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Regulation of development and differentiation by the extracellular matrix

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SUMMARY

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INTRODUCTION

Differentiation is a continuously regulated process and interactions between the cell and its environment play a major role in maintaining stable expression of differentiation-specific genes (Blau and Baltimore, 1991). An important component of the cellular environment is the extracellular matrix (ECM), which is composed of glycoproteins, proteoglycans and glycosaminoglycans that are secreted and assembled locally into an organised network to which cells adhere (Hay, 1981). An ECM is present within mammalian embryos from the two-cell stage and is a component of the environment of all cell types, although the composition of the ECM and the spatial relationships between cells and ECM differ between tissues. Cells may be completely surrounded by ECM, as is the case for chondrocytes, or may contact the ECM only at one surface, as exemplified by epithelial and endothelial cells. In some tissues only a proportion of the cells are exposed to ECM: for example, in stratified epithelia. The ECM offers structural support for cells, and can also act as a physical barrier or selective filter to soluble molecules.

It has been clear for many years (Grobstein, 1954; Bissell et al., 1982) that the ECM plays a role in regulating the differentiated phenotype of cells (reviewed by Watt, 1986), but the mechanisms involved remained largely mysterious until recently, when cell-binding sites within individual ECM glycoproteins and specific ECM receptors were identified. The cell-binding sites were mapped by using proteolytic fragments and synthetic peptides to define the minimal sequences responsible for adhesive activity. In the case of fibronectin, the primary determinant of cell-binding activity for many cell types resides in the sequence GRGDSP, which occurs in one of the type III repeats that form the central domain of the molecule (Ruoslahti and Pierschbacher, 1987). Subsequently, RGD-containing sequences have been found in other matrix proteins, and additional short linear adhesive sequence motifs have been defined, although it is clear that the three-dimensional structure of matrix proteins is also an important determinant of adhesive activity (reviewed by Humphries, 1990). Affinity chromatography techniques, together with adhesion-perturbing antibodies that recognise specific plasma membrane glycoproteins, allowed the identification of ECM receptors, many of which belong to the integrin family of α/β heterodimers (Ruoslahti and Pierschbacher, 1987; Hynes, 1987).

In this review, we will summarise some of the evidence that ECM components regulate differentiation and development, describe the regulatory mechanisms involved and, finally, discuss the intracellular events that may transduce signals between ECM receptors and the nucleus.

EVIDENCE THAT CELL-EXTRACELLULAR MATRIX INTERACTIONS REGULATE DEVELOPMENT AND DIFFERENTIATION

Developmental mutations

Evidence for the importance of cell-matrix interactions

during development has come from mutations affecting extracellular matrix proteins and their receptors in a range of organisms. In *Caenorhabditis elegans* mutations in over 50 genes that affect the gross morphology of the organism have been identified and several of these genes are now known to encode extracellular matrix proteins. The *C. elegans* cuticle is composed primarily of covalently cross-linked collagens and a number of the mutations affecting morphology map to collagen genes. Mutations in *dyp-13* result in a short, chunky body shape (von Mende et al., 1988). Mutations in *Sqt-1*, another collagen gene, cause lengthening, shortening or twisting of the entire worm (Kramer et al., 1988). Single nucleotide alterations within a third collagen gene, *clb-2*, which encodes the α1 chain of type IV collagen found in basement membranes, cause gross morphological defects and are lethal during late embryogenesis (Guo et al., 1991). Finally, mutations in another *C. elegans* gene, *unc-6*, cause defects in neuronal development; the product of this gene is related to laminin and is required for axon guidance (Ishii et al., 1992).

In *Drosophila*, mutations that result in developmental defects have been identified in both extracellular matrix proteins and receptors. A null mutation in the laminin A chain gene is a recessive lethal in late embryogenesis (Hortsch and Goodman, 1991). Mutations in the gene *Scabrous* alter the spacing pattern of R8 photoreceptor cells in the eye: the gene product has homology with vertebrate fibrinogen and is proposed to act as a lateral inhibitor of R8 differentiation (Baker et al., 1990). Mutations in subunits of the *Drosophila* integrins, the PS antigens, lead to developmental abnormalities of the wings, eyes and muscle (Leptin et al., 1989; Wilcox et al., 1989; Brower and Jaffe, 1990; Volk et al., 1990; Zusman et al., 1990).

The *Toll* gene product in *Drosophila* is a transmembrane protein that is an important mediator of dorsal-ventral polarity in the embryo (Anderson et al., 1985 a,b). While the cytoplasmic domain of the Toll protein has some characteristics of a growth factor receptor (eg. Heguy et al., 1992), the extracellular domain has sequence homology with the proteoglycan, decorin, and with human platelet glycoprotein 1b, a receptor that binds von Willebrand factor and thrombin (Hashimoto et al., 1988; Keith and Gay, 1990). Expression of Toll protein in a non-adhesive *Drosophila* cell line promotes intercellular adhesion (Keith and Gay, 1990).

The majority of ECM gene mutations mapped in vertebrates have been identified in humans, where a number of heritable disorders have been studied. Mutations in type I collagen result in fragility of bone and other tissues that are rich in type I collagen, while mutations in type II collagen result in disorders of cartilage. The phenotype of each mutation depends on its effect on the structural integrity of the protein and the extent to which the abnormal collagen chains are incorporated into the extracellular matrix (Byers, 1990). Alport syndrome, a hereditary glomerulonephritis often accompanied by loss of hearing, involves ultrastructural defects in the glomerular basement membrane due to mutations in the α5 chain of type IV collagen (Barker et al., 1990). Mutations in fibrillin lead to Marfan's syndrome, a disorder of connective tissue that affects the ocular, skeletal and cardiovascular systems (Dietz et al., 1991; Maslen et al., 1991). Transgenic mice provide an experimental model for some of these human diseases: for example, mutations in the α1 chain of type II collagen result in transgenic animals with a range of skeletal defects including abnormal limb and craniofacial development (Metsäranta et al., 1992; Garofalo et al., 1991; Vandenberg et al., 1991). Transgenic mice expressing a truncated α1 chain of type X collagen have abnormalities in tissues that undergo a transition from cartilage to bone during endochondral ossification; this is consistent with the expression of type X collagen by hypertrophic chondrocytes (Lu Valle et al., 1993).

Techniques for preventing expression of specific ECM genes allow a systematic analysis of the role of extracellular matrix proteins and receptors during mouse development. The effect of preventing type I collagen gene expression in mice was documented several years ago, when viral integration within the α1 type I collagen chain gene was found to block its transcription and cause perinatal death of homozygous embryos (Schnieke et al., 1983). Gene knock-out through homologous recombination is now the preferred approach; the first null mutation to be described is in the tenascin gene and, surprisingly, the mutant mice are phenotypically normal (Saga et al., 1992).

Experimental perturbation of embryonic development

Another approach to analysing the role of cell-ECM interactions in development has been to investigate the consequences of perturbing such interactions by injection of specific antibodies or peptides. In vertebrates, the role of cell adhesion to fibronectin has been most thoroughly characterised (reviewed by Dufour et al., 1988). Fibronectin appears before or at the onset of gastrulation in all vertebrates examined and is abundant at times and sites of cell migration: during gastrulation, neural crest cell migration and the migration of primordial germ cells. Direct evidence that cell adhesion to fibronectin is required for morphogenetic cell movements comes from the inhibition of gastrulation that occurs when antibodies to fibronectin are injected into the blastocoel cavity (Boucaut et al., 1984a). More general evidence for the importance of cell-ECM interactions has come from the injection of RGD-containing peptides: these inhibit gastrulation in salamander (*Pleurodeles*) embryos and perturb neural crest cell migration in avian embryos (Boucaut et al., 1984b). In addition, Fab fragments of antibodies to β_1 integrins arrest gastrulation in *Pleurodeles* embryos (Darribère et al., 1988). At later stages of development, microinjection of RGD peptides randomises the development of right/left asymmetry of the heart and gut in *Xenopus* embryos (Yost, 1992).

The role of fibronectin in *Drosophila* early development is less clear. RGD-containing peptides were originally reported to inhibit gastrulation and the establishment of the dorsoventral axis (Naidet et al., 1987). However, the known *Drosophila* integrins are not required for these processes (Leptin et al., 1989) and more recent experiments have failed to show an effect of RGD peptides (Leptin et al., 1992). An explanation for these discrepancies may lie in the fact that fibronectin and other RGD-containing matrix glycoproteins have not been definitively identified in *Drosophila* (Hortsch and Goodman, 1991).

Table 1. Examples of positive and negative regulation of differentiation by ECM proteins in vitro

Matrix component	Cell type	Reference
POSITIVE		
Laminin	epithelial conversion of kidney mesenchyme	Klein et al., 1988
	neurite outgrowth	Sanes, 1989
	albumin synthesis by hepatocytes	Caron 1990
	milk protein production by mammary epithelial cells	Streuli et al., 1991
	tubule formation by endothelial cells	Kubota et al., 1988 Grant et al., 1989
	process formation by osteoblasts	Vukicevic et al., 1990
	myoblast fusion	von der Mark and Öcalan, 1989
Thrombospondin	neurite outgrowth	Neugebauer et al., 1991 O'Shea et al., 1991
Fibronectin	erythroblast differentiation	Patel and Lodish, 1987
Collagens	mammary epithelial morphogenesis	Hall et al., 1982 Lee et al., 1985
	colonic epithelial morphogenesis	Pignatelli and Bodmer, 1988
	tubule formation by endothelial cells	Montesano et al., 1983
Vitronectin	neurite outgrowth	Neugebauer et al., 1991
Tenascin	neurite outgrowth	Chiquet, 1989
	chondrocyte differentiation	Mackie et al., 1987
NEGATIVE		
Fibronectin	myoblast fusion	Podleski et al., 1979 von der Mark and Öcalan, 1989
	keratinocyte terminal differentiation	Adams and Watt, 1989
	chondrocyte differentiation	West et al., 1979
	adipocyte differentiation	Spiegelman and Ginty, 1983

Finally, there are examples of cell-matrix interactions that do not involve the RGD sequence yet are required for embryonic morphogenesis. Injection of heparin, which interferes with heparan sulphate proteoglycan-mediated interactions, retards gastrulation and affects neural development in *Xenopus* (Mitani, 1989), while injection of heparitinase randomises the development of right/left asymmetry (Yost, 1992). In sea urchin embryos, the major protein of the external extracellular matrix is hyalin; a monoclonal antibody to hyalin inhibits gastrulation and arm formation by inhibiting cell-matrix adhesion (Adelson and Humphreys, 1988).

Cell and organ cultures

Although experiments with whole embryos establish that cell-matrix interactions are required for normal development, the results are consistent with a purely structural role for the ECM: given, for example, that type II collagen is a major component of cartilage ECM, it is hardly surprising that mutations that prevent its correct assembly result in joint defects. Evidence for an instructive role of matrix components has come, instead, from in vitro experimental models in which positive and negative effects on the differentiation of specific cell types are observed. A number of examples are listed in Table 1.

Whole-embryo experiments have demonstrated a role of fibronectin in morphogenetic movements (see above), and cell culture provides a means of assaying the effects of fibronectin and other matrix proteins on the migration of individual cell types. Cells of mouse blastocysts attach and migrate on fibronectin, laminin, vitronectin, collagen and thrombospondin; for substrata other than laminin and thrombospondin, these processes are sensitive to inhibition by RGD peptides or anti-integrin antibodies (Armant et al., 1986a,b; Richa et al., 1985; Sutherland et al., 1988; O'Shea et al., 1990). As one would predict from the presence of fibronectin within the pathways of neural crest cell migration, explanted neural crest cells migrate preferentially on fibronectin-coated substrata (Newgreen et al., 1982), from which they are detached by anti-integrin antibodies (Bronner-Fraser, 1985). Similar types of assays have demonstrated a role for laminin and other matrix components in myoblast migration and fusion and in neurite outgrowth (reviewed by Sanes, 1989 and see Table 1).

The ECM also plays a key role in the morphological differentiation of epithelia, as the following examples illustrate. In organ cultures of developing kidney, anti-laminin antibodies inhibit cell polarisation and epithelial conversion of the mesenchyme; this activity has been mapped to the E3 and E8 domains of the long arm of laminin (Klein et al., 1988; Ekblom et al., 1990) and is mediated by the $\alpha_6\beta_1$ integrin (Sorokin et al., 1990). Mammary epithelial cells only assume a polarised phenotype and secrete milk proteins apically when cultured on reconstituted basement membrane or floating collagen gels (Hall et al., 1982; Lee et al., 1985); one effect of exogenous ECM is to regulate the production and organisation of ECM by the cells themselves (Streuli and Bissell, 1990).

One difficulty in interpreting the results of experiments involving epithelial sheets, or indeed any tissue, is to distinguish direct effects of the ECM from indirect effects caused by changes in cell shape or intercellular adhesion. The use of synthetic peptides corresponding to adhesive sequence motifs to manipulate differentiation goes some way towards resolving these problems, since soluble peptides can block differentiative responses to intact ECM glycoproteins (Menko and Boettiger, 1987; Pignatelli and Bodmer, 1988) and, in some instances, this is achieved at peptide concentrations that do not prevent cell adhesion (Menko and Boettiger, 1987). For osteoblasts, only two out of three peptides corresponding to laminin adhesive sequences trigger differentiation (Vukicevic et al., 1990). However, the clearest evidence for direct effects of ECM components on differentiation comes from studies in which keratinocytes and mammary epithelial cells are cultured in suspension under conditions in which cell-cell adhesion is prevented and the cells remain rounded: in these assays, matrix components regulate differentiated gene expression in individual cells (Fig. 1; Adams and Watt, 1989; Streuli et al., 1991). Thus ECM signals for differentiated gene

expression can indeed be separated from secondary adhesive events, such as cell spreading or polarisation.

REGULATORY MECHANISMS

There are at least three mechanisms by which the extracellular matrix can regulate cell behaviour. One is through the composition of the extracellular matrix. The second is through synergistic interactions between growth factors and matrix molecules. The third is through the cell surface receptors that mediate adhesion to extracellular matrix components.

