Communication plays an important role in the rapid progress of modern society. We live in an age when information is transmitted through many different pathways and in many different forms, to influence our daily decisions. However, though we live in communities, we still treasure our individuality. By analogy, the survival of multicellular organisms depends on each cell type retaining its individuality, eventhough all cellular activities must be coordinated with other cells. Organisms have evolved multiple strategies to achieve this goal, which include long range interactions mediated by neural or endocrine mechanism or short range interactions that include direct physical or cell-cell contact. While the first strategy involves interactions at a distance, one mode of direct communication involves the cell to cell or cell to extracellular matrix transmission of molecules through channels. There is an intimate cooperation between the cell adhesion events and signalling processes that control the transfer of information between cells (Kumar and Gilula, 1996).

Cell adhesion is crucial for the assembly of individual cells into the three-dimensional tissues of animals. Cells do not simply "stick" together to form tissues, but rather are organized into very diverse and highly distinctive patterns. A variety of cell adhesion mechanisms are responsible for assembling cells together and along with their connections to the internal cytoskeleton, determine the overall architecture of the tissue. Thus, cell adhesion systems should be regarded as mechanisms that help translate basic genetic information into complex three-dimensional pattern of cells in tissues.

The functional units of cell adhesion are typically multiprotein complexes made up of three general class of proteins; the cell adhesion molecules/adhesion receptors, the extracellular matrix (ECM) proteins and cytoplasmic plaque/peripheral membrane proteins. The cell adhesion receptors are usually transmembrane glycoproteins that mediate binding interactions at the extracellular surface and determine the specificity of cell-cell and cell-ECM recognition. They include members of integrins, cadherins, immunoglobulins, selectins and proteoglycans. At the extracellular surface, the cell adhesion receptors recognize and interact with either other cell adhesion receptors on neighbouring cells or with proteins of the ECM. ECM proteins are typically large glycoproteins, including the collagens, fibronectins, laminins and proteoglycans that assemble into fibrils or other complex macromolecular arrays. Owing to their binding to adhesion receptors, they can also be tightly associate with the cell surface. At the intracellular
surface of the plasma membrane, cell adhesion receptors associate with cytoplasmic plaques or peripheral membrane proteins. Cytoplasmic plaque proteins serve to link the adhesion molecules to transduce signals initiated at the cell surface by the adhesion receptors (Gumbiner, 1996).

Cell adhesion is an important biological process that is implicated in tissue pattern formation, organogenesis and embryogenesis. It is also crucial for the recovery and maintenance of normal body functions during many diseased conditions such as inflammation, hemostasis, and wound healing. Only recently, the function of cell adhesion in angiogenesis, proteolysis, motility and tumor cell invasions was realized. The molecular mediators of various cell adhesion processes are termed as adhesion molecules and comprise at least five prominent families i.e cadherins, selectins, immunoglobulins, integrins and proteoglycans (Tang and Honn, 1994).

1.1 Cadherins

Cadherins comprise of a family of Ca^{2+} dependent adhesion molecules that function to mediate cell-cell binding critical to the maintenance of tissue structure and morphogenesis. The classical cadherins, E-, N- and P-Cadherin, each consists of a large extracellular domain (characterized by a series of five homologous N-terminal repeats, the most distal of which is thought to be responsible for binding specificity) a transmembrane domains and a carboxyl-terminal intracellular domain. The relatively short intracellular domains interact with a variety of cytoplasmic proteins, such as β catenin, to regulate Cadherin function. More recently described members of this family of adhesion proteins include Cadherin k, R-Cadherin, B-Cadherin, E/P Cadherin and Cadherin-5 (Takeichi, 1991).

1.2 Selectins

The selectins are a family of cell-cell adhesion proteins that participate in leukocyte-endothelial and leukocyte-platelet interactions. The selectins were named for the C type lectin domain at their amino terminal and their function in recognition of specific cell surface carbohydrates, followed by a single epidermal growth factor like domain, a series of consensus repeats similar to those of complement regulator protein, a single transmembrane and a carboxyl terminal cytoplasmic domain (Arnaout, 1993). This family consists of three members; P-selectin, expressed on activated endothelium; L selectin, expressed on circulating leukocytes and E-selectin found on endothelial cells.
1.3 Immunoglobulin Superfamily of adhesion receptors

A diverse array of cell adhesion receptors are included in the immunoglobulin (Ig) Superfamily (CAMS). Proteins of this family are defined by the presence of one or more copies of the Ig fold and a compact structure of 60-100 amino acids arranged as a 3-stranded β sheet facing a 4 stranded β sheet. In most cases, the structure of Ig family adhesion receptors includes an amino-terminal extracellular domain, a single transmembrane segment, and a cytoplasmic tail. CAMS are involved in cell adhesion events in a variety of cell types. Thus, T-lymphocytes express several Ig superfamily receptors including CD2, CD4, CD8, CEA, DCC (CD56), ICAM-1, ICAM-2 and ICAM-3, VCAM and PECAM (CD3) and the T-cell receptor (TCR) itself. These receptors play critical role in antigen recognition, cytotoxic T cell function and lymphocyte recirculation and also serve as endothelial ligands for leukocytes. Recently, a number of transmembrane phosphatases have been identified to belong to this Ig superfamily and seem to be involved in cell adhesion (Walton and Dixon, 1993, Arnaout, 1993).

1.4 Integrins

The integrins are a family of cell surface glycoproteins that act as receptors for extracellular matrix proteins, or for membrane bound counter-receptors on other cells. Each integrin is a heterodimer that contains α and β subunits with each subunit having a large extracellular domain, a single transmembrane region and in most cases a short cytoplasmic domain. The Integrin receptor family of vertebrates includes at least 15 distinct α subunits and 8 or more β subunits which can associate to form at least 21 distinct integrins (Hynes, 1992). The α/β pairings specify the ligand binding abilities of the integrin heterodimers. Some integrins, such as α5β1, the classic fibronectin receptor bind to a single ECM protein. More commonly, an individual integrin will recognize several distinct extracellular matrix proteins. Cells often display multiple integrins capable of interacting with a particular ECM protein. Some integrin α subunits undergo alternative splicing in a tissue specific and developmentally regulated manner. The α subunit cytoplasmic domain is involved in cytoskeletal organization and cell mobility, in modulation of focal contact formation or in activating integrins. Some integrins seem to form a complex with focal contact proteins including α-actinin and talin via the binding site in the β subunit cytoplasmic domains. These interactions could be important
in cytoskeletal organization and may also play a role in signaling processes (Rosales et al., 1995).

1.5 Proteoglycans (PG)

Proteoglycans are proteins that carry unusual carbohydrates, glycosaminoglycans (GAGs). The glycosaminoglycans are large carbohydrates that are composed of repeating disaccharide units and exist in four main forms: heparin sulphate & heparin, chondroitin sulphate & dermatan sulphate, keratan sulphate and hyaluronic acid. The first three are protein bound glycosaminoglycans in their natural form, and they all contain sulphate; hyaluronic acid is found as a free glycosaminoglycan and lacks sulphate. Each of the glycosaminoglycans, the sulphated ones in particular, have a strong negative charge. This charge makes it possible for glycosaminoglycans to bind many substances, including some growth factors and cytokines.

Due to the fact that proteoglycans are abundant and ubiquitous tissue components, they are likely to be able to act as reservoirs for growth factors in many tissues. Characterization of the biological significance of interaction between heparin sulfate and GAG-binding growth factor has been demonstrated for the fibroblast growth factors (FGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), amphiregulin and heparin binding-epidermal growth factor (HB-EGF). Binding of growth factors to proteoglycans via GAG moieties has been proposed to provide a mechanism for growth factor recruitment at cell surface, presentation to specific receptor and regulation of their action on target cells (Ruoslathi and Yamaguchi, 1991).

