Hyaluronan-binding proteins in development, tissue homeostasis, and disease

CHERYL B. KNUDSON AND WARREN KNUDSON

Departments of Biochemistry and Pathology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612, USA

ABSTRACT **The high molecular weight glycosaminoglycan hyaluronan plays an important role in tissue remodeling during development, normal tissue homeostasis, and disease. The interaction of hyaluronan with matrix hyaluronan-binding proteins and cell-surface hyaluronan receptors regulates many aspects of cell behavior such as cell migration, cell-cell adhesion, and cell differentiation. Hyaluronan-binding proteins have been grouped together as a family termed hyaladherins—further subdivided in matrix and cell-surface hyaladherins (receptors). Specific hyaluronan-hyaladherin interactions that affect cell behavior are the focus of this review. Both clearance and turnover of hyaluronan involve hyaluronan receptor-mediated endocytosis. Pericellular matrix assembly and retention on many cells, especially chondrocytes, are mediated by hyaluronan receptors, in coordination with other matrix hyaladherins. Hyaluronan can also have an independent, direct effect on cell-to-cell adhesion as well as migration, again mediated by specific cell-surface hyaluronan receptors. This is especially apparent in tumor cells, where metastatic potential is correlated with hyaluronan receptor expression. As migrating cells encounter new environments enriched in matrix hyaladherins, the capacity for matrix assembly may terminate cell migration. Thus, the temporal/spatial deposition of particular matrix hyaladherins also serves as signals or matrix cues to alter cell behavior.—Knudson, C. B., Knudson, W. Hyaluronan-binding proteins in development, tissue homeostasis, and disease. *FASEB J.* 7: 1233–1241; 1993.**

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HYALURONAN (HA)[1] A HIGH MOLECULAR WEIGHT polysaccharide found in the extracellular matrix of most animal tissues, has received considerable attention in recent years due to its profound influence on cell behavior (1, 2). Tissue extracellular matrices become enriched in HA coincident with periods of rapid cell proliferation and migration. Such events have been well documented in developing, regenerating, and remodeling tissues, and in tissues undergoing malignant tumor cell invasion (2, 3). HA levels within a matrix have also been shown to promote or inhibit the state of differentiation of several mesenchymal progenitor cell types (2, 4) and to participate directly in cell-cell aggregation events (2, 5). These matrix-induced effects on cells are in turn supported and directed by a wide variety of HA-binding proteins termed hyaladherins (2, 4). Some of these HA-binding proteins interact with HA within the extracellular matrix proper, whereas others interact with HA at the plasma membrane of cells, as cell-surface matrix "receptors." The ability of HA and HA-binding proteins to influence cell physiology and behavior may occur via an alteration of the hydrodynamic and physical properties of the matrix itself or via direct interaction of HA (or its degradation products) with cell-surface protein receptors. This review details how HA binding proteins, cell-surface HA receptors in particular, coordinate the functions of this remarkable polysaccharide during development, tissue homeostasis, and disease.

HYALURONAN-BINDING PROTEINS—THE HYALADHERINS

The widespread occurrence of HA-binding proteins indicates that the recognition of HA is important to tissue organization and the control of cellular behavior. Toole (4) has proposed that the family of HA-binding proteins be termed the "hyaladherins." The hyaladherin family would include structural matrix HA-binding proteins as well as cell-surface HA receptors that exhibit high affinity binding of HA.

Matrix hyaladherins

HA in several tissues exists in complexes with other matrix macromolecules that exhibit strong HA-binding capacity. Matrix hyaladherins found within cartilage matrix have been well characterized (6). The large, aggregating chondroitin sulfate proteoglycan of cartilage has been termed aggrecan because of its high affinity binding to HA. Link protein, a glycoprotein with isoforms of $M_r \sim$ 45–48 kDa, also demonstrates strong, specific binding for HA. The noncovalent interaction of aggrecan and link protein with HA serves to retain aggrecan within cartilage in the form of a supramolecular ternary complex, often with more than 100 aggrecan and link protein molecules bound to a single filament of HA. Proteoglycan/HA aggregates generate the viscoelastic properties of cartilage tissue. Other proteins that also bind HA have been detected in cartilage matrix, such as PG-M (7), type VI collagen (8), and a 102-kDa protein (9). These proteins, although present in smaller proportions, may participate in cartilage matrix assembly and maintenance of matrix integrity. HA-binding proteins found in noncartilaginous tissues also form strong structural complexes with HA. Versican, a proteoglycan synthesized by fibroblasts (10), hyaluronectin, a proteoglycan widely distributed in nervous tissue and soft connective tissues (11), glial-hyaluronan binding protein in the central nervous system (12), and neurocan, a chondroitin sulfate proteoglycan of brain (13), are examples of matrix hyaladherins. Link proteins have also been detected in noncartilaginous tissues and may stabilize the interaction of neurocan or versican with

[1]Abbreviations: HA, hyaluronan; HA_6, hyaluronan hexasaccharides; HARC, HA receptor complex; LEC, liver endothelial cell.

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HA (13). All matrix hyaladherins contain a sequence of amino acids homologous to the tandem-repeated B loops of cartilage link protein, a structural motif that is predicted to contain the HA-binding domain (for references see refs 4, 6). In some tissues these matrix HA-binding proteins modulate HA functions; in other tissues such as cartilage, HA functions primarily to retain and organize proteoglycans within the extracellular matrix.

Cell-surface receptor hyaladherins

Cell-surface hyaladherins, or HA receptors, have been detected on several cell types from a wide variety of tissues (4, 14). This distribution usually, but not always, mimics tissue distribution of HA (15, 16). Recent reports suggest that some HA receptors are related or identical to the CD44 family of lymphocyte homing receptors (for references see refs 14, 17). Independent work on the CD44 family of receptors in distinct functional areas have used the terms Pgp-1, Hermes antigen, H-CAM, ECMRIII, and others to name apparently the same protein on different cells or in different species (14, 17). The first HA receptor described was the 85-kDa protein present on the surface of SV-40 transformed 3T3 cells, also identified in BHK cells (for references, see ref 15). A monoclonal antibody (K-3) was shown to block the binding of HA to the 85-kDa HA receptor. Using the K-3 antibody, the presence of this 85-kDa receptor has been immunohistochemically documented in the hamster on many types of epithelia (with notable exceptions of endothelium and mesothelium) and on members of the mononuclear phagocytic family of cells (15). The K-3 antibody was later used to demonstrate the identity of the 85-kDa HA receptor as CD44 (16, 18). Sequence homology of the distal extracellular domain of CD44 with one of the two B loop motifs of link protein is consistent with the ability of CD44 to bind to HA (4, 14). Multiple isoforms of CD44 have been discovered, suggesting that different forms of CD44 mediate different cellular functions (14, 17, 19). CD44 binds other ligands in addition to HA such as collagen types I and VI and the endothelial glycoprotein, mucosal vascular addressin (14, 19). The binding of one ligand may also modulate the binding of CD44 to another ligand. Thus, together with modulation by glycosylation and alternative splicing, several mechanisms may be available to cells to regulate CD44 binding to extracellular macromolecules.