Extracellular matrix diversity

Diversity in the composition of ECM in different tissues and at different stages of development arises not only through expression of different matrix molecules, but also from the existence of multiple forms of individual molecules (Table 2). Although it has not yet been demonstrated that variant forms of specific matrix molecules differ in their ability to regulate differentiation, other assays, facilitated by the availability of recombinant reagents, have demonstrated differences in function. Thus, alternative splicing may alter the potential for interaction with other ECM molecules, as shown by the binding of tenascin splice variants to fibronectin (Chiquet-Ehrismann et al., 1986, 1991); or confer the ability to bind to a specific cell surface receptor, as demonstrated for the V+ splice variant of fibronectin (Wayner et al., 1989; Guan and Hynes, 1990); or affect more complex processes, as shown by the differing abilities of agrin splice variants to mediate acetylcholine receptor clustering (Ferns et al., 1992; Ruegg et al., 1992). Different laminin heterotrimers differ in their adhesivity towards specific cell types (Calof and Lander, 1991; Hunter et al., 1992).

Post-translational modifications also affect the ways in which ECM components interact with each other and with cells. The degree of glycosylation of fibronectin (Jones et al., 1986) and laminin (Dean et al., 1990) and the amount of calcium bound by thrombospondin (Lawler et al., 1988) have all been shown to modulate cell adhesion. Self-aggregation of laminin-nidogen complexes is dependent on calcium ions (Paulsson, 1988) while fibronectin becomes incorporated into ECM through transglutaminase-catalysed cross-linking (Barry and Mosher, 1988). Self-assembly or cross-linking to other matrix components might affect cell adhesive activity by increasing the local concentration of cell-binding sites or, conversely, obscuring the sites. Non-covalent interactions between matrix molecules can affect the activity of adhesive glycoproteins such as fibronectin: such observations have led to the categorisation of thrombospondin, tenascin, SPARC (osteonectin) and, in some circumstances, laminin, as ‘anti-adhesive’ matrix glycoproteins (Sage and Bornstein, 1991; Calof and Lander, 1991). In addition, soluble proteoglycans can inhibit cell adhesion to collagen and fibronectin (Ruoslahti, 1989).

The composition of the ECM is not static, and changing patterns of expression of individual components are observed during development (eg. Laurie et al., 1989; Leivo and Engvall, 1988; Inaguma et al., 1988; Sanes et al., 1990; Hunter et al., 1992), as are alterations in the pattern of expression of splice variants (eg. ffrench-Constant and Hynes, 1989; Prieto et al., 1990; Weller et al., 1991). The existence of all these sources of variation makes it clear that, at a given time and place, the extracellular matrix has the potential to provide specific environmental information to cells.

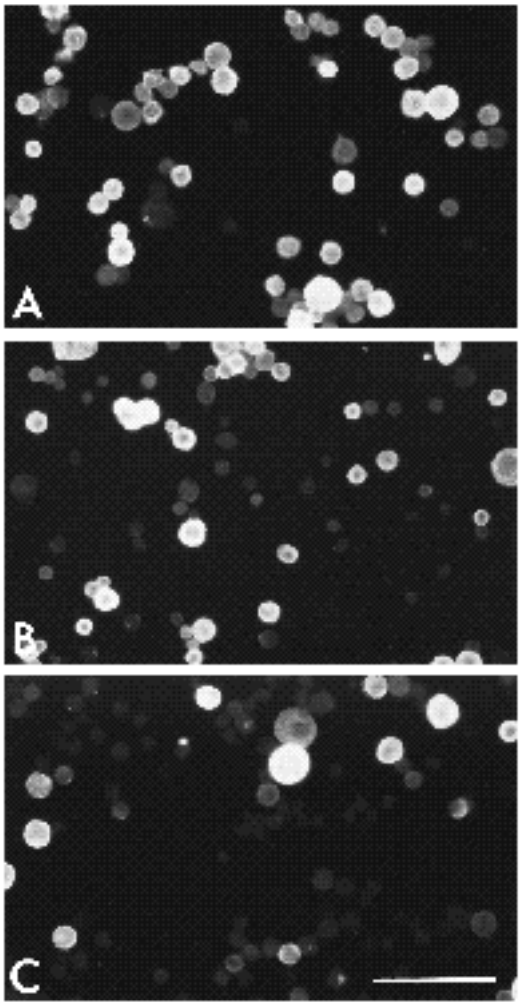

Fig. 1. Suspension-induced terminal differentiation of human keratinocytes. Keratinocytes were suspended for 24 hours in methyl cellulose containing (A) 100 μg/ml BSA (B) 100 μg/ml fibronectin (C) 0.5 mg/ml IgG isolated from anti-β1 integrin antiserum. Cells were stained for immunofluorescence with an antibody to involucrin, a marker of terminal differentiation. The percentage of involucrin-positive cells is lower in B and C than in A. Scale bar = 100 μm.

Interactions of growth factors with the extracellular matrix

Growth factors and extracellular matrix molecules interact in a number of ways to regulate cell behaviour (reviewed by Nathan and Sporn, 1991). One type of interaction is the binding of growth factors to the ECM, which affects the local concentration and biological activity of the growth factors. A second type of interaction involves regulation of gene expression: growth factors can have profound effects on the production of ECM proteins and their recep-

Table 2. Sources of structural variation in extracellular matrix proteins

Tissue-specific ECM components		
Examples:	Epiligrin and kalinin (stratified squamous epithelia)	Carter et al., 1991 Rousselle et al., 1991
	Claustrin (brain)	Cole and McCabe, 1991
	Restrictin (CNS)	Nörenberg, 1992
Isoforms		
Examples:	Laminin family (A/B1/B2; merosin-A/B1/B2; A/B2/S)	Leivo and Envgall, 1988 Ehrig et al., 1990 Hunter et al., 1989
	Thrombospondin family (TSP-1, TSP-2, TSP-3)	Lawler and Hynes, 1986 Bornstein et al., 1991 Vos et al., 1992
	Type IV collagen (α1 to α5 (IV))	Hudson et al., 1989
Alternative splicing		
Examples:	Elastin	Pollock et al., 1990
	Fibronectin	Kornblihtt et al., 1985
	Tenascin	Jones et al., 1989
	Type VI collagen	Doliana et al., 1990
	Agrin	Ruegg et al., 1992
Post-translational modifications		
Examples:	Glycosylation (laminin, fibronectin)	Jones et al., 1986; Dean et al 1990
	Glycosaminoglycan chain composition (aggrecan)	Bayliss et al., 1983
	Transglutaminase cross-linking (fibronectin)	Barry and Mosher, 1988
	Cation-dependent aggregation (laminin) or conformation (thrombospondin)	Paulsson, 1988 Lawler et al., 1988

Table 3. Examples of association of growth factors with extracellular matrix proteins

Binding to heparin/heparan sulphate chains	
FGFs (aFGF, bFGF, KGF, int-2)	Klagsbrun, 1990, Kiefer et al., 1991
IL-3	Roberts et al., 1988
Scatter factor/hepatocyte growth factor	Rosen et al., 1989
GM-CSF	Gordon, 1988
Schwann cell growth factor	Ratner et al., 1988
Purpurin	Berman et al., 1987
Platelet factor 4	Rusolahti and Yamaguchi, 1991
PDGF-B	La Rochelle et al., 1991
Pleiotrophin	Li et al., 1990
HB- EGF	Higashiyama et al., 1991
Binding to chondroitin sulphate chains	
Platelet factor 4	Périn et al., 1988
Binding to proteoglycan core proteins	
TGF-β (betaglycan, decorin)	Andres et al., 1989 Yamaguchi et al., 1990
Binding to ECM glycoproteins	
TGF-β (fibronectin)	Fava and McClure, 1987
TGF-β (thrombospondin)	Murphy-Ullrich et al., 1992
PDGF-A, PDGF-B (SPARC)	Raines et al., 1992
β-endorphin (vitronectin)	Hildebrand et al., 1989

tors, and there is also growing evidence for effects of the ECM on synthesis of growth factors and growth factor receptors. Finally, ECM molecules can themselves be mitogenic or can influence the responsiveness of cells to growth factors.

Growth factors can bind to the ECM via the glycosaminoglycan side chains or the protein cores of specific matrix molecules (Table 3). Many growth factors contain clusters of basic amino acids within regions of α-helical structure and these motifs mediate binding to the negatively charged heparan sulphate side chains of proteoglycans (Cardin and Weintraub, 1989; Ruoslahti and Yamaguchi, 1991). A number of growth factors are expressed in forms that differ in ECM binding. Examples include CSF-1, LIF and PDGF (Rathjen et al., 1990; La Rochelle et al., 1991). In the case of PDGF, the A form is secreted into the culture medium whereas the B form is held at the cell surface. Both the A and B forms of PDGF contain a cluster of basic residues, but that of the A form is at a proteolytic cleavage site; a PDGF-B molecule that is no longer retained at the cell surface can be created by substituting in the basic sequence from PDGF A (La Rochelle et al., 1991). Similarly, peptides corresponding to the A or B basic sequences cause release of PDGF from the cell surface (Raines and Ross, 1992).

ECM binding of growth factors can have a number of biological consequences. By limiting diffusion, the ECM provides a local store of growth factor that persists after growth factor production has ceased; for example, matrix-bound FGF is degraded more slowly than free FGF, prolonging its activity (Klagsbrun, 1990). In contrast, TGFβ bound to decorin, or PDGF bound to SPARC, are inactive (Yamaguchi et al., 1990; Raines et al., 1992). Matrix-bound growth factors can be released by proteolysis of proteoglycans, although it is not clear how this mechanism operates physiologically (Saksela and Rifkin, 1990; Isha-Michaeli et al., 1990; Klagsbrun and Baird, 1991).

There are numerous examples of growth factor-ECM interactions in the regulation of specific gene expression. Adhesion of neutrophils to fibronectin leads to increased TNF production (Nathan and Sporn, 1991). IL-3, IL-5 and GM-CSF stimulate proteoglycan synthesis in eosinophils (Rothenberg et al., 1988) while TGFβ upregulates transcription of a variety of matrix components (Ignotz and Massagué, 1986), alters expression of adhesive receptors (Ignotz and Massagué, 1987; Heino et al., 1989; Elenius et al., 1992) and regulates expression of collagenase and the metalloproteinase inhibitor, TIMP (Edwards et al., 1987). Induction of fibronectin expression is a primary response to EGF stimulation of mouse embryo fibroblasts (Blatti et al., 1988).

Some ECM proteins possess intrinsic growth factor activity. Many ECM proteins contain repeated epidermal growth factor (EGF)-like sequences (Engel, 1989; Argraves et al., 1990; Maslen et al., 1991; Ferns et al., 1992; Hardingham and Fosang, 1992) and, of these, laminin, tenascin and thrombospondin-1 have been reported to possess mitogenic activity (Panayotou et al., 1989; Majack et al., 1986; Chiquet-Ehrismann et al., 1986). In the case of laminin, the activity localises to the domain containing the EGF-like repeats; however, since laminin and EGF do not compete for cell binding, it is not clear whether laminin acts via the EGF receptor (Panayatou et al., 1989), and a subsequent study has localised mitogenic activity at the carboxy-ter-

minal end of the A chain (Kubota et al., 1992). In thrombospondin-1, mitogenic activity is not located within the EGF repeats, but rather in the amino-terminal heparin-binding domain (Majack et al., 1986).

The extracellular matrix can regulate the mitogenic response of cells to growth factors in a general fashion by regulating cell shape (Folkman and Moscona, 1978; Watt, 1986) but there is also evidence of specificity. Thus polymorphonuclear leukocytes respond to TNF when adherent on matrix proteins but not on uncoated tissue culture plastic (Nathan et al., 1989). Activin A and bFGF are survival factors for EC cells grown on tissue culture plastic, but are mitogens for EC cells adherent on laminin or fibronectin (Schubert and Kimura, 1991). Overexpression of individual adhesive receptors also causes changes in cell growth properties (Giancotti and Ruoslahti, 1990; Leppä et al., 1992).

All of these observations support the idea that growth factors and ECM proteins collaborate in creating distinct cellular environments or 'niches' that regulate proliferation and differentiation, a concept originally formulated for stem cells in self-renewing tissues (Schofield, 1978). Further evidence has come from a number of experimental models of differentiation. Thus the binding of bFGF to cell surface heparan sulphate proteoglycans is necessary for interaction with its high affinity receptor, and both these interactions are required for inhibition of myoblast terminal differentiation by bFGF (Klagsbrun and Baird, 1991; Rapraeger et al., 1991). In bone marrow, differentiation of progenitor stem cells along separate lineages is directed by growth factors, several of which are presented in a functionally active form by matrix components secreted by fibroblasts of the bone marrow stroma (Gordon, 1988). Thrombospondin and c-kit ligand synergistically promote the adhesion and growth of particular lineages from progenitor cell populations (Long et al., 1992). Ciliary neurotrophic factor (CNTF) induces O-2A progenitor cells isolated from newborn optic nerve to differentiate transiently into type 2 astrocytes in vitro, but stable astrocytic differentiation cannot be achieved unless the progenitor cells are grown on ECM derived from 9- to 12-day cultures of newborn optic nerve cells (Lillien et al., 1990). NBT-II carcinoma cells transdifferentiate to a mesenchymal phenotype in response to aFGF (Vallés et al., 1990); however, in order to respond, the cells must be cultured on a substratum, such as collagen, upon which they can move (Tucker et al., 1991). TGFβ stimulates tubule formation by endothelial cells embedded in collagen (Madri et al., 1988), while scatter factor/HGF increases the motility of MDCK epithelial cells on tissue culture plastic, but stimulates the formation of tubules by cells embedded in collagen gels (Montesano et al., 1991).

Extracellular matrix receptors

As outlined in the Introduction, the identification of cell-binding sites within extracellular matrix molecules was a key step towards identifying direct, receptor-mediated interactions of ECM components with cells. As well as the RGD sequence motif, other peptide sequences have also been identified as cell-binding sites (reviewed by Humphries, 1990; Hynes, 1992); and it is becoming clear that each ECM glycoprotein contains multiple cell-binding sites. Since many ECM proteins are multimeric, they are also multivalent with respect to individual cell-binding sites.