1.5.1 The small leucine rich proteoglycans (SLRPs)

SLRPs encompass a family of secretory proteoglycans that include five structurally related but genetically distinct members, (1) decorin (2) biglycan (3) fibromodulin (4) lumican and (5) epiphycan. These proteoglycans share the unique feature of being composed primarily of leucine-rich tandem repeats that confer most of their biological functions. The overall protein core structure reveals that it consists of three main regions: an amino terminal region which contains the negatively charged GAGs or tyrosine sulphate, a central domain with varying number of Leucine rich repeats (LRRs) and a carboxyl domain. In all cases, the central domain is flanked by cysteine-rich clusters. In decorin, biglycan and epiphycan, the amino-terminal region harbours 1-2 GAG chains that can be either dermatan or chondroitin
sulphate, which could provide an analogous negative charge. This region of the molecule might be directly involved in interactions with cationic domains of extracellular matrix or cell surface proteins.

1.5.2 The Modular proteoglycans

Modular proteoglycans are works of art produced by an assembly of protein fragments in unlikely or unexpected juxta-position. It is a sort of protein collage, the major unifying feature of all modular proteoglycans. This family of macromolecules can be further divided into two sub families; the hyalectans, an acronym for hyaluronan-and lectin-binding proteoglycans, which comprises of versican, aggrecan, neurocan, and brevican. This family is characterized by a similar amino terminal that bind hyaluronan, an extended central domain that harbours the GAG chains and a carboxyl terminus that is homologous to selectin family. The nonhyaluronan-binding proteoglycans are the members of the other sub family which comprises of syndecan, betaglycan, perlecan and agrin, two large PGs that carry primarily heparan sulphate (HS) chains, and testican, an HS/Chondroitin sulphate-carrying proteoglycan isolated from seminal fluid (Iozzo and Murdoch, 1996).

1.6 Molecules that bind heparin and heparin sulphate

Heparin sulphate, initially distinguished from heparin by a difference in the extent of sulphation (Jorpes and Gardell, 1948), contains greater structural variability than any other GAG. Both HS and chondroitin sulphate are bound to serine residues in proteins but a universal consensus sequence for GAG attachment is not known. Heparin sulphates and heparin contain the most negatively charged structures produced by vertebrate cells and widely distributed throughout the animal kingdom (Nader et al., 1988). Heparan sulphate is found intracellularly, principally within storage vesicles of various secretory cells and possibly in the nucleus; at the cell surface as part of integral membrane PGs and extracellularly in the pericellular matrix and basement membrane.

Heparan sulphates bind diverse proteins found in the cellular microenvironment, including extracellular matrix components, peptide growth factors, cell adhesion molecules, lipolytic enzymes, protease inhibitors and circulating lipoproteins. Some of these interactions are required for the function of these proteins. Other proteins that are bound include viral coat proteins, nucleases, DNA and RNA polymerases and transcription factors. Heparan sulphate
binds proteins with relatively high affinity, having Kd as low as 1-5 nM. The binding is predominantly via electrostatic interactions between the highly anionic sulphate groups on the GAG and clusters of basic amino acids arranged in a three-dimensional array on the protein. Hydrogen bonding involving carboxylate groups may also be involved and there is a high degree of cooperativity among the anionic sites. While the local conformation of the HS chain may be altered by interaction with protein, proteins can change their conformation upon binding heparin/HS. For example, fibronectin, antithrombin III, and bFGF, each undergo a conformational change when they bind heparin (Jackson et al., 1991).

1.7 Molecules that Bind Hyaluronan

Hyaluronan (hyaluronic acid, HA) is a high molecular mass, broadly distributed extracellular matrix glycosaminoglycan (GAG) polymer, synthesized by HA synthetase expressed in plasma membranes of a variety of cell types (Laurent & Fraser, 1992). For a long time, HA had been considered as a space filler with little biological significance. This view began to change when it was discovered that HA is specifically bound by several cell surface and matrix proteins. HA-binding proteins or hyaladherins (Toole, 1990), can be subdivided into mainly two classes : the first comprises those that are part of the extracellular matrix (ECM), (Knudson & Knudson, 1993) and the second class represents cell surface HA-binding proteins. All matrix hyaladherins contain a sequence of amino acids homologous to the tandem-repeated β loops of cartilage-link protein, a structural motif that has been predicted to contain the HA-binding domain. The HA-binding motif has recently been defined as BX₇B, where B is either Arg or Lys, and X₇ contains no acidic residues and at least one basic amino acid (Yang et al., 1994). This motif has been identified in all ECM and cell surface hyaladherins characterized to date (Yang et al., 1994). An important functional difference between the ECM and cell surface hyaladherins is that ECM hyaladherins require at least a deca- or dodecamer of HA for binding, whereas HA hexamers constitute the minimal sequence that is recognized by cell surface HA receptors (Knudson, 1993). The affinity of cell surface hyaladherins for HA increases with increasing polymer length of HA, which suggests that multivalent interaction of HA with multiple cell surface receptor molecules (Toole, 1990; Underhill, 1992). Thus, several properties facilitate the distinction between specific binding of HA to cell surface hyaladherins and specific aggregation of HA with ECM hyaladherins.
Increased HA production can be observed in developing cells, at sites of inflammation and wound healing; and in the vicinity of tumour invasion (Toole, 1981), underscoring the involvement of HA in multiple biological processes.

1.8 Extracellular matrix HA-binding proteins

1.8.1 Link protein

Link protein is an essential constituent of cartilage extracellular matrix and together with HA and the proteoglycan aggrecan, forms large multimolecular aggregates that provide the stability. It, therefore, plays a vital role in tissues with its capacity in maintaining the integrity of the cells to bear load and resist deformation. Link protein consists of three functional domains; the Ig fold domain which binds to a similar domain in aggrecan and a pair of proteoglycan tandem repeat (PTR) domains, both of which bind to HA (Goetinek et al., 1987; Doege et al., 1986; Dudhia and Hardingham, 1989).

1.8.2 Brain enriched hyaluronan binding protein (BEHAB)

Brain enriched hyaluronan binding protein (BEHAB) is a 38 kDa glycoprotein found in the ECM of mammalian central nervous system (Jaworski et al., 1994). The predicted amino acid sequence obtained from cDNA encoding BEHAB revealed that BEHAB is a member of PTR family of HA-binding proteins, having substantial degree of homology with other members (rat neurocan 48% Rauch et al., 1992; rat LP (42%), Doege et al., 1986, versican (46%) Zimmerman, 1989) of the PTR family having characteristic PTR and Ig domain. The size and sequence similarity to LP suggested that BEHAB could serve the LP function in the stabilization of proteoglycan HA aggregation. BEHAB also associate with other Ig loop containing protein like cell adhesion molecules (N-CAM) and signal transducing molecules (tyrosine phosphatases) and participate in cell-cell or cell-matrix interactions.

1.8.3 Versican

The largest member of this family is versican, a proteoglycan first cloned from a fibroblast cDNA library (Zimmermann and Ruoslahti, 1989) that is widely expressed in vascular and avascular connective tissues (Zimmermann et al., 1994). The N-terminal globular domain contains the hyaluronan-binding region that lies within characteristic loops that are tandemly repeated. Recombinant forms of this domain bind to hyaluronan with high affinity (Kd~4 nM), thereby establishing a strong functional correlation with aggrecan (LeBaron et al.,
1992). On this structural basis alone, one could predict that the other members of this family would also bind hyaluronan. The second domain in versican encompasses two alternatively spliced exons that harbor the GAG attachment regions, which have been designated GAG-α and GAG-β (Naso et al., 1994; Dours and Zimmermann, 1994). These regions are free of cysteine residues, but are enriched in acidic amino acids and carry up to ~30 binding sites for GAG chains as well as several binding sites for O- and N-linked oligosaccharides. Four possible variants of versican can exist and this has been shown by analysis of both mRNA and protein isoforms (Naso et al., 1994). The carboxyl terminus of versican contains a series of structural motifs characteristically found in the selectin family: two EGF repeats, a C-type lectin domain and a CRP-like motif.