Other cell-surface HA receptors have been reported that do not belong to the CD44 family. Turley and co-workers (20–23) have characterized a novel HA receptor that mediates HA-induced cell locomotion. The receptor protein is part of an HA receptor complex (HARC) that occurs both at the cell surface of fibroblasts and released as soluble proteins of M_r = 72, 68, 58, and 52 kDa. The 58-kDa protein of HARC contains the HA-binding component, and has been termed RHAMM, for receptor for HA-mediated motility. By cDNA sequence comparison, it is unrelated to the hyaladherins that contain the B loop motif in aggrecan, link protein, versican, neurocan, hyaluronectin, and CD44 (6), and is not recognized by anti-CD44 antibodies (14). Two domains containing clusters of basic amino acids within a region of 35 amino acids near the carboxyl terminus of RHAMM mediate its binding to HA (23).

Other hyaladherins that function as cell-surface HA receptors is a group of cell-surface antigens recognized by a monoclonal antibody raised by Banerjee and Toole (24), termed IVd4. These antibodies were raised against a soluble hyaladherin present in embryonic chick brain (unrelated to hyaluronectin and glial-hyaluronan binding protein) and selected by their capacity to block binding of ^{3}H-labeled HA to dot blots of brain extracts. Although these antibodies were raised against soluble antigens, they recognize with cell-surface HA-binding proteins present on many cell types. Three dominant proteins in the range of M_r ~ 50,000–95,000 have been detected (24, 25). The relationship between the IVd4-group of hyaladherin receptors and CD44 is not yet clear. Nonetheless, when detected on cells in tissue sections or in culture, these hyaladherin receptors exist most often in an occupied state rather than as unoccupied receptors, suggesting their importance in mediating cell-HA interactions.

Another family of HA receptors, apparently not related to CD44, is involved in the clearance of HA from the circulation and is primarily localized on endothelial cells of liver sinusoids (26, 27). Hence, these HA receptors have been given the name liver endothelial cell (LEC) receptors.

Although the original definition of the "hyaladherins family" by Toole (4) includes only those HA-binding proteins that contain the B loop motif of link protein, we would like to extend the definition of hyaladherins to include other HA-binding proteins (such as RHAMM, LEC, and possibly the IVd4-group) that do not contain this structural motif. It will be interesting to compare the biological functions of the hyaladherins with different structural HA-binding motifs as these motifs become identified.

Although similarities between cell-surface hyaladherins and matrix hyaladherins are clearly present (i.e., the expression of a link protein B loop motif), there are nonetheless several important distinctions between these two classes of hyaladherins. Most of the cell-surface receptor hyaladherins (CD44, IVd4-group, and LEC) display effective competition for the binding of native HA by HA hexasaccharides (HA_6). Thus, HA_6 represents the minimum size of an HA oligosaccharide that interacts with these cell-surface hyaladherins. All of the matrix hyaladherins described require at least a deca- or dodecasaccharide of HA (HA_{10-12}) for competition (for references, see ref 28). The affinity of binding HA to cell-surface hyaladherins also increases with increasing polymer length of HA. These results have suggested a multivalent interaction of HA with several cell-surface receptors to obtain the high binding affinity for HA reported ($K_d \cong 10^{-9}$ M) (for references, see refs 4, 14). In addition, the binding of HA to cell-surface hyaladherins increases with increasing buffer ionic strength, and binding to the CD44-like HA receptors is stable to mild glutaraldehyde fixation. These properties help to differentiate the specific binding of HA to cell-surface receptor hyaladherins from the specific aggregation of HA with matrix hyaladherins.

HYALADHERINS REGULATE THE FUNCTION OF HYALURONAN WITHIN THE EXTRACELLULAR MATRIX

The temporal and spatial patterning of HA during embryogenesis, tissue remodeling, and tumor invasion suggest that the timing of synthesis and removal of HA are important regulatory factors in tissue reconstruction. Changes in the composition and organization of HA within a matrix are controlled by a variety of hyaladherins.

Clearance and catabolism of hyaluronan within tissues

The removal of HA from an extracellular matrix occurs via local cellular catabolism and/or, in adult tissues, by drainage

into the lymphatic system for catabolism in regional lymph nodes. After entry into the circulation, HA is cleared by cells of liver sinusoids. In both systems catabolism apparently occurs intracellularly after receptor-mediated endocytosis. This would be expected because most mammalian tissues exhibit only a low pH, lysosomal form of hyaluronidase (2, 29, 30).

Clearance of matrix hyaluronan in development

Rapid reductions in the level of extracellular HA have been reported in many embryonic organs undergoing morphogenesis (i.e., cornea, kidney, lung, limb). The decrease in HA is correlated with an increase in cellular hyaluronidase and a narrowing of the overall intercellular space (for references, see ref 2). Receptor-mediated endocytosis of HA by local cells is the primary mechanism for the clearance of this HA. The HA-enriched matrix of the cardiac jelly in the embryonic heart promotes the invasion of endocardial cells (29). During that cellular migration and subsequent organogenesis, HA is depleted from the matrix and heparan sulfate proteoglycans as well as chondroitin sulfate proteoglycans become incorporated in it. The invading endocardial cells express the capacity to bind to HA but lack the ability to internalize and degrade HA. It is the cells of the myocardium that have the capacity for the timely removal of HA from the cardiac jelly via receptor-mediated endocytosis and degradation (29). The type of HA receptor responsible for this internalization has not yet been determined.

During limb morphogenesis, high levels of matrix HA are rapidly depleted in the central core of the limb, which serves to promote core condensation leading to chondrogenesis and myogenesis (5, 28, 31). At precisely this stage of condensation, presumptive chondroblasts begin expressing cell-surface protein binding sites specific for HA, i.e., HA receptors (32). Early limb myoblasts also exhibit a capacity to bind HA (5, 32). It is not known yet whether these cells are responsible for the clearance of HA. However, the timely expression of HA receptors at the onset of HA depletion from the tissue, the depletion of HA only in the central region of the limb undergoing condensation, and the documented capacity of mature chondrocytes to bind and internalize HA (discussed below) strongly suggest the role of these resident cells in the removal of HA.

Underhill et al. (33) have shown a correlation of tissue macrophages that express CD44 and show HA in endosomes with a decrease in tissue HA content during critical stages of mouse lung development. Thus endocytosis by macrophages may result in focal depletion of HA within a tissue. CD44-mediated uptake of HA for subsequent degradation in the lysosomal compartment has been demonstrated on hamster alveolar macrophages as well as simian virus-transformed 3T3 cells (34). Resident macrophages and cells such as fibroblasts, using CD44/HA receptors, may thus play a significant role in the local clearance of HA in adult tissues such as lung, intestinal mucosa, and skin.

