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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 ß-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 (Ruoslahti, 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 (ß-dystroglycan) and 50 kDa (adhalin). The complex also contains an intracellular peripheral membrane protein of 59 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

Muscle formation in Drosophila

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.*, 1973; see also Fig. 1). 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 h of embryonic development. As germ band shortens, so-called founder cells of individual muscles segregate out from the mesoderm (Bate, 1990). A *Drosophila* member of the myogenic regulatory factor family (dMyd 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-competent myoblasts to form so-called muscle precursors, which prefigure the final muscle pattern. The embryonic somatic muscle is used by the larvae for crawling. Adult muscles must meet other needs and are largely formed *de novo* from different cells during later stages of development.

ECM molecules in Drosophila

A beginning has been made in understanding cell-matrix interactions in *Drosophila*. Current methods and some of the results were recently reviewed by Fessler *et al.* (1994). Identified ECM molecules in *Drosophila* include vertebrate homologs to collagen IV, laminin, syndecan and tenascin (Baumgartner *et al.*, 1994; Fessler *et al.*, 1994; Spring *et al.*, 1994). Interestingly, there are also proteins only identified in *Drosophila* such as glutactin, peroxidasin and tiggrin (Fessler *et al.*, 1994). Except for the recently described embryonic lethal mutants in the laminin α1 chain locus, no other mutations in *Drosophila* ECM genes have been described (Henchcliffe *et al.*, 1993). The inability to detect an effect on neuronal or muscle development up to the time of death during late embryogenesis in the *lamA* null mutant initially indicated that redundant mechanisms might be operative in *Drosophila*. Except for the recently characterized laminin α1 chain, no other α chain has so far been described in *Drosophila* (Kusche-Gullberg *et al.*, 1992). Closer examination of the *lamA* null mutants has recently indicated embryonal defects in somatic muscle and heart development that might explain such lethality (Yarnitzky and Volk, 1995).

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 ß chain ($ß_{PS}$ integrin) found to be homologous to vertebrate ß1 integrin was originally identified by monoclonal antibodies by Wilcox and Brower. In addition, a separate ß chain ($ß_v$) has recently been identified by the use of homology PCR (Yee and Hynes, 1993). The $ß_{PS}$ integrin chain has been found to be associated with three a chains: $α_{PS1}$, which during embryogenesis is expressed on ectodermally and endodermally derived cells, $α_{PS2}$, which is expressed on mesodermal derivatives such as somatic and visceral muscles and $α_{PS3}$ (for a review see Gotwals *et al.*, 1994a). The distribution of $α_{PS3}$ is so far unknown. $ß_v$ 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 $ß_v$ has not been identified. The two ligands known for the *Drosophila* integrins are the RGD-containing protein tiggrin (binds $α_{PS2}$) and the basement membrane protein laminin (binds $α_{PS1}$) (Fogerty *et al.*, 1994; Gotwals *et al.*, 1994a). The $α_{PS2}$ 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 $α_{PS2}$ integrin heterodimers with different affinities for their ligand. Recent experiments with cells transfected with the two $α_{PS2}$ splice variants that 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: $ß_{PS}$ *(myospheroid ((l)mys))*, $α_{PS1}$ *(multiple edematous wings (mew))* and $α_{PS2}$ *(inflated (if))* (MacKrell *et al.*, 1988; Brown 1994; Brower *et*

al., 1995). *In vitro* analysis of *mys* myotubes have implied a role for integrins in sarcomere stability (Volk *et al.,* 1991). *In vivo* analysis of the above mentioned mutations have indicated a role for *Drosophila* $ß_{PS}$ 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 co-receptor 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 (Florini *et al.,* 1991). The changing conditions also extend to the nature and composition

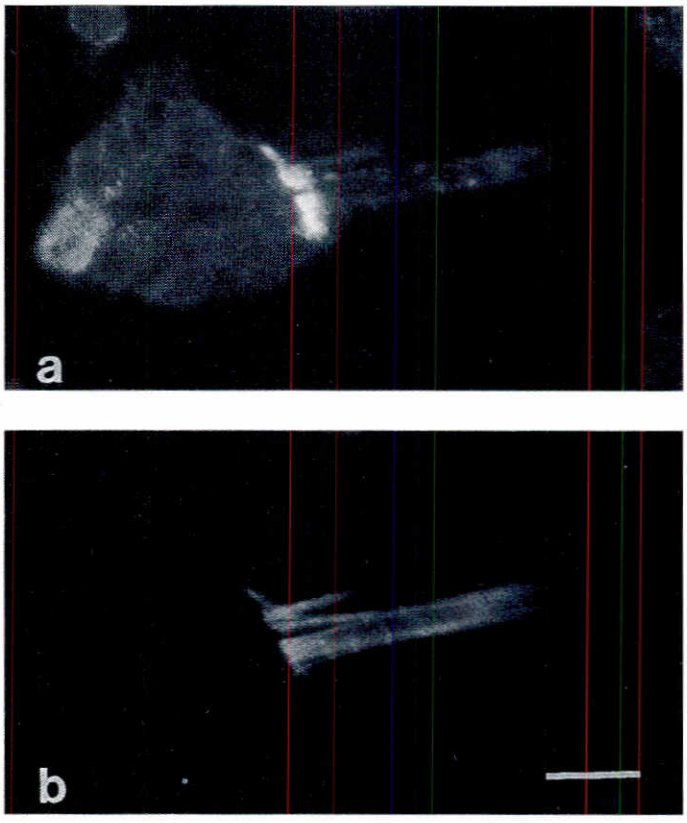

Fig. 1. Illustration of $ß_{PS}$ integrin and actin distribution in primary embryonic *Drosophila* cell cultures. *Primary Drosophila embryo cultures were grown on laminin substrates in the presence of serum for 36 h. Cells were fixed and double-labeled for $ß_{PS3}$ integrin* ***(a)*** *and actin* ***(b).*** *Note the staining for integrin at cell-cell junctions and at striations in the myotube. Bar, 15 μm. From Gullberg et al. (1994).*