The versican protein contains an N-terminal domain homologous to the hyaluronic acid-binding domains of link protein and cartilage aggrecan; a C-terminal domain containing two epidermal growth factor (EGF)-like repeats and a lectin-like sequence; and a complement regulatory protein (CRP)-like domain. These domains are separated by a region containing up to 15 potential attachment sites for chondroitin sulphate chains.

1.8.4 Aggrecan

Aggrecan structure is quite similar to versican, with a few exceptions. First, it contains a second globular domain at the N-terminus, whose function is not clearly understood so far, as it does not bind hyaluronan. Second, it contains a GAG-β of versican but that harbors many more Ser-Gly consensus sequences. Thus, a finally glycosylated aggrecan would contain more than three fold the number of side chains than the largest isoform of versican, ~100 vs 30 chains, respectively. Third, in the carboxyl end of aggrecan, the two EGF repeats can be alternatively spliced in a significant portion of the molecules (Roughley and Lee, 1994; Fulop et al., 1993). These structural differences would have significant consequences in the functional properties of the hyalectans. Recombinant forms of the C-type lectin domain from both versican and aggrecan bind various carbohydrates, including galactose and fucose, in a calcium-dependent manner this further emphasizes the functional relationship between hyalectan domains in different PGs.

Aggrecan is the main proteoglycan component in the extracellular matrix of cartilage.
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It contains at its N-terminus two globular domains referred to as G1 and G2 which are separated by a 90-residue inter globular domain (IGD). The G1 domain mediates the binding of aggrecan to hyaluronan (HA), thereby allowing the formation of multimolecular complexes which absorb water and contribute to the resilience of the tissue. The G1 domain has a structure consisting of three disulphide-bonded loops. Loop $\alpha$ has structural characteristics common to members of the IgG family (Perkins et al., 1989; Bonnet et al., 1986) and is, by analogy with link protein, involved in the interaction between aggrecan and link protein (Perin et al., 1987). Loops $\beta$ and $\beta$ are involved in binding to HA (Yang et al., 1994). They form a structural motif referred to as the proteoglycan tandem repeat (PTR) which appears to be unique to HA-binding proteins. Aggrecan is heavily substituted with both chondroitin sulphate (CS) and keratan sulphate (KS) and the size, distribution and degree of sulphation of the KS chains increase with age. KS is a glycosaminoglycan occuring widely in connective tissues.

1.8.5 Neurocan

The another member of this family is neurocan, a major CS-PG found in early postnatal brain (Rauch et al., 1992). As with other members of the hyalectan family, neurocan harbors amino and carboxyl-terminal domains that share more than 40 and 60% identity to the hyaluronan binding region and the selectin region of versican and aggrecan. The central domain, which contains upto seven GAG attachment sites, and shows no significant homology to other sequenced PGs (Margolis and Margolis, 1994). Neurocan is developmentally regulated and it appears that the adult form, which lacks the amino-terminal domain, is generated by an endoproteolytic cleavage of the protein core (Rauch et al., 1992).

1.8.6 Brevican

Brevican, is another brain-specific CS-PG (Yamada et al., 1994). The most prominent feature of brevican and hence its name, is the remarkably short central domain. This nonhomologous region is enriched in acidic residues, especially glutamic acid, which may be used to bind cationic substances and minerals (Yamada et al., 1994). As in the case of neurocan, brevican is found as two major products; a full-length proteoglycan and a GAG-deficient protein core that is generated by proteolytic cleavage within the central domain. Since a cleaved version of brevican exists in brain extracts and a sizeable proportion of aggrecan molecules are C-terminally truncated in cartilage extracts, it is possible that
proteolytic processing of the hyalectans is a general feature required for their specialized function in tissues.

1.9 Cell surface HA-binding proteins

1.9.1 CD44

CD44 is a broadly expressed polymorphic type I transmembrane glycoprotein generally 80 to 90 kDa Mr. CD44 molecule contains three major domains: the cytoplasmic domain, which mediates the interaction with the cytoskeleton; the middle domain, which is responsible for lymphocyte homing; and the N-terminal domain which binds to HA. Many isoforms of CD44 are generated by alternative splicing from a single gene. CD44 is implicated to serve as an adhesion molecule in the cell-surface and cell-cell interaction including lymphocyte homing to mucosal lymphoid tissue, hemopoiesis, NK-cell mediated killing macrophage and lymphocyte activation, tumor metastasis and binding of certain cytokines to endothelium. CD44 contains two BX,B motifs in the extracellular domain (Yang et al., 1994; Stamenkovic et al., 1989), which contribute to HA-binding. Normally lymphocytes express a relatively high level of CD44 but bind HA poorly. Upon activation by antigen, mitogens, mAbs, or certain cytokines, a several-fold increase in binding is observed (Murakami et al., 1990, 1994; Lesley et al., 1994). The observed enhancement in binding does not appear to be the result of an increase in receptor number, but some alteration of receptor conformation. Alterations in both N- and O-linked glycosylation may provide a potential mechanism for regulating the affinity of CD44 for HA (Bartolazzi et al., 1994). The intracellular domain of CD44 has been proposed to be associated with cytoskeleton proteins (Lacy & Underhill, 1987), Ankyrin (Lokeshwar et al., 1994) and the ezrin/moesin/radixin (EMR) family (Tsukita et al., 1994). The cytoplasmic domain of CD44 is required for HA-binding on cell surface (Thomas et al., 1992), supporting a functional role for CD44-cytoskeletal interaction. Although HA-binding is a major property of CD44, it also recognizes other ligands such as collagens type I and VI (Carter & Wayner, 1988), fibronectin (Jalkanen & Jalkanen, 1992) and chondroitin sulphate (Naujokas et al., 1993). Recent evidence suggests that binding to hyaluronan triggers specific CD44-mediated signal transduction events (Webb et al., 1990; Noble et al., 1993).

1.9.2 Receptor for hyaluronan mediated motility (RHAMM)

A distinct HA receptor expressed in fibroblast has been recently identified and given the acronym RHAMM. It is expressed as 52 and 58 kDa isoforms of a protein encoded by a
single gene on the surface of ras-transformed fibroblasts, where it forms hyaluronan receptor complex (HARC) with at least two other molecules of 68 and 72 kDa (Hardwick et al., 1992). RHAMM is expressed on the cell membrane and the perinuclear region of rapidly migrating fibroblasts and has been shown to bind HA. It mediates cell locomotion through a pathway involving tyrosine phosphorylation within the focal adhesion region (Turley et al., 1991; Hardwick et al., 1992). Interestingly, RHAMM is not homologous to CD44 but contains two BX_B motifs (residues 401-411 and 423-432) that contribute equally to HA-binding (Yang et al., 1993, 1994). The sequence analysis of RHAMM isoforms do not encode a transmembrane domain or a signal sequence. Entwistle (1995) proposed that RHAMM may resemble the high affinity elastin/laminin receptor (Yow et al., 1988) and associate with the cell surface via an integral docking protein. A candidate for RHAMM docking protein has been identified as the HA synthase (Klewes et al., 1993).

