Extracellular matrix and its receptors during development

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ABSTRACT Extracellular matrix (ECM) components are essential for morphogenesis of virtually all tissues. The ECM interacts with the cell surface by binding to specific receptors. The first family of receptors for the ECM that was identified was the integrin family. Integrins are composed of an α and a β-chain, both of which are single pass transmembrane proteins. In muscle cells the dystroglycan complex forms another important receptor system for ECM. It is a complex composed of many proteins. Recent studies have shown that dystroglycan is expressed by embryonic epithelial cells as well. The nature of constituents of the dystroglycan complex is well known for muscle, whereas the detailed composition of the dystroglycan complex in embryonic epithelium is not yet well known. We here review the evidence that binding of ECM to integrins and the dystroglycan complex could be essential for muscle and epithelial cell development and function. It is likely that integrins and the dystroglycan complex have distinct roles during development. It will be an interesting task to study the signal transduction pathways elicited by the interactions between ECM and the two receptor systems during muscle and epithelial morphogenesis.

KEY WORDS: laminin, fibronectin, integrin, dystroglycan, muscle, epithelium

Introduction

The view of the extracellular matrix (ECM) as a biologically inert support has changed with the identification of glycoproteins in the ECM that interact with cells and with the characterization of signal transducing receptors for the ECM. Much of the early work on cell-interactive ECM components focused on the glycoproteins fibronectin and the laminins, but in recent years several additional cell adhesive glycoproteins have been identified. The first family of receptors for the ECM that was identified was the integrin family. The name integrin was given to denote the importance of these receptors for the integrity of both the cytoskeleton and the ECM. Somewhat later it became clear that a group of cell-surface associated proteoglycans, named syndecans, might act as co-receptors for the ECM and above all for certain growth factor receptors. More recently dystroglycans have emerged as another type of receptors that link the cytoskeleton to the ECM.

Integrins

The first integrin to be discovered bound to fibronectin (Ruoslaiti, 1988) but it was soon found that several ECM components bind to similar types of receptors (Hynes, 1992). Integrins contain two membrane glycoproteins, an α and a β chain. Generally the subunits possess a small intracellular domain, a single transmembrane spanning region, and a large extracellular domain. The structure and ligand specificity of the characterized integrins have now been studied in great detail.

More than 20 integrins have been found, and new integrins are still to be discovered. With new techniques such as PCR more integrins are likely to be identified. For instance, a new major integrin, the α9 integrin, was recently characterized by homology PCR (Palmer et al., 1993). Immunostaining revealed high levels of expression restricted to differentiated cells in vivo and also revealed a selective expression in cultured cell lines, which is probably the reason why this integrin was not identified earlier. It is likely that additional integrins are still to be identified on specialized cells in a similar way.

Recent work has revealed that integrins can be activated, and that the level of integrins does not necessarily always correlate with functional integrins. The role of integrins in biological systems as developing organisms is thus potentially very complex. Transgenic mice, knock-out mice and establishment of cell lines lacking desired integrin genes are likely to generate valuable information (Hynes, 1994; Fässler et al., 1995). However, the role of different in vitro systems for organ development to complement these systems should not be underestimated.

Studies of integrin function in simpler organisms such as Drosophila, C. elegans and Xenopus are also likely to generate valuable information. Analysis of the data that has been obtained from these systems so far has generated information about some developmental themes that also are to be recognized in higher vertebrates.

Abbreviations used in this paper: ECM, extracellular matrix; FN, fibronectin; VN, vitronectin; LN, laminin.

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Syndecans

Syndecans are cell surface associated proteoglycans composed of a core protein intercalated in the plasma membrane with glycosaminoglycan chains attached extracellularly (reviewed in David, 1993; Jalkanen et al., 1993; Salmivirta and Jalkanen, 1995). Some data indicate that syndecans can modulate the activity of integrins in cell adhesion (Woods et al., 1986; LeBaron, 1988). It has been recently shown that syndecan-4 localizes to focal contacts in vitro (Woods and Couchman, 1994). An increasing amount of data also indicate that syndecans can act as co-receptors for growth factor receptors.