Local turnover of matrix hyaluronan in adult cartilage

Cartilage matrix turnover, even in minor amounts, must be carefully controlled and coordinated to ensure tissue integrity. HA plays a key structural role in the organization of cartilage extracellular matrix, serving as a backbone structure for the fixation of aggrecan within the tissue. Recent studies in our laboratories have demonstrated that HA is, in fact, endocytosed locally within the tissue by resident chondrocytes and that the endocytosis is mediated via cell-surface CD44/HA receptors (35). Intense binding and accumulation of either fluorescein- or ^{3}H-labeled HA on cultured chondrocytes was visualized on the cell surface and within small, discrete intracellular vesicles. Binding and endocytosis of labeled HA was completely blocked by the addition of excess unlabeled HA or HA_6. Binding and endocytosis was also blocked by the addition of anti-CD44 monoclonal antibodies. Characterization of endocytosed ^{3}H-labeled HA demonstrated that a significant portion of the HA was degraded to small-sized oligosaccharides by the chondrocytes. Chloroquine treatment reduced by 85% the amount of extensively degraded HA, indicating that the intracellular degradation of HA after endocytosis occurs via lysosomal enzymes. Therefore, HA receptor-mediated endocytosis and degradation of HA may provide a critical link to the maintenance and homeostasis of cartilage tissue.

Clearance of hyaluronan via the lymphatic and circulatory systems

Adult tissues also maintain proper matrix HA levels by lymphatic system clearance. Upon entering the regional lymph nodes, as much as 90% of the lymph HA is removed and degraded (1). The small percentage that does enter the circulation is cleared, within a half-life of minutes, by hyaladherin receptors present on liver cells. Receptors for HA have been isolated from rat liver sinusoidal endothelial cells by different techniques and different research groups. Proteins of M_r = 100,000 (36) and of M_r = 175,000 and 166,000 (37) have been described. They all show Ca^{2+}-independent binding and uptake via clathrin-coated pits, and increasing affinity with increased HA polymer length. These hyaladherin receptors differ from all other hyaladherins by displaying a similar capacity to bind and internalize chondroitin sulfate as well as HA. Diseases or malfunctions of the liver, such as cirrhosis, and liver transplant rejection result in very high serum levels of HA as well as elevated levels of HA within tissues (for references, see ref 1).

Hyaluronan biosynthesis

Little is known about the exact mechanism (or mechanisms) that regulate HA synthesis. Laurent and Fraser (30) have outlined several factors known to influence HA synthesis, most of which are related to activation of adenylate cyclase or activation of cell proliferation. On chondrocytes, the presence of high concentrations of exogenous HA can have a negative effect on its own synthesis as well as on the synthesis of aggrecan (9). Where HA inhibition of HA synthesis occurs, it most likely represents a feedback signal resulting from the saturation of cell-surface HA receptors such as CD44. It has been proposed by Prehm (38) that the binding of elongating nascent HA to an as yet undefined HA receptor controls chain length and elongation. Thus, total saturation of receptors may inhibit HA synthesis by "stalling" termination of nascent chains or by transmembrane signal transduction, which results in an intracellular response governing HA and proteoglycan synthesis.

MATRIX ASSEMBLY

Besides controlling the levels of HA within a tissue, HA-binding proteins, cell-surface HA receptors in particular, regulate assembly and patterning of the extracellular matrix. Although HA receptors may be thought of as a means to anchor fibroblasts, epithelia, and hematopoietic cells to the extracellular matrix, they should also be viewed as tethering the extracellular matrix to the cells. Initial assembly of extracellular matrix occurs within a zone immediately adjacent

to the cell surface termed the cell-associated or pericellular matrix. In many connective tissues the pericellular matrix may serve as a template structure to facilitate the organization of the intracellular matrix. Extensive pericellular matrices of varying thicknesses have been visualized by a particle exclusion assay surrounding a wide variety of eukaryotic cells in vitro (1, 2, 28, 31) suggesting that matrix assembly and patterning occur close to the cell surface and are common features of many tissues. The principal feature that is known about the composition of these pericellular matrices visualized on cultured cells is that they are scaffolded on filaments of HA anchored to the cell surface via HA receptors (5, 17, 28, 39).

Do such pericellular matrices exist in vivo? This is difficult to determine because the means to isolate single cells from a tissue typically requires the destruction of pericellular matrix. To address this question, we isolated chondrocytes directly from embryonic chick tibiae by a 10-min treatment with purified collagenase in the presence of 20% horse serum (40). After a rapid cytospin procedure, "splatted" freshly isolated chondrocytes exhibited prominent pericellular matrices (**Fig. 1*A***) similar in size to those observed around cultured chondrocytes (28, 31). However, these pericellular matrices had been assembled and patterned in vivo. Further, these pericellular matrices could be displaced by subsequent 3-h incubation in medium containing HA_6 (Fig. 1*B*), suggesting that the matrix was, in fact, anchored to the cell surface via HA receptors. The expression of pericellular matrices visualized around chondrocytes in vitro correlates well with their primary cellular function: to generate and organize an extracellular matrix capable of withstanding load forces.

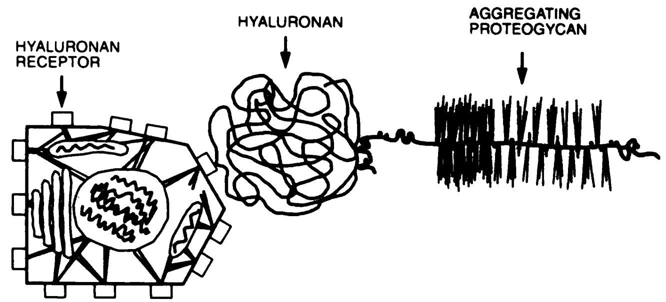

Figure 2. Model for pericellular matrix assembly. Our hypothesis that a minimum of three components are necessary for initial matrix assembly and patterning: a cell-surface hyaladherin receptor, hyaluronan, and a matrix hyaladherin. A proteoglycan matrix hyaladherin with a functional hyaluronan-binding domain and intact glycosaminoglycan-containing domain apparently is required.

