Regulation of development and differentiation by the extracellular matrix

Josephine C. Adams¹ and Fiona M. Watt²,*

¹Vascular Research Division, Department of Pathology, Brigham and Women’s Hospital, Harvard Medical School, Room LM-414, 221 Longwood Avenue, Boston, MA 02115, USA
²Keratinocyte Laboratory, Imperial Cancer Research Fund, PO Box 123, Lincoln’s Inn Fields, London WC2A 3PX, UK

*Author for correspondence

SUMMARY

Introduction
Evidence that cell-ECM interactions regulate differentiation and development
  Developmental mutations
  Experimental perturbation of embryonic development
  Cell and organ cultures

Regulatory mechanisms
  ECM diversity
  Interactions of growth factors with the ECM
  ECM receptors

Non-integrin receptors
  Integrins
  Signal transduction
    Cytoskeleton
    Second messengers
    ‘ECM-response elements’

Conclusion

Key words: extracellular matrix, integrins, proteoglycans, signal transduction, growth factors, differentiation

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-permitting 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 collagen 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 sub-units 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 normal 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 tenasin 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 β1 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).
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 (Arma 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 α6β1 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

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

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

**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

<table>
<thead>
<tr>
<th>Tissue-specific ECM components</th>
<th>Examples:</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiligrin and kalinin</td>
<td>Carter et al., 1991</td>
<td></td>
</tr>
<tr>
<td>(stratified squamous epithelia)</td>
<td>Rousselle et al., 1991</td>
<td></td>
</tr>
<tr>
<td>Claustrin (brain)</td>
<td>Cole and McCabe, 1991</td>
<td></td>
</tr>
<tr>
<td>Restrictin (CNS)</td>
<td>Nörenberg, 1992</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isoforms</th>
<th>Examples:</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminin family (A/B1/B2)</td>
<td>Leivo and Envang, 1988</td>
<td></td>
</tr>
<tr>
<td>Thrombospondin family (TSP-1, TSP-2, TSP-3)</td>
<td>Lawler and Hynes, 1986</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bornstein et al., 1991</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vos et al., 1992</td>
<td></td>
</tr>
<tr>
<td>Type IV collagen (α1 to α5 (IV))</td>
<td>Hudson et al., 1989</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alternative splicing</th>
<th>Examples:</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastin</td>
<td>Pollock et al., 1990</td>
<td></td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Kombliht et al., 1985</td>
<td></td>
</tr>
<tr>
<td>Tenascin</td>
<td>Jones et al., 1989</td>
<td></td>
</tr>
<tr>
<td>Type VI collagen</td>
<td>Doliama et al., 1990</td>
<td></td>
</tr>
<tr>
<td>Agrin</td>
<td>Ruegg et al., 1992</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post-translational modifications</th>
<th>Examples:</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosylation (laminin, fibronectin)</td>
<td>Jones et al., 1986; Dean et al, 1990</td>
<td></td>
</tr>
<tr>
<td>Glycosaminoglycan chain composition (aggrecan)</td>
<td>Bayliss et al., 1983</td>
<td></td>
</tr>
<tr>
<td>Transglutaminase cross-linking (fibronectin)</td>
<td>Barry and Mosher, 1988</td>
<td></td>
</tr>
<tr>
<td>Cation-dependent aggregation (laminin) or conformation (thrombospondin)</td>
<td>Paulsson, 1988; Lawler et al., 1988</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Binding to heparin/heparan sulphate chains</th>
<th>FGFs (aFGF, bFGF, KGF, int-2)</th>
<th>Klagsbrun, 1990, Kiefer et al., 1991</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-3</td>
<td>Roberts et al., 1988</td>
<td></td>
</tr>
<tr>
<td>Scatter factor/hepatocyte growth factor</td>
<td>Rosen et al., 1989</td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Gordon, 1988</td>
<td></td>
</tr>
<tr>
<td>Schwann cell growth factor</td>
<td>Ratner et al., 1988</td>
<td></td>
</tr>
<tr>
<td>Purpurin</td>
<td>Berman et al., 1987</td>
<td></td>
</tr>
<tr>
<td>Platelet factor 4</td>
<td>Rusolaihti and Yamaguchi, 1991</td>
<td></td>
</tr>
<tr>
<td>PDGF-B</td>
<td>La Rochelle et al., 1991</td>
<td></td>
</tr>
<tr>
<td>Pleiotrophin</td>
<td>Li et al., 1990</td>
<td></td>
</tr>
<tr>
<td>HB- EGF</td>
<td>Higashiyama et al., 1991</td>
<td></td>
</tr>
</tbody>
</table>

| Binding to chondroitin sulphate chains   | Platelet factor 4             | Périn et al., 1988 |