Dystroglycan

Through the study of the cytoskeletal protein dystrophin in skeletal muscle it became clear that this cytoskeletal protein takes part in a linkage between the cytoskeleton and the muscle basement membrane. The third link in this chain was subsequently identified as a dystrophin associated glycoprotein complex, composed of an extracellular peripheral membrane protein of 156 kDa (α-dystroglycan) that binds laminin-2, transmembrane proteins of 25 kDa, 35 kDa, 43 kDa (β-dystroglycan) and 50 kDa (adhalin). The complex also contains an intracellular peripheral membrane protein of 58 kDa (Hoffman et al., 1987; Campbell, 1995). The dystroglycan complex is an important receptor system for ECM in muscle, but there is recent evidence that other cell types also express some of the proteins of this complex.

Cell-matrix interactions during development

Virtually every developing cell encounters some form of extracellular matrix and we have good reasons to believe that their interaction is of importance for the development of the cells (Adams and Watt, 1993; Hay, 1993). It is not possible to cover all tissues, and we will restrict ourselves here to a large extent to cover only the two systems we are particularly familiar with, myogenesis and epithelial morphogenesis. One common feature in these two systems is the formation of a basement membrane type of an ECM. As will be evident, the cells use the same types of receptors to bind to the basement membrane.

Myogenesis

Competence for muscle differentiation in Drosophila develops at gastrulation. Cells from gastrulae can be grown in tissue culture to develop contractile myofibers (Secof et al., 1984). Three types of embryonic musculature can be recognized: somatic body wall muscle, visceral muscle and heart (dorsal vessel) muscle. The heart and visceral muscle in Drosophila consists of mononucleated cells. Unlike smooth muscle in vertebrates, Drosophila visceral muscle is striated and the Z-disc is perforated. The somatic muscles are derived from the ventrolateral portion of the somitic mesoderm, which becomes segmented at 6 hrs of embryonic development. As germ band shortens, so-called founder cells of individual muscles segregate out from the somoderm (Bate, 1990). A Drosophila member of the myogenic regulatory factor family (dMyoD or nautilus) is expressed in these founder cells (Michelson et al., 1990; Paterson et al., 1991). At the appropriate location these cells can fuse with fusion-competing myoblasts to form so-called muscle precursors, which pre-figure the final muscle pattern. The embryonic somatic muscle is used by the larva for crawling. Adult muscles must meet other needs and are largely formed de novo from different cells during later stages of development.

