HYALURONAN-CELL INTERACTIONS IN LIMB DEVELOPMENT

Bryan Toole*, Shib Banerjee*, Raymond Turner*,
Syeda Munaim*, and Cheryl Knudson+

*Department of Anatomy and Cellular Biology, Tufts University Health Science Schools, Boston, MA 02111, USA
+Departments of Biochemistry and Pathology, Rush/Presbyterian/St. Luke's Medical School, Chicago, IL 60612, USA

Hyaluronan is a ubiquitous component of the extracellular matrices in which cells migrate and proliferate during embryonic development (reviewed in Toole, 1981). Its physical and chemical properties contribute to an extracellular milieu which is important both to the structural integrity of embryonic tissues and to the morphogenetic processes that take place within them. One way in which hyaluronan participates in tissue structure arises from its ability to form meshworks that exert osmotic pressure (Comper and Laurent, 1978; Meyer, 1983). The resultant swelling pressure within the tissue can lead to separation of cellular or fibrous structures or deformation of the tissue, possibly facilitating cell and tissue movements (Toole, 1981; Morris-Wiman and Brinkley, 1990). In addition, some embryonic cells exhibit large, hyaluronan-dependent, pericellular coats (Knudson and Toole, 1985) that may influence cell-cell adhesion (Underhill and Toole, 1981; Knudson, 1990a), cell-substratum adhesion (Barnhart et al., 1979), cell proliferation (Brecht et al., 1986), migration (Turley et al., 1985; Schor et al., 1989) or differentiation (Kujawa et al., 1986).

Interaction of hyaluronan with the cell surface is mediated by membrane-bound receptors (reviewed in Toole, 1990). Recent work has demonstrated that the hyaluronan receptor of baby hamster kidney and transformed 3T3 cells is a glycoprotein of molecular weight, ~85kDa (Underhill et al., 1987) and is closely related to the CD44 lymphocyte homing receptor (Aruffo et al., 1990; Lesley et al., 1990; Miyake et al., 1990). We have recently prepared a monoclonal antibody, MAb 4D4, that recognizes chick embryo hyaluronan-binding proteins of molecular weight 93, 91 and 69kDa; these proteins are widely distributed in embryonic tissues, and are probably related to the 85kDa receptor since the antibody blocks binding of hyaluronan to transformed 3T3 cells (Banerjee and Toole, 1990). The antibody also inhibits production of hyaluronan-dependent pericellular coats (Yu et al., 1990), implying that interaction of hyaluronan with the receptor proteins is necessary for coat formation.

MESODERMAL HYALURONAN-DEPENDENT PERICELLULAR COATS

Mesodermal cells in the early chick embryo limb bud are separated by extensive hyaluronan-rich spaces (Singley and Solursh, 1981) and these cells

Developmental Patterning of the Vertebrate Limb
Edited by J.R. Hinchliffe *et al.*, Plenum Press, New York, 1991