1.9.3 Tumor necrosis factor stimulated gene 6 (TSG-6)

TSG-6 a secretory glycoprotein, is a member of hyaladherin family (Lee et al., 1992; Toole, 1990). It binds to HA and may be found as a component of the ECM. In the N-terminal half, the predicted amino acid sequence has 36-40% homology with complement component C1r. This homology region forms CUB domain which is a motif found in proteins involved in the development processes. TSG-6 protein has been detected in the synovial fluids from individuals with arthritis. It is not present in normal individuals and it was found to be constitutively expressed in synoviocytes in the rheumatoid arthritis patients. It, thus, seems likely that TSG-6 is produced locally in inflamed joints and may serve as a useful marker in arthritis (Kohda et al., 1996). Although the protein itself is 35 kDa under fully glycosylated condition it readily forms a complex of 130 kDa with inter-α-inhibitor (IαI)(Wisniewski et al., 1994).

1.9.4 Intercellular adhesion molecule-1 (ICAM-1)

The mechanism for clearing HA from the bloodstream is receptor mediated endocytosis by liver endothelial cells (LEC; McGary et al., 1989). The HA receptor on LEC (also known as HARLEC) is a 85-100 kDa protein that is not related to CD44 (McGary et al., 1989). Corneal endothelial cells as well as nonendothelial thymic reticular cells are specifically recognized by anti-HARLEC mAb. Purification and protein sequencing of rat HARLEC
revealed that its amino acid sequence is identical to that of ICAM-I (McCourt et al., 1994). The extracellular domain of ICAM-I is composed of 5-Ig subdomains. So far, none of the Ig superfamily members have been found to bind HA. However, ICAM-I contains several near-BX_B motifs, differing from the canonical motif by the presence of at least one acidic residue in the X_7 sequence. This suggests that the HA-binding motif may need to be redefined and that other sequences may be involved in binding.

1.9.5 HA Clearance receptor

Plasma HA is cleared by the liver sinusoidal endothelial cells (EC) through a Ca^{2+} independent class II endocytic receptors (McGary et al., 1989). Binding and endocytosis of HA to the receptors can be competed with heparin and chondroitin sulphate, but not with chondroitin or with dextran sulphate (Raja et al., 1988). Endocytosis and degradation demonstrated that the HA receptor is specific for a subset of glycosaminoglycans. Biochemical data suggest that the LEC HA receptor is a thermally labile integral membrane protein. Recently, McGary et al (1989) as proposed that the LEC HA receptor is a disulphide bound heterodimer Mr 175,000 and 160,000) with a Mr of approximately 340,000 (Yannariello et al., 1992). The LEC HA receptors mediate multiple rounds of internalization of HA through clathrin coated pit (McGary et al., 1989). After endocytosis, the internalized HA are released back into the culture medium.

1.9.6 CD38

CD38 is a 42 kDa type II transmembrane glycoprotein with a short N-terminal cytoplasmic domain, a single membrane spanning region and a long C-terminal extracellular domain. It is expressed widely on hematopoietic cells. CD38 been shown to have wide range of effects, including, induction of B and T cell proliferation, protection of B cells from apoptosis, inhibition of B lymphopoeisis and enhancement of antigen-presenting function in macrophages (Malvasi et al., 1994). CD38 is also thought to function as cell adhesion molecule, possibly through its ability to bind hyaluronan (Nishina et al., 1994). Recently, a high molecular mass form of CD38 (Mr 190 kDa) was identified in retionic acid (RA)-induced human leukemia cells. The high molecular weight form is a result of transglutaminase catalysed post translational cross linking of CD38. Recently, it was also demonstrated that CD38 is a bifunctional enzyme capable of catabolizing nicotinamide adenine dinucleotide
(NADT) to cyclic ADP ribose (cADPR) and then, rapidly and efficiently hydrolyzing cADPR to adenosine diphosphoribose (ADPR). cADPR is a novel Ca\textsuperscript{2+} mobilizing agent that acts independently of the Ins (1,4,5) P\textsubscript{3} pathway of Ca\textsuperscript{2+} mobilization, this enzyme is thought to require the cofactor calmodulin to induce Ca\textsuperscript{2+} release via Ca\textsuperscript{2+} channels (Lee et al., 1989). ADPR is a reactive moiety that can covalently attach to proteins either in a non-enzymatic manner or in the presence of an ADP-ribosyl transferase, in a reaction termed as ADP-ribosylation. Thus, CD38 may also modulate cell function via ADP ribosylation of intracellular signal-transducing mediators.

1.9.7 Cdc 37

Cdc37 is an essential component of cell cycle regulation in yeast (Reed, 1980, 1992; Ferguson et al., 1986) and it may also play a role in certain differentiation events (Simon et al., 1991; Cutforth and Rubin, 1994). The biochemical function of Cdc37 is unknown, but genetic evidence strongly suggests that Cdc37 influences the activity of p34cdc2 kinase and consequently cell cycle progression (Reed, 1992; Boschelli, 1993; Cutforth and Rubin, 1994). The chick homologue of Cdc37 exhibits characteristic properties of a GAG-binding protein. The putative GAG binding regions of the chick Cdc37 are well conserved in the Drosophila protein but only partially in yeast. Thus, considering the phylogenetic distance between these organisms, it seems reasonable to suppose that binding of GAGs to Cdc37 may have a significant physiological role, at least in the metazoan species. Using a monoclonal antibody, IVd4, that recognizes a novel group of hyaluronan-binding proteins (Banerjee et al., 1991). Grammatikakis et al., (1995) have immunoscreened a cDNA library constructed from embryonic chick heart muscle in RNA. One of the cDNAs isolated from the library, encodes a 29.3 kDa protein homologous to Cdc37, an essential cell cycle regulatory factor previously characterized genetically in yeast and Drosophila. The molecular mass of the major proteins recognized by mAbIVd4 are of ~35, 50, 70 and 90 kDa. The relative amounts of these forms varies from tissue to tissue.

A great deal of evidence has been published indicating that GAGs are present, at least transiently, in the cytoplasm and in the nucleus. The types of GAGs shown to be present in these cellular compartments include hyaluronan, chondroitin sulfate, dermatan sulfate and heparan sulfate (Furukawa and Terayama, 1977; Fedarko and Conrad, 1986; Ishihara et al., 1986; Ripellino et al., 1988, 1989). Of particular interest is the observation that targetting of
a specific sub-population of heparan sulfate to the nucleus of rat hepatoma cells increases markedly under conditions of reduced growth rate and decreases on stimulation of cell division (Ishihara and Conrad, 1989; Fedarko et al., 1989). It has also been shown that heparin and related polysaccharides inhibit the action of Fos and Jun on transcription events involved in cell cycle progression. Bush et al. (1992) have suggested that endogenous nuclear heparan sulfate may exhibit this regulatory role in vivo. Heparan sulphate and heparan are targeted to the nucleus and elicits similar effects to the above, even when added extracellularly (Fedarko et al., 1989; Pukac et al., 1990). It seems likely that GAGs in the cytoplasm or nucleus are involved in cell cycle regulation and possibly other intracellular events. Binding of GAGs to Cdc37 may mediate one or more of these events.

1.10 Enzyme HA-binding proteins

1.10.1 Hyaluronan synthase

Hyaluronan (HA) is a glycosaminoglycan synthesised by essentially all organisms from bacteria to mammals. Unlike other GAGs, HA synthesis occurs at the inner surface of the plasma membrane with the growing HA chains extruding through the pore like structures into the extracellular space. HA and chitin are structurally related, while HA is a repeating disaccharide polymer of GlcA (β1-->3) GlcNAc(β1-->4) residues, chitin is a homopolymer of GlcNAc (β1-->4). Therefore, HA synthases have β- N-acetylglucosaminyltransferase activity in common. The sequence data base search revealed that the genes for Rhizobium NodC, (Bulawa, 1993), yeast chitin synthases, the Xenopus developmental protein OG42 (Rosa et al., 1988) and streptococcus hyaluronan synthase HasA (Spicer et al., 1996) bear substantial similarities in sequence with murine synthases and human Has 1 and Has 2, but are not identical. Induction of synthase activity by growth factors has also been shown to be correlated with phosphorylation events. The differential transcriptional regulation of Has1 and Has2 genes control the rate of hyaluronan synthesis (Watanabe and Yamaguchi, 1996).