Integrins in Drosophila

Research initiated by Michael Wilcox and Daniel Brower has significantly contributed to the current knowledge about Drosophila integrins (Wilcox et al., 1981; Brower et al., 1984). The Drosophila integrin β chain (βPS integrin) found to be homologous to vertebrate β1 integrin was originally identified by monoclonal antibodies by Wilcox and Brower. In addition, a separate β chain (β2) has recently been identified by the use of homology PCR (Yee and Hynes, 1993). The βPS integrin chain has been found to be associated with three α chains: αPS1, which during embryogenesis is expressed on ectodermally and endodermally derived cells, αPS2, which is expressed on mesodermal derivatives such as somatic and visceral muscles and αPS3 (for a review see Gotwals et al., 1994a). The distribution of αPS3 is so far unknown. βPS shows a remarkable tissue-specific distribution during embryogenesis in that it is only expressed in endodermal cells around the midgut. The α-chain associated with βPS has not been identified. The two ligands known for the Drosophila integrins are the RGD-containing protein tiggrin (binds αPS2) and the basement membrane protein laminin (binds αPS1) (Fogerty et al., 1994; Gotwals et al., 1994a). The αPS2 was the first integrin chain described to show developmentally regulated splicing in the postulated ligand binding extracellular domain (Brown et al., 1989). This was hypothesized to generate αPS2 integrin heterodimers with different affinities for their ligand. Recent experiments with cells transfected with the two αPS2 spliced variants which were allowed to interact with tiggrin, support this splicing-regulated affinity modulation (Fogerty et al., 1994). Lethal alleles exist in Drosophila where different integrins are affected: βPS (mospheroid (I/mys)), αPS1 (multiple edematous wings (new)) and αPS2 (intiliated (if)) (MacKrell et al., 1988; Brown 1994; Brower et
al., 1995). In vitro analysis of mys myotubes have implied a role for integrins in sarcoramic stability (Volk et al., 1991). In vivo analysis of the above mentioned mutations have indicated a role for Drosophila βPS integrins in muscle attachments and the attachment of epithelial cells to basement membranes in the adult wing and eye (Brabant and Brower, 1993; Brown, 1994; Brower et al., 1995). Gastrulation and cell differentiation appears normal in the mys embryos but at the time of the first muscle contraction in the embryos, visceral and somatic muscles come loose from their attachments and the embryos become spheroid in shape (Wright, 1960). Integrins in Drosophila thus function as an important link between the cytoskeleton and the basement membrane. In vertebrates the importance of integrins for muscle integrity has not yet been evaluated. However, deficiencies in two other components involved in the transmission of force, the cytoskeletal protein dystrophin and the ECM protein laminin-2, both cause muscle dystrophies (Hoffman et al., 1987; Xu et al., 1994). Drosophila should continue to be an interesting system to study integrin function during development. In summary, the Drosophila system shows that integrins are needed at sites of strong cell adhesion and that alternative splicing in the ligand binding domain can be used to generate integrins with different affinities with consequences for integrin function during developmental processes.

Syndecans in Drosophila
Homology PCR has led to the identification of one type of syndecan in Drosophila (Spring et al., 1994). Expression analysis shows that syndecan expression follows that of two Drosophila fibroblast growth factor receptor homologs, implying a role for syndecan as a coreceptor for growth factor receptors. The role of syndecan for muscle differentiation has not been analyzed.

Dystroglycan in Drosophila
That non-integrin receptors for the ECM exist in Drosophila muscle is indicated by the fact that myogenic cells from (l)mys embryos can differentiate in vitro on laminin. In vertebrates the dystroglycan complex has recently been identified as a non-integrin laminin receptor on skeletal muscle cells (see below). Homologs of dystroglycan complex components have not yet been identified in Drosophila although a candidate for a Drosophila homolog to the dystroglycan binding protein dystrophin has been identified (Volk, 1992). It will be interesting to determine whether Drosophila variants of dystroglycans exist and if so, what is the relative importance of integrins and dystroglycans for muscle stability.

Myogenesis in vertebrates
The study of myogenic transcription factors during myogenic differentiation has increased our understanding of the transcriptional regulation during myogenesis (Olson, 1992; Buckingham, 1994a,b). Relatively little is known about how external stimuli influence myogenesis. As cells undergo myogenesis in vivo they are exposed to different conditions depending on the time of development when myogenic differentiation occurs (Miller, 1992; Cusella-De Angelis et al., 1994). The type and levels of growth factors and the expression of corresponding receptors are tightly regulated during development (Fiorini et al., 1991). The changing conditions also extend to the nature and composition of the ECM. Somitic, embryonic, fetal and newborn myoblasts that differ in their expression patterns of muscle regulatory factors have been shown to respond differently to the extracellular matrix during in vitro differentiation (Foster et al., 1987; Smith et al., 1993).