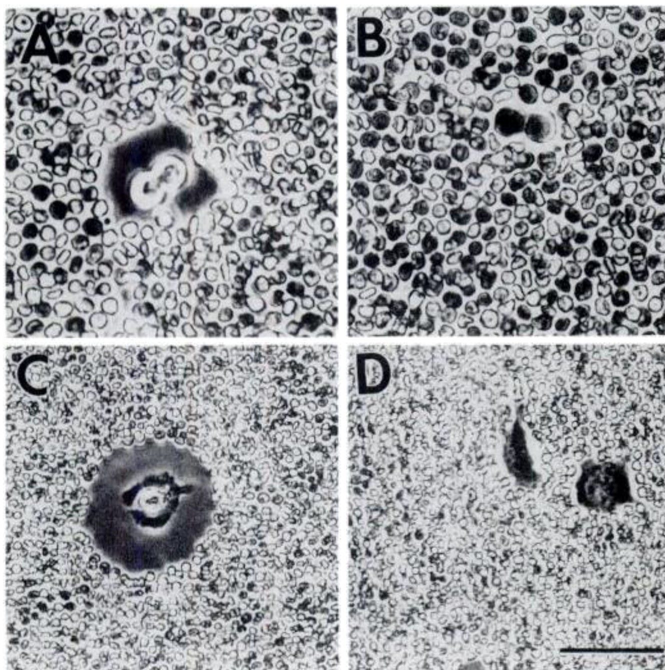

Figure 1. Visualization of hyaluronan-dependent pericellular matrices. *A)* Chondrocyte pericellular matrix assembled in vivo. Chondrocytes were liberated from embryonic chick tibial cartilage by a 10-min treatment with purified collagenase. After cytospin, a native pericellular matrix was visualized by its capacity to exclude fixed erythrocytes. *B)* Displacement of in vivo matrix by hyaluronan hexasaccharides HA_6. Chondrocytes, released from tibial cartilage as in *A*, were incubated in suspension for 2 h in the presence or absence of 50 μg/ml HA_6. In the absence of HA_6 the matrix was intact (as shown in *A*.) In the presence of HA_6, competitive inhibitors of hyaluronan/receptor binding, the matrix was displaced indicating that hyaluronan receptors anchor the matrix. *C)* Matrix assembly with exogenous macromolecules. HCV-29T bladder carcinoma cells express hyaluronan receptors (53) but do not exhibit pericellular matrices. However, cells incubated in the presence of exogenously added hyaluronan and aggrecan exhibited prominent pericellular matrices. *D)* Effect of HA_6 on pericellular matrix assembly with exogenous macromolecules. HCV-29T cells incubated in the presence of hyaluronan, aggrecan, and excess HA_6 resulted in no pericellular matrix assembly. Thus blocking the interaction of hyaluronan with its cell-surface receptor prevents pericellular matrix assembly. Bar, 50 μm.

Role of hyaladherins in pericellular matrix assembly

As described previously, embryonic chondrogenic and myogenic limb mesenchyme express HA receptors precisely at the stage of chondrogenic condensation and the local removal of matrix HA (5, 32). However, chondrogenic cells isolated from embryonic limb tissue at this stage of development do not exhibit pericellular matrices in culture (31). HA as well as HA receptors are present on these cells, suggesting that other matrix components are required for pericellular matrix assembly. Newly differentiated chondrocytes, isolated from a subsequent stage of development, exhibit prominent pericellular matrices similar to adult chondrocytes (28, 31). These cells have now also begun to express type II collagen and aggregating proteoglycan (aggrecan), suggesting that receptor-bound HA must be decorated with matrix hyaladherins in order to establish a pericellular matrix of sufficient structural integrity to exclude particles. This led to the hypothesis that a minimum of three components was necessary for initial matrix assembly and patterning: a cell-surface hyaladherin receptor, HA, and a matrix hyaladherin (**Fig. 2**).

Are these three components alone sufficient for assembly of a pericellular matrix around cells, and presumably the intercellular matrix as well? To explore this question, we added combinations of HA and aggregating proteoglycan to cells in culture that expressed HA receptors, but did not synthesize these matrix components. Tumor cells derived from malignant human carcinomas that express HA receptors, but have little matrix, were one example. When purified HA and aggregating proteoglycan were added to these cells exogenously, the epithelial tumor cells assembled prominent

pericellular matrices similar in size to those of chondrocytes (39; Fig. 1*C*). Addition of HA alone, proteoglycan alone, or HA and proteoglycan in the presence of HA_6 (Fig. 1*D*) resulted in a failure of matrix assembly. This work was repeated on other tumor cells (39), glutaraldehyde-fixed tumor cells (39), glutaraldehyde-fixed, matrix-free chondrocytes (28), and COS cells transfected with CD44-H as well as CD44-E cDNA (17), all with similar results. Cells derived from noninvasive human papillomas that do not express HA receptors (39), COS cells prior to transfection, or transfected with control vector minus CD44 inserts (28), as well as pretrypsinized chondrocytes or tumor cells (28, 39), failed to assemble pericellular matrices in the presence of HA and aggregating proteoglycan.

What types of HA receptors are involved? Matrix assembly on CD44-transfected COS cells as well as chondrocytes could be inhibited with anti-CD44 antibodies (17, 41), so CD44-like HA receptors are clearly involved in matrix assembly and patterning. However, Toole (2) reported that IVd4 antibodies also block matrix assembly on embryonic chick limb chondrocytes as well as other cell types. IVd4-stained tissue sections of the developing chick nervous system exhibit cell-surface receptor sites apparently occupied by HA (24), perhaps in complex with other matrix hyaladherins, such as hyaluronectin. Thus these binding proteins likely are also involved in matrix assembly, especially during development.

What types of matrix hyaladherins are required? Aggrecan proteoglycans isolated from rat chondrosarcoma, bovine nasal, or bovine articular cartilages were equivalent in their capacity to support this type of matrix assembly (39). However, reduced/alkylated aggrecan, the HA-binding region of aggrecan, or the chondroitin sulfate-rich-region of aggrecan were unable to support assembly (28). Thus, a functional HA-binding domain and an intact glycosaminoglycan-containing domain apparently are required. Other matrix hyaladherins that decorate HA filaments may also support this type of matrix assembly. For example, we have found that addition of conditioned medium from fibrosarcoma cells is sufficient for matrix assembly (39). This conditioned medium contains high levels of HA but also versican-type proteoglycans. In emerging patterns in the extracellular matrix during the development of the brain and loose connective tissue in a variety of organs, the colocalization of hyaluronectin or neurocan with HA suggests that these hyaladherins may also function in matrix assembly.

Matrix assembly during chondrogenesis

When added to prechondrogenic micromass cultures established from embryonic chick limb bud mesoderm, HA_6 inhibits chondrogenesis, a process believed to be mediated via HA receptors (5). When HA_6 was added, matrix accumulation was reduced and $^{35}SO_4$-proteoglycan was displaced from the cartilage micromass nodules (42). These results suggest that HA receptors mediate matrix assembly during chondrogenesis via their interaction with HA/proteoglycan complexes. The IVd4 monoclonal antibody to HA receptors, in a manner analogous to HA_6 inhibits chondrogenesis in micromass cultures (2). Through the interaction of these matrix receptors with extracellular components, prechondrogenic mesenchyme may establish and maintain the chondrocyte phenotype with a differentiated morphology and pattern of matrix biosynthesis (for references, see ref 28).

DIRECT EFFECT OF HYALURONAN ON CELL BEHAVIOR

Cell-to-cell adhesion mediated by hyaluronan/hyaladherin receptor interactions

Cells can be cross-bridged via the multivalent interaction of HA with receptors on adjacent cells (4), thus mediating homotypic cell adhesion. Fragments of HA too small to bind to multiple receptors inhibit this cell aggregation by occupying individual HA receptors. Excess HA also inhibits this aggregation by receptor saturation. In addition, it has been proposed that prominent pericellular matrices reduce hyaladherin-mediated interactions between homologous cells — in other words, they modulate cell-cell aggregation (5, 15).

Hyaluronan-mediated leukocyte aggregation

Aggregation of lymphoma cells and macrophages can be induced by small concentrations of exogenous HA. These cells aggregate via the multivalent interaction of HA with CD44-HA receptors. Peritoneal macrophages that undergo aggregation phenomena in vivo were demonstrated to exhibit aggregation induced by HA in vitro that was blocked by the K-3 antibody to CD44 (43). When HA was added to several T lymphoma or B cell lines that all express Pgp-1/CD44, only some formed cell aggregates; this aggregate formation was blocked with anti-Pgp-1 antibodies (44). However, it is interesting that not all cells expressing the epitopes recognized by these same antibodies are able to undergo cell aggregation by HA.