<table>
<thead>
<tr>
<th>Binding to proteoglycan core proteins</th>
<th>TGF-β (betaglycan, decorin)</th>
<th>Andres et al., 1989; Yamaguchi et al., 1990</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF-A, PDGF-B (SPARC)</td>
<td>Raines et al., 1992</td>
<td></td>
</tr>
<tr>
<td>β-endorphin (vitronectin)</td>
<td>Hildebrand et al., 1989</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Binding to ECM glycoproteins</th>
<th>TGF-β (fibronectin)</th>
<th>Fava and McClure, 1987</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β (thrombospondin)</td>
<td>Murphy-Ulrich et al., 1992</td>
<td></td>
</tr>
<tr>
<td>PDGF-A, PDGF-B (SPARC)</td>
<td>Raines et al., 1992</td>
<td></td>
</tr>
<tr>
<td>β-endorphin (vitronectin)</td>
<td>Hildebrand et al., 1989</td>
<td></td>
</tr>
</tbody>
</table>

...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; Rusoalhti 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 (Sakela and Rifkin, 1990; Ishimiachi 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 (Ignutz and Massagué, 1986), alters expression of adhesive receptors (Ignutz 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 (Panayotou 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-bind-
ing 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 differenti-
ation 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 fac-
tors, 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 new-
born 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 inter-
actions 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 multiva-
tent 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 (Greenwald 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 mol-
ecules at the cell surface, syndecan may assemble a signal-
alling complex or may serve as an accessory signalling molecule in combination with other receptors (Salminen et al., 1992). A family of syndecan-related proteoglycans has now been identified, which have different ectodomains but identical cytoplasmic domains, suggesting that the cyto-
plasmic domain may fulfil some conserved signalling func-
tion (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 (Bern-
field 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 proper-
ties: 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 αβ 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 dif-
ferent 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 com-
plexity is provided by the existence of mRNA splice vari-
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 β1 integrins changes as myoblasts fuse to form myotubes (Damsky et al., 1985). In the developing kidney, the αβ1 laminin receptor is expressed by epithelia and not by unduced mesenchyme (Sorokin et al., 1990), and the α, α6 and α5 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 β1 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 β2 subgroup and αβ4, 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 αβ1 (Dedhar et al., 1991). Mouse ES cells express αβ1 containing the αβ splice variant of αβ, but the αβA form is induced upon differentiation (Cooper et al., 1991). Fibronectin receptor expression is decreased in erythroleukaemia cells induced to undergo terminal differentiation and this correlates with lack of adhesion to fibronectin (Patel and Lodish, 1987). αmβ2 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 (β1 subgroup) but not by antibodies to the vitronectin receptor (α, 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 β1 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 β1 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 β4 subunit, integrin α and β subunits have short cytoplasmic domains, of up to 53 amino acids. The high interspecies conservation of the β1 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 β1 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, αβ1 and αβ6, 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 $\alpha$ cytoplasmic domains in signal transduction has come from molecular genetic experiments. In the case of the platelet integrin IIb/IIIa (IIb/IIIa), 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 $\beta_3$ subunit does not impair normal function but deletion of the $\alpha$IIb cytoplasmic domain or substitution with the $\alpha_5$ cytoplasmic domain causes constitutive activation of the receptor (O’Toole et al., 1991). Similarly, chimeras made between the $\alpha_2$ subunit and the cytoplasmic domains of other $\alpha$ subunits show collagen-binding activity equivalent to the wild-type $\alpha_5\beta_1$ integrin, but have differing abilities to contract collagen gels (Chan et al., 1992). These data suggest that $\alpha$ 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 cultures (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 $\beta_1$ cytoplasmic domain is of particular functional significance for cytoskeletal association. Thus, transfected $\beta_1$ molecules that lack the cytoplasmic domain associate with endogenous $\alpha$ 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 $\beta_1$, but not the $\alpha_5$, 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 $\beta_1$ 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 $\beta_1$ 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 $\beta_1$ or $\beta_3$ cytoplasmic domains specifically bind $\alpha$-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 $\alpha$-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 $\alpha_\omega\beta_1$ 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-
attenocytes 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 β1 integrins by ligand (Adams and Watt, 1989; Fig. 1). Terminal differentiation of keratinocytes in suspension can be inhibited by antibodies to β1 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 alkalisation, due to activation of the sodium/hydrogen antiporter (Inger 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 β2 and β3 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 β1 and β2 subunits contain a tyrosine residue within a consensus phosphorylation site; however, although constitutive phosphorylation of the β1 subunit in Rous sarcoma virus transformed cells correlates with reduced integrin/fibronectin/talin binding in vitro (Tapley et al., 1989), β1 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 α2β1 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.
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 $\beta_1$ 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-$\beta_1$ 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 $\beta$-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 $\beta$ 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.

We thank Wendy Senior for typing the manuscript. J. C. A. gratefully acknowledges the financial support of the International Human Frontiers of Science Program Organisation. F. M. W. is supported by the Imperial Cancer Research Fund.

REFERENCES


(Accepted 8 January 1993)