Integrins
It is likely that the varying response to the ECM is in part due to the changing integrin repertoire on myoblasts during development. Experiments with murine myogenic cells isolated from newborn mice have indicated that fibronectin promotes dedifferentiation whereas laminin promotes differentiation (von der Mark and Ocakan, 1989). A major laminin receptor found on muscle cells is the α7β1 integrin (Song et al., 1992). Embryonic myoblasts express the α6β1 integrin (Terpe et al., 1994). Analysis of the in vivo expression of α7β1 integrin during myogenesis has indicated that it is expressed concomitantly with the formation of a laminin-rich basement membrane and that later it is concentrated at the myotendinous junction (Bao et al., 1993). Recent experiments have indicated that α7β1 is subject to complex alternative splicing suggesting this is a mechanism to generate an integrin heterodimer with diverse functions during different stages of development (Collo et al., 1993; Song et al.,
The nature of the receptor mediating the dedifferentiating capacity of FN on murine myoblasts in vitro has not been established.

Analysis of fibronectin receptors during myogenic differentiation has established a role for α4β1 during secondary myogenesis (Rosen et al., 1992). During secondary myogenesis α4β1 seems to interact with the counter receptor VCAM-1 and not fibronectin. During in vitro differentiation antibodies to α4β1 can inhibit myoblast fusion. The role of α3β1, α5β1 and different αv containing receptors during myogenesis is less clear.

Our own analyses of the role of integrins during human fetal myogenesis have revealed that in the human fetal muscle at 10 weeks gestational age, fibronectin (FN) and laminins (LN) are present in the ECM. At this developmental stage the differentiated muscle cells in vivo express α5 and α6 integrins but not α3, α1 and α3 integrins. However, in vitro cultured myoblasts isolated from the same gestational age express αv, α1, and α3 integrins in addition to α5 and α6 integrins. A more detailed analysis of the marked vitronectin (VN) receptor expression in culture shows that the localization of different αv heterodimers into focal contacts is regulated in a different way. αvβ1 and αvβ3 are present at focal contacts throughout in vitro myogenesis whereas αvβ5 appears to depend on an endogenously produced factor to localize to focal contacts. The αvβ1, αvβ5 and α3β1 heterodimers, often reported not to focalize, did form focal contacts in human fetal muscle cells, indicating that these myoblasts possess components that facilitate the formation of cytoskeletal linkages containing these integrins. αvβ6 co-localized with FN in myoblast cultures, whereas myotubes lacked both FN and αvβ6 on the cell surface (Gullberg et al., 1995a). Further studies will be needed to assess the relative importance of the FN and VN binding integrins for the differentiation process, in comparison with the laminin binding integrins α6 and α7, also present on these cells. We have also recently described the identification of a novel β1 integrin associated α-chain that is upregulated during in vitro myogenesis of human fetal myotubes (Gullberg et al., 1995b). A previous study of the integrin repertoire on myotubes in human adult skeletal muscle indicated that unidentified β1 integrin heterodimers might be present in the sarcolemma (Virtanen et al., 1990). Later studies confirmed this notion with the identification of α7 and α9 integrin chains on adult skeletal muscle (Song et al., 1992; Palmer et al., 1993). It is unclear whether yet unidentified integrins are present in adult skeletal muscles. In our studies we found that immunoprecipitation of β1 integrins from surface iodinated and metabolically labeled human fetal muscle cells typically showed a 5-fold induction of αv-integrin containing receptors upon differentiation. Under non-reducing conditions this β1-associated protein migrated as 145 kDa. The β1 integrin associated cell surface protein present in myotubes remained associated with the β1 subunit in the presence of 1% Triton X-100 and 0.5 M NaCl. Like integrin α-chains, the protein dissociated from the β1 integrin subunit at low pH. Immunodepletion with integrin α-chain antibodies to α1, α3, α4, α5, α6 and αv integrin chains could not deplete the β1-integrin associated protein, indicating that it did not interact with any of these integrin heterodimers known. Upon treatment with reducing agents, the β1 integrin associated protein migrated in SDS-PAGE as a 155 kDa protein. The decreased mobility in SDS-PAGE upon reduction is a feature shared with α1, α2 and α9 integrin α-chains. Antibodies to α1 immunoprecipitated an integrin heterodimeric protein of 155 kDa. Antibodies to α2 and α9 failed to immunoprecipitate proteins from human fetal myotubes and northern blot analysis likewise failed to detect messages for these two integrin α-chains (Gullberg et al., 1995b).