Non-integrin receptors

Non-integrin receptors form a diverse group of molecules that includes cell surface proteoglycans, CD36, a collagen and thrombospondin-binding glycoprotein (Greenwalt et al., 1992), and certain laminin-binding proteins (Mecham, 1991). The status of the latter as true laminin receptors is in doubt because they lack transmembrane domains. Of the cell surface proteoglycans, syndecan and CD44 have been most thoroughly studied as adhesive receptors.

Syndecan has both chondroitin sulphate and heparan sulphate side chains and, in addition to binding collagens, fibronectin and thrombospondin, binds bFGF (reviewed by Bernfield and Sanderson, 1990). The glycosaminoglycan composition of syndecan varies between tissues and may modulate ligand recognition since syndecan isolated from tooth mesenchyme is unique in binding tenascin and thrombospondin binds only to the heparan sulphate side chains of syndecan. By colocalising growth factor and ECM molecules at the cell surface, syndecan may assemble a signalling complex or may serve as an accessory signalling molecule in combination with other receptors (Salmivarta et al., 1992). A family of syndecan-related proteoglycans has now been identified, which have different ectodomains but identical cytoplasmic domains, suggesting that the cytoplasmic domain may fulfil some conserved signalling function (Bernfield and Sanderson, 1990; Gould et al., 1992). A signalling role for syndecan is also suggested by its expression pattern in developing tissues, where it follows morphogenetic rather than histological boundaries (Bernfield and Sanderson, 1990).

CD44, also known as PgP-1, or Hermes antigen, is a transmembrane glycoprotein which carries N- and O-linked sugars and glycosaminoglycan side chains. Tissue-specific forms of the mature protein exhibit both alternative splicing of the core protein and differences in post-translational modifications (Brown et al., 1991). CD44 binds collagens I and IV and hyaluronic acid and is also implicated in cell-cell adhesion (reviewed by Hardingham and Forsang, 1992). Expression of certain variants has been correlated with unique functions and altered cellular adhesive properties: for example, one of the higher molecular weight epithelial-specific forms does not bind HEV cells (Stamenkovic et al., 1991), while another form confers metastatic potential upon carcinoma cells (Gunthert et al., 1991).

Integrins

Integrins comprise a large family of α/β heterodimeric cell surface glycoproteins (Hynes, 1987, 1992; Hemler, 1990). The family has been classified into subgroups according to the identity of the β subunit, based on the finding that different α subunits in combination with the same β subunit form receptors of different specificity. However, some α subunits partner several β subunits, and these combinations also alter ligand specificity; indeed, ligand cross-linking experiments have indicated that ligand contact sites are found on both α and β subunits. A further level of complexity is provided by the existence of mRNA splice vari-

ants for the cytoplasmic domains of certain α and β subunits. Finally, the ligand specificity of certain integrins appears to be cell-type dependent: the $\alpha_2\beta_1$ integrin acts as a collagen receptor in platelets, but is a collagen and laminin receptor in endothelial cells (reviewed by Hynes, 1992).

Multiple integrins recognise each of the major ECM glycoproteins (eg. Hall et al., 1990) but the functional significance of this apparent redundancy is presently unclear. One simple model is that each site is recognised by a different adhesive receptor, which has different functions. For example, in the case of fibronectin, the EILDV adhesive sequence is recognised by the $\alpha_4\beta_1$ integrin (Wayner et al., 1989; Guan and Hynes, 1990) and the RGD site is recognised by the $\alpha_5\beta_1$ integrin (Ruoslahti and Pierschbacher, 1987). However, the RGD site of fibronectin is also recognised by at least five other integrins, and individual cells can simultaneously express more than one RGD-binding integrin (Humphries, 1990); furthermore, $\alpha_4\beta_1$ recognises two other sequences related to EILDV within fibronectin (Mould and Humphries, 1991). Therefore, a more likely explanation for the multiplicity of receptors is that occupancy of different receptors by the same cell-binding site may convey different information to the cell (see below).

Many developmentally regulated changes in integrin expression have been described in vivo. During development of human epidermis a single layer of keratinocytes gives rise to the mature tissue through a process of stratification which is associated with changes in the types of integrins expressed and their location (Hertle et al., 1991). The surface expression of β_1 integrins changes as myoblasts fuse to form myotubes (Damsky et al., 1985). In the developing kidney, the $\alpha_6\beta_1$ laminin receptor is expressed by epithelia and not by uninduced mesenchyme (Sorokin et al., 1990), and the α_2, α_3 and α_6 integrin subunits all exhibit distinct, localised expression patterns in different segments of the adult nephron (Korhonen et al., 1990). In *Drosophila*, developmentally regulated alternative splicing of the PS2α integrin subunit has been observed (Brown et al., 1989). In *Xenopus* synthesis of β_1 integrin from maternal mRNA is observed throughout the pregastrula phase, but until the late blastula only small amounts are processed to the mature form (Gawantka et al., 1992). Although certain integrins, such as the β_2 subgroup and $\alpha_6\beta_4$, have a limited tissue distribution, most integrins are expressed by a variety of cell types. Thus, where integrin-mediated events regulate differentiation, ligation of common cell surface receptors leads to cell-type-specific responses.

In vitro, multipotent ES and EC cells can be induced to differentiate along multiple lineages by a variety of agents (Watt, 1991) and changes in the types of integrins expressed have been correlated with these events. Thus, retinoic acid induced neural differentiation of P19 cells correlates with induction of $\alpha_v\beta_1$ (Dedhar et al., 1991). Mouse ES cells express $\alpha_6\beta_1$ containing the α_6B splice variant of α_6, but the α_6A form is induced upon differentiation (Cooper et al., 1991). Fibronectin receptor expression is decreased on erythroleukaemia cells induced to undergo terminal differentiation and this correlates with lack of adhesion to fibronectin (Patel and Lodish, 1987). $\alpha_m\beta_2$ expression is altered during myeloid differentiation (Hickstein et al., 1989). In most cases, the functional significance of these changes in integrin expression is not yet clear, but presumably they alter cell adhesive behaviour and transmit different signals from the ECM.

The signalling roles of integrins, suggested by the activities of synthetic peptides corresponding to adhesive sequences (see above), have been substantiated by experiments in which monoclonal antibodies to integrins are used to manipulate differentiation. Such experiments have now been carried out in a variety of systems and several conclusions have emerged. First, adhesion-perturbing antibodies may either prevent differentiative events (Menko and Boettiger, 1987; Dedhar, 1989; Sorokin et al., 1990; Streuli et al., 1991) or may mimic the effects of normal ligand/integrin binding (Adams and Watt, 1989). Secondly, integrin specificity can be demonstrated. For example, osteocytic differentiation of MG-63 cells in response to IL-1 can be prevented by antibodies to the fibronectin receptor (β_1 subgroup) but not by antibodies to the vitronectin receptor (α_v subgroup; Dedhar, 1989).

Differentiation is not only associated with changes in integrin expression, but also with down-regulation of receptor function, probably involving changes in receptor conformation (reviewed by Hynes, 1992). Thus, E7 and E11 chick retinal neurons express equivalent numbers of laminin-binding sites, yet only E7 cells adhere to laminin. Adhesiveness of E11 cells can, however, be restored in the presence of an antibody to the β_1 subunit, which presumably switches the laminin-binding integrins back into an active conformation (Neugebauer and Reichardt, 1991). When epidermal keratinocytes become committed to undergo terminal differentiation, the ability of the cells to adhere to matrix proteins is rapidly lost; this does not correlate with loss of β_1 integrins from the cell surface (Adams and Watt, 1990) but with modulation of preexisting receptors in the plasma membrane (Hotchin and Watt, 1992).

SIGNAL TRANSDUCTION

In order for cell-ECM interactions to cause changes in differentiated gene expression, ECM receptors must be able to transduce signals to the nucleus. A series of experiments have focussed upon the roles of integrin cytoplasmic domains in signal transduction. With the exception of the β_4 subunit, integrin α and β subunits have short cytoplasmic domains, of up to 53 amino acids. The high interspecies conservation of the β_1 subunit (82% to 90% between vertebrates) suggests that it may have some conserved role in signalling (see below), while the low (20% to 30%) sequence homologies of the different α subunits that form heterodimers with β_1 suggest that they may confer specificity of signal transduction (Hemler, 1990). Although no accessory proteins specific to different heterodimers have yet been identified, there is evidence that occupancy of different heterodimers by the same ligand may result in different functional consequences. For example, the RGD-dependent vitronectin receptors, $\alpha_v\beta_3$ and $\alpha_v\beta_5$, are both expressed by melanoma cells and are both involved in initial attachment to vitronectin, yet subsequently segregate to different cellular locations (Wayner et al., 1991). In cells

adherent upon fibronectin, which coexpress $\alpha_5\beta_1$ and $\alpha_3\beta_1$, only $\alpha_5\beta_1$ localises to focal contacts (Elices et al., 1991).

Direct evidence for the importance of α cytoplasmic domains in signal transduction has come from molecular genetic experiments. In the case of the platelet integrin IIb/IIIa (αIIb/β_3), ligand-binding activity normally requires platelet activation, but is stimulated in vitro by various monoclonal antibodies to IIb/IIIa. Deletion of the cytoplasmic domain of the β_3 subunit does not impair normal function but deletion of the αIIb cytoplasmic domain or substitution with the α_5 cytoplasmic domain causes constitutive activation of the receptor (O'Toole et al., 1991). Similarly, chimeras made between the α_2 subunit and the cytoplasmic domains of other α subunits show collagen-binding activity equivalent to the wild-type $\alpha_2\beta_1$ integrin, but have differing abilities to contract collagen gels (Chan et al., 1992). These data suggest that α subunit sequences are involved in the transmission of distinct intracellular signals, and also indicate that feedback from these events can affect the function of integrin extracellular domains. Current evidence suggests that the signalling pathways downstream of integrins involve both the cytoskeleton and the second messenger pathways that, classically, are associated with growth factor receptors.

Involvement of the cytoskeleton

ECM glycoproteins, integrins and cell surface proteoglycans such as syndecan colocalise with cytoskeletal proteins in the focal adhesions that form at the ends of actin microfilament bundles in adherent stationary cells (reviewed by Burridge et al., 1988). On individual ECM glycoprotein substrata, the appropriate integrin colocalises with its ligand, while other integrins remain diffusely distributed. These observations suggest a physical association between integrins and the actin cytoskeleton.

Biochemical and molecular biological studies have shown that the β_1 cytoplasmic domain is of particular functional significance for cytoskeletal association. Thus, transfected β_1 molecules that lack the cytoplasmic domain associate with endogenous α subunits, are sorted to the cell surface and bind fibronectin, but do not localise to focal contacts (Solowska et al., 1989; Hayashi et al., 1990; Marcantonio et al., 1990). The β_1, but not the α_5, cytoplasmic domain contains sufficient information to target a chimeric molecule with the extracellular and transmembrane domains of the IL2 receptor to focal contacts (LaFlamme et al., 1992). Internal deletions and point mutations within the β_1 cytoplasmic domain indicate that three regions contribute to focal contact localisation (Reszka et al., 1992).

Equilibrium gel filtration assays and antibody co-capping experiments have demonstrated that β_1 integrins bind the focal contact component talin (Horwitz et al., 1986; Burn et al., 1988), although the affinity of integrin for talin is low, at least in vitro (Horwitz et al., 1986). In addition, synthetic peptide affinity matrices corresponding to integrin β_1 or β_3 cytoplasmic domains specifically bind α-actinin (Otey et al., 1990). In general, the physical relationships between the different focal contact molecules remain to be clarified; however, vinculin is known to bind to talin and paxillin to vinculin (reviewed by Turner and Burridge, 1991). To date there is very little evidence for heterogeneity in the cytoplasmic components of focal contacts, although keratinocyte focal contacts vary according to whether or not they contain α-actinin (Kubler et al., 1991) and dystrophin is a focal contact component exclusive to myoblasts (Turner and Burridge, 1991).

Although actin polymerisation does not appear to be necessary for stable ligand-integrin binding it is clear that the actin cytoskeleton is involved in secondary events such as cell spreading (Orlando and Cheresh, 1991), and that the association of integrins with the cytoskeleton can be regulated. When activated by TPA, macrophages acquire adhesiveness to laminin; this correlates with increased phosphorylation of the $\alpha_6\beta_1$ integrin and increased linkage of this integrin to the cytoskeleton, as defined by resistance to detergent extraction (Shaw et al., 1990). Similarly, colocalisation of integrins and talin can be stimulated by TPA treatment of peripheral blood lymphocytes (Burn et al., 1988).

The dynamic association of integrin cytoplasmic domains with the actin cytoskeleton and the changes in cell shape that accompany many developmental and differentiative processes (reviewed by Watt, 1986) make it attractive to suggest that the cytoskeleton plays a role in signal transduction. Certainly, experimentally induced changes in cell shape alter the ability of cells to proliferate (Folkman and Moscona, 1978) or differentiate (Watt et al., 1988). The idea that the state of assembly of the cytoskeleton is important has been put forward by a number of workers and is central to the concepts of 'tensegrity' (Ingber and Folkman, 1989; Ingber, 1991) and 'dynamic reciprocity' (Bissell et al., 1982; Bissell and Barcellos-Hoff, 1987). Tensegrity stresses the importance of mechanical forces in regulating cell behaviour, with the ECM as the site at which these forces are transmitted to and from the cell (Ingber, 1991). There is experimental evidence that physical stimuli applied to the cell surface via the ECM can result in changes in the polymerisation and organisation of the cytoskeleton, which, in turn, can alter the distribution and function of plasma membrane proteins, including cell surface receptors. Many elements of the metabolic machinery of the cell, such as polyribosomes and mitochondria, are associated with the cytoskeleton and changes in their position or organisation could result in changes in function (reviewed by Singer, 1992). In addition, mechanical forces could be transmitted directly to the nucleus from the ECM since intermediate filaments physically link the plasma membrane to the nuclear envelope and thus external physical forces could result in changes in nuclear size and DNA packaging. The importance of the cytoskeleton in mechanical signal transduction is also central to the dynamic reciprocity concept, which emphasises a reciprocity between the ECM and the nucleus: the ECM influences gene expression and changes in expression of genes encoding matrix proteins in turn alter the composition of the ECM (Bissell et al., 1982).