In a study of heterotypic cell adhesion, HA and CD44 were found to mediate the adhesion between B lineage hybridoma cells and bone marrow stromal cells (45). Because treatment of the stromal cells with *Streptomyces* hyaluronidase reduced the B cell adherence and treatment of the lymphoid cells anti-Pgp-1/CD44 antibody blocked the adhesion to stromal cells, the authors suggested that the adhesion may be a receptor-ligand interaction. Thus, it is possible that CD44-positive lymphocytes may be sequestered to a site of inflammation where HA levels are elevated (1). Thus, pericellular HA may be used to cross-bridge cells if unoccupied HA receptors are expressed on adjacent cells.

Cell-cell interactions during development

Adhesive interactions are also important in the regulation of morphogenesis. One example is the formation of precartilage cellular condensations, an important step in chondrogenesis (for references, see ref 28). Chondrogenic cells in the core of the embryonic limb not only deplete HA from the core matrix but also use some of the residual HA to form essential cell-to-cell contacts prior to chondrogenesis. We found that the potential for intercellular adhesion via HA receptors was correlated with the onset of cellular condensation in vivo (32). Intercellular adhesion of limb mesoderm was found to be stage-dependent, trypsin-sensitive, and dependent on the presence of HA (C. B. Knudson, unpublished results). The addition of HA_6 to high-density micromass cultures of limb mesoderm inhibited the formation of precartilage cell aggregations by 50% as well as markedly reduced chondrogenic differentiation.

Cell migration

Increased matrix deposition of HA has been correlated with cell migration in embryogenesis, limb regeneration, wound healing, and tumor invasion (for references see refs 2–5, 15, 20, 46). As discussed, elevated matrix HA provides an expanded, highly hydrated extracellular milieu conducive to cell migration. Migrating cells use a variety of cell type-specific extracellular matrix receptors (i.e., laminin/elastin receptors [47]; integrins, etc.) to direct their migration. In addition, expression of unoccupied HA receptors may allow cells to adhere directly to HA and to be translocated through HA-enriched extracellular matrices. Since the intracellular domain of CD44 HA receptors has been shown to associate with the cytoskeletal protein ankyrin (48), these same receptors may have the capacity to transduce the signal of HA binding and affect reorganization of the actin cytoskeleton necessary for cell migration. Thus, multiple matrix recognition signals have the potential to direct the complex migration behavior of cells involved in morphogenesis, tissue repair, and neoplastic invasion.

Some cell types express HARC, which mediate a locomotory response to HA (20–23). This response involves activation of various intracellular pathways resulting in ruffling of plasma membrane, detachment from substratum, and cell locomotion. The HARC proteins are expressed only in the leading lamellae and perinuclear regions of locomoting fibroblasts. One protein of the HARC, termed RHAMM, binds to HA with high affinity. The cDNA encoding RHAMM does not include a putative transmembrane hydrophobic region, and is thus predicted to be a peripheral protein associated with other transmembrane HARC proteins. This organization is similar to model of the 67-kDa elastin/laminin receptor, in which the ligand-binding sites are in a peripheral protein that also associates with an actin-binding transmembrane docking protein (47). In *c*-H-*ras* transformed fibroblasts, RHAMM appears to be complexed with a membrane-bound intracellular tyrosine protein kinase (21). Addition of a tyrosine kinase inhibitor (genistein) before the addition of HA inhibited the typical HA stimulatory locomotion response. However, if HA was added 10 min before the genistein, locomotion occurred, suggesting that HA stimulates locomotion via a rapid tyrosine kinase signal transduction pathway in these cells. Thus, cells may use one type of cell-surface hyaladherin (RHAMM/HARC) to induce migratory responses to a HA-enriched matrix and other HA receptors (CD44-like, IVd4-like) to adhere and translocate on HA as an extracellular matrix substratum.

Migration of cardiac septal mesenchyme

During embryonic heart development, cells of the epithelial endocardium elongate, detach and take on characteristics of mesenchymal cells as they migrate into a HA-enriched extracellular matrix, the cardiac jelly. Cells undergoing this epithelial-mesenchymal transformation and migration express the capacity to bind to HA (29), which may promote or directly facilitate this cell migration. The cell-surface, HA-binding characteristics are similar to CD44-, IVd4-like HA receptors. In addition, HARC proteins are expressed on embryonic heart fibroblasts (20), some of which may include these mesenchymal cells derived from the epithelial endocardium.

Endothelial cell migration

Morphogenesis of the endothelium also appears to require cell migrations mediated via the interaction of HA with cell-surface receptors. High concentrations of matrix HA are associated with new capillary sprouts, but matrix levels of HA decrease subsequently with vessel formation. On the other hand, high molecular weight HA inhibits endothelial cell proliferation (for references, see refs 25, 49, 50). Endothelial cells exhibit high-affinity, cell-surface HA binding activity and express three proteins (M_r = 95, 77, and 50 kDa) that are recognized by the IVd4 antibody (25). By immunolocalization the IVd4 epitopes were localized to the leading lamellipodia of motile endothelial cells, consistent with their role in cell migration. Blocking the interaction of HA with these receptors with either high concentrations of HA_6 or the IVd4 antibody inhibited endothelial cell migration in wounded monolayers as well as the formation of capillary-like tubules in gels of collagen and basement membrane proteins. Other investigators have demonstrated that soluble HA oligosaccharides, of a defined size range, stimulate not only a migratory response of endothelial cells, but directed migration (e.g., chemotaxis). Low concentrations of HA oligosaccharides (6–20 monosaccharides) stimulate endothelial proliferation, are angiogenic in CAM assays, and stimulate chemotactic migration in Boyden chamber assays (50). Soluble native HA and HA oligosaccharides of larger sizes have no chemotactic influence on these cells. Whether such HA oligosaccharides exist in tissues is only speculation; but the disparate influence of HA of different molecular weights supports the hypothesis that the monovalent interaction of HA oligosaccharides may affect different signals from those resulting from the multivalent interactions of HA with multiple receptors. Whether this chemotactic response is mediated via IVd4, CD44, or other HA receptors is not conclusive.

Neural crest cell migrations

The complex dispersion of the neural crest is the classic paradigm for cell-matrix interactions in cell migration during embryogenesis (for references, see refs 2, 51). Multiple cellular signals and motility activation factors are clearly involved because multiple matrix adhesion proteins promote neural crest migration, and antibodies directed toward these matrix macromolecules or their extracellular matrix receptors inhibit migration. Matrix HA has been correlated with neural crest cell pathways (**Fig. 3*A***). HA again may provide a hydrated milieu conducive to cellular migration or directly facilitate neural cell migration via interaction with cell-surface HA receptors. The temporal and spatial pattern of these multiple signals may be crucial to initiate and promote neural crest cell migration.