Based on these characteristics, we propose that the induced protein is a hitherto unidentified integrin α-chain on myotubes that we name αm (mt from myotube). We suggest that αmβ1 is involved in early human fetal myogenesis and our data support the hypothesis that different integrin α-chains play different roles in myogenesis during different developmental stages. Our data also imply that previous data on the role of different integrins dur-
ing myogenesis have to be re-considered in the light of our finding of a novel integrin α-chain on myotubes.

**Syndecans**

Inhibition of proteoglycan sulphation in myoblasts has profound effects on the differentiation capacity of the myoblasts. Sulphation inhibited myoblasts rapidly withdraw from the cell cycle and form myotubes. This has been interpreted as an effect on cell surface proteoglycans that, when defective, are unable to activate mitogenic growth factor receptor signals (Rapraeger et al., 1991).

**The dystroglycan complex**

Although integrins seem to be of major importance in cell-matrix interactions during muscle development, there are additional receptors for ECM in muscle.

The dystroglycan complex is an important receptor complex. In vitro binding studies have shown that α-dystroglycan interacts with the ECM proteins agrin and laminin of muscle. The binding to laminin-2 might occur via lectin-binding properties of dystroglycan. Intracellularly, the dystroglycan complex binds to dystrophin (Campbell, 1995). In two genetic diseases affecting muscle, the dystrophic mdx mice, and the human Duchenne muscular dystrophy, the defect was identified in dystrophin. In another mouse mutant with a muscle disorder, the α2 chain of laminin-2 is mutated (Xu et al., 1994). A protein that can compensate for dystrophin and associate with dystroglycan has been named utrophin or dystrophin related protein (Love et al., 1989). In cardiac muscle and small caliber skeletal muscle in the mdx mouse utrophin is induced and this has been suggested to prevent muscle wasting in these particular muscle groups.

It is interesting to note that muscle differentiation in the embryo does not seem to be seriously affected by abnormal laminin-2 or lack of dystrophin. Based on the genetic disease with lesions occurring after birth, it could be suggested that laminin-2 in the extracellular space and dystrophin in the intracellular space are mainly involved in the maintenance of muscle stability rather than in embryonic muscle development. Whether the dystrophin-dystroglycan complex is involved in the transmission of force or whether they are important for muscle stability in more indirect ways, is still unclear. The dystroglycan complex is apparently a main linkage between muscle laminin and dystrophin. It remains to be seen whether genetic manipulation of the genes for the dystroglycan complex will affect differentiation of muscle in the embryo or muscle stability after birth.

**Differentiation of epithelial cells in vertebrates**

In most developing tissues epithelial cells emerge by a process called branching epithelial morphogenesis. In these organs, one can distinguish early a small epithelial rudiment which is surrounded by a cuff of mesenchymal cells. The epithelium then begins to grow and branch into the mesenchyme. The branching pattern varies slightly from tissue to tissue but many of the basic features of the branching process are similar in all organs. In all tissues the branching of the epithelium requires the presence of adjacent mesenchymal cells (Grobstein, 1967; Theiseletal., 1995).

The molecular basis of the epithelial-mesenchymal interactions have not been well clarified yet. Growth factors and their receptors are involved, but it is also becoming clear that ECM is of crucial importance in epithelial-mesenchymal interactions. There is some evidence that proteoglycans of the basement membrane are involved in branching epithelial morphogenesis (Bernfield et al., 1972, 1984), but other components of the basement membrane are apparently also involved. Laminin was identified as a major glycoprotein of basement membranes some 15 years ago. Recent studies have shown that it was only one member of a large family of morphoregulatory proteins (Tryggvason, 1993; Timpl and Brown, 1994). We will here review the evidence that the first discovered member of the family, laminin-1, is required for epithelial morphogenesis in many embryonic organs.

