cell surfaces [33,34]. The best-characterized matrix-associated hyaladherins are aggrecan and link protein of articular cartilage, where HA interaction is mediated by a 'link module'. This domain appears in all matrix hyaladherins, as well as in some cell-surface hyaladherins, including CD44 [35]. CD44 was originally described as a lymphocyte differentiation marker and is now considered to be the principal cell-surface-associated hyaladherin. Although it can bind HA hexasaccharides, the interaction is weak in comparison with its affinity for larger HA molecules. It also binds, via its chondroitin sulphate side chains, to other ligands, including collagens I and VI [34] and its HA-binding capacity is modified by: (i) phorbol ester treatment; (ii) its interaction with cytoskeletal actin [36], and (iii) its cell-surface clustering.

Toole [37] had proposed previously that local increases in intercellular HA and, specifically, its interaction with cells expressing HABPs could facilitate cell separation. On this basis, we hypothesized that this interaction was essential for efficient cavitation. This was supported by immunolabelling studies, which showed that, in developing human embryonic limbs, CD44 expression was very marked in cells at sites of cavitation [26]. We have also demonstrated that several HABPs, namely CD44, RHAMM (receptor for HA-mediated motility; [38]) and epitopes recognized by IVd4 (antibody raised against an epitope capable of blocking HA binding in chick brain cell extracts) are all expressed at sites of joint formation in the embryonic chick. Most notably, however, CD44 showed a marked joint-line-selective differential labelling, which exhibited a clear pericellular distribution at all cavitation stages. In contrast, IVd4 and RHAMM expression were more uniform and intracellularly localized [39]. These findings suggest that local CD44, but not RHAMM or IVd4, plays a role in cavitation. However, they provide little information on local HA-binding status. Accordingly, we have used a range of approaches to determine whether interaction between HA and CD44 are essential for joint cavity formation.

First, using biotin-labelled HA we have shown that cells within developing joint interzones, but not those in fibrocartilaginous articular surfaces of fully cavitated joints, exhibit evidence for HA-binding ability [39]. This is in accordance with the virtually non-existent labelling for 'free HA' in fibrocartilaginous surfaces, in comparison with the intense labelling of all other tissues lining the joint cavity [28]. Thus cells associated with the loss of cohesion at the joint line show abundant expression of CD44 with functional HA-binding capacity and are associated with a 'free HA'-rich ECM. In contrast, cells in the articular fibrocartilage show less CD44 expression with less HA-binding capacity, and are associated with little free HA.

Secondly, based on studies suggesting that HABPs' interaction with actin cytoskeletal elements is required for effective ligand binding and that this is regulated by a family of actin-capping proteins (ezrin, radixin and moesin), collectively known as ERM proteins [40,41], we examined both their distribution and that of polymerized actin during joint cavitation. We found that interzone cells in presumptive areas of cavitation are labelled with pan-specific ERM antibodies, and that moesin specifically shows a strong joint-line-selective differential expression in both forming and fully formed joints. Our confocal microscopic studies using fluorescein isothiocyanate-conjugated phalloidin revealed a specific alignment of polymerized actin filaments parallel with developing joint surfaces, also within cells directly bordering sites of cavitation. In contrast, neighbouring chondrocytes show very low, if any, actin and moesin labelling. Thus the co-distribution of moesin and polymerized actin also indicates that this CD44 is likely to be engaged in binding to HA at sites of joint cavitation.

Finally, it is well established that oligosaccharides of HA can block HA's interaction with
cell-surface-associated HABPs [42]. Therefore, using a slow-release bead-delivery system *in ovo*, we examined the effect of introducing oligosaccharides of HA to sites of presumptive joint formation. We found that HA oligosaccharide treatment prevented joint cavity formation, and that this was associated both with local decreases in CD44 and moesin expression, and most markedly with local decreases in UDPGD activity [39]. Together, these findings suggest that HA:CD44 interactions constitute an essential component of the joint cavitation process, and that a failure to cavitate is associated with local decreases in UDPGD activity, suggesting that cellular supply of UDP-glucuronate may reflect a limited HA synthetic potential in these cells, and that this also is essential for joint cavity formation.

**Mechanomodulatory influences in joint space formation and maintenance**

Failures in joint formation have also been induced by long-term paralysis *in ovo*, and many studies emphasize an essential role for movement in the initial cavitation and in the joint’s subsequent maintenance [22]. These studies showed that limb maintenance in culture, or immobilization using botulinum toxin, neurectomy, decamethonium bromide or succinylcholine *in ovo*, resulted in the prevention of cavitation in as yet uncavitated joints and the regression of those previously cavitated. This provided verification of the original paradigm concerning movement’s requirement, but did not, however, provide a mechanistic basis for its role in cavitation.