1.10.2 Hyaluronidase

PH-20, a 34 kDa glycosyl phosphatidyl inositol (GPI)- anchored glycoprotein is present on the plasma membrane of sperm. The main function of PH-20 is to mediate sperm-zona pellucida adhesion. The PH-20 has become a member of HA-binding protein family (Gmachl
et al., 1993; Lathrop et al., 1990), the hyaladherins because of a having defined HA-binding motif in the polypeptide sequence and well documented hyaluronidase activity. The high degree of PH-20 sequence homology in rodents, primates and mammals suggests that they may have a conserved function and immunogenicity. As sperm antigens are of great interest for developing target antigen contraceptive vaccines, this observation suggests the feasibility of human PH-20 as an antigen for human contraceptive vaccine without any autoimmune responses (Lin et al., 1993).

1.10.3 Hemopexin

Hemopexin is a heme binding serum glycoprotein. Its function is, scavenging heme from blood. Hemopexin has also been found to have hyaluronidase activity from partial amino acid sequence and cDNA sequence of porcine liver hyaluronidase which bore a 70% sequence similarity to human, rabbit and rat hemopexins (Zhu et al., 1994). Substrate gel assay with HA, immunological cross reactivity and the presence of HA-binding motif proved that hemopexin is a hyaluronidase as well as a HA-binding protein (Takahashi et al., 1985). One interesting observation is the presence of hemopexin domain, a short conserved peptide sequence that occurs once in each half of the hemopexin proteins found in serum derived ECM associated enzymes like collagenases, gelatinase and stromelysin. This domain may serve either to promote recognition of the ECM substrate or to modulate intrinsic enzyme activity (Zhu et al., 1994).

1.10.4 Group B streptococcal HA Lyase (GBS HA lyase)

*Streptococcus pyogenes* is human pathogenic bacterium that produces a hyaluronate capsule, an ubiquitous eukaryotic component of the extracellular matrix. Hyaluronate is a major virulence factor that inhibits phagocytosis and masks bacterial antigens from immunological defence (Wessels et al., 1991, 1994). The pathogenesis of rheumatic fever is not clear, but there are indications that it arises from immunological mimicry between bacterial and human antigens. Recently, Prehm et al, (1995) showed that proteins of hyaluronate synthase complex are immunologically cross-reactive and exposed on the eukaryotic cell surface so as to be accessible to circulating antibodies. 56 kDa HA synthase is shed from growing *Streptococci* into the culture medium and it is, therefore likely, that it is released in infected patients to induce an immunological response. The cross- reactive
antigen to the *streptococcal* 56 kDa protein has been identified as a 52 kDa protein that forms complex with the hyaluronate receptor RHAMM and shows some homology to a eukaryotic initiation factor, eIF-2 (Salmen, 1993).

Several bacterial products are believed to be important in pathogenesis. One of them is an extracellular hyaluronate lyase, which has been recently demonstrated was being incorrectly identified as a neuraminidase for many years (Pritchard and Lin, 1993). Strains producing high levels of the enzyme were frequently found to be the most virulent strains. Lin et al, (1993) recently, reported that the GBS hyaluronate lyase degrades hyaluronan by a unique mode of action. Rather than randomly cleaving hyaluronan chains yielding a mixture of oligosaccharide fragments like other known hyaluronidases, the GBS enzyme appears to progressively move along the hyaluronan chains, continuously releasing disaccharide units as it travels. Little is known about the role of GBS hyaluronate lyase in pathogenesis. It may simply facilitate the invasion of tissues by the bacteria. However, there is a strong possibility that the enzyme may also subvert some normal host defence mechanisms. Hyaluronan and molecules that bind to it are involved in numerous immune system functions.

1.11 HA-binding proteins covalently linked to HA

1.11.1 Inter α Trypsin inhibitors (IαTI)

Members of the Inter-α-trypsin inhibitor family of glycoproteins are composed of varying combination of the following subunits: a light chain (bikunin) which is where the protease inhibitory activity resides and three closely related heavy chains. Human inter-α-trypsin inhibitor consists of bikunin and two heavy chains (HC1, HC2) linked covalently via a chondroitin sulfate like component. Recently, Huang et al, (1993) have shown that HA interacts strongly with the heavy chain of bovine and human inter-α-trypsin inhibitor (HC2). They found that this binding interaction, although relatively slow, was highly resistant to ionic strength and pH, suggesting a possible covalent linkage between HA and HC2. It has been proposed that IαTI functions as a "protease shuttle" by complexing loosely with proteases and transferring them to other protease inhibitors for subsequent clearance of secondary enzyme inhibitory complex. The physiological relevance of a HA-binding capacity of IαTI family members has been established with various cell type that display HA containing pericellular coat. The involvement of IαTI family members have been shown in
the process of oocyte maturation (Chen et al., 1995). The IαTI family members involvement in the physiological control of cumulus-oocyte complex is likely to originate from the blood.

1.11.2 Plasma hyaluronan binding protein (PHBP)

A novel hyaluronan-binding protein has been purified from human plasma. PHBP is a heterodimer composed of 50 kDa and 17 kDa subunits, bridged by a disulphide linkage. Both subunits have novel N-terminal amino acid sequences, indicating that PHBP is a novel hyaluronan-binding protein in human plasma. The amino acid sequence deduced from the nucleotide sequence of the cloned PHBP cDNA exhibited significant homology with that of hepatocyte growth factor activator (HGFA) (Mizuno et al., 1994). The protein is also expressed in liver, kidney and pancreas. The predicted structure of PHBP showed three epidermal growth factor (EGF) domains, a kringle domain and a serine protease domain, from its N-terminals, although HGFA has a fibronectin type II domain, an EGF domain, a fibronectin type I domain, an EGF domain, a kringle domain and a serine protease domain, from its N-terminus. Regarding the ECM-stabilizing activity of the IαTI family, the heavy chains of the family are cleaved and bind to HA firmly (Dietl et al., 1979). PHBP might be a candidate of enzyme concerned in this process, because PHBP exhibits HA-binding properties and is cleaved from HC2 of IαTI molecules (Choi-Miura et al., 1996).

1.11.3 Serum HA-binding proteins

It has already been documented that hyaluronate from pathological synovial fluid is firmly bound to proteins. These proteins have been identified as IgG, IgM, acute phase proteins such as α1 protease inhibitors, IαTI and haptoglobin. Recently, studies also suggest that hyaluronate can be bound to serum proteins such as antibodies, which are covalently coupled to hyaluronate by a unknown inflammatory reaction. The linkages of IgG to hyaluronate may create new antigenic sites and elicit antibody response. When these antibodies in turn recognize their target antigen on hyaluronate, they are likely to be coupled covalently to hyaluronate and become antigenic themselves. Thus, an acute inflammation leading into the chronic phase occurs, during which antibodies are formed against the hyaluronate-IgG complex which binds C1q to initiate the complement cascade. Activation of the complement system can involve the utilization of the 12 serum complement components in a cascade fashion, via triggering of either the classical or the alternative pathways. The
system is one of the major immune effector mechanisms in the blood and its activation leads to the generation of inflammation, the killing and clearance of pathogenic microorganisms and the elimination of immune complexes (Prehm, 1995).