**Conversion of mesenchyme to epithelium**

Evidence for a role of laminin-1 in epithelial morphogenesis was first obtained from studies on the embryonic mouse kidney.
In the embryonic kidney, branching epithelial morphogenesis leads to the development of the ureter and the collecting ducts. In addition, all other epithelial cells of the kidney form by a conversion of mesenchyme to epithelium. Close to the first epithelium, some mesenchymal cells will be induced to begin a differentiation process into epithelial cells. This mode of epithelial development is rather unique, but can be used to study the role of ECM in epithelial development. It is a very good system to study the role of basement membrane components in development. The cells do not initially produce a basement membrane, but within a few days, a new basement membrane forms (Grobstein, 1956; Saxén et al., 1968). The appearance of proteins specific for the basement membrane can be precisely monitored (Ekblom et al., 1980, 1981), and the role of these components can be studied by blocking antibodies.

If the kidney mesenchyme is induced to differentiate in vitro, the first signs of epithelial morphogenesis can be seen on day 2 of culture. On day 3 polarized epithelial cells form. When the kidney mesenchyme is induced, expression of laminin α1 and γ1 mRNA increases early on day 1 of in vitro development, but the expression of laminin α1 chain remains low. When epithelial cell polarization begins on day 2 of in vitro development, laminin α1 chain mRNA expression increases (Ekblom et al., 1980). The increased expression at sites where epithelial cell polarization begins has been verified by in situ hybridization (Ekblom et al., 1990) and by immunofluorescence by using either polyclonal (Klein et al., 1986) or monoclonal antibodies against the α1 chain polypeptide (Sorokin et al., 1992). It is noteworthy that the expression of both integrin α6 subunit and E-cadherin also increases at the same time (Vestweber et al., 1985; Sorokin et al., 1990).

Role of integrin α6β1 in kidney tubulogenesis

The expression results suggest a role for both E-cadherin and laminin-1 for the formation of kidney tubules. However, antibodies against E-cadherin have failed to perturb kidney tubule development in vitro (Vestweber et al., 1985). It is therefore still unclear whether E-cadherin is required for kidney development. One possibility is that kidney epithelial cells express many cadherins (Hatta et al., 1987; Xiang et al., 1994) and it might be insufficient to apply antibodies to only one cadherin in order to perturb the cadherin-mediated cell-cell attachments in the developing kidney. In contrast, it has been possible to perturb kidney development with antibodies to both laminin-1 and its receptors. Defined fragments of laminin can be prepared by enzymatic treatment of laminin-1, and antibodies against these fragments have been prepared. In organ cultures of the embryonic kidney antibodies against E8 fragment of laminin-1 perturb formation of kidney tubules (Klein et al., 1988). A very similar inhibition was obtained with a monoclonal antibody against integrin α6 subunit (Sorokin et al., 1990). The results suggest a role for laminin-1 in epithelial development, and that laminin-1 might in part act by binding to integrin α6β1.

Role of dystroglycan in kidney tubulogenesis

Although the integrin α6β1 seems to be a major receptor for laminin-1 in kidney tubules, other receptors apparently exist. Antibodies against laminin-1 fragment E3 can also perturb kidney tubule development in vitro (Klein et al., 1988; Sorokin et al., 1992). E3 fragment is the carboxyterminus of laminin α1 chain and α1 and γ1 chains do not contribute to E3 fragment (Sasai et al., 1988). The E3 fragment of laminin-1 chain should thus have a receptor on the epithelial cell surface. The dystroglycan complex could be such a receptor. It is well known that the dystroglycan complex binds to laminin-2 in muscle (Campbell, 1995), but recent evidence suggests that dystroglycan might also bind to laminin-1. It has now been found that dystroglycan mRNA is expressed at high levels by epithelial cells in the embryonic kidney, and is thus coexpressed with laminin α1 chain. By immunofluorescence, dystroglycan can be seen on the basal side of the epithelial cells, which is compatible with the hypothesis that it acts as a receptor for laminin-1. Moreover, antibodies known to affect dystroglycan-laminin can inhibit kidney development in vitro (Durbeej et al., 1995).