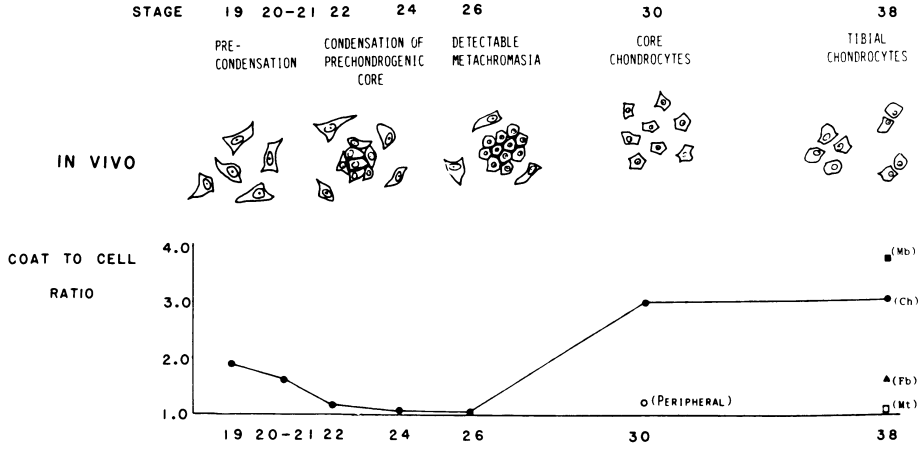

Figure 1. **Comparison of pericellular coats in vitro with cell organization in vivo**. The sizes of pericellular coats are represented as mean coat-to-cell ratios (ratios of coat perimeter to cell perimeter) obtained with cells cultured from limbs at the stages indicated. Prior to condensation, the mesodermal cells in vivo are widely separated by hyaluronan-rich matrix and in vitro they exhibit large hyaluronan-dependent coats. When the mesoderm becomes condensed in vivo, the cells lack coats in vitro. On differentiating, chondrocytes again elaborate large hyaluronan-dependent coats in vitro and an extensive matrix in vivo. Myoblasts exhibit coats prior to fusion but lose them on fusion. Ch, chondrocytes; Fb, fibroblasts; Mb, myoblasts; Mt, myotubes. (From Knudson & Toole, 1985, with permission).

in culture produce large, hyaluronan-dependent, pericellular coats (Knudson and Toole, 1985). When the chondrogenic and myogenic areas of the limb bud become condensed in vivo, i.e. these cells are separated by a smaller volume of matrix, the isolated mesodermal cells do not exhibit visible coats in culture. This change in the ability to express coats is accompanied by a large decrease in the ratio of hyaluronan to chondroitin sulfate-proteoglycan produced by the condensation-stage mesoderm, as compared to the pre-condensation mesoderm (Knudson and Toole, 1985).

During differentiation of chondrocytes, which are again separated by extensive spaces in vivo, large pericellular coats are re-expressed in culture. Chondrocyte coat structure is still dependent on hyaluronan even though proteoglycan is now a quantitatively more prominent component (Goldberg and Toole, 1984; Knudson and Toole, 1985). In contrast to chondrogenesis, fusion of myoblasts is accompanied by loss of pericellular coats (Orkin et al, 1985), apparently a necessary step towards differentiation since myoblasts cultured on a hyaluronan substratum fail to fuse (Kujawa et al., 1986). Thus there is a close correlation between the presence of large intercellular spaces in vivo and the expression of large hyaluronan-dependent coats in vitro (see Fig. 1).

CHANGES IN HYALURONAN-CELL INTERACTIONS DURING CONDENSATION

An important stage in differentiation of limb mesoderm is the above-mentioned condensation of cells that precedes final cytodifferentiation of muscle and cartilage. In parallel to the loss of ability to express

hyaluronan-dependent coats and the changes in glycosaminoglycan synthesis that occur at this stage, membrane-associated binding sites for hyaluronan are expressed (Knudson and Toole, 1987). Hyaluronan-binding sites are known, in other systems, to be involved in endocytosis en route to degradation of hyaluronan (Laurent et al., 1986; McGuire et al., 1987; McGary et al., 1989), and so it is reasonable to suppose that the appearance of hyaluronan-binding sites at the time of condensation represents the onset or increase in receptor-mediated endocytosis of hyaluronan. Decreased hyaluronan synthesis and coat production together with increased endocytosis and degradation of hyaluronan would lead to a dramatic reduction in volume of matrix between the mesodermal cells, thus allowing them to "condense".