Articulation normally associated with movement clearly engenders several mechanical sequelae within joints. It must engender physiological static loading of joints, by virtue of the sustained contraction of particular muscles, but it will also engender dynamic mechanical stimuli resulting from the intermittent and discontinuous nature of such contractions. To establish the role of these ‘dynamic’ and ‘static’ components of the mechanical milieu, we have examined how short-term (3 days) flaccid and spastic paralysis might each independently influence developing joints. We found that a short period of either spastic paralysis (removes only the dynamic component) or flaccid paralysis (removes both dynamic and static components) was sufficient to halt the initial cavity formation process. The common influence of the distinct immobilization regimes, before cavitation, suggests that the dynamic component of articulation exerts a dominant effect during these early stages. In contrast, the effect of spastic and flaccid paralysis on cavity maintenance differed, with regression of previously cavitated joints being more pronounced in limbs subjected to flaccid paralysis. This suggests that the static component of loading, which spastic paralysis sustains, can make a unique contribution to joint cavity maintenance [43].

Intriguingly, both forms of paralysis selectively decreased local joint-line-associated UDPGD activity, CD44 and moesin expression and free HA content, in a manner related to their cavitation-inhibitory influence. This strengthens the notion that local HA synthesis and its association with CD44 are intimately involved in the initial cavitation and also contribute to maintaining the cavity thereafter. They also provide the first, although indirect, evidence regarding the mechanistic basis of movement’s role [43]. However, it remains possible that movement influences events by some mechanism that does not rely upon the sensitivity of these joint-line cells to their mechanical environment. To examine this directly, we have developed a system in which controlled mechanical strains of specific duration, character and magnitude can be applied to cells isolated from embryonic chick articular surfaces *in vitro*.

**The effect of mechanical strain in vitro**

The use of *in vitro* techniques to address this particular question is novel, yet they do have extensive experimental support in other tissues, particularly bone. Thus short (<10 min) daily periods of dynamic mechanical loading applied to functionally isolated avian ulnae *in vitro* do not only suppress the resorption induced by such isolation, but also stimulate increased bone formation [44]. Furthermore, a single period of such loading converted quiescent bone surfaces into active formation 5 days later [1]. Thus it is clear that this provides a foundation for the mechanistic consequences of specific, single temporally defined stimuli to be examined *in vitro*.

In order to establish a basis for these studies, we initially undertook cell characterization studies. In common with cells in chick fibrocartilaginous articular surfaces *in situ*, cells derived from these sites and maintained *in vitro* exhibited collagen type I, CD44 and moesin.
expression, and high UDPGD activity per cell and, importantly, did not express chondrocyte differentiation markers, such as collagen II and chondroitin/heparan sulphate staining [45]. Having characterized these chick articular fibrocartilage cells (isolated from stage 42 tibiotarsi by collagenase digestion), we subjected the strips on to which cells had been plated to: (i) uniaxial binding, and that a failure to bind HA efficiently or (ii) for 10 min at 1 Hz (‘dynamic’ strain); (iii) medium perturbation alone (‘flow’, 1 Hz); or (iv) no perturbation (‘static’) cells. We found that 24 h after stimulation, cells subjected to either dynamic or static strain, but not flow, exhibited marked increases in UDPGD activity per cell, UDPGD protein content per cell and HA release. In contrast, using Alcian Blue staining and microdensitometry to quantify and distinguish between cell-associated GAG content, we found that only dynamic strain, but not static strain or flow, induced marked increases in relative HA content. These effects were not accompanied by changes in sulphated GAG content or release, or cell proliferation [46]. Furthermore, we found that the total number of HA-binding sites, expressed using hyaluronidase treatment and biotinylated HA, were increased only after the application of dynamic strain. Thus it appears that both static and dynamic strain increase HA synthesis and release, whereas dynamic strain alone induces increases in cellular binding of HA.

Thus dynamic strains may contribute to joint cavitation specifically by modulating HA binding, and that a failure to bind HA efficiently is a consequence of both forms of paralysis, which thus abrogate the cavitation process. Although this suggests that HA binding and CD44 expression are key elements of cavitation, it is also evident, on the other hand, that once cavities are present, static strain (sustained by spastic paralysis) might contribute to cavity maintenance by promoting HA synthesis and release, without necessarily increasing cell-surface HA binding further.

These findings suggest some extensions to the initial hypothesis that variations in extracellular HA levels promote either aggregation (at low concentrations) or separation (at high concentrations) of adjacent cells expressing HABPs by a process involving HABP saturation [37]. First, differential regulation of CD44 expression might also promote separation. Secondly, cells that actively separate are themselves responsible for the increased levels of HA synthesized. Finally, while initial separation does indeed require increases in both HA content and binding (via CD44), the maintenance of such spaces may only require continued stimulation of HA synthesis and release.