Neural crest cells also use "stop signals" to terminate their migration and initiate cytodifferentiation at their destination site. As neural crest cells migrate into particular destination sites, they encounter new extracellular matrix components that serve as stop signal cues. It has been proposed by Perris and Johansson (51) that neural crest cell migration is inhibited when motility-promoting matrix adhesion molecules become in lesser proportion to HA-aggregating proteoglycans; in other words, HA-proteoglycan complexes inhibit migration. These investigators found that cartilage aggrecan inhibited neural crest cell migration on fibronectin substrata, and that this inhibition was abrogated when HA was released from the culture system by *Streptomyces* hyaluronidase treatment. Delpech and co-workers (46, 52) have observed similar effects on invading tumor cells. Mammary tumors and gliomas of higher invasive grade have a lower ratio of hyaluronectin to HA. Conversely, their data suggest that tumor cells show less invasion when the levels of hyaluronectin are high. Thus, HA-hyaluronectin complexes

may also serve as stop signals. In our laboratory, we have documented that cells expressing HA receptors, exposed to HA and an aggregating proteoglycan, assemble prominent pericellular matrices (Fig. 1*C*). No such matrices form in the presence of HA alone, at any concentration, or proteoglycan alone. We would therefore propose that when neural crest cells encounter new matrices enriched in both matrix hyaladherins as well as HA, pericellular matrices would assemble. These pericellular matrices would then sterically inhibit the interaction of cells with other migration signals (Fig. 3*B*). To test this hypothesis, neural crest cells were obtained from chick embryo neural tube explants. No native endogenous pericellular matrices were detected on these neural crest cells by a particle exclusion assay (**Fig. 4*A***), even in the presence of exogenous HA (data not shown). However, in the presence of HA and aggrecan, prominent pericellular matrices formed around individual "pioneer" neural crest cells (Fig. 4*B*) as well as groups of migrating neural crest cells (Fig. 4*C*). The assembly of these matrices could be blocked using HA_6, indicating the involvement of cell-surface HA receptors. Neural crest cells may thus use these same HA receptors both for migration through HA-enriched extracellular matrices and in the assembly of HA/versican, PG-M, or hyaluronectin containing pericellular matrices, blocking their interaction with other matrix adhesion proteins, to terminate migration (Fig. 3*B*).

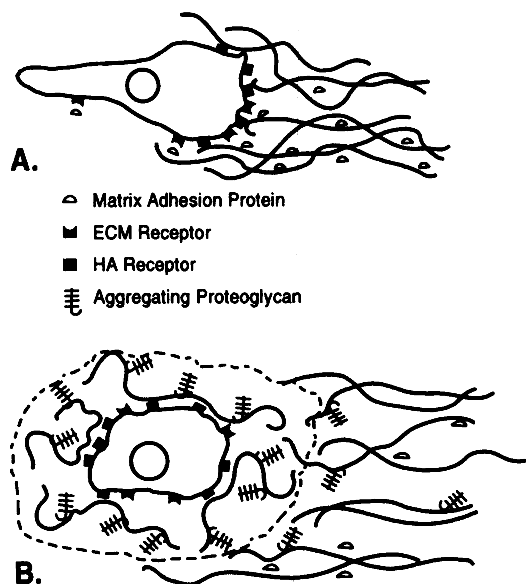

Figure 3. Cell migration promoted or inhibited by differing extracellular matrix environment. *A)* Multiple cellular signals and motility activation factors in the extracellular matrix are clearly involved in cell migration. Hyaluronan may provide a hydrated milieu conducive to cellular migration or directly facilitate cell migration via interaction with cell-surface hyaluronan receptors. The interaction of hyaluronan with HARC results in a chemokinetic response. Expression of unoccupied CD44-like hyaluronan receptors may allow cells to adhere to and be translocated through hyaluronan-enriched extracellular matrices. *B)* When migrating cells encounter new matrices enriched in hyaluronan as well as matrix hyaladherins, the assembly of new pericellular matrices results. Such matrices may result in a steric hinderance of the interaction with migration signals. Individual cells may remain isolated in matrix cocoons within connective tissues or may initiate new patterns of cytodifferentiation within this new location.

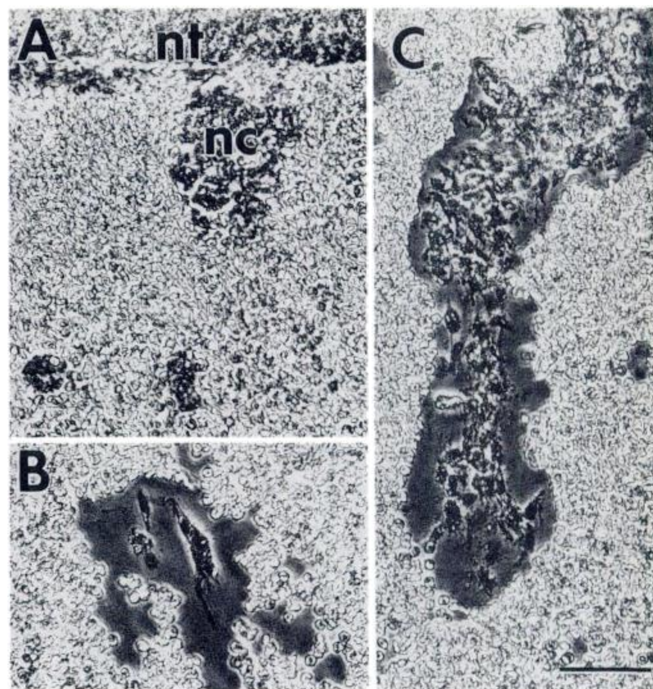

Figure 4. Neural crest cell migration and assembly of pericellular matrices. *A)* Neural crest (nc) cells emigrating from chick embryo neural tube (nt) explants do not exhibit native endogenous pericellular matrices. *B)* However, in the presence of hyaluronan and aggrecan, prominent pericellular matrices form around individual "pioneer" neural crest cells, revealed by the capacity of this new matrix to exclude erythrocytes. *C)* Pericellular matrix assembly by groups of migrating neural crest cells also occurs in the presence of hyaluronan and aggrecan. Bar, 50 μm. The assembly of these matrices can be blocked by the addition of hyaluronan hexasaccharides, indicating the involvement of cell-surface hyaluronan receptors. Neural crest cells may thus use hyaluronan receptors both to facilitate migration through hyaluronan-enriched extracellular matrices and to halt migration due to matrix assembly when additional hyaladherins such as versican, PG-M, or hyaluronectin are encountered.

Tumor invasion and metastasis

Like embryonic extracellular matrices, tumor-associated stroma are often highly enriched in HA (3). Invasive tumor cells utilize a combination of HARC/RHAMM and/or CD44-like HA receptors to promote their invasion through soft connective tissues (Fig. 3*A*). For example, in earlier work we demonstrated that cell lines derived from invasive human bladder carcinomas (HCV-29T and HU-456) exhibit high-affinity, high-specificity, cell-surface HA-binding sites, whereas cells derived from a noninvasive human bladder papilloma (RT-4) do not exhibit binding activity (53). Similarly, metastatic cell lines derived from a rat pancreatic adenocarcinoma express the CD44v isoform whereas no expression was detected on the nonmetastatic derivatives of the same parental tumor (54). CD44-transfection of lymphoma cells enhanced tumor formation as well as the metastatic potential of these cells (55). Stable transfectants of melanoma cells expressing CD44 displayed an increased motility on HA-coated coverslips, a motility that required the extracellular HA-binding domain as well as the cytoplasmic domain, presumably for interaction with the actin cytoskeleton (56). Posttranslational modifications of CD44 in lymphoma cells increase the binding of CD44 to ankyrin, although they are not required for interaction with ankyrin (48).