1.12 34 kDa HA-binding protein

During the last few years, our laboratory is engaged in understanding the molecular nature and the functional significance of one HA-binding protein. The preliminary investigation demonstrated the purification of a 68 kDa HA-binding protein from rat brain and liver (D’Souza and Datta, 1985, 1986a) and the specific binding of this protein towards HA among other GAGs was subsequently reported (D’Souza and Datta, 1986b). Later, Gupta et al. (1991a) also purified this protein (a homodimer of 34 kDa subunits) from rat kidney, showed its glycoprotein nature and positive co-operative binding interactions with other well characterized extracellular proteins like fibronectin, laminin; implying its role in structural organisation. Interestingly, they demonstrated the cell surface localization and secretory nature of this HA-binding protein. Using AK-5 tumor cell line, a transplantable histiocytema, the role of this protein in cell adhesion and tumor invasion was delineated (Gupta and Datta, 1991b). It was shown that this protein binds specifically to 41 and 37 kDa polypeptides of the fibrosarcoma plasma membrane (Gupta et al., 1993). Recently, we sequenced the gene encoding this HA-binding protein in human skin fibroblasts (Deb and Datta, 1996). The presence of HA-binding protein on the spermatozoa has been demonstrated and its involvement in sperm functions like sperm maturation, motility and fertilization have been elucidated (Ranganathan et al., 1994). Our speculation is that the HA-binding protein, being a phosphoprotein may have a role in signal transducing events. Recently, it has been found that this protein is present in lower concentrations in teratozoospermic and asthenozoospermic patients in comparison to the normozoospermic spermatozoa, with enhanced phosphorylation in fertile spermatozoa as compared to the lower level of phosphorylation in infertile spermatozoa, thus, assigning a marker status to this protein in assessing human infertility (Bharadwaj, 1995). Conformational studies of this protein suggest a reversible folding pathway, which is initiated by the dissociation of native dimer into denatured monomers through a compact monomeric intermediate state (Kumar, 1995).
1.13 C1q Receptor

C1q (Mr 460 kDa) is a collagen-like, structurally complex, cationic (γ2 mobility) glycoprotein consisting of 18 similar but distinct polypeptide chains (6A, 6B and 6C) (Reid, 1985). Each chain (Mr, A = 28 kDa, B = 25 kDa, and C = 24 kDa) consists of 81-amino-acids, collagen-like N-terminal portion and a C-terminal globular region of 136 amino acids. In normal plasma, C1q circulates as a subunit of the first component of complement, C1, which is a Ca2+ dependent pentamolecular complex with a structural formula of C1qC1r2C1s2 (Ziccardi and Cooper, 1977). During activation of the classical pathway of complement, C1q within the C1 macromolecule functions as a recognition unit by virtue of its ability to recognize and bind to particle-bound IgG or IgM (Plaut, 1972). Immune complex-bound C1q, then undergoes a conformational change which in turn induces autocatalytic activation of C1r. Finally, activated C1r effects the proteolytic conversion of C1s to C1s', thus completing the activation of C1. Activated C1 is readily controlled by C1 inhibitor (C1-INH), which firmly binds to and dissociates C1r and C1s from particle-bound C1. Under these conditions, the collagenous tail of the C1q molecule becomes exposed and potentially available for interaction with C1q binding particles and cell surface C1q receptors (C1q-R) (Ghebrehiwet, 1987). The C1q receptor has been shown to mediate cellular cytotoxicity, inhibit collagen-induced platelet aggregation, enhance phagocytosis of C3b/iC3b coat, or IgG opsonized target particles, modulate synthesis of Ig by B cells, inhibit IL-1 production by B cells, and suppress the proliferation of tumor cells (Ghebrehiwet, 1993). The interaction of C1q with its receptor triggers a variety of cellular responses and these receptors are suspected to involve certain signalling pathways. Platelets, for instance, can be activated by aggregated C1q. This activation, which is accompanied by a significant increase in the release of inositol 1,4,5-triphosphate (IP3) results in the expression of α11b/β3 integrins (GPIIb/IIIα) and procoagulant activity and may contribute to thrombogenic inflammatory reactions. The ability of C1q to induce proliferative or antiproliferative responses depend upon the nature of the C1q or the state of cell expressing the C1qR.

1.14 Collectins

Collectins are humoral lectins found in mammals and birds. They are oligomers whose subunits comprise three polypeptide chains each containing a collagenous section and a
C-terminal lectin domain. They are related structurally and functionally to the first component of the classical complement pathway, C1q and seem to serve important roles in innate immunity through opsonization and complement activation. The lectin domains bind carbohydrates on microorganisms, while the collagenous regions are ligands for the collectin receptor on phagocytes and also mediate C1q-independent activation of the classical complement pathway (Holmskov, 1994).

1.15 Collectin receptors

The collectin receptor was first described as a receptor for the complement protein C1q (Arvieux, 1984; Erdei, 1988). It has been observed on many cell types, including most leukocytes, platelets, endothelial cells, fibroblasts and specialized epithelia. Mannose binding protein (MBP), conglutinin and Surfactant-A (SP-A) were shown to bind to the same receptor as C1q (Malhotra et al., 1990). Competition studies, utilizing the binding of radioiodinated C1q receptor to different immobilized ligands, showed that all of these ligands compete, with similar affinity, for binding to the receptor and this was confirmed using cells bearing the receptor. Subsequent studies indicate that CL-43 also binds to the same receptor, but it is not yet clear whether Surfactant-D (SP-D) shares this specificity. As the C1q receptor recognizes multiple ligands it was renamed as the collectin receptor (Malhotra et al., 1992). The partial amino acid sequence of the collectin receptor shows a high degree of identity with calreticulin (Malhotra et al., 1993). Studies on the structural and antigenic properties, and on the cellular localization of the collectin receptor and of calreticulin, indicate that the two proteins are related but not identical. Further, a group of proteins some of which are characterized as calcium binding protein, have N-terminal sequences similar to those of the collectin receptor and calreticulin (Malhotra et al., 1993).

1.16 The relationship of the HA-binding proteins with C type lectins

The HA interactions are often mediated by a common protein domain termed as Link module, also known as a proteoglycan tandem repeat (Perkins et al., 1989; Hardingham and Fosang, 1992; Bork and Bairoch, 1995). This module is approximately 100 amino acids in length and has a characteristic consensus sequence, containing four disulphide-bonded cysteines (Neame et al., 1986; Neame and Barry, 1993). Link modules have been described in extracellular matrix molecules (link protein, aggrecan, versican, neurocan, and brevican),
the HA receptor CD44 and the arthritis-associated protein tumor necrosis factor (TNF)-stimulated gene-6 (TSG-6). The PTR sequence indicate the occurrence of α helices (A) and β stand (B) in the sequence BABABBB. This is very similar to consensus secondary structure prediction for 129 CRD (carbohydrate recognition domain ) sequence. The PTR and CRD superfamily are associated with carbohydrate binding, where the PTR binds hyaluronate and the CRD binds a variety of oligosaccharide ligands. The CRD residues form the Ca²⁺ and carbohydrate binding sites 1 and 2 in mannose binding protein (MBP) and E-selectin, and are found in the β region. The link module does not bind Ca²⁺ and this occurs due to the absence of these CRD residues in the PTR. The structural similarity between the link module and the C type lectin domain suggest that these have common evolutionary origin, which provides a possible explanation for the similar roles of CD44 and the selectins in leukocyte extravasation at sites of inflammation (Kohda et al., 1996).