Second messengers

Although many developmental and differentiative processes involve changes in cell shape, integrin-mediated changes in gene expression can occur in the absence of changes in cell morphology or overt reorganisation of actin microfilaments (Werb et al., 1989). Inhibition of spreading in adherent ker-

atinocytes acts as a trigger for terminal differentiation (Watt et al., 1988), but it is not cell shape per se that regulates the initiation of terminal differentiation, but the occupancy of functional β_1 integrins by ligand (Adams and Watt, 1989; Fig. 1). Terminal differentiation of keratinocytes in suspension can be inhibited by antibodies to β_1 integrins in the absence of receptor clustering or polymerisation of microfilaments and microtubules (Adams and Watt, 1989; Adams, Kubler and Watt, unpublished observations). These observations suggest that signal transduction pathways distal to ECM receptors cannot be dependent solely on the state of assembly of the cytoskeleton. Indeed, there is increasing evidence for overlap in the second messenger pathways downstream of ECM receptors and growth factor receptors.

Changes in the intracellular milieu that are observed both in response to growth factor-receptor interactions and in response to integrin occupancy include cytoplasmic alkalinisation, due to activation of the sodium/hydrogen antiporter (Ingber et al., 1990; Schwartz et al., 1991a,b), alterations in cAMP levels (Nathan and Sanchez, 1990), increases or decreases in the intracellular concentration of calcium ions (Jaconi et al., 1991; Ng-Sikorski et al., 1991; Miyauchi et al., 1991) and alterations in the phosphorylation state of intracellular proteins (Kornberg et al., 1991; Guan et al., 1991; Shattil and Brugge, 1991). Conversely, growth factors such as PDGF cause membrane ruffling and reorganization of the actin cytoskeleton (Hammacher et al., 1989).

Serine/threonine phosphorylation events involve both ECM receptors and various intracellular proteins and may regulate cytoskeletal organisation. Activation of β_2 and β_3 integrins has been correlated with phosphorylation of their cytoplasmic domains (Buyon et al., 1990; Valmu et al., 1991). CD44 contains phosphoserine (Isacke et al., 1986), but it is not clear if this modification is regulated or functionally significant. In intact cells, activation of protein kinase C or cAMP-dependent kinases often correlates with changes in actin microfilament organisation (Jaken et al., 1989; Turner et al., 1989). Various focal contact components are substrates in vitro for these kinases (Burridge et al., 1988): MARCKS (for myristoylated, alanine-rich C kinase substrate), is a calmodulin- and actin-binding protein which, upon phosphorylation, relocates to the cytosol (Hartwig et al., 1992; Aderem, 1992) and VASP is a substrate for cAMP- and cGMP-dependent protein kinases (Reinhard et al., 1992).

In the case of growth factor receptors, tyrosine phosphorylation results either from the intrinsic tyrosine kinase activity of the receptors, or from the association of activated, non-kinase receptors with intracellular tyrosine kinases such as c-src (reviewed by Cantley et al., 1991). The cytoplasmic domains of the integrin β_1 and β_2 subunits contain a tyrosine residue within a consensus phosphorylation site; however, although constitutive phosphorylation of the β_1 subunit in Rous sarcoma virus transformed cells correlates with reduced integrin/fibronectin/talin binding in vitro (Tapley et al., 1989), β_1 function does not appear to depend on tyrosine phosphorylation in nontransformed cells (Solowska et al., 1989; Hayashi et al., 1990; Guan et al., 1991; Kornberg et al., 1991). The 34 amino acid cytoplasmic domain of syndecan contains three tyrosine residues; it is not known if these are phosphorylated in a regulated manner (Bernfield and Sanderson, 1990).

There is better evidence that other cytoskeleton-linked components are regulated by tyrosine phosphorylation. Phosphotyrosine-containing proteins including v-src and other nonreceptor tyrosine kinases are concentrated in focal contacts (Maher et al., 1985) and cell spreading on fibronectin, or antibody-mediated integrin clustering, correlates with tyrosine phosphorylation of several focal contact proteins including FAK (Focal Adhesion Kinase), a tyrosine kinase that is a substrate of the v-src kinase (Guan et al., 1991; Guan and Shalloway, 1992; Shattil and Brugge, 1991; Schaller et al., 1992; reviewed by Burridge et al., 1992). A myristoylated tyrosine kinase substrate protein enhances $\alpha_2\beta_1$ integrin-mediated cell adhesion to collagen (Pullman and Bodmer, 1992). There is as yet no evidence that matrix components activate tyrosine phosphatases; however, it is intriguing that some transmembrane tyrosine phosphatase molecules contain fibronectin type III repeats in their extracellular domains and thus may have the potential to act as adhesive receptors (Tonks, 1991).

A variety of intracellular proteins including the focal contact component, tensin, contain an amino acid sequence domain termed SH2, which is homologous to a non-kinase domain of the src family of tyrosine kinases (reviewed by Koch et al., 1991). Biochemical and molecular genetic evidence indicates that SH2 domains interact with phosphotyrosine residues on proteins, and deletion of the SH2 domain from v-src prevents its association with the cytoskeleton (Fukui et al., 1991). Thus, tyrosine phosphorylation of cytoskeletal proteins may facilitate their interaction with tyrosine kinases or other components of signal transduction pathways, and so cause the transient formation of protein complexes near the plasma membrane. SH2 domains from different proteins appear to be capable of distinguishing phosphotyrosine in different sequence contexts, offering potential for specificity of signalling. Another protein sequence domain common to the src family of kinases, termed SH3, is also found in other cytoplasmic proteins, including various cytoskeletal proteins (Koch et al., 1991; Musacchio et al., 1992). The function of the SH3 domain is unclear, but it may mediate protein-protein interactions with cytoskeletal components, and so may also be involved in the formation of multiprotein complexes.

There are additional lines of evidence that cytoskeletal proteins and components of the growth-factor-activated signal transduction pathways can associate, and it appears that these interactions have feedback consequences for both pathways. Thus, ligation of CD36, the platelet collagen and thrombospondin receptor, causes it to become associated with src family kinases (Huang et al., 1991). Actin-binding proteins such as profilin and gelsolin bind to phosphatidylinositol bis phosphate (PIP2; reviewed by Hartwig and Kwiatkowski, 1991); this decreases the ability of profilin to bind actin monomers or cap actin filaments, whilst PIP2 bound to profilin is hydrolysed less well by phospholipase C (Stossel, 1989; Goldschmidt-Clermont et al., 1991). Genetic evidence from yeast indicates that profilin may also interact with adenylate cyclase (Vojtek et al., 1991). Diacylglycerols, which are generated by hydrolysis

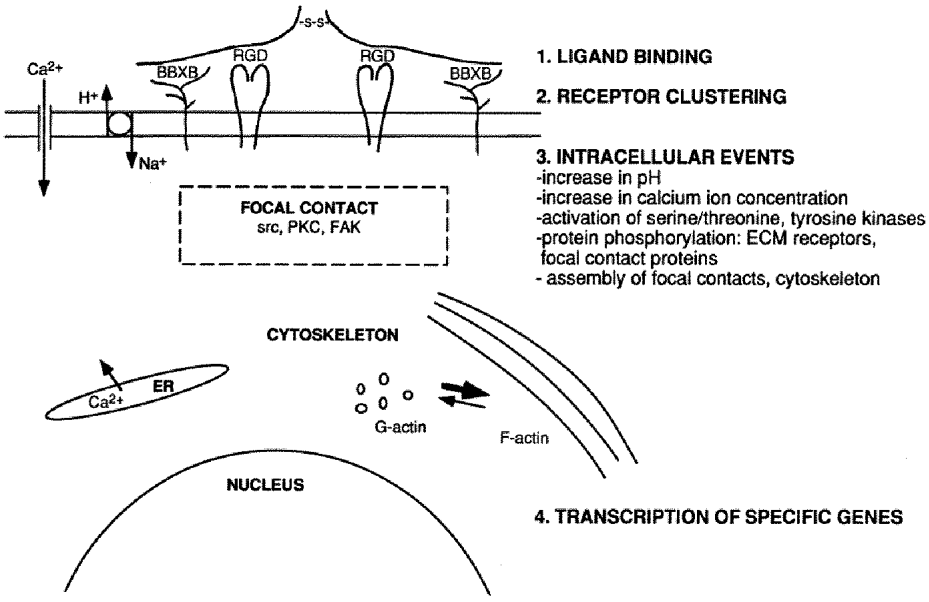

Fig. 2. Signal transduction pathways between the extracellular matrix and the nucleus. Integrins are shown binding to the RGD site of fibronectin and syndecan is shown binding to the BBXB glycosaminoglycan-binding site (where B is a basic amino acid and X is any amino acid; see Ruoslahti,1989).

of phospholipids catalysed by phospholipase C, stimulate actin polymerisation (Shariff and Luna, 1992). Guanine-nucleotide-binding proteins are also involved in the regulation of cell adhesion and cytoskeletal organisation (Symons and Mitchison, 1992): specifically, rho regulates stress fibre formation and focal contact organisation (Paterson et al., 1990; Ridley and Hall, 1992) and rac regulates membrane ruffling (Ridley et al., 1992). Other signal transducing molecules, for example, c-mos, a tyrosine kinase, associate with microtubules (Zhou et al., 1991). Thus the extracellular reciprocity between matrix components and growth factors is mirrored by linked intracellular signalling pathways, which also cross-regulate each other.

There is some evidence that second messenger-mediated events are involved in differentiative processes. For example, differentiation of F9 cells correlates with changes in phosphorylation of the β_1 integrin subunit (Dahl and Grabel, 1989) and myoblast terminal differentiation can be prevented by inhibitors of G proteins (Kelvin et al., 1989). However, this type of experiment cannot distinguish between events normally triggered by growth factors or by ECM proteins. Again, simple systems for examining biochemical differentiation may be of value for investigating signal transduction pathways in more detail.

Fig. 2 summarises some of the components that are implicated in the transduction of signals between the extracellular matrix and the nucleus. Although integrin clustering appears to be an important event in signal transduction in a variety of cells (reviewed by Kornberg and Juliano, 1992), it is not necessarily a requirement in all situations, as the ability of Fab fragments of anti-β_1 integrin antiserum to inhibit keratinocyte terminal differentiation illustrates (Adams and Watt, 1989). A final point to note is that signalling via integrins is bidirectional (reviewed by Hynes, 1992): in keratinocytes, for example, commitment to terminal differentiation results in functional downregulation of $\alpha_5\beta_1$ (inside-out signalling; Adams and Watt, 1990), but one stimulus for terminal differentiation is lack of occupancy of $\alpha_5\beta_1$ by fibronectin (outside-in signalling; Adams and Watt, 1989).

'ECM-response elements'

Whatever the signal transduction mechanisms involved, it is clear that cell-ECM interactions can regulate gene expression at the transcriptional level. Matrix-stimulated transcription of differentiation-specific genes has been demonstrated, for example, in hepatocytes and mammary epithelial cells (Caron, 1990; Schmidhauser et al., 1990, 1992; DiPersio et al., 1991; Liu et al., 1991). The goal now is to define the DNA regulatory sequences that are required for the matrix response and to identify the transcription factors that bind to them. An intriguing question is whether there are ECM-response elements, analogous to the serum-response element that is common to many immediate-early genes activated by mitogens (Treisman, 1990).

Two genes that are activated in response to ECM are serum albumin in hepatocytes and β-casein in mammary epithelial cells. Binding of a number of transcription factors to the serum albumin enhancer is induced under conditions that promote hepatocyte differentiation and two transcription factors are specifically regulated in response to cultivation on a collagen gel in serum-free medium (Liu et al., 1991; DiPersio et al., 1991). A 160 base pair enhancer upstream of the β casein gene contains responsive elements for positive regulation by prolactin and extracellular matrix; although the ECM response has not been separated from the prolactin response in terms of sequence requirements, ECM and prolactin can stimulate enhancer function independently (Schmidhauser et al., 1990, 1992).

Transcriptional activation of the same gene by different stimuli need not, however, involve different DNA response elements. The mitogenic response of cells to growth factors includes a rapid and transient increase in c-fos and c-jun expression and binding of fos-jun heterodimers to a DNA sequence known as AP1 (Halazonetis et al., 1988; Nakabeppu et al., 1988). The same events occur when PC12

cells are stimulated to proliferate by binding to laminin or a peptide corresponding to a carboxy terminal portion of the laminin A chain (Kubota et al., 1992). Clearly, the matrix-responsiveness of many more genes must be examined before any general conclusions can be drawn about the existence, or otherwise, of ECM-response elements.

CONCLUSION

Research on cell-matrix interactions has entered an exciting phase. Many ECM molecules and their receptors have now been identified and this has revealed levels of complexity that had not been anticipated, resulting from the diversity of ECM components and the multiplicity of their receptors. The ECM is an important part of the cellular microenvironment and, in collaboration with growth factors, plays a central role in regulating differentiation and development. One of the major challenges now is to unravel the signal transduction pathways by which ECM ligand-receptor binding causes transcriptional activation of specific sets of genes in different cell types.

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REFERENCES

Adams, J. C. and Watt, F. M. (1989). Fibronectin inhibits the terminal differentiation of human keratinocytes. *Nature* **340**, 307-309.

Adams, J. C. and Watt, F. M. (1990). Changes in keratinocyte adhesion during terminal differentiation: reduction in fibronectin binding precedes $\alpha_5\beta_1$ integrin loss from the cell surface. *Cell* **63**, 425-435.

Adelson, D. L. and Humphreys, T. (1988). Sea urchin morphogenesis and cell-hyalin adhesion are pertubed by a monoclonal antibody specific for hyalin. *Development* **104**, 391-402.

Aderem, A. (1992). The MARCKS brothers: a family of protein kinase C substrates. *Cell* **71**, 713-716.

Andres, J. L., Stanley, K., Cheifetz, S. and Massagué, J. (1989). Membrane-anchored and soluble forms of betaglycan, a polymorphic proteoglycan that binds transforming growth factor-β. *J. Cell Biol.* **109**, 3137-3145.

Anderson, K. V., Jürgens, G. and Nüsslein-Volhard, C. (1985a). Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the *Toll* gene product. *Cell* **42**, 779-789.

Anderson, K. V., Bokla, L. and Nüsslein-Volhard, C. (1985b). Establishment of dorsal-ventral polarity in the Drosophila embryo: the induction of polarity by the *Toll* gene product. *Cell* **42**, 791-798.