Tumor cells that express HA receptors, like invading neural crest cells, also have a capacity to assemble HA-enriched pericellular matrices (39, 53). It is not known whether these pericellular matrices occur in vivo around tumor cells. These pericellular matrix halos may function to reduce cell-cell adhesion or provide a layer of protection from immune surveillance (1). Alternatively, the assembly of pericellular matrices may represent a protective response of the host to introduce "stop signals" (Fig. 3*B*). In this way, individual tumor cells may remain isolated in matrix cocoons within connective tissues. During the infiltration of basal cell carcinomas, part of the intense stromal response at the advance of the invading tumor cells includes the elevated deposition of hyaluronectin, synthesized by the stromal fibroblasts (57). As discussed previously, lower levels of stromal hyaluronectin were associated with breast carcinomas and gliomas of higher invasive grade (46, 52). Thus, this capacity for matrix assembly raises interesting possibilities for metastatic cell migration, as well as embryonic cell migration, during which a particular cell type may come into new matrix environments.

During development, tissue homeostasis, and disease, complex changes in cell behavior are often triggered and patterned by alterations in extracellular matrices. The contribution of HA and hyaladherins to these changes is beginning to unfold and promises to be enlightening. FJ

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REFERENCES

1. Laurent, T. C., and Fraser, J. R. E. (1992) Hyaluronan. *FASEB J.* **6**, 2397-2404
2. Toole, B. P. (1991) Proteoglycans and hyaluronan in morphogenesis and differentiation. *Cell Biology of Extracellular Matrix* (Hay, E. D., ed) pp. 305-339, Plenum Press, New York
3. Knudson, W., Biswas, C., Li, X. Q., Nemec, R. E., and Toole, B. P. (1989) The role and regulation of tumour-associated hyaluronan. *The Biology of Hyaluronan, Ciba Foundation Symposium 143* (Evered, D., and Whelan, J., eds) pp. 150-169, John Wiley and Sons, Chichester, U.K.
4. Toole, B. P. (1990) Hyaluronan and its binding proteins, the hyaladherins. *Curr. Opin. Cell Biol.* **2**, 839-844
5. Toole, B. P., Munian, S. I., Wells, S., and Knudson, C. B. (1989) Hyaluronate-cell interactions and growth factor regulation of hyaluronate synthesis during limb development. *The Biology of Hyaluronan, Ciba Foundation Symposium 143* (Evered, D., and Whelan, J., eds) pp. 138-149, John Wiley and Sons, Chichester, U.K.
6. Hardingham, T. E., and Fosang, A. J. (1992) Proteoglycans: many forms and many functions. *FASEB J.* **6**, 861-870
7. Shinomura, T., Nishida, Y., and Kimata, K. (1992) A large chondroitin sulfate proteoglycan (PG-M) and cartilage differentiation. *Articular Cartilage and Disease* (Kuettner, K. E., Schleyerbach, R., Peyron, J. G., and Hascall, V. C., eds) pp. 35-44, Raven Press, New York
8. Kielty, C. M., Whittaker, S. P., Grant, M. E., and Shuttleworth, C. A. (1992) Type VI collagen microfibrils: evidence for a structural association with hyaluronan. *J. Cell Biol.* **118**, 979-990
9. Mason, R. M., Crossman, M. V., and Sweeney, C. (1989) Hyaluronan and hyaluronan-binding proteins in cartilaginous tissues. *The Biology of Hyaluronan, Ciba Foundation Symposium 143* (Evered, D., and Whelan, J., eds) pp. 107-120, Wiley Press, Chichester, U.K.
10. LeBaron, R. G., Zimmermann, D. R., and Ruoslahti, E. (1992) Hyaluronate binding properties of versican. *J. Biol. Chem.* **267**, 10003-10010
11. Delpech, B., Delpech, A., Bruckner, G., Girard, N., and Maingonnat, C. (1989) Hyaluronan and hyaluronectin in the nervous system. *The Biology of Hyaluronan, Ciba Foundation Symposium 143* (Evered, D., and Whelan, J., eds) pp. 208-232, Wiley Press, Chichester, U.K.
12. Perides, G., Lane, W., Andrews, D., Dahl, D., and Bignami, A. (1989) Isolation and partial characterization of a glial hyaluronate binding protein. *J. Biol. Chem.* **264**, 5981-5987
13. Rauch, U., Karthikeyan, L., Maurel, P., Margolis, R. U., and Margolis, R. K. (1992) Cloning and primary sequence of neurocan, a developmentally regulated, aggregating chondroitin sulfate proteoglycan of brain. *J. Biol. Chem.* **267**, 19536-19547
14. Underhill, C. B. (1992) CD44: the hyaluronan receptor. *J. Cell Sci.* **103**, 293-298
15. Underhill, C. B. (1989) The interaction of hyaluronate with the cell surface: the hyaluronate receptor and the core protein. *The Biology of Hyaluronan, Ciba Foundation Symposium 143* (Evered, D., and Whelan, J., eds) pp. 87-106, John Wiley and Sons, Chichester, U.K.
16. Aruffo, A., Stamenkovic, I., Melnick, M., Underhill, C. B., and Seed, B. (1990) CD44 is the principal cell surface receptor for hyaluronate. *Cell* **61**, 1303-1313
17. Knudson, W., Bartnik, E., and Knudson, C. B. (1993) Assembly of pericellular matrices by COS-7 cells transfected with CD44 homing receptor genes. *Proc. Natl. Acad. Sci. USA* **90**, 4003-4007
18. Culty, M., Miyake, K., Kincade, P. W., Silorski, E., Butcher, E. C., and Underhill, C. B. (1990) The hyaluronate receptor is a member of the CD44 (H-CAM) family of cell surface glycoproteins. *J. Cell Biol.* **111**, 2765-2774
19. Haynes, B. F., Hua-Xin, L., and Patton, K. L. (1991) The transmembrane hyaluronate receptor (CD44): multiple functions, multiple forms. *Cancer Cells* **3**, 347-350
20. Turley, E. A. (1989) The role of a cell-associated hyaluronan-binding protein in fibroblast behaviour. *The Biology of Hyaluronan, Ciba Foundation Symposium 143* (Evered, D., ed) pp. 121-137, Wiley Press, Chichester, U.K.
21. Turley, E. A., Austen, L., Vandeligt, K., and Clary, C. (1991) Hyaluronan and a cell associated hyaluronan binding protein regulate the locomotion of ras-transformed cells. *J. Cell Biol.* **112**, 1041-1047
22. Hardwick, C., Hoare, K., Owens, R., Hohn, H. P., Hook, M., Moore, D., Cripps, V., Austen, L., Nance, D. M., and Turley, E. A. (1992) Molecular cloning of a novel hyaluronan receptor that mediates tumor cell motility. *J. Cell Biol.* **117**, 1343-1350
23. Yang, B., Zhang, L., and Turley, E. A. (1993) Identification of two hyaluronan-binding domains in the hyaluronan receptor RHAMM. *J. Biol. Chem.* **268**, 8617-8623
24. Banerjee, S. D., and Toole, B. P. (1991) Monoclonal antibody to chick embryo hyaluronan-binding protein: changes in distribution of binding during early brain development. *Dev. Biol.* **146**, 186-197
25. Banerjee, S. D., and Toole, B. P. (1992) Hyaluronan-binding protein in endothelial cell morphogenesis. *J. Cell Biol.* **119**, 643-652
26. Smedsrod, B., Pertoft, H., Eriksson, S., Fraser, J. R. E., and Laurent, T. C. (1984) Studies in vitro on the uptake and degradation of sodium hyaluronate in rat liver endothelial cells. *Biochem. J.* **223**, 617-626
27. McGary, C. T., Raja, R. H., and Weigel, P. H. (1989) Endocytosis of hyaluronic acid by rat liver endothelial cells, evidence for receptor recycling. *Biochem. J.* **257**, 875-884
28. Knudson, C. B. (1993) Hyaluronan receptor-directed assembly of chondrocyte pericellular matrix. *J. Cell Biol.* **120**, 825-834
29. Bernanke, D. H., and Orkin, R. W. (1984) Hyaluronate binding and degradation by cultured embryonic chick cardiac cushion and myocardial cells. *Dev. Biol.* **106**, 360-367
30. Laurent, T. C., and Fraser, J. R. E. (1986) The properties and turnover of hyaluronan. *Functions of the Proteoglycans, Ciba Foundation Symposium 124* (Evered, D., and Whelan, J., eds) pp. 9-29, Wiley Press, Chichester, U.K.
31. Knudson, C. B., and Toole, B. P. (1985) Changes in the pericellular matrix during differentiation of limb bud mesoderm. *Dev. Biol.* **112**, 308-318
32. Knudson, C. B., and Toole, B. P. (1987) Hyaluronate-cell interactions during differentiation of chick embryo limb mesoderm. *Dev. Biol.* **124**, 82-90
33. Underhill, C. B., Nguyen, H. A., Shizari, M., and Culty, M. (1993) CD44 positive macrophages take up hyaluronan during lung development. *Dev. Biol.* **155**, 324-336
34. Culty, M., Nguyen, H. A., and Underhill, C. B. (1992) The hyaluronan receptor (CD44) participates in the uptake and degradation of hyaluronan. *J. Cell Biol.* **116**, 1055-1062
35. Hua, Q., Knudson, C. B., and Knudson, W. (1993) Internalization of hyaluronan by chondrocytes occurs via receptor-mediated endocytosis. *J. Cell Sci.* In press
36. Forsberg, N., and Gustafson, S. (1991) Characterization and purification of the hyaluronan-receptor on liver endothelial cells. *Biochem. Biophys. Acta* **1078**, 12-18
37. Yannariello-Brown, J., Frost, S. J., and Weigel, P. H. (1992) Identification of the Ca^{2+}-independent endocytic hyaluronan receptor in rat liver sinusoidal endothelial cells using a photoaffinity cross-linking reagents. *J. Biol. Chem.* **267**, 20451-20456
38. Prehm, P. (1989) Identification and regulation of the eukaryotic hyaluronate synthase. In *The Biology of Hyaluronan, Ciba Foundation Symposium 143* (Evered, D., and Whelan, J., eds) pp. 21-40, Wiley Press, Chichester, U.K.
39. Knudson, W., and Knudson, C. B. (1991) Assembly of a chondrocyte-