1.17 Signal transduction by cell adhesion Receptors

One of the most important advances made recently is the realization that adhesion molecules, like conventional growth factors receptors, are capable of mediating various signal transductions. A unifying feature is beginning to emerge that two requisite signals, one delivered by growth factors and the other transduced by cell adhesion receptors, communicate and often counter balance the survival and the homeostasis of cells.

A cell-surface receptor that is either a tyrosine kinase (RTK) (e.g. receptors for epidermal growth factor, nerve growth factor, and platelet-derived growth factor, Ulrich and Schlessinger, 1990) or that is associated with such a kinase (e.g. T-cell receptor or interleukin-6 receptor, Weiss and Litmann, 1994; Kishimoto et al., 1994) and continues all the way upto the nucleus. The proto-oncogene protein Ras is central to this pathway, therefore this signaling cascade is also known as the Ras pathway. Ras signaling pathways seem to be present in all eukaryotic organisms from yeast to humans (Marx, 1993).

The receptor tyrosine kinase pathway starts with the binding of a growth factor to its tyrosine kinase receptor. This ligand interaction induces the receptor to cluster in the plane of the membrane resulting in activation of the receptor's kinase domain and autophosphorylation of specific tyrosine residues (Ullrich and Schlessinger, 1990). Adaptor proteins, such as Grb2/Sem5, bind to a Ras-activator protein (mSos1) (Suen et al., 1993; Chardin et al., 1993)
bringing it to the receptor to form a stable complex (Rosakis-Adcock et al., 1993; Simon et al., 1991). Activation of Ras is induced by the exchange of GDP for GTP. On the other hand, inactivation is mediated by GTPase activating proteins (GAPs), that stimulate the intrinsic GTPase, which in turn determines the level of activity of Ras in a particular cell. Once activated, Ras has effects on cytoplasmic serine/threonine kinase cascades (Wood et al., 1992), that involve Raf-1 (Li et al., 1991), MEK (also named MAP kinase kinase, Kyriakis et al., 1992; Dent et al., 1992), mitogen-activated protein (MAP) kinase (also named ERKs, Boulton et al., 1991) and possibly also ribosomal S6 kinases (Rsk, Sturgill and Wu, 1991). MAP kinases can migrate from the cytosol into the nucleus after growth factor stimulation (Lenormand et al., 1993) finally delivering the signal into the nucleus by phosphorylating transcription factors (Jackson, 1992; Chen et al., 1993). The sequential event of Growth factor receptor and cell adhesion receptor mediated signalling reaction is illustrated in Fig 1.1.

1.18 Regulation of signaling by the cytoskeleton

Evidence supporting the idea that there are bi-directional connections between signaling pathways and the organization of the cytoskeleton is emerging. For example, the Ras-related small GTP-binding proteins Rho and Rac are known to be involved in controlling actin polymerization (Ridley and Hall, 1992; Koch et al., 1991). It is also clear that growth factor receptors are coupled via Ras to Rho and Rac, and thus influence cytoskeletal assembly (Ridley and Hall, 1992). Recently, it has been shown that Rho activates phosphatidylinositol-3-kinase in preparations of platelets (Zhang et al., 1993) and also phosphatidylinositol-5-kinase in mammalian cells (Chong et al., 1994), suggesting a mechanism by which Rho could regulate cytoskeletal reorganization. Activation of the phosphatidylinositol 5-kinase also affects the availability of substrates (PIP$_2$) involved in growth factor signaling (Chong et al., 1994).

A number of other reports indicate that the cytoskeleton can be influenced by several signal transduction pathways. For example, proteins involved in actin cross-linking have been identified as substrates for protein kinase C (Aderem, 1992; Hartwig et al., 1992). Conversely, cytoskeletal proteins can regulate signal transduction by several different mechanisms. For example, actin filaments modulate enzymatic activities through direct interaction with phosphatases and kinases (Carraway et al., 1993; Lokeshwar and Bourguignon, 1992). L-plastin, an actin binding protein, has been implicated in the IP$_3$-independent mechanism of
Figure 1.1 Potential signal transduction pathways in response to ECM cell surface receptor interactions (reproduced from Raghove, FASEB J. 8, 823-831, 1994)
Ca$^{2+}$ release from intracellular stores after Fc receptor ligation in leukocytes (Rosales et al., 1994). The cytoskeletal protein ankyrin has been found to block the IP$_3$ for the IP$_3$ receptor (Bourguignon et al., 1993). All these reports indicate that, in some cases, the cytoskeleton is clearly involved in the process of signal transduction. Proteins are organized spatially in the cell via interactions with the cytoskeleton, as exemplified by the formation of focal contacts after integrin interaction with extracellular matrix proteins (Burridge and Fath, 1989). The actin cytoskeleton may be a site where signals are organized by bringing together molecular complexes.

Integrins bind directly to two cytoskeletal proteins, α-actin and talin, which in turn links integrins to cytoskeletal matrix comprising actin filaments and other actin-associated proteins, including vinculin, zyxin and paxillin (Clark and Brugge, 1995). This matrix both strengthens the ECM-integrin interaction and forms a protein scaffold for assembly of a signaling network comprising focal adhesion kinase, components of the pathways (e.g., SOS and Grb2) and GTP binding proteins (Clark and Brugge, 1995). A number of integrin-triggered signaling processes have been observed in various cells, where the relationship to integrin-mediated tyrosine phosphorylation is currently unclear. Integrin clustering or integrin-mediated adhesion has been reported to activate the Na$^+$/H$^+$ antiporter (Schwartz et al., 1991); induce Ca$^{2+}$ transients (Leavesley et al., 1993; Schwartz, 1993) activate calcineurin (Hendey et al., 1992); play a role in leukotriene production (Graham et al., 1993); affect Ca$^{2+}$ activated proteases and the distribution of PI-3-kinase (Fox et al., 1993; Zhang et al., 1992) modulate a K$^+$ channel via pertussis toxin sensitive G proteins (Arcangeli et al., 1993), activate phospholipase A2 (PLA2) (Cybulsky et al., 1993)

and affect cAMP levels. These reports suggest that different integrins may utilize several distinct biochemical pathways to deliver signals to the cell interior; this diversity may, in part, be responsible for the specificity in the response to various integrins.

The role of calcium in integrin signaling events is particularly interesting. Cell spreading on fibronectin arises from calcium influx through voltage independent channels (Schwartz, 1993). This calcium influx is regulated by a 50 kDa transmembrane integrin-associated protein (IAP) with channel-like structure previously identified as a protein that binds to the β3 integrin subunit (Brown et al., 1990; Lindberg et al., 1993). In another
set of interesting observations, a protein of 60 kDa that is homologous to the calcium binding protein, calrecticulin, has been reported to bind to the KXGFFKR motif found in the cytoplasmic domains of integrin α chains. A similar motif is found in steroid hormone receptors leading to the interesting but controversial hypothesis that integrin ligation may affect these nuclear receptors (Dedhar et al., 1994).

Cadherin-mediated cell adhesion also results in localized assembly of cytoskeletal and signaling networks. A family of related cytoplasmic proteins (β catenin, plakoglobin and p120) bind tightly to the cytoplasmic domains of cadherins. These proteins bind α-catenin, which has some homology to vinculin, indicating that α-catenin may link the cadherin-catenin complex to the cytoskeleton. In vitro studies show that α-catenin binds to actin (Rimm et al., 1995) and fordin (Lombardo et al., 1994). α actinin is also a component of the cadherin-catenin complex and may provides an additional link between the cadherin-catehin complex and actinin (Knudseneal, 1995). Formation of complexes between cadherin-catenin and the cytoskeleton strengthens cell adhesion and provide a protein scaffold for a signaling network comprising kinases (src and yes, Tsulkita et al., 1991) and a protein phosphatase (PTDp, Brady-kalnay et al., 1995). This signaling network may regulate cadherin-catenin interactions with the actin cytoskeleton and signaling through β catenin components of Ras signaling pathway and small GTP binding protein also associate with the cadherin-catenin complex. Activation of protein kinase C appears to accelerate cell-cell adhesion (Lewis et al., 1994) and small GTP-binding proteins (eg Rho) localize to cell-cell contacts in some epithelial cells (Adamson et al., 1992). Together, these observations indicate that phosphorylation may be a positive or negative regulator of functions of the cadherin-catenin complex in cell adhesion, assembly of the cytoskeleton and signal transduction.