Argraves, W. S., Tran, H., Burgess, W. H. and Dickerson, K. (1990). Fibulin is an extracellular matrix and plasma glycoprotein with repeated domain structure. *J. Cell Biol.* **111**, 3155-3164.

Armant, D. R., Kaplan, H. A. and Lennarz, W. J. (1986a). Fibronectin and laminin promote in vitro attachment and outgrowth of mouse blastocysts. *Dev. Biol.* **116**, 519-523.

Armant, D. R., Kaplan, H. A., Mover, H. and Lennarz, W. J. (1986b). The effect of hexapeptides on attachment and outgrowth of mouse blastocysts cultured in vitro: evidence for the involvement of the cell-recognition tripeptide, Arg-Gly-Asp. *Proc. Natl. Acad. Sci. USA* **83**, 6751-6755.

Baker, N. E., Mlodzik, M. and Rubin, G. M. (1990). Spacing differentiation in the developing *Drosophila* eye: a fibrinogen-related lateral inhibitor encoded by *scabrous*. *Science* **250**, 1370-1377.

Barker, D. F., Hostikka, S. L., Zhou, J., Chow, L. T., Oliphant, A. R., Gerken, S. C., Gregory, M. C., Skolnick, M. H., Atkin, C. L. and Tryggvason, K. (1990). Identification of mutations in the COL4A5 collagen gene in Alport syndrome. *Science* **248**, 1224-1227.

Barry, E.L.R. and Mosher, D.F. (1988). Factor XIII cross-linking of fibronectin at cellular matrix assembly sites. *J. Biol. Chem.* **263**, 10464-10469.

Bayliss, M.T., Venn, M. and Ali, S.Y. (1983). Structure of proteoglycans from different layers of human articular cartilage. *Biochem. J.* **209**, 387-400.

Berman, P., Gray, P., Chen, E., Keyser, K., Ehrlich, D., Karten, H., LaCorbiere, M., Esch, F. and Schubert, D. (1987). Sequence analysis, cellular localisation, and expression of a neuroretina adhesion and cell survival molecule. *Cell* **51**, 135-142.

Bernfield, M. and Sanderson, R. D. (1990). Syndecan, a developmentally regulated cell surface proteoglycan that binds extracellular matrix and growth factors. *Phil. Trans. R. Soc. Lond.* ***Series B,*** **327**, 171-186.

Bissell, M.J. and Barcellos-Hoff, M.H. (1987). The influence of extracellular matrix on gene expression: is structure the message? *J. Cell Sci* **8 Supplement,** 327-343.

Bissell, M. J., Hall, G. and Parry, G. (1982). How does the extracellular matrix direct gene expression? *J. Theor. Biol.* **99**, 31-68.

Blatti, S. P., Foster, D. N., Ranganathan, G., Moses, H. L. and Getz, M. J. (1988) Induction of fibronectin gene transcription and mRNA is a primary response to growth-factor stimulation of AKR-2B cells. *Proc. Natl. Acad. Sci. USA* **85**, 1119-1123.

Blau, H. M. and Baltimore, D. (1991). Differentiation requires continuous regulation. *J. Cell Biol.* **112**, 781-783.

Bornstein, P., O'Rourke, K., Wikstrom, K., Wolf, F. W., Katz, R., Li, P. and Dixit, V. M. (1991). A second, expressed thrombospondin gene (Thb2) exists in the mouse genome. *J. Biol. Chem.* **266**, 12821-12824.

Boucaut, J. C., Darribère, T., Boulekbache, H. and Thiery, J. P. (1984a). Prevention of gastrulation but not neurulation by antibodies to fibronectin in amphibian embryos. *Nature* **307**, 364-367.

Boucaut, J. C., Darribère, T., Poole, T. J., Aoyama, H., Yamada, K.M. and Thiery, J.P. (1984b). Biologically active synthetic peptides as probes of embryonic development: a competitive peptide inhibitor of fibronectin function inhibits gastrulation in amphibian embryos and neural crest cell migration in avian embryos. *J. Cell Biol.* **99**, 1822-1830.

Bronner-Fraser, M. (1985). Alterations in neural crest migration by a monoclonal antibody that affects cell adhesion. *J. Cell Biol.* **101**, 610-617.

Brower, D. L. and Jaffe, S. M. (1990). Requirement for integrins during *Drosophila* wing development. *Nature* **342**, 285-287.

Brown, N. H., King, D. L., Wilcox, M. and Kafatos, F. C. (1989). Developmentally regulated alternative splicing of Drosophila integrin PS2 α transcripts. *Cell* **59**, 185-195.

Brown, T.A., Bouchard, T., St John, T., Wayner, E. and Carter, W.G. (1991). Human keratinocytes express a new CD44 core protein (CD44E) as a heparan-sulphate intrinsic membrane proteoglycan with additional exons. *J. Cell Biol.* **113,** 207-221.

Burn, P., Kupfer, A. and Singer, S. J. (1988). Dynamic membrane-cytoskeletal interactions: specific association of integrin and talin arises *in vivo* after phorbol ester treatment of peripheral blood lymphocytes. *Proc. Natl. Acad. Sci. USA* **85**, 497-501.

Burridge, K., Fath, K., Kelly, T., Nuckolls, G. and Turner, C. (1988). Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. *Ann. Rev. Cell Biol.* **4**, 487-525.

Burridge, K., Petch, L.A. and Romer, L.H. (1992). Signals from focal adhesions. *Current Biol.* **2,** 537-539.

Buyon, J. P., Slade, S. G., Reibman, J., Abramson, S. B., Philips, M. R., Weissmann, G. and Winchester, R. (1990). Constitutive and induced phosphorylation of the α- and β-chains of the CD11/CD18 leukocyte integrin family. Relationship to adhesion-dependent functions. *J. Immunol.* **144**, 191-197.

Byers, P. H. (1990). Brittle bones - fragile molecules: disorders of collagen gene structure and expression. *Trends in Genetics* **6**, 293-300.

Calof, A. L. and Lander, A. D. (1991). Relationship between neuronal migration and cell-substratum adhesion: laminin and merosin promote olfactory neuronal migration but are anti-adhesive. *J. Cell Biol.* **115**, 779-794.

Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R. and Soltoff, S. (1991). Oncogenes and signal transduction. *Cell* **64**, 281-302.

Cardin, A.D. and Weintraub, H. J. R. (1989). Molecular modeling of protein-glycosaminoglycan interactions. *Atherosclerosis* **9**, 21-32.

Caron, J. M. (1990). Induction of albumin gene transcription in hepatocytes by extracellular matrix proteins. *Molec. Cell. Biol.* **10**, 1239-1243.

Carter, W. G., Ryan, M. C. and Gahr, P. J. (1991). Epiligrin, a new cell adhesion ligand for integrin α3β1 in epithelial basement membranes. *Cell* **65**, 599-610.

Chan, B. M. C., Kassner, P. D., Schiro, J. A., Byers, H. R., Kupper, T. S. and Hemler, M. E. (1992). Distinct cellular functions mediated by different VLA integrin α subunit cytoplasmic domains. *Cell* **68**, 1051-1060.

Chiquet, M. (1989). Tenascin/JI/cytotactin: the potential function of hexabrachion proteins in neural development. *Dev. Neurosci.* **11**, 266-275.

Chiquet-Ehrismann, R., Mackie, E. J., Pearson, C. A. and Sakakura, T. (1986). Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. *Cell* **47**, 131-139.

Chiquet-Ehrismann, R., Matsuoka, Y., Hofer, U., Spring, J., Bernasconi, C. and Chiquet, M. (1991). Tenascin variants: differential binding to fibronectin and distinct distribution in cell cultures and tissues. *Cell Regulation* **2**, 927-938.

Cole, G. J. and McCabe, C. F. (1991). Identification of a developmentally regulated keratan sulphate proteoglycan that inhibits cell adhesion and neurite outgrowth. *Neuron.* **7**, 1007-1018.

Cooper, H. M., Tamura, R. N. and Quaranta, V. (1991). The major laminin receptor of mouse embryonic stem cells is a novel isoform of the $\alpha_6\beta_1$ integrin. *J. Cell Biol.* **115**, 843-850.

Dahl, S. C. and Grabel, L. B. (1989). Integrin phosphorylation is modulated during the differentiation of F-9 teratocarcinoma stem cells. *J. Cell Biol.* **108**, 183-190.

Damsky, C. H., Knudsen, K. A., Bradley, D., Buck, C. A. and Horwitz, A. F. (1985). Distribution of the cell substratum attachment (CSAT) antigen on myogenic and fibroblastic cells in culture. *J. Cell Biol.* **100**, 1528-1539.

Darribère, T., Yamada, K. M., Johnson, K. E. and Boucault, J. C. (1988). The 140kDa fibronectin receptor complex is required for mesodermal cell adhesion during gastrulation in the amphibian *Pleurodeles waltlii*.*Dev. Biol.* **126**, 182-194.

Dean III, J.W., Chanrasekaran, S. and Tanzer, M.L. (1990). A biological role of the carbohydrate moieties of laminin. *J.Biol. Chem.* **265**, 12553-12562.

Dedhar, S. (1989). Signal transduction via the β_1 integrins is a required intermediate in interleukin-1β induction of alkaline phosphatase activity in human osteosarcoma cells. *Exp. Cell Res.* **183**, 207-214.

Dedhar, S., Robertson, K. and Gray, V. (1991). Induction of expression of the $\alpha_v\beta_1$ and $\alpha_v\beta_3$ integrin heterodimers during retinoic acid-induced neuronal differentiation of murine embryonal carcinoma cells. *J. Biol. Chem.* **266**, 21846-21852.

Dietz, H. C., Cutting, G. R., Pyeritz, R. E., Maslen, C. L., Sakai, L. Y., Corson, G. M., Puffenberger, E. G., Hamosh, A., Nanthakumar, E. J., Curristin, S. M., Stetten, G., Meyers, D. A. and Francomano, C. A. (1991). Marfan syndrome caused by a recurrent *de novo* missense mutation in the fibrillin gene. *Nature* **352**, 337-339.

DiPersio, C. M., Jackson, D. A. and Zaret, K.S. (1991). The extracellular matrix coordinately modulates liver transcription factors and hepatocyte morphology. *Mol. Cell Biol.* **11**, 4405-4414.

Doliana, R., Bonaldo, P. and Colombatti, A. (1990). Multiple forms of chicken α3(VI) collagen chain generated by alternative splicing in type A repeated domains. *J. Cell Biol.* **111**, 2197-2205.

Dufour, S., Duband, J-L., Kornblihtt, A. R. and Thiery, J. P. (1988). The role of fibronectins in embryonic cell migrations. *Trends in Genetics* **4**, 198-203.

Edwards, D. R., Murphy, G., Reynolds, T. J., Whitman, S. E., Docherty, A. T. P., Angel, P. and Heath, J.K. (1987). Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. *EMBO J.* **6**, 1899-1904.

Ehrig, K., Leivo, I., Argraves, W. S., Ruoslahti, E. and Engvall, E. (1990). Merosin, a tissue-specific basement membrane protein, is a laminin-like protein. *Proc. Natl. Acad. Sci. USA* **87**, 3264-3268.

Ekblom, M., Klein, G., Mugrauer, G., Fecker, L., Deutzmann, R., Timpl, R. and Ekblom, P. (1990). Transient and locally restricted expression of laminin A chain mRNA by developing epithelial cells during kidney organogenesis. *Cell* **60**, 337-346.

Elenius, K., Määttä, A., Salmivirta, M. and Jalkanen, M. (1992). Growth factors induce 3T3 cells to express b-FGF-binding syndecan. *J. Biol. Chem.* **267**, 6435-6441.

Elices, M. J,. Urry, L. A. and Hemler, M. E. (1991). Receptor functions for the integrin VLA-3: fibronectin, collagen and laminin-binding are differentially influenced by Arg-Gly-Asp peptide and by divalent cations. *J. Cell Biol.* **112**, 169-181.

Engel, J. (1989). EGF-like domains in extracellular matrix proteins: localised signals for growth and differentiation. *FEBS Letts.* **251**, 1-7.

Fava, R. A. and McClure, D. B. (1987). Fibronectin associated transforming growth factor. *J. Cell Physiol.* **131**, 184-189.

Ferns, M., Hoch, W., Campanelli, J. T., Rupp, F., Hall, Z. W. and Scheller, R. H. (1992). RNA splicing regulates agrin-mediated acetylcholine receptor clustering activity on cultured myotubes. *Neuron* **8**, 1079-1086.

ffrench-Constant, C. and Hynes, R.O. (1989). Alternative splicing of fibronectin is temporally and spatially regulated in the chicken embryo. *Development* **106**, 375-388.

Folkman, J. and Moscona, A. (1978). Role of cell shape in growth control. *Nature* **273**, 345-349.

Fukui, Y., O'Brien, M. C. and Hanafusa, H. (1991). Deletions in the SH2 domain of $p60^{v\text{-}src}$ prevent association with the detergent-insoluble cellular matrix. *Molecular and Cellular Biology* **11**, 1207-1213.

Garofalo, S., Vuorio, E., Metsaranta, M., Rosati, R., Toman, D., Vaughan, J., Lozano, G., Mayne, R., Ellard, J., Horton, W. and de Crombrugghe, B. (1991). Reduced amounts of cartilage collagen fibrils and growth plate anomalies in transgenic mice harboring a glycine-to-cysteine mutation in the mouse type II procollagen α_1-chain gene. *Proc. Natl. Acad. Sci. USA* **88**, 9648-9652.

Gawantka, V., Ellinger-Ziegelbauer, H. and Hausen, P. (1992). β_1-integrin is a maternal protein that is inserted into all newly formed plasma membranes during early *Xenopus* embryogenesis. *Development* **115**, 595-605.

Giancotti, F. G. and Ruoslahti, E. (1990). Elevated levels of the $\alpha_5\beta_1$ fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. *Cell* **60**, 849-859.

Goldschmidt-Clermont, P. J., Kim, J. W., Machesky, L. M., Rhee, S. G. and Pollard, T. D. (1991). Regulation of phospholipase C-γ1 by profilin and tyrosine phosphorylation. *Science* **251**, 1231-1233.