These results strongly suggest that dystroglycan is an epithelial receptor for basement membrane components in the developing kidney (Durbeej et al., 1995). It has not been shown that laminin-1 is the only ligand for dystroglycan in the epithelium, but dystroglycan does not bind nidoerin, type IV collagen or fibronectin (Ervasti and Campbell, 1993). Dystroglycan-like molecules from brain bind to E3 fragment of laminin-1 (Gee et al., 1993). A similar binding may occur during development of kidney epithelium and we thus suggest that the dystroglycan complex could be a long sought epithelial receptor for fragment E3 of laminin-1. E3 fragment might bind to many other proteins. It has been shown that some forms of syndecan bind to E3 fragment of laminin-1 (Salminen et al., 1994) and proteoglycans including syndecan-1 are expressed in embryonic kidney (Vainio et al., 1992; Laah et al., 1983). It is still unclear whether syndecan-1 has a role in kidney development, but there is some recent evidence from other experimental systems that it could be involved in the maintenance of epithelial cell polarity (Kato et al., 1995).

In conclusion, there is so far evidence for a role of 2 independent receptor systems, the integrins and the dystroglycan complex in the attachment of developing epithelial cells to laminin-1. It is likely that these receptors have distinct functions. Both dystroglycan (Durbeej et al., 1995) and integrins containing the α6 subunit (Sonnenberg et al., 1990; Sorokin et al., 1990; De Curtis and Reichardt, 1992; Terpe et al., 1994; Zuk and Hay, 1994) are expressed by epithelial cells in many tissues. The presented 2-receptor system (Fig. 3) is thus likely to be important for the early stages of morphogenesis of a large number of epithelial cell types. When epithelial differentiation proceeds further, additional receptors may appear, and the receptor repertoire apparently varies depending on the cell type. A large number of studies describing the dynamic expression patterns of various integrin subunits in different epithelial cells are available, but will not be covered here. These studies suggest that fine tuning of epithelial morphogenesis and the control of epithelial morphology in the adult may be controlled by cell-matrix interactions. Basement membranes have been shown to prevent apoptosis (Meredith et al., 1983; Frisch and Francis, 1994), so it is fully possible that adult epithelial cells must continuously sense the basement membrane in order to survive.

Branching epithelial morphogenesis

The embryonic kidney is a special case since large parts of the mesenchyme convert into epithelium. Several other embry-
Fig. 4. Expression of laminin chains and integrin chains α6 and β1 during branching epithelial morphogenesis of submandibular gland. Monoclonal antibodies against E3 fragment of laminin α1 chain stains only basement membranes of epithelia (A), whereas polyclonal antibodies detecting laminin chain α1, β1 and γ1 detect basement membranes of both endothelial and endothelial cells (B). Monoclonal antibodies against integrin α6 subunit stain epithelial and endothelial cell membranes, with strong expression on the basal side (C), whereas antibodies against β1 integrin stain the sections much more broadly, with staining in epithelial, mesenchymal and endothelial cells (D). From Kadoya et al. (1995).

Epithelial tissues can be used to study the more common form of epithelial morphogenesis, branching epithelial morphogenesis. One much used organ culture model is the submandibular gland of mouse embryos (Grobstein, 1953, 1967; Bernfield et al., 1984). Laminin-1 and its receptor integrin α6β1 are expressed by the epithelial cells of early embryonic submandibular gland (Fig. 4). It has recently been shown that monoclonal antibody 200 against E3 fragment of laminin-1 (Sorokin et al., 1992) perturbs branching morphogenesis of the submandibular gland. The antibodies against E3 fragment might have acted by disrupting the formation of basement membranes at the tip of the growing epithelium. It was also demonstrated that antibodies against integrin α6 subunit perturbed branching epithelial morphogenesis. An interesting difference was that the antibodies against α6 integrin subunit did no lead to clear defects of the basement membrane (Kadoya et al., 1995). The antibody perturbation experiments in organ cultures of submandibular gland suggest that the E3 fragment could initiate epithelial basement membrane assembly. Perhaps receptors for E3 provide a nucleation site for polymerization of the basement membrane. It is likely that dystroglycan is an E3 receptor also in the submandibular gland, but this has not yet been studied.