1.19 Regulation of gene expression by cell adhesion receptors

There are a large number of observations in the literature indicating that adhesive interactions with extracellular matrix can affect cell differentiation and gene expression. A good example of integrin mediated gene induction occurs in monocytes (Haskill et al., 1988; Sporn et al., 1990). This system has allowed new insights into the relationships between matrix proteins, integrins, protein kinase, transcription factors and gene regulation (Juliano and Haskill, 1993). When peripheral blood monocytes are plated onto tissue culture plastic or onto
substrata coated with extracellular matrix ligands such as fibronectin, collagen or laminin, there is a rapid and profound induction of a number of so-called immediate-early (IE) genes including transcription factors such as c-fos, c-jun, Ikβ and MAD-6/A20, as well as cytokines such as IL-1β, IL-8 and TNFα (Haskill et al., 1991). There is some selectivity of gene expression since plating the cells onto different ECM proteins caused preferential expression of some of the IE genes (Sporn et al., 1990).

In contrast to the situation with integrins, there is a relative paucity of information on gene induction mediated by cell-cell adhesion molecules such as cadherins, selectins and Ig family CAMs. However, this is clearly an area of great interest, and some work is beginning to emerge. One report examined the role of NCAM in regulating metalloproteinase expression in glial cells (Edvardsen et al., 1993). A report concerning a role for selectins in gene expression has appeared recently (Laudana et al., 1994). Sulfatides have been established as ligands for L-selectin. When neutrophils were treated with sulfatides, a transient increase in cytosolic calcium was observed; this was followed by increased expression of messages for TNFα and IL-8. It seems clear that signals transmitted by Ig family CAMs or by selectins can have a major impact on regulation of gene expression in some types of cells.

1.20 Cell adhesion receptors: a role in cancer

Most of our progress in understanding molecular aspects of cancer has been made by studying the stages involved in cellular transformation, the process that converts a normal cell into a cell which under conditions of sufficient density can grow as a tumor in an isogenic host. This process is driven by dominant oncogenes and the loss of recessive tumor suppressor genes, and essentially involves the alteration of cell cycle control. The cells become growth-factor-independent, do not obey contact inhibition and lose features of differentiation.

The development of invasive and metastatic properties is probably not completely independent from cell transformation. Transformation is, of course, a precondition for metastatic spreading. At the same time, transformed cells appear to develop properties which are also required for metastasis formation. It has been known for a long time that transformed cells look phenotypically different as compared to their normal counterparts. Fibroblasts, for example, round up, are less adherent to neighboring cells, and change their cytoskeletal
organization (Bar-Sagi and Feramisco, 1985; Kamps et al., 1988). Furthermore, transformed cells can reorganize the ECM. In particular, they produce elevated levels of various proteases including metalloproteases which are currently thought to endow the cells with invasive properties. Thus, while cell cycle control appears to be the primary target of carcinogenesis, transformation may overlap with other stages of tumor progression. In order to form distant metastatic colonies, cancer cells have to overcome many obstacles. These include combating immune surveillance, loss of original tissue contacts, moving through the ECM (e.g., the basement in lymph nodes, entering the blood stream, extravasating and settling in other tissues). Tumor cells, thus, need to acquire a wide range of molecular properties to complete the whole metastatic process most of which are likely to be mediated by cell-surface proteins.

One of the most important advances made recently is the realization that adhesion molecules, like conventional growth factor receptors, are capable of mediating various signal transduction events. A unified picture is beginning to emerge that two requisite signals, one delivered by growth factors and the other transduced by adhesion receptors, communicate and often counterbalance for the survival and homeostasis of cells. Most adhesion receptors (e.g., integrins, cadherins, some Ig family members, CD44) are transmembrane proteins that are connected intracellularly to the cytoskeleton; therefore, many signaling functions have been ascribed to various adhesion molecules. Thus, L-selectin is involved in inducing calcium fluctuation in human neutrophils (Laudanna et al., 1994), VCAM-1 mediates the induction of 72 kDa gelatinase in T cells upon adhesion to endothelial cells. The ICAM-1 activation has been shown to induce tyrosine phosphorylation of the cytoskeleton-associated protein, cortactin, in microvessel endothelial cells (Durieu-Trautman et al., 1994). Integrins are the best characterized adhesion molecules with regard to their role in signal transduction. All integrin receptors are α/β heterodimeric proteins that link extracellular matrix ligand proteins (such as laminin and fibronectin) to cytoskeletal elements (primarily, actin microfilaments). Intracellularly, integrins establish connections with the cytoskeleton through other bridging molecules including α-actinin, vinculin, talin, tensin, zyxin and paxillin (Burridge and Fath, 1989). The unique structural feature of integrin receptors enables them to participate in a diverse cytoskeleton-related signaling functions encompassing the triggering of Ca²⁺ transient, inducing tyrosine phosphorylation and modulating gene expression. Integrin-transduced
biochemical and molecular signals are closely involved in tumor metastasis. For example, signaling pathways motivated by integrin αVβ3 induce the generation of collagenases which promote tumor cell motility. The same integrin receptor is also involved in modulating endothelial cell phenotypes and angiogenesis (Davis et al., 1993 and Brooks et al., 1994), an important parameter of metastasis. Adhesion receptor signaling also appears to play an essential role in maintaining cellular homeostasis. For example, adhesion mediated by integrins or other adhesion molecules such as αLβ2-ICAM-1 and α4β1-VCAM-1 prevents programmed cell death (apoptosis) (Bates et al., 1994; Koopman et al., 1994). In contrast, inhibition of cell-cell contact or disruption of cell-matrix interactions with antibodies against integrins such as αVβ3 triggers apoptosis (Bates et al., 1994). These experimental observations suggest that interference with the expression and function of specific adhesion molecules may not only disrupt the physical association between spreading cancer cells and host elements, but may also block the biochemical signals transduced by these adhesion receptors and thus promote apoptosis of tumor cells. While a large body of work has sought to evaluate the role of adhesion receptors in the invasive and metastatic behaviour of tumor cells, relatively little attention has been given to the role of these molecules in regulating tumor cell proliferation. Much of the focus, instead has centered on studying signaling pathways of soluble growth promoters and growth inhibitors. This has proved to be a fruitful area as many proto-oncogenes and tumor suppressors have been shown to function in signaling pathways (Bishop, 1991). A consensus view of cancer is that mutational activation of dominant oncogenes or inactivation of proliferative pathways leading to uncontrolled growth. However, this is an incomplete view of cancer since normal cells, in addition to soluble factors, also require attachment to a substratum for proliferation (anchorage dependent growth). Tumor cells lose this requirement for anchorage, they are less adhesive and often display a less well organized cytoskeleton than their normal counterparts. Over expression of adhesion receptors or focal contact protein involved in ECM-cell adhesion restores cytoskeletal organization and reduces tumorigenicity. Also, cell adhesion receptors have been identified as tumor suppressor genes, most notably in colon carcinomas. These insights, coupled with the emergence of cell adhesion receptors as signaling receptors has prompted fresh interest in the role of cell adhesion receptor molecules in regulating growth of normal and malignant cells (Rosales et al., 1995).