Despite this sensitivity to isolated mechanical events, the cellular mechanisms responsible for their control and regulation remain largely unaddressed. Our studies suggest that this involves several discrete stages, including: (i) the transduction of mechanical events into cellular signals (mechanotransduction); (ii) the cellular communication of these events, or signals, to establish a pattern of local mechanical requirements; and (iii) the co-ordination of ECM turnover, which alters or maintains the joints’ functional competence. That this model has physiological relevance in developing limbs is evidenced by our recent findings, which show by direct measurement of chick movement in ovo using video microscopy that, during normal development, periods of movement are brief (9 ± 3 s) and intermittent (0.8 ± 0.6 periods/min). As this is normally sufficient to promote HA-related joint-line differentiation, it is clear that our combined in vitro and in ovo studies may explain at least one mechanism by which movement participates in joint formation.

**Mechanotransduction events in the joint cavitation process**

This model highlights several areas where our knowledge is limited. One such area is the initial mechanotransduction stage, during which short-term mechanical stimulation is transduced into cellular signals that promote a long-term influence. In endothelial cells, this appears to involve selective, and often sustained, activation of mitogen-activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK [47]). Using an antibody specific to chick phosphorylated (p42, Thr383/Tyr385) MAPK (phospho-ERK-1), we have observed joint-line-selective labelling for ERK-1 during cavitation, with little if any labelling within neighbouring epiphyses. Immunolabelling for this constitutively active ERK-1 was absent in limbs subjected to the cavitation-inhibitory influence of either spastic or flaccid paralysis. Furthermore, Western blotting suggests that ERK-1 expression increases 24 h after application (10 min) of dynamic or static strain, when compared with static ‘control’ cells [46]. Thus it is possible that selective MAPK phosphorylation at sites of cavitation contributes to joint formation.
Along these lines we have preliminary evidence in cultured embryonic chick fibrocartilage cells that CD44 and moesin exhibit strain-induced dephosphorylation [45]. These seemingly paradoxical findings suggest that activated MAPK may be required for maintenance of CD44 and moesin in their dephosphorylated state.

Further, analogy with mechanotransduction events in other cells [48] led to the investigation of nitric oxide (NO) production in vitro. These studies indicated that immediately upon dynamic strain application there are marked increases in NO release, and that this is a transient response, which diminishes rapidly following the removal of strain. In other connective tissues, such as bone, it has been shown that the inhibition of these acute mechanically induced increases in NO release in vivo results in the complete abrogation of loading-related osteogenesis [1]. In close agreement with this proposed role for NO in mechanotransduction events, we have preliminary evidence that indicates that non-selective inhibitors of NO production block strain-related increases in HA synthesis and its association with CD44 and moesin in cultured embryonic chick articular surface cells in vitro. Together, these studies indicate that strain-related NO release may also constitute a key event in modulating differential increases in HA synthesis, release and cell-surface-associated binding.

Conclusions
On the basis of in vivo interventional studies and in vitro elucidation of the response to mechanical strain, a combination of immunolabelling, quantitative cytochemical and histological labelling studies allow us to conclude that differential increases in local HA synthesis and its association with CD44 constitute an essential and early event in the cavitation process. These interactions appear to be a prerequisite for efficient joint cavitation and they are at least partly responsible for the maintenance of these cavities, once formed. Finally, we have presented preliminary findings that suggest that specific signalling pathways, including strain-related NO production and MAPK activation, might be involved in the mechanomodulatory influence of movement during joint formation.

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7 Laurent, T. C. and Fraser, J. R. E. (1992) FASEB J. 6, 2397–2404
Receptor for hyaluronan-mediated motility (RHAMM), a hyaladherin that regulates cell responses to growth factors

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Introduction

Hyaluronan (HA) is a relatively simple polysaccharide that is composed of repeating disaccharide units of N-acetylglucosamine and glucuronic acid. In spite of this simplicity, HA binds with high affinity ($K_d = 10^{-12}$ M), and often high specificity, to a group of disparate proteins.

Abbreviations used: AP-1, activator protein-1; ERK, extracellular signal-regulated kinase; HA, hyaluronan; IHABP, intracellular hyaluronic-acid-binding protein; MAP, mitogen-activated protein; MEK, MAP kinase kinase; PDGF, platelet-derived growth factor; RACE, rapid amplification of cDNA ends; RHAMM, receptor for HA-mediated motility; RT, reverse transcriptase.

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That have been termed hyaladherins [1]. These proteins can be classed into groups that include cell-associated hyaladherins, extracellular hyaladherins and soluble hyaladherins. Most of the proteins within these groups bear little homology with one another other than at the site to which they bind HA. However, at least one other protein that is homologous with CD44 has been reported recently [2] suggesting that, at least in the case of CD44, a family of related proteins may exist. At present, and for the most part, hyaladherins resemble each other only in that they bind to HA. Recent studies have implicated two structures in binding of HA to hyaladherins and these include a peptide known as the link module [3] and basic amino acid motifs [4].