Gordon, M. Y. (1988). Extracellular matrix of the marrow microenvironment. *Br. J. Haemat.* **70**, 1-4.

Gould, S. E., Upholt, W. B. and Kosher, R. A. (1992). Syndecan 3: a member of the syndecan family of membrane-intercalated proteoglycans that is expressed in high amounts at the onset of chicken limb cartilage differentiation. *Proc. Natl. Acad. Sci. USA* **89**, 3271-3275.

Grant, D. S., Tashiro, K-I., Segui-Real, B., Yamada, Y., Martin, G. R. and Kleinman, H. K. (1989). Two different laminin domains mediate the differentiation of human endothelial cells into capillary-like structures in vitro. *Cell* **58**, 933-943.

Greenwalt, D. E., Lipsky, R. H., Ockenhouse, C. F., Ikeda, H., Tandon, N. N. and Jamieson, G. A. (1992). Membrane glycoprotein CD36: A review of its roles in adherence, signal transduction and transfusion medicine. *Blood* **80**, 1105-1115.

Grobstein, C. (1954). Tissue interaction in the morphogenesis of mouse embryonic rudiments *in vitro.* In *Aspects of Synthesis and Order in Growth*. (ed. O. Rudnik) pp. 233-256. Princeton: Princeton University Press.

Guan, J-L. and Hynes, R.O. (1990). Lymphoid cells recognise an alternatively spliced segment of fibronectin via the integrin receptor $\alpha_4\beta_1$. *Cell* **60**, 53-61.

Guan, J-L., Trevithick, J. E. and Hynes, R. O. (1991). Fibronectin/integrin interaction induces tyrosine phosphorylation of a 120 kDa protein. *Cell Regulation* **2**, 951-964.

Guan, J.-L. and Shalloway, D. (1992). Regulation of focal adhesion-associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation. *Nature* **358**, 690-692.

Günthert, U., Hofmann, M., Rudy, W., Reber, S., Zöller, M., Haußmann, I., Matzku, S., Wenzel, A., Ponta, H. and Herrlich, P. (1991). A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell* **65**, 13-24.

Guo, X., Johnson, J.J. and Kramer, J.M. (1991). Embryonic lethality caused by mutations in basement membrane collagen of *C. elegans. Nature* **349**, 707-709.

Halazonetis, T.D., Georgopoulos, K., Greenburg, M.E. and Leder, P. (1988). c-Jun dimerizes with itself and with c-fos, forming complexes of different DNA binding affinities. *Cell* **55**, 917-924.

Hall, D. E., Reichardt, L. F., Crowley, E., Holley, B., Moezzi, H.,

Sonnenberg, A. and Damsky, C. H. (1990). The α_1/β_1 and α_6/β_1 integrin heterodimers mediate cell attachment to distinct sites on laminin. *J. Cell Biol.* **110**, 2175-2184.

Hall, H. G., Farson, D. A. and Bissell, M. J. (1982). Lumen formation by epithelial cell lines in response to collagen overlay: a morphogenetic model in culture. *Proc. Natl. Acad. Sci. USA* **79**, 4672-4678.

Hammacher, A., Mellström, K., Heldin, C-H. and Westermark, B. (1989). Isoform-specific induction of actin reorganisation by platelet-derived growth factor suggests that the functionally active receptor is a dimer. *EMBO J.* **8**, 2489-2495.

Hardingham, T. E. and Fosang, A. J. (1992). Proteoglycans: many forms and functions. *FASEB J.* **6**, 861-870.

Hartwig, J. H. and Kwiatkowski, D. J. (1991). Actin binding proteins. *Curr. Opinion in Cell Biol.* **3**, 87-97.

Hartwig, J. H., Thelen, M., Rosen, A., Janmey, P. A., Nairn, A. C. and Aderem, A. (1992). MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. *Nature* **356**, 618-622.

Hashimoto, C., Hudson, K. L. and Anderson, K. V. (1988). The *Toll* gene of *Drosophila*, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. *Cell* **52**, 269-279.

Hay, E. D. (1981). (Ed). *Cell Biology of Extracellular Matrix.* New York: Plenum Press.

Hayashi, Y., Haimovich, B., Reszka, A., Boettiger, D. and Horwitz, A. (1990). Expression and function of chicken integrin β1 subunit and its cytoplasmic domain mutants in mouse NIH 3T3 cells. *J. Cell Biol.* **110**, 175-184.

Heguy, A., Baldari, C. T., Macchia, G., Telford, J. L. and Melli, M. (1992). Amino acids conserved in interleukin-1 receptors (IL-IRs) and the Drosophila protein are essential for IL-1R signal transduction. *J. Biol. Chem.* **267**, 2605-2609.

Heino, J., Ignotz, R. A., Hemler, M. E., Crouse, C. and Massagué, J. (1989). Regulation of cell adhesion receptors by transforming growth factor-β. *J. Biol. Chem.* **264**, 380-387.

Hemler, M. E. (1990). VLA proteins in the integrin family: structures, functions and their role on leukocytes. *Annu. Rev. Immunol.* **8**, 365-400.

Hertle, M.D., Adams, J. C. and Watt, F. M. (1991). Integrin expression during human epidermal development *invivo* and *in vitro*. *Development* **112**, 193-206.

Hickstein, D. D., Back, A. L. and Collins, S. J. (1989). Regulation of expression of the CD11b and CD18 subunits of the neutrophil adherence receptor during human myeloid differentiation. *J. Biol. Chem.* **264**, 21812-21817.

Higashiyama, S., Abraham, J. A., MIller, J., Fiddles, J. C. and Klagsbrun, M. (1991). A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. *Science* **251**, 936-939.

Hildebrand, A., Preissner, K. T., Müller-Berghaus, G. and Teschemacher, H. (1989). A novel β-endorphin binding protein. *J. Biol. Chem.* **264**, 15429-15434.

Hortsch, M. and Goodman, C. S. (1991). Cell and substrate adhesion molecules in *Drosophila*. *Ann. Rev. Cell Biol.* **7**, 505-557.

Horwitz, A., Duggan, E., Buck, C., Beckerle, M. C. and Burridge, K. (1986). Interaction of plasma membrane fibronectin receptor with talin-a transmembrane linkage. *Nature* **320**, 531-533.

Hotchin, N. A. and Watt, F. M. (1992) Transcriptional and post-translational regulation of β_1 integrin expression during keratinocyte terminal differentiation. *J. Biol. Chem.* **267**, 14852-14858.

Huang, M-M., Bolen, J. B., Barnwell, J. W., Shattil, S. J. and Brugge, J. S. (1991). Membrane glycoprotein IV (CD36) is physically associated with Fyn, Lyn and Yes protein tyrosine kinases in human platelets. *Proc. Natl. Acad. Sci. USA* **88**, 7844-7848.

Hudson, B. G., Wieslander, J., Wisdom, B. J. and Noelken, M. E. (1989). Goodpasture syndrome: molecular architecture and function of basement membrane antigen. *Lab. Invest.* **61**, 256-269.

Humphries, M. J. (1990). The molecular basis and specificity of integrin-ligand interactions. *J. Cell Sci.* **97**, 585-592.

Hunter, D. D., Shah, V., Merlie, J. P. and Sanes, J. R. (1989). A laminin-like adhesive protein concentrated in the synaptic cleft of the neuromuscular junction. *Nature* **338**, 229-234.

Hunter, D. D., Murphy, M. D., Olsson, C. V. and Brunken, W. J. (1992). S-laminin expression in adult and developing retinae: a potential cue for photoreceptor morphogenesis. *Neuron* **8**, 399-413.

Hynes, R. O. (1987). Integrins: a family of cell surface receptors. *Cell* **4**, 549-554.

Hynes, R. O. (1992). Integrins: versatility, modulation and signalling in cell adhesion. *Cell* **69**, 11-25.

Ignotz, R. A. and Massagué, J. (1986). Transforming growth factor-β stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J. Biol. Chem.* **261**, 4337-4345.

Ignotz, R. A. and Massagué, J. (1987). Cell adhesion protein receptors as targets for transforming growth factor-β action. *Cell* **51**, 189-197.

Inaguma, Y., Kusakabe, M., Mackie, E. J., Pearson, C. A., Chiquet-Ehrismann, R. and Sakakura, T. (1988). Epithelial induction of stromal tenascin in the mouse mammary gland: from embryogenesis to carcinogenesis. *Developmental Biology* **128**, 245-255.

Ingber, D. (1991). Integrins as mechanochemical transducers. *Curr. Op. Cell Biol.* **3**, 841-848.

Ingber, D.E. and Folkman, J. (1989). Tension and compression as basic determinants of cell form and function: utilization of a cellular tensegrity mechanism. In *Cell Shape: Determinants, Regulation, and Regulatory Role* (ed. W.D. Stein and F. Bronner), pp. 3-31.New York: Academic Press.

Ingber, D. E., Prusty, D., Frangioni, J. V., Cragoe, Jr., E. J., Lechene, C. and Schwartz, M. A. (1990). Control of intracellular pH and growth by fibronectin in capillary endothelial cells. *J. Cell Biol.* **110**, 1803-1811.

Isacke, C. M., Sauvage, C. A., Hyman, R., Lesley, J., Schulte, R. and Trowbridge, I. S. (1986). Identification and characterisation of the human Pgp-1 glycoprotein. *Immunogenetics* **23**, 326-332.

Isha-Michaeli, R., Edlar, A. and Vlodavsky, I. (1990). Heparanase activity expressed by platelets, neutrophils and lymphoma cells releases active fibroblast growth factor from extracellular matrix. *Cell Reg.* **1**, 833-842.

Ishii, N., Wadsworth, W. G., Stern, B. D., Culotti, J. G., Hedgecock, E. M. (1992). UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in C. elegans. *Neuron* **9**, 873-881.

Jaconi, M. E. E., Theler, J. M., Schlegel, W., Appel, R. D., Wright, S. D. and Lew, P. D. (1991). Multiple elevations of cytosolic-free Ca^{2+} in human neutrophils: initiation by adherence receptors of the integrin family. *J. Cell Biol.* **112**, 1249-1257.

Jaken, S., Leach, K. and Klauck, T. (1989). Association of type 3 protein kinase C with focal contacts in rat embryo fibroblasts. *J. Cell Biol.* **109**, 697-704.

Jones, F. S., Hoffman, S., Cunningham, B. A. and Edelman, G. M. (1989). A detailed structural model of cytotactin: protein homologies, alternative mRNA splicing and binding regions. *Proc. Natl. Acad Sci. USA* **86**, 1905-1909.

Jones, G.E., Arumugham, R.G. and Tanzer, M.L. (1986). Fibronectin glycosylation modulates fibroblast adhesion and spreading. *J. Cell Biol.* **103**, 1663-1670.

Keith, F. J. and Gay, N. J. (1990). The *Drosophila* membrane receptor Toll can function to promote cellular adhesion. *EMBO J.* **9**, 4299-4306.

Kelvin, D. J., Simard, G., Tai, H. H., Yamaguchi, T. P. and Connolly, J. A. (1989). Growth factors, signaling pathways, and the regulation of proliferation and differentiation in BC3H1 muscle cells. I. A pertussis toxin-sensitive pathway is involved. *J. Cell Biol.* **108**, 159-167.

Kiefer, P., Peters, G. and Dickson, C. (1991). The *Int-2/Fgf-3* oncogene product is secreted and associates with extracellular matrix: implications for cell transformation. *Molecular and Cellular Biology* **11**, 5929-5936.

Klagsbrun, M. (1990). The affinity of fibroblast grown factors for heparin: FGF-heparan sulphate interactions in cells and extracellular matrix. *Curr. Opin. in Cell Biol.* **2**, 857-863.

Klagsbrun, M. and Baird, A. (1991). A dual receptor system is required for basic fibroblast growth factor activity. *Cell* **67** 229-231.

Klein, G., Langegger, M., Timpl, R. and Ekblom, P. (1988). Role of laminin A chain in the development of epithelial cell polarity. *Cell* **55**, 331-341.

Koch, C. A., Anderson, D., Moran, M. J., Ellis, C. and Pawson, T. (1991). SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. *Science* **252**, 668-674.

Korhonen, M., Ylänne, J., Laitinen, L. and Virtanen, I. (1990). The α_1-α_6 subunits of integrins are characteristically expressed in distinct segments of developing and adult human nephron. *J. Cell Biol.* **111**, 1245-1254.

Kornberg, L. J., Earp, H. S., Turner, C. E., Prockop, C. and Juliano, R. L. (1991). Signal transduction by integrins: increased protein tyrosine phosphorylation caused by clustering of β_1 integrins. *Proc. Natl. Acad. Sci. USA* **88**, 8392-8396.

Kornberg, L. and Juliano, R.L. (1992). Signal transduction from the extracellular matrix: the integrin-tyrosine kinase connection. *TiPS* **13**, 93-95.

Kornblihtt, A. R., Umezawa, K., Vibe-Pedersen, K. and Baralle, F. E. (1985). Primary structure of human fibronectin: differential splicing may generate at least 10 polypeptides from a single gene. *EMBO J.* **4**, 1755-1759.

Kramer, J. M., Johnson, J. J., Edgar, R. S., Basch, C. and Roberts, S. (1988). The *sqt*-1 gene of C. elegans encodes a collagen critical for organismal morphogenesis. *Cell* **55**, 555-565.

Kubler, M.-D., Jordan, P. W., O'Neill, C. H. and Watt, F. M. (1991). Changes in the abundance and distribution of actin and associated proteins during terminal differentiation of human epidermal keratinocytes. *J. Cell Sci.* **100**, 153-165.

Kubota, Y., Kleinman, H. K., Martin, G. R. and Lawley, T.J. (1988). Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures. *J. Cell Biol.* **107**, 1589-1598.

Kubota, S., Tashiro, K. and Yamada, Y. (1992). Signaling site of laminin with mitogenic activity. *J. Biol. Chem.* **267**, 4285-4288.

LaFlamme, S. E., Akiyama, S. K. and Yamada, K. M. (1992). Regulation of fibronectin receptor distribution. *J. Cell. Biol.* **117**, 437-447.

LaRochelle, W. J., May-Siroff, M., Robbins, K. C. and Aaronson, S. A. (1991). A novel mechanism regulating growth factor association with the cell surface: identification of a PDGF retention domain. *Genes Dev.* **5**, 1191-1199.