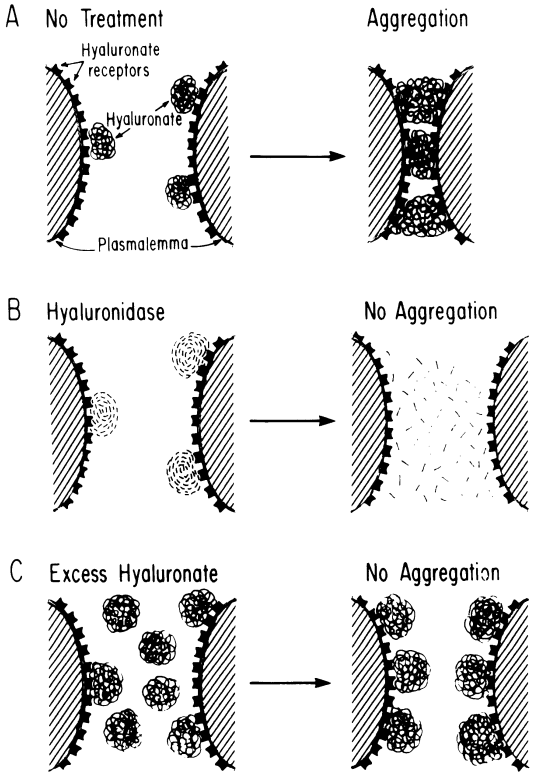

Figure 2. **Hyaluronan-mediated aggregation of cells.** Hyaluronan can crossbridge cells via a calcium-independent, multivalent interaction with receptors on adjacent cells. This aggregation is inhibited by treatment of cells with hyaluronidase (B), or excess hyaluronan (C); the latter causes saturation of the receptors, inhibiting crossbridging. If hyaluronan receptors are present but there is no hyaluronan on the surface of the cells, as occurs with some lymphocytes (e.g. see Green et al., 1988; Lesley et al., 1990), then addition of hyaluronan will induce aggregation. If the receptors are partially occupied, as occurs with some virally transformed cells (e.g. see Underhill and Toole, 1981; Green et al. 1988) and condensation-stage mesodermal cells (see Knudson, 1990a), then the cells will spontaneously aggregate. If the cells have large hyaluronan-dependent coats such as pre-condensation-stage mesodermal cells (Knudson, 1990a), aggregation will not occur. (From Toole, 1981, with permission).

In addition to the events leading to reduction in matrix volume, condensation may also involve direct cell interactions (e.g. see Knudsen et al., 1990; Bee and von der Mark, 1990). One such interaction is likely to be cross-bridging of cells via multivalent binding of hyaluronan to the cell surface binding sites that are expressed at this stage (Knudson and Toole, 1987). We have shown previously that Ca^{++}-independent self-aggregation of transformed cell lines is due to crossbridging by hyaluronan of binding sites on adjacent cells; removal of cell surface hyaluronan or saturation of the binding sites inhibits this crossbridging (Underhill and Toole, 1981; Underhill, 1982) (see Fig. 2). Knudson (1990a) has recently demonstrated that mesodermal cells from condensation stage limbs, but not cells from pre-condensation limbs, aggregate in vitro via such hyaluronan-mediated crossbridging.

Thus we propose that condensation may be explained in large part by the following events (see Fig. 3A vs B):
1. Receptor-mediated endocytosis of pre-existing pericellular hyaluronan, to permit condensation to begin;
2. Decreased hyaluronan synthesis and cessation of coat assembly, to permit condensation to continue;
3. Hyaluronan-mediated crossbridging of cells, to stabilize the condensate.

HYALURONAN-CELL INTERACTIONS IN CARTILAGE AND MUSCLE DIFFERENTIATION

Recent studies in our laboratory (Turner et al., 1990) have shown that interference with binding of hyaluronan, presumably at the surface of mesodermal cells, blocks chondrogenesis as assessed in the micromass culture

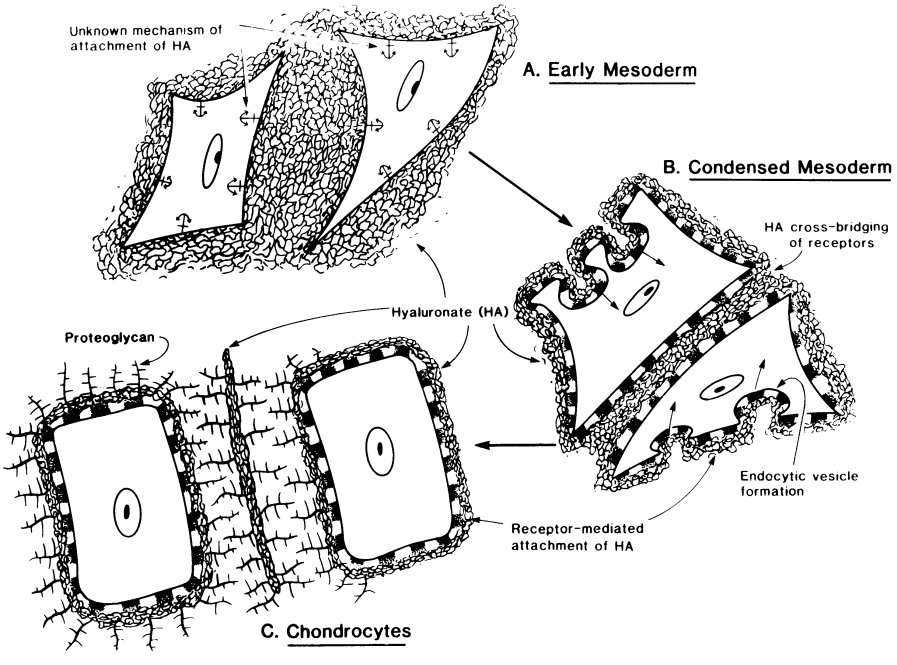

Figure 3. **The hypothesized role of hyaluronan-cell interactions in mesodermal condensation and chondrocyte differentiation.** (From Toole et al., 1989, with permission).