There is evidence that laminin-1 is also important for branching epithelial morphogenesis of lung (Schuger et al., 1990a,b, 1991). Domain-specific antibodies against laminin-1 were applied to organ culture of embryonic lung. The antibodies against the central part of laminin-1 partially perturbed lung epithelial morphogenesis. In contrast, antibodies against the distal part of the long arm did not do so (Schuger et al., 1990b, 1991). This does not necessarily mean that lung epithelial morphogenesis requires domains other than kidney and salivary gland epithelial morphogenesis. It would be of importance to test the antibodies used in the kidney and salivary gland organ cultures in the lung organ cultures. Conversely, those used in the lung organ cultures should be tested in the kidney and salivary gland organ cultures.
Epithelial-mesenchymal interactions

Epithelial morphogenesis requires the presence of mesenchymal cells. This has been known since the classical studies of Grobstein (1953, 1967). It is therefore notable that mesenchymal compartments in the embryo express a large number of basement membrane components (Ekblom et al., 1980, 1994; Hogan et al., 1982; Warburton et al., 1984; Klein et al., 1990; Kücherer-Ehret et al., 1990; Simon-Assmann et al., 1990; Fleischmajer et al., 1993; Thomas and Dziadek, 1993). The physiological role of this expression is not yet fully clear. However, there is now some evidence that these mesenchymal ECM components are required for epithelial morphogenesis (Ekblom et al., 1994). Specific antisera that block the interaction between laminin γ1 chain and nidogen have recently been generated (Mayer et al., 1993). These antisera were found to perturb kidney and lung epithelial branching morphogenesis in vitro. In contrast, control antibodies against adjacent EGF-like repeats on fragment P1 have no effect on branching epithelial morphogenesis (Ekblom et al., 1994). Nidogen might thus be one of the long sought mesenchymal factors required for epithelial morphogenesis.

ECM components produced by mesenchyme might be important for epithelial morphogenesis although they will not be incorporated into the epithelial basement membrane, which is the case for nidogen. Antibodies against tenascin-C and epi-morphin have also been shown to perturb branching epithelial morphogenesis in other systems (Hirai et al., 1992; Young et al., 1994) and both these proteins are expressed by embryonic mesenchyme (Chiquest-Ehrismann et al., 1986; Hirai et al., 1992).

Concluding remarks

During muscle and epithelial cell development a basement membrane type of an ECM will form early. In both cases, integrins are major cell receptors for the basement membrane components. The nature of the basement membrane varies slightly from cell type to cell type, and so do the integrin receptors. A large body of evidence has shown that these interactions are crucial for morphogenesis of muscle and epithelial cells. Future studies should be aimed at clarifying the signal transduction mechanisms initiated or maintained by these interactions. More recent studies have shown yet another similarity in the cell-matrix interactions of these two cell types; they both use the dystroglycan complex to bind to laminins. The complex has been well studied in muscle, and many of the associated proteins are well known. In the epithelium, we only know so far that dystroglycan is present, and many of the associated proteins may be different. It will be a very interesting task to compare the dystroglycan complex of epithelium and muscle.

Finally, it should be pointed out that basement membrane assembly both in muscle and epithelium requires interactions between the adjacent cells. In both systems, the adjacent cell in part acts by producing some of the basement membrane components. With the necessary tools to study individual protein-protein interactions now available, it is becoming possible to dissect these cell-cell interactions at the molecular level. These types of studies already now have clarified some long standing issues of developmental biology.

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References