Laurie, G. W., Horikoshi, S., Killen, P. D., Segui-Real, B. and Yamada, Y. (1989). In situ hybridization reveals temporal and spatial changes in cellular expression of mRNA for a laminin receptor, laminin, and basement membrane (type IV) collagen in the developing kidney. *J. Cell Biol.* **109**, 1351-1362.

Lawler, J. and Hynes, R. O. (1986). The structure of human thrombospondin, an adhesive glycoprotein with multiple calcium-binding sites and homologies with several different proteins. *J. Cell Biol.* **103**, 1635-1648.

Lawler, J., Weinstein, R. and Hynes, R.O. (1988). Cell attachment to thrombospondin: The role of ARG-GLY-ASP, calcium and integrin receptors. *J.Cell Biol.* **107**, 2351-2361.

Lee, E. Y-H. P., Lee, W-H., Kaetzel, C. S., Parry, G. and Bissell, M. J. (1985). Interaction of mouse mammary epithelial cells with collagen substrata: regulation of casein gene expression and secretion. *Proc. Natl. Acad. Sci. USA* **82**, 1419-1423.

Leivo, I. and Engvall, E. (1988). Merosin, a protein specific for basement membrane of Schwann cells, striated muscle, and trophoblasts, is expressed late in nerve and muscle development. *Proc. Natl. Acad. Sci. USA* **85**, 1544-1548.

Leppä, S., Mali, M., Miettinen, H. M. and Jalkanen, M. (1992). Syndecan expression regulates cell morphology and growth of mouse mammary epithelial tumor cells. *Proc. Natl. Acad. Sci. USA* **89**, 932-936.

Leptin, M., Bogaert, T., Lehmann, R. and Wilcox, M. (1989). The function of PS integrins during Drosophila embryogenesis. *Cell* **56**, 401-408.

Leptin, M., Grunewald, B. and Stein, D. (1992). No effect of RDGS peptides. *Nature* **355**, 777.

Li, Y-S., Milner, P. G., Chauhan, A. K., Watson, M. A., Hoffman, R. M., Kodmer, C. M., Milbrandt, J. and Deuel, T.F. (1990). Cloning and expression of a developmentally regulated protein that induces mitogenic and neurite outgrowth activity. *Science* **250**, 1690-1694.

Lillien, L. E., Sendtner, M. and Raff, M. C. (1990). Extracellular matrix-associated molecules collaborate with ciliary neurotrophic factor to induce type-2 astrocyte development. *J. Cell Biol.* **111**, 635-644.

Liu, J-K., DiPersio, C. M. and Zaret, K. S. (1991). Extracellular signals that regulate liver transcription factors during hepatic differentiation in vitro. *Mol. Cell. Biol.* **11**, 773-784.

Long, M.W., Briddell, R., Walter, A.W., Bruno, E. and Hoffman, R. (1992). Human hematopoietic stem cell adherence to cytokines and matrix molecules. *J. Clin. Invest.* **90**, 251-255.

Lu Valle, P., Jacenko, O., Iwamoto, M., Pacifici, M. and Olsen, B. R. (1993). From cartilage to bone - the role of collagenous proteins. In *Molecular Basis of Morphogenesis. 51st Annual Symposium of the Society for Developmental Biology* (ed. M. Bernfield). New York: John Wiley and Sons (in press).

Mackie, E. J., Thesleff, I. and Chiquet-Ehrismann, R. (1987). Tenascin is associated with chondrogenic and osteogenic differentiation *in vivo* and promotes chondrogenesis *in vitro*. *J. Cell Biol.* **105**, 2569-2579.

Madri, J. A., Pratt, B. M. and Tucker, A. M. (1988). Phenotypic modulation of endothelial cells by transforming growth factor-β depends upon the composition and organization of the extracellular matrix. *J. Cell Biol.* **106**, 1375-1381.

Maher, P.A., Pasquale, E. B., Wang, J. Y. J. and Singer, S. J. (1985). Phosphotyrosine-containing proteins are concentrated in focal adhesions and intercellular junctions in normal cells. *Proc. Natl. Acad. Sci. USA* **82**, 6576-6580.

Majack, R. A., Cook, S.C. and Bornstein, P. (1986). Regulation of vascular smooth muscle cell growth by the extracellular matrix: an autocrine role for thrombospondin. *Proc. Natl. Acad. Sci. USA.* **83**, 9050-9054.

Marcantonio, E. E., Guan, J. L., Trevithick, J. E. and Hynes, R. (1990). Mapping of the functional determinants of the integrin β1 cytoplasmic domain by site-directed mutagenesis. *Cell Reg.* **1**, 597-604.

Maslen, C. L., Corson, G. M., Maddox, B. K., Glanville, R. W. and Sakai, L.Y. (1991). Partial sequence of a candidate gene for the Marfan syndrome. *Nature* **352**, 334-337.

Mecham, R. P. (1991). Laminin receptors. *Ann. Rev. Cell Biol.* **7**, 71-91.

Menko, A. S. and Boettiger, D. (1987). Occupation of the extracellular matrix receptor, integrin, is a control point for myogenic differentiation. *Cell* **51**, 51-57.

Metsäranta, M., Garofalo, S., Decker, G., Rintala, M., de Crombrugghe, B. and Vuorio, E. (1992). Chondrodysplasia in transgenic mice harboring a 15-amino acid deletion in the triple helical domain of Proα1(II) collagen chain. *J. Cell Biol.* **118**, 203-212.

Mitani, S. (1989). Retarded gastrulation and altered subsequent development of neural tissues in heparin-injected *Xenopus* embryos. *Dev.* **107**, 423-435.

Miyauchi, A., Alvarez, J., Greenfield, E. M., Teti, A., Grano, M., Colucci, S., Zambonin-Zallone, A., Ross, F. P., Teitelbaum, S. L., Cheresh, D. and Hruska, K. A. (1991). Recognition of osteopontin and related peptides by an $\alpha_v\beta_3$ integrin stimulates immediate cell signals in osteoclasts. *J. Biol. Chem.* **266**, 20369-20374.

Montesano, R., Orci, L. and Vassalli, P. (1983). In vitro rapid organisation of endothelial cells into capillary-like networks is promoted by collagen matrices. *J. Cell Biol.* **97**, 1648-1652.

Montesano, R., Schaller, G. and Orci, L. (1991). Induction of epithelial tubular morphogenesis in vitro by fibroblast-derived soluble factors. *Cell* **66**, 697-711.

Mould, A. P. and Humphries, M. J. (1991). Identification of a novel recognition sequence for the integrin $\alpha_4\beta_1$ in the COOH-terminal heparin-binding domain of fibronectin. *EMBO J.* **10**, 4089-4095.

Murphy-Ullrich, J. E., Schultz-Cherry, S. and Höök, M. (1992). Transforming growth factor-β complexes with thrombospondin. *Mol. Biol. of the Cell* **3**, 181-188.

Musacchio, A., Gibson, T., Lehto, V-P. and Saraste, M. (1992). SH3- an abundant protein domain in search of a function. *FEBS Lett.* **307**, 55-61.

Naidet, C., Semeriva, M., Yamada, K. M. and Thiery, J. P. (1987). Peptides containing the cell-attachment recognition sequence Arg-Gly-Asp prevent gastrulation in *Drosophila* embryos. *Nature* **325**, 348-350.

Nakabeppu, Y., Ryder, K. and Nathans, D. (1988). DNA binding activities of three murine jun proteins: stimulation by fos. *Cell* **55**, 907-915.

Nathan, C. and Sanchez, E. (1990). Tumor necrosis factor and CD11/CD18 (β_2) integrins act synergistically to lower cAMP in human neutrophils. *J. Cell Biol.* **111**, 2171-2181.

Nathan, C. and Sporn, M. (1991). Cytokines in context. *J. Cell Biol.* **113**, 981-986.

Nathan, C., Srimal, S., Farber, C., Sanchez, E., Kabbash, L., Asch, A., Gailit, J. and Wright, S. D. (1989). Cytokine-induced respiratory burst of human neutrophils: dependence on extracellular matrix proteins and CD11/CD18 integrins. *J. Cell Biol.* **109**, 1341-1349.

Newgreen, D. F., Gibbons, I. L., Sauter, J., Wallenfels, B. and Wutz, R. (1982). Ultrastructural and tissue-culture studies on the role of fibronectin, collagen and glycosaminoglycans in the migration of neural crest cells in the fowl embryo. *Cell Tissue Res.* **221**, 521-549.

Neugebauer, K. M. and Reichardt, L. F. (1991). Cell-surface regulation of β_1-integrin activity on developing retinal neurons. *Nature* **350**, 68-71.

Neugebauer, K. M., Emmett, C. J., Venstrom, K. A. and Reichardt, L. F. (1991). Vitronectin and thrombospondin promote retinal neurite

outgrowth: developmental regulation and role of integrins. *Neuron* **6**, 345-358.

Ng-Sikorski, J., Andersson, R., Patarroyo, M. and Andersson, T. (1991). Calcium signaling capacity of the CD11b/CD18 integrin on human neutrophils. *Exp. Cell Res.* **195**, 504-508.

Nörenberg, U., Wille, H., Wolff, J. M., Frank, R. and Rathjen, F. G. (1992). The chicken neural extracellular matrix molecule restrictin: similarity with EGF-, fibronectin type III-, and fibrinogen-like motifs. *Neuron* **8**, 849-863.

Orlando, R. A. and Cheresh, D. A. (1991). Arginine-Glycine-Asparatic acid binding leading to molecular stabilisation between integrin $\alpha_v\beta_3$ and its ligand. *J. Biol. Chem.* **266**, 19543-19550.

O'Shea, K. S., Liu, L-H. J., Kinnunen, L. H. and Dixit, V. M. (1990). Role of the extracellular matrix protein thromobospondin in the early development of the mouse embryo. *J. Cell Biol.* **111**, 2713-2723.

O'Shea, K. S., Liu, L-H. J. and Dixit, V. M. (1991). Thrombospondin and a 140kD fragment promote adhesion and neurite outgrowth from embryonic central and peripheral neurons and from PC12 cells. *Neuron* **7**, 231-237.

O'Toole, T. E., Mandelman, D., Forsyth, J., Shattil, S. J., Plow, E. F. and Ginsberg, M. H. (1991). Modulation of the affinity of integrin $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) by the cytoplasmic domain of α_{IIb}. *Science* **254**, 845-847.

Otey, C. A., Pavalko, F. M. and Burridge, K. (1990). An interaction between α-actinin and the β_1 integrin subunit in vitro. *J. Cell Biol.* **111**, 721-729.

Panayotou, G., End, P., Aumailley, M., Timpl, R. and Englel, J. (1989). Domains of laminin with growth factor-activity. *Cell* **56**, 93-101.

Patel, V. P. and Lodish, H. F. (1987). Fibronectin matrix is required for differentiation of murine erythroleukemia cells intro reticulocytes. *J. Cell Biol.* **105**, 3105-3118.

Paterson, H. F., Self, A. J., Garrett, M. D., Just, I., Aktories, K. and Hall, A. (1990). Microinjection of recombinant $p21^{rho}$ induces rapid changes in cell morphology. *J. Cell Biol.* **111**, 1001-1007.

Paulsson, M. (1988). The role of Ca^{2+} binding in the self-aggregation of laminin -nidogen complexes. *J. Biol Chem.* **263,** 5425-5430.

Périn, J-P., Bonnet, F., Maillet, P. and Jollès, P. (1988). Characterization and N-terminal sequence of human platelet proteoglycan. *Biochem. J.* **255**, 1007-1013.

Pignatelli, M. P. and Bodmer, W. F. (1988). Genetics and biochemistry of collagen binding triggered glandular differentiation in a human colon carcinoma cell line. *Proc. Natl. Acad. Sci. USA* **85**, 5561-5565.

Podleski, T. R., Greenberg, I., Schlessinger, J. and Yamada, K. M. (1979). Fibronectin delays the fusion of L6 myoblasts. *Exp. Cell Res.* **122**, 317-326.

Pollock, J., Baule, V. J., Rich, C. B., Ginsberg, C. D., Curtiss, S. W. and Forster, J. A. (1990). Chicken tropoelastin isoforms. From the gene to the extracellular matrix. *J. Biol. Chem.* **265**, 3697-3702.

Prieto, A. L., Jones, F. S., Cunningham, B. A., Crossin, K. L. and Edelman., G.M. (1990). Localization during development of alternatively spliced forms of cytotactin mRNA by in situ hybridization. *J. Cell. Biol.* **111**, 685-698.

Pullman, W. E. and Bodmer, W. F. (1992). Cloning and characterisation of a gene that regulates cell adhesion. *Nature* **356**, 529-532.

Raines, E. W., Lane, T. F., Iruela-Arispe, M. L., Ross, R. and Sage, E. H. (1992). The extracellular glycoprotein SPARC interacts with platelet-derived growth factor (PDGF)-AB and -BB and inhibits the binding of PDGF to its receptors. *Proc. Natl. Acad. Sci. USA* **89**, 1281-1285.

Raines, E. W. and Ross, R. (1992). Compartmentalization of PDGF on extracellular binding sites dependent on exon-6 encoded sequences. *J. Cell Biol.* **116**, 533-543.

Rapraeger, A. C., Krufka, A. and Olwin, B. B. (1991). Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science* **252**, 1705-1708.

Rathjen, P. D., Toth, S., Willis, A., Heath, J. K. and Smith, A. G. (1990). Differentiation inhibiting activity is produced in matrix-associated and diffusable forms that are generated by alternate promoter usage. *Cell* **62**, 1105-1114.

Ratner, N., Hong, D., Lieberman, M. A., Bunge, R. P. and Glaser, L. (1988). The neuronal cell-surface molecule mitogenic for Schwann cells is a heparin-binding protein. *Proc. Natl. Acad. Sci. USA* **85**, 6992-6996.

Reinhard, M., Halbrügge, M., Scheer, U., Wiegand, C., Jockusch, B. M. and Walter, U. (1992). The 46/50 kDa phosphoprotein VASP purified from human platelets is a novel protein associated with actin filaments and focal contacts. *EMBO J.* **11**, 2063-2070.