system of Solursh and colleagues (1978). In these studies, chondrogenesis was blocked by treatment of mesodermal cells either with the monoclonal antibody, MAb 4D4, to chick embryo hyaluronan-binding protein or with hyaluronan hexasaccharide that also disrupts binding of polymeric hyaluronan to binding protein. However, further experiments are necessary to distinguish whether hyaluronan and hyaluronan-binding proteins are directly involved in cell aggregation or differentiation in addition to their role in matrix assembly, discussed below.

Many studies indicate that hyaluronan plays an important role in matrix assembly by mature chondrocytes; for example, the well-documented interaction of hyaluronan with link protein and proteoglycan is central to the structure of cartilage matrix (Hascall and Hascall, 1981). Recent evidence indicates that retention of hyaluronan-proteoglycan aggregates in the pericellular matrix by binding to other hyaluronan-binding proteins is also important. As mentioned above, chondrocytes in culture exhibit pericellular coats that have provided a useful in vitro model for studying the pericellular matrix of chondrocytes. Using this system it has been shown, first, that chondrocyte coats are destroyed by treatment with hyaluronan-specific hyaluronidase, and this loss of coats is accompanied by loss of much of the proteoglycan associated with the chondrocyte cell layer, thus indicating that it was retained in the cell layer by interaction with hyaluronan (Goldberg and Toole, 1984; McCarthy and Toole, 1989). Second, production of these pericellular coats by chondrocytes is inhibited by hyaluronan hexasaccharide (Knudson, 1990b) or the antibody to hyaluronan-binding protein, MAb 4D4. The 4D4 antibody reacts strongly with embryonic cartilage but only after treatment of the tissue with hyaluronan-specific hyaluronidase, implying that the binding protein is also occupied by hyaluronan in vivo. The relationship of the proteins recognized by MAb 4D4 with other hyaluronan-binding proteins in cartilage (McCarthy and Toole, 1989; Crossman and Mason, 1990) is not yet known. Third, pericellular coats can be rebuilt around chondrocytes by addition of hyaluronan and proteoglycan to chondrocytes stripped of their coats by treatment with hyaluronidase, but this process is inhibited in the presence of hyaluronan hexasaccharide (Knudson, 1990b). Since hyaluronan hexasaccharides do not inhibit hyaluronan-link protein or hyaluronan-proteoglycan binding (Tengblad, 1981) but do inhibit hyaluronan interaction with the proteins recognized by MAb 4D4 (Banerjee and Toole, 1990), we conclude that hyaluronan-proteoglycan aggregates are retained in the pericellular matrix or at the chondrocyte surface by interaction with the hyaluronan-binding proteins recognized by MAb 4D4 (see Fig. 3C).

Whereas hyaluronan is clearly an important structural component of differentiated cartilage, there is no evidence that hyaluronan plays a central role in the structure of differentiated muscle. However, hyaluronan may be important in early events prior to cytodifferentiation. Exposure of myoblasts to a substratum to which hyaluronan is conjugated inhibits their differentiation and maintains the cells in a proliferative state (Kujawa et al., 1986). It is known that fibroblast-myoblast interactions are important in regulating basement membrane formation in muscle (Sanderson et al., 1986). We have shown in addition that chick embryo muscle fibroblasts in culture exhibit a hyaluronan-rich apical matrix that inhibits myoblast differentiation and a basal matrix that promotes differentiation (Welles and Toole, 1987). These findings suggest that matrix constituents, including hyaluronan, produced by neighboring mesodermal cells or fibroblasts are important in regulating myoblast differentiation. Myoblasts themselves, but not myotubes, exhibit hyaluronan-dependent coats (Orkin et al., 1985) and membrane-associated hyaluronan-binding sites (Knudson and Toole, 1987), characteristics that may be important in their response to cellular interactions prior to differentiation.