like pericellular matrix on non-chondrogenic cells. *J. Cell Sci.* **99,** 227–235
40. Knudson, C. B. (1991) Comparison of pericellular matrix assembly on chondrocytes grown in suspension and in monolayer culture. *J. Cell Biol.* **115,** 297a (abstr.)
41. Hua, Q., Knudson, C. B., and Knudson, W. (1993) Hyaluronan receptors mediate distribution of proteoglycans within the chondrocyte pericellular matrix. *Orthop. Trans.* **18,** 70 (abstr.)
42. Maleski, M. P., and Knudson, C. B. (1992) Matrix assembly during chondrogenesis occurs via hyaluronan-cell surface interactions. *Orthop. Trans.* **17,** 105 (abstr.)
43. Green, S. J., Tarone, G., and Underhill, C. B. (1988) Aggregation of macrophages and fibroblasts is inhibited by a monoclonal antibody to the hyaluronate receptor. *Exp. Cell Res.* **178,** 224–232
44. Lesley, J., Schulte, R., and Hyman, R. (1990) Binding of hyaluronic acid to lymphoid cell lines is inhibited by monoclonal antibodies against Pgp-1. *Exp. Cell Res.* **187,** 224–233
45. Miyake, K., Underhill, C. B., Lesley, J., and Kincade, P. W. (1990) Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. *J. Exp. Med.* **172,** 69–75
46. Delpech, B., Maingonnat, C., Girand, N., Chauzy, C., Maunoury, R., Olivier, A., Tayot, J., and Creissard, P. (1993) Hyaluronan and hyaluronectin in the extracellular matrix of human brain tumour stroma. *Eur. J. Cancer* **29A,** 1012–1017
47. Mecham, R. P. (1991) Receptors for laminin on mammalian cells. *FASEB J.* **5,** 2538–2546
48. Lokeshwar, V. B., and Bourguignon, L. Y. W. (1991) Post-translational protein modification and expression of ankyrin-binding site(s) in GP85 (Pgp-1/CD44) and its biosynthetic precursors during T-lymphoma membrane biosynthesis. *J. Biol. Chem.* **266,** 17983–17989
49. 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
50. West, D. C., and Kumar, S. (1989) Hyaluronan and angiogenesis. In *The Biology of Hyaluronan, Ciba Foundation Symposium 143* (Evered, D., and Whelan, J., eds) pp. 187–207, Wiley Press, Chichester, U.K.
51. Perris, R., and Johansson, S. (1990) Inhibition of neural crest cell migration by aggregating chondroitin sulfate proteoglycans is mediated by their hyaluronan-binding region. *Dev. Biol.* **137,** 1–12
52. Bertrand, P., Girard, N., Delpech, B., Duval, C., D'Anjou, J., and Dauce, J. P. (1992) Hyaluronan (hyaluronic acid) and hyaluronectin in the extracellular matrix of human breast carcinomas: comparison between invasive and non-invasive areas. *Int. J. Cancer* **52,** 1–6
53. Nemec, R. E., Toole, B. P., and Knudson, W. (1987) The cell surface hyaluronate binding sites of invasive human bladder carcinoma cells. *Biochem. Biophys. Res. Commun.* **149,** 249–257
54. Gunthert, U., Hofmann, M., Rudy, W., Reber, S., Zoller, M., Haussmann, I., Matzku, S., Wenzel, A., Ponta, H., and Herrlich, P. (1991) A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell* **65,** 13–24
55. Sy, M. S., Guo, Y. J., and Stamenkovic, I. (1991) Distinct effects of two CD44 isoforms on tumor growth in vivo. *J. Exp. Med.* **174,** 859–866
56. Thomas, L., Byers, H. R., Vink, J., and Stamenkovic, I. (1992) CD44H regulates tumor cell migration on hyaluronate-coated substrate. *J. Cell Biol.* **118,** 971–977
57. Delpech, A., Delpech, B., Girard, N., Boullie, M. C., and Lauret, P. (1982) Hyaluronectin in normal human skin and in basal cell carcinoma. *Br. J. Dermatol.* **106,** 561–568