Reszka, A. A., Hayashi, Y. and Horwitz, A. F. (1992). Identification of amino acid sequences in the integrin β_1 cytoplasmic domain implicated in cytoskeletal association. *J. Cell Biol.* **117**, 1321-1330.

Richa, J., Damsky, C. H., Bick, C. A., Knowles, B. B. and Solter, D. (1985). Cell surface glycoproteins mediate compaction, trophoblast attachment and endoderm formation during early mouse development. *Dev. Biol.* **108**, 513-521.

Ridley, A. J., Paterson, H.F., Johnston, C. L., Diekmann, D. and Hall, A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* **70**, 401-410.

Ridley, A. J. and Hall, A. (1992). The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* **70**, 389-399.

Roberts, R., Gallagher, J., Spooncer, E., Allen, T. D., Bloomfield, F. and Dexter, T. M. (1988). Heparan sulphate bound growth factors: A mechanism for stromal cell mediated haemopoiesis. *Nature* **332**, 376-378.

Rosen, E. M., Goldberg, I. D., Kacinski, B. M., Buckholz, T. and Vinter, D. W. (1989). Smooth muscle releases an epithelial cell scatter factor which binds to heparin. *In Vitro Cellular and Developmental Biology* **25**, 163-173.

Rothenberg, M.E., Pomerantz, J.L., Owen Jr, W.F., Avraham, S., Soberman, R.J., Austen, K.F. and Stevens, R.L. (1988). Characterization of a human eosinophil proteoglycan, and augmentation of its biosynthesis and size by interleukin 3, interleukin 5, and granulocyte/macrophage colony stimulating factor. *J. Biol. Chem.* **263**, 13901-13908.

Rousselle, P., Lunstrum, G. P., Keene, D. R. and Burgeson, R. E. (1991). Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. *J. Cell Biol.* **114**, 567-576.

Ruegg, M. A., Tsim, K. W. K., Horton, S. E., Kröger, S., Escher, G., Gensch E. M. and McMahan, U.J. (1992). The agrin gene codes for a family of basal lamina proteins that differ in function and distribution. *Neuron* **8**, 691-699.

Ruoslahti, E. (1989). Proteoglycans in cell regulation. *J. Biol. Chem.* **264**, 13369-13372.

Ruoslahti, E. and Pierschbacher, M. D. (1987). New perspectives in cell adhesion: RGD and integrins. *Science* **238**, 491-497.

Ruoslahti, E. and Yamaguchi, Y. (1991). Proteoglycans as modulators of growth factor activities. *Cell* **64**, 867-869.

Saga, Y., Yagi, T., Ikawa, Y., Sakakura, T. and Aizawa, S. (1992). Mice develop normally without tenascin. *Genes Dev.* **6**, 1821-1831.

Sage, E.H. and Bornstein, P. (1991). Extracellular proteins that modulate cell-matrix interactions. *J. Biol. Chem.* **266**, 14831-14834.

Saksela, O. and Rifkin, D. B. (1990). Release of basic fibroblast growth factor-heparan sulphate complexes from endothelial cells by plasminogen activator mediated proteolytic activity. *J. Cell Biol.* **110**, 767-775.

Salmivirta, M., Heino, J. and Jalkanen, M. (1992). Basic fibroblast growth factor - syndecan complex at cell surface or immobilised to matrix promotes growth. *J. Biol. Chem.* **267**, 17606-17610.

Sanes, J. R. (1989). Extracellular matrix molecules that influence neural development. *Ann. Rev. Neurosci.* **12**, 491-516.

Sanes, J. R., Engvall, E., Butkowski, R. and Hunter, D. D. (1990). Molecular heterogeneity of basal laminae: isoforms of laminin and collagen IV at the neuromuscular junction and elsewhere. *J. Cell Biol.* **111**, 1685-1699.

Schaller, M. D., Borgman, C. A., Cobb, B. S., Vines, R. R., Reynolds, A. B. and Parsons, J. T. (1992). $pp125^{FAK}$, a structurally distinctive protein-tyrosine kinase associated with focal adhesions. *Proc. Natl. Acad. Sci. USA* **89**, 5192-5196.

Schmidhauser, C., Bissell, M. J., Myers, C. A. and Casperson, G. F. (1990). Extracellular matrix and hormones transcriptionally regulate bovine β-casein 5′ sequences in stably transfected mouse mammary cells. *Proc. Natl. Acad. Sci. USA* **87**, 9118-9122.

Schmidhauser, C., Casperson, G.F., Myers, C.A., Sanzo, K.T., Bolten, S. and Bissell, M.J. (1992). A novel transcriptional enhancer is involved in the prolactin- and extracellular matrix-dependent regulation of β-casein gene expression. Mol. Biol. *Cell* **3**, 699-709.

Schnieke, A., Harbers, K. and Jaenisch, R. (1983). Embryonic lethal mutation in mice induced by retrovirus insertion into the α1 (I) collagen gene. *Nature* **304**, 315-320.

Schofield, R. (1978). The relationship between the spleen-colony forming cell and the haematopoietic stem cell. *Blood Cells* **4**, 7-25.

Schubert, D. and Kimura, H. (1991). Substratum-growth factor

collaborations are required for the mitogenic activities of activin and FGF on embryonal carcinoma cells. *J. Cell Biol.* **114**, 841-846.

Schwartz, M. A., Ingber, D. E., Lawrence, M., Springer, T. A. and Lechene, C. (1991a). Multiple integrins share the ability to induce elevation of intracellular pH. *Exp. Cell Res.* **195**, 533-535.

Schwartz, M. A., Lechene, C. and Ingber, D. E. (1991b). Insoluble fibronectin activates the Na/H antiporter by clustering and immobilizing integrin α5β1, independent of cell shape. *Proc. Natl. Acad. Sci. USa* **88**, 7849-7853.

Shariff, A. and Luna, E.J. (1992). Diacylglycerol-stimulated formation of actin nucleation sites at plasma membranes. *Science* **256**, 245-247.

Shattil, S. J. and Brugge, J. S. (1991). Protein tyrosine phosphorylation and the adhesive functions of platelets. *Cell Biol.* **3**, 869-879.

Shaw, L. M., Messier, J. M. and Mercurio, A. M. (1990). The activation dependent adhesion of macrophages to laminin involves cytoskeletal anchoring and phosphorylation of the $\alpha_6\beta_1$ integrin. *J. Cell Biol.* **110**, 2167-2174.

Singer, R. H. (1992). The cytoskeleton and mRNA localisation. *Curr. Opin. Cell Biol.* **4**, 15-19.

Solowska, J., Guan, J-L., Marcantonio, E. E., Trevithick, J. E., Buck, C. A. and Hynes, R. O. (1989). Expression of normal and mutant avian integrin subunits in rodent cells. *J. Cell Biol.* **109**, 853-861.

Sorokin, A., Sonnenberg, A., Aumailley, M., Timpl, R. and Ekblom, P. (1990). Recognition of the laminin E8 cell-binding site by an integrin possessing the α_6 subunit is essential for epithelial polarization in developing kidney tubules. *J. Cell Biol.* **111**, 1265-1273.

Spiegelman, B. M. and Ginty, C. A. (1983). Fibronectin modulation of cell shape and lipogenic gene expression in 3T3-adipocytes. *Cell* **35**, 657-666.

Stamenkovic, I., Aruffo, A., Amiot, M. and Seed, B. (1991). The haemopoietic and epithelial foms of CD44 are distinct polypeptides with different adhesion potentials for hyaluronate-bearing cells. *EMBO J.* **10**, 343-348.

Stossel, T. P. (1989). From signal to pseudopod: how cells control cytoplasmic actin assembly. *J. Biol. Chem.* **264**, 18261-18264.

Streuli, C. H. and Bissell, M.J. (1990). Expression of extracellular matrix components is regulated by substratum. *J. Cell Biol.* **110**, 1405-1415.

Streuli, C. H., Bailey, N. and Bissell, M. J. (1991). Control of mammary epithelial differentiation: basement membrane induces tissue-specific gene expression in the absence of cell-cell interaction and morphological polarity. *J. Cell Biol.* **115**, 1385-1395.

Sutherland, A. E., Calarco, P-G. and Damsky, C. H. (1988). Expression and function of cell surface extracellular matrix receptors in mouse blastocyst attachment and outgrowth. *J. Cell Biol.* **106**, 1331-1348.

Symons, M. H. and Mitchison, T. J. (1992). A GTPase controls cell-substrate adhesion in *Xenopus* XTC fibroblasts. *J. Cell Biol.* **118**, 1235-1244.

Tapley, P., Horwitz, A., Buck, C., Duggan, K. and Rohrschneider, L. (1989). Integrins isolated from Rous sarcoma virus-transformed chicken embryo fibroblasts. *Oncogene* **4**, 325-333.

Tonks, N. K. (1991). Plague, pox and tyrosine dephosphorylation. *Current Biology* **1**, 259-261.

Treisman, R. (1990). The SRE: a growth factor responsive transcriptional regulator. *Seminars in Cancer Biol.* **1**, 47-58.

Tucker, G. C., Boyer, B., Vallés, A. M. and Thiery, J. P. (1991). Combined effects of extracellular matrix and growth factors on NBT-II rat bladder carcinoma cell dispersion. *J. Cell Sci.* **100**, 371-380.

Turner, C. E., Pavalko, F.M. and Burridge, K. (1989). The role of phosphorylation and limited proteolytic cleavage of talin and vinculin in the disruption of focal adhesion integrity. *J. Biol. Chem.* **264**, 11938-11944.

Turner, C. E. and Burridge, K. (1991). Transmembrane molecular assemblies in cell-extracellular matrix interactions. *Curr. Opinion in Cell Biol.* **3**, 849-853.

Valmu, L., Autero, M., Siljander, P., Patarroyo, M. and Gahmberg, C. G. (1991). Phosphorylation of the β-subunit of CD11/CD18 integrins by protein kinase C correlates with leukocyte adhesion. *Eur. J. Immunol.* **21**, 2857-2862.

Vallés, A. M., Boyer, B., Badet, J., Tucker, G. C., Barritault, D. and Thiery, J. P. (1990). Acidic fibroblast growth factor is a modulator of epithelial plasticity in a rat bladder carcinoma cell line. *Proc. Natl. Acad. Sci. USA* **87**, 1124-1128.

Vandenberg, P., Khillan, J. S., Prockop, D. J., Helminen, H., Kontusaari, S. and Ala-Koffo, L. (1991). Expression of a partially deleted gene of human type II procollagen (COL2A1) in transgenic mice produces a chondrodysplasia. *Proc. Natl. Acad. Sci. USA* **88**, 7640-7644.

Vojtek, A., Haarer, B., Field, J., Gerst, J., Pollard, T. D., Brown, S. and Wigler, M. (1991). Evidence for a functional link between profilin and CAP in the yeast S. cerevisiae. *Cell* **66**, 497-505.

Volk, T., Fessler, L. I. and Fessler, J. H. (1990). A role for integrin in the formation of sarcomeric cytoarchitecture. *Cell* **63**, 525-536.

von der Mark, K. and Öcalan, M. (1989). Antagonistic effects of laminin and fibronectin on the expression of the myogenic phenotype. *Differentiation* **40**, 150-157.

von Mende, N., Bird, D. M., Albert, P. S. and Riddle, D. L. (1988). *dpy-13*: A nematode collagen gene that affects body shape. *Cell* **55**, 567-576.

Vos, H-L., Devarayalu, S., de Vries, Y. and Bornstein, P. (1992). Thrombospondin 3 (Thb3), a new member of the thrombospondin gene family. *J. Biol. Chem.* **267**, 12192-12196.

Vukicevic, S., Luyten, F. P., Kleinman, H. K. and Reddi, A. H. (1990). Differentiation of canalicular cell processes in bone cells by basement membrane matrix components: regulation by discrete domains of laminin. *Cell* **63**, 437-445.

Watt, F. M. (1986). The extracellular matrix and cell shape. *TIBS* **11**, 482-485.

Watt, F.M. (1991). Cell culture models of differentiation. *FASEB J.* **5**, 287-294.

Watt, F. M., Jordan, P.W. and O'Neill, C. H. (1988). Cell shape controls terminal differentiation of human epidermal keratinocytes. *Proc. Natl. Acad. Sci. USA* **85**, 5576-5580.

Wayner, E. A., Garcia-Pardo, A., Humphries, M. J., McDonald, J. A. and Carter, W.G. (1989). Identification and characterisation of the lymphocyte adhesion receptor for an alternative cell attachment domain in plasma fibronectin. *J. Cell Biol.* **109**, 1321-1330.

Wayner, E. A., Orlando, R. A. and Cheresh, D. A. (1991). Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ contribute to cell attachment to vitronectin but differentially distribute on the cell surface. *J. Cell Biol.* **113**, 919-929.

Weller, A., Beck, S. and Ekblom, P. (1991). Amino acid sequence of mouse tenascin and differential expression of two tenascin isoforms during embryogenesis. *J. Cell Biol.* **112**, 355-362.

West, C. M., Lanza, R., Rosenbloom, J., Lowe, M. and Holtzer, H. (1979). Fibronectin alters the phenotypic properties of cultured chick embryo chondroblasts. *Cell* **17**, 491-501.

Werb, Z., Tremble, P. M., Behrendtsen, O., Crowley, E. and Damsky, C. H. (1989). Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. *J. Cell Biol.* **109**, 877-889.

Wilcox, M., DiAntonio, A. and Leptin, M. (1989). The function of PS integrins in *Drosophila* wing morphogenesis. *Development* **107**, 891-897.

Yamaguchi, Y., Mann, D. M. and Ruoslahti, E. (1990). Negative regulation of transforming growth factor β by the proteoglycan decorin. *Nature* **346**, 281-284.

Yost, H. J. (1992). Regulation of vertebrate left-right asymmetries by extracellular matrix. *Nature* **357**, 158-161.

Zhou, R., Oskarsson, M., Paules, R. S., Schulz, N., Cleveland, D. and Vande Woude, G. F. (1991). Ability of the *c-mos* product to associate with and phosphorylate tubulin. *Science* **251**, 671-675.

Zusman, S., Patel-King, R. S., ffrench-Constant, C. and Hynes, R. O. (1990). Requirements for integrins during Drosophila development. *Development* **108**, 391-402.

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