REGULATION OF HYALURONAN SYNTHESIS AND COAT FORMATION

A positive relationship may exist between hyaluronan synthesis and cell proliferation (Brecht et al., 1986), two prominent activities during the earliest stages of limb development. Basic FGF is a major growth factor in the early developing limb. The amount of this factor is highest in pre-condensation stage limb buds, when hyaluronan synthesis and cell proliferation are maximal, and decreases during subsequent condensation and differentiation of the mesoderm (Munaim et al., 1988; Seed et al., 1988). Basic FGF stimulates hyaluronan synthesis and hyaluronan-dependent coat formation in cultures of limb mesoderm (Munaim et al., 1990). Thus FGF may be important in the coordinated regulation of cell proliferation, hyaluronan synthesis and pericellular matrix assembly in the pre-condensation limb bud mesoderm. Diffusible growth factors, including FGF, have been implicated in the growth regulatory effects of the apical ectodermal ridge and zone of polarizing activity of the early limb bud (Bell and McLachlan, 1985; Aono and Ide, 1988). Therefore it will be of interest to determine whether these regions of the limb also influence hyaluronan-rich matrix assembly, and whether this potential effect contributes to their morphogenetic roles.

Interaction of limb mesoderm and ectoderm is important in the regulation of morphogenesis and differentiation in the limb bud. The subectodermal mesoderm remains non-condensed during premyogenic and prechondrogenic condensation, retaining mesenchymal morphology and large hyaluronan-rich spaces between cells (Singley and Solursh, 1981). This regionalization of condensed and non-condensed mesoderm appears to be under the control of the ectoderm since factors produced by the ectoderm prevent differentiation of nearby mesoderm to cartilage and cause the retention of mesenchymal characteristics, including hyaluronan-rich intercellular spaces (Solursh et al, 1981; Hurle et al., 1989; Solursh, 1990). We have found that ectodermal cells in culture produce a factor or combination of factors that stimulate hyaluronan synthesis and formation of hyaluronan-dependent coats in condensation-stage mesodermal cells (Knudson and Toole, 1988). Antibody raised against TGF-beta, but not antibodies against several other growth factors, inhibits these effects (Toole et al., 1989). TGF-beta itself stimulates hyaluronan-dependent coat formation and hyaluronan synthesis in these mesodermal cells but, unlike the ectodermal factor, it also stimulates chondroitin sulfate synthesis (Munaim et al., 1990). Carcinoma cells, but not normal adult epithelial cells, also produce a factor that stimulates hyaluronan synthesis in limb mesodermal cells (Knudson and Knudson, 1990). It seems likely that this factor would be similar to that produced by the limb ectoderm but its effect is not blocked by neutralizing antibody to TGF-beta (Knudson et al., 1989). We conclude from these studies that the ectoderm produces a factor that causes subjacent mesoderm to maintain a high rate of hyaluronan synthesis relative to the central condensed mesoderm; however its relationship to TGF-beta requires further investigation.

Thus a combination of regulatory factors, including factors related to FGF and TGF-beta, may regulate regional matrix production during condensation and other morphogenetic events in the limb bud. Cooperative effects between factors related to FGF and TGF-beta have been demonstrated in other morphogenetic systems (see Smith, 1989; Whitman and Melton, 1989), and these agents interact with and influence the composition of extracellular matrices. It is anticipated, then, that future research will continue to focus on their important role in regulation of early morphogenetic events in the limb.

REFERENCES

Aono, H., and Ide, H., 1988, A gradient of responsiveness to the growth-promoting activity of ZPA (zone of polarizing activity) in the chick limb bud, **Develop. Biol.** 128:136-141.

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.

Banerjee, S.D., and Toole, B.P., 1990, Monoclonal antibodies to chick embryo hyaluronan-binding proteins, submitted for publication.

Barnhart, B.J., Cox, S.H., and Kraemer, P.M., 1979, Detachment variants of Chinese hamster cells. Hyaluronic acid as a modulator of cell detachment, **Exp. Cell Res.** 119:327-332.

Bee, J.A., and von der Mark, K., 1990, An analysis of chick limb bud intercellular adhesion underlying the establishment of cartilage aggregates in suspension culture, **J. Cell Sci.** 96:527-536.

Bell, K.M., and McLachlan, J.C., 1985, Stimulation of division in mouse 3T3 cells by coculture with embryonic chick limb tissue, **J. Embryol. Exp. Morphol.** 86:219-226.

Brecht, M., Mayer, U., Schlosser, E., and Prehm, P., 1986, Increased hyaluronate synthesis is required for fibroblast detachment and mitosis, **Biochem. J.** 239:445-450.

Comper, W.D., and Laurent, T.C., 1978, Physiological function of connective tissue polysaccharides, **Physiol. Rev.** 58:255-315.

Crossman, M.V., and Mason, R.M., 1990, Purification and characterization of a hyaluronan-binding protein from rat chondrosarcoma, **Biochem. J.** 266:399-406.

Goldberg, R.L., and Toole, B.P., 1984, Pericellular coat of chick embryo chondrocytes: structural role of hyaluronate, **J. Cell Biol.** 99:2114-2122.

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.

Hascall, V.C., and Hascall, G.K., 1981, Proteoglycans, in: **Cell Biology of Extracellular Matrix**, E.D. Hay, ed., Plenum, New York, pp 39-63..

Hurle, J.M., Ganan, Y., and Macias, D., 1989, Experimental analysis of the in vivo chondrogenic potential of the interdigital mesenchyme of the chick leg bud subjected to local ectodermal removal, **Develop. Biol.** 132:368-374.

Knudsen, K.A., Myers, L., and McElwee, S.A., 1990, A role for the Ca^{2+}-dependent adhesion molecule, N-cadherin, in myoblast interaction during myogenesis, **Exp. Cell Res.** 188:175-184.

Knudson, C.B., 1990a, Cell-cell adhesion of limb bud mesoderm mediated by hyaluronan, **Development**, in press.

Knudson, C.B., 1990b, Role of hyaluronan in the assembly of chondrocyte pericellular matrix, submitted for publication.

Knudson, C.B., and Knudson, W., 1990, Similar epithelial-stromal interactions in the regulation of hyaluronate production during limb morphogenesis and tumor invasion, **Cancer Lett.** 52:113-122.

Knudson, C.B., and Toole, B.P., 1985, Changes in the pericellular matrix during differentiation of limb bud mesoderm, **Develop. Biol.** 112:308-318.

Knudson, C.B., and Toole, B.P., 1987, Hyaluronate-cell interactions during differentiation of chick embryo limb mesoderm, **Develop. Biol.** 124:82-90.

Knudson, C.B., and Toole, B.P., 1988, Epithelial-mesenchymal interaction in the regulation of hyaluronate production during limb development, **Biochem. Intl.** 17:735-745.

Knudson, W., Biswas, C., Li, X-Q., Nemec, R.E., and Toole, B.P., 1989, The role and regulation of tumour-associated hyaluronan, **Ciba Found. Symp.** 143:150-169.

Kujawa, M.J., Pechak, D.G., Fiszman, M.Y., and Caplan, A.I., 1986, Hyaluronic acid bonded to cell culture surfaces inhibits the program of myogenesis, **Develop. Biol.** 113:10-16.

Laurent, T.C., Fraser, J.R.E., Pertoft, H., and Smedsrod, B., 1986, Binding of hyaluronate and chondroitin sulphate to liver endothelial cells, **Biochem. J.** 234:653-658.

Lesley, J., Schulte, R., and Hyman, R., 1990, Binding of hyaluronic acid to lymphoid cell lines is inhibited by monoclonal antibodies against Pgp1, **Exp. Cell Res.** 187:224-233.

McCarthy, M.T., and Toole, B.P., 1989, Membrane-associated hyaluronate-binding activity of chondrosarcoma chondrocytes, **J. Cell. Physiol.** 141:191-202.

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.

McGuire, P.G., Castellot, J.J., and Orkin, R.W., 1987, Size-dependent hyaluronate degradation by cultured cells, **J. Cell. Physiol.** 133:267-276.

Meyer, F.A., 1983, Macromolecular basis of globular protein exclusion and of swelling pressure in loose connective tissue (umbilical cord), **Biochim. Biophys. Acta** 755:388-399.

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.

Morris-Wiman, J., and Brinkley, L.L., 1990, Changes in mesenchymal cell and hyaluronate distribution correlate with in vivo elevation of the mouse mesencephalic neural folds, **Anat. Rec.** 226:383-395.

Munaim, S.I., Klagsbrun, M., and Toole, B.P., 1988, Developmental changes in fibroblast growth factor in the chicken embryo limb bud, **Proc. Natl. Acad. Sci. USA** 85:8091-8093.

Munaim, S.I., Klagsbrun, M., and Toole, B.P., 1990, Hyaluronan-dependent pericellular coats of chick embryo limb mesodermal cells: induction by basic fibroblast growth factor, **Develop. Biol.**, in press.

Orkin, R.W., Knudson, W., and Toole, B.P., 1985, Loss of hyaluronate-dependent coat during myoblast fusion, **Develop. Biol.** 107:527-530.

Sanderson, R.D., Fitch, J.M., Linsenmayer, T.F., and Mayne, R., 1986, Fibroblasts promote the formation of a continuous basal lamina during myogenesis in vitro, **J. Cell Biol.** 102:740-747.

Schor, S.L., Schor, A.M., Grey, A.M., Chen, J., Rushton, G., Grant, M.E., and Ellis, I., 1989, Mechanism of action of the migration stimulating factor produced by fetal and cancer patient fibroblasts: Effect on hyaluronic acid synthesis, **In Vitro Cell Develop. Biol.** 25:737-746.

Seed, J., Olwin, B.B., and Hauschka, S.D., 1988, Fibroblast growth factor levels in the whole embryo and limb bud during chick development, **Develop. Biol.** 128:50-57.

Singley, C.T., and Solursh, M., 1981, The spatial distribution of hyaluronic acid and mesenchymal condensation in the embryonic chick wing, **Develop. Biol.** 84:102-120.

Smith, J.C., 1989, Mesoderm induction and mesoderm-inducing factors in early amphibian development, **Development** 105:665-677.

Solursh, M., 1990, The role of extracellular matrix molecules in early limb development, **Seminars Develop. Biol.** 1:45-53.

Solursh, M., Ahrens, P.B., and Reiter, R.S., 1978, A tissue culture analysis of the steps in limb chondrogenesis, **In Vitro** 14:51-61.

Solursh, M., Singley, C.T., and Reiter, R.S., 1981, The influence of epithelia on cartilage and loose connective tissue formation by limb mesenchyme cultures, **Develop. Biol.** 86:471-482.

Tengblad, A., 1981, A comparative study of the binding of cartilage link protein and the hyaluronate-binding region of the cartilage proteoglycan to hyaluronate-substituted Sepharose gel, **Biochem. J.** 199:297-305.

Toole, B.P., 1981, Glycosaminoglycans in morphogenesis, in **Cell Biology of Extracellular Matrix**, E.D. Hay, ed., Plenum, New York, pp. 259-294.

Toole, B.P., 1990, Hyaluronan and its binding proteins, the hyaladherins, **Current Opinion Cell. Biol.** 2: in press.

Toole, B.P., Munaim, S.I., Welles, S., and Knudson, C.B., 1989, Hyaluronate-cell interactions and growth factor regulation of hyaluronate synthesis during limb development, **Ciba Found. Symp.** 143:138-149.

Turley, E.A., Bowman, P., and Kytryk, M.A., 1985, Effects of hyaluronate and hyaluronate binding proteins on cell motile and contact behavior, **J. Cell Sci.** 78:133-145.

Turner, R., Banerjee, S.D., and Toole, B.P., 1990, Role of hyaluronan-binding protein in chondrogenesis, submitted for publication.

Underhill, C.B., 1982, Interaction of hyaluronate with the surface of simian virus 40-transformed 3T3 cells: Aggregation and binding studies, **J. Cell Sci.** 56:177-189.

Underhill, C.B., and Toole, B.P., 1981, Receptors for hyaluronate on the surfaces of parent and virus-transformed cell lines. Binding and aggregation studies, **Exp. Cell Res.** 131:419-423.

Underhill, C.B., Green, S.J., Comoglio, P.M., and Tarone, G., 1987, The hyaluronate receptor is identical to a glycoprotein of M_r 85,000 (gp85) as shown by a monoclonal antibody that interferes with binding activity, **J. Biol. Chem.** 262:13142-13146.

Welles, S., and Toole, B.P., 1987, Muscle fibroblast extracellular matrix modulates myoblast differentiation in vitro, **J. Cell Biol.** 105:139a (abstract).

Whitman, M., and Melton, D.A., 1989, Growth factors in early embryogenesis, **Annu. Rev Cell. Biol.** 5:93-117.

Yu, Q., Banerjee, S.D., and Toole, B.P., 1990, Inhibition of pericellular coat production by antibody to hyaluronan-binding protein, submitted for publication.