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 Öcalan, 1989). A major laminin receptor found on muscle cells is the α7ß1 integrin (Song *et al.,* 1992). Embryonic myoblasts express the α6ß1 integrin (Terpe *et al.,* 1994). Analysis of the *in vivo* expression of α7ß1 integrin during myogenesis has indicated that it is expressed concomitant 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 α7ß1 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.,*

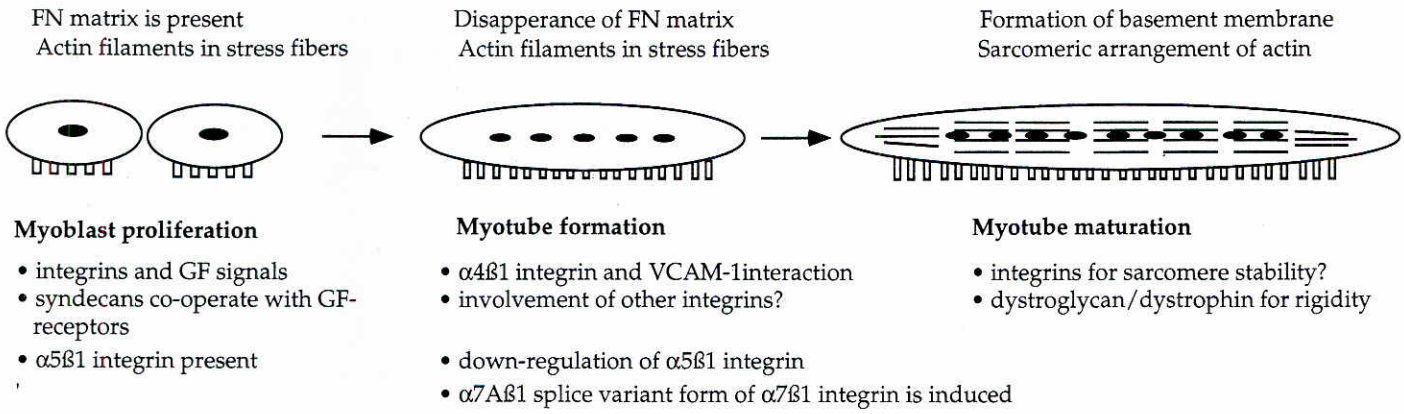

Fig. 2. Schematic representation of *in vitro* myogenesis. *As mononuclear skeletal myoblasts fuse in vitro to form multinucleated myotubes a number of changes take place. Growth factor (GF) and integrin signals most likely contribute to generate mitogenic signals. α4ß1 integrin binding to the counter-receptor VCAM-1 is a recognition system that plays a role in the events leading up to myoblast fusion. In a number of systems it has been shown that myotubes are not in contact with a fibronectin-rich matrix. This seems to correlate with the down-regulation of α5β1 integrins on myotubes. In the presence of non-muscle cells a basement membrane is elaborated on the myotube surface. α7β1 integrin is one laminin receptor on myotubes. The dystroglycan complex can also bind laminin and may have a major function in stabilizing the sarcolemma-basement membrane contact. Intracellularly the actin cytoskeleton is initially organized into so-called stress-fibers. In mature myotubes a sarcomeric arrangement is assumed that might be stabilized by integrins.*

1993; Ziober *et al.*, 1993). 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 α4ß1 during secondary myogenesis (Rosen *et al.*, 1992). During secondary myogenesis α4ß1 seems to interact with the counter receptor VCAM-1 and not fibronectin. During *in vitro* differentiation antibodies to α4ß1 can inhibit myoblast fusion. The role of α3ß1, α5ß1 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 αv, 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. αvß1 and αvß3 are present at focal contacts throughout *in vitro* myogenesis whereas αvß5 appears to depend on an endogenously produced factor to localize to focal contacts. The αvß1, αvß5 and α3ß1 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. α5ß1 colocalized with FN in myoblast cultures, whereas myotubes lacked both FN and α5ß1 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 ß1 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 ß1 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 $ß_1$ integrins from surface iodinated and metabolically labeled human fetal muscle cells typically showed a 5-fold induction of a $ß_1$ integrin associated protein upon differentiation. Under non-reducing conditions this $ß_1$ associated protein migrated as 145 kDa. The $ß_1$ integrin associated cell surface protein present in myotubes remained associated with the $ß_1$ subunit in the presence of 1% Triton X-100 and 0.5 M NaCl. Like integrin α-chains, the protein dissociated from the $ß_1$ 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 $ß_1$-integrin associated protein, indicating that it did not interact with any of these integrin heterodimers known. Upon treatment with reducing agents, the ß1 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 heterodimer distinct from the 155 kDa protein. 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 $α_{mt}$ (mt from myotube). We suggest that $α_{mt}ß1$ 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; Thesleff *et al.*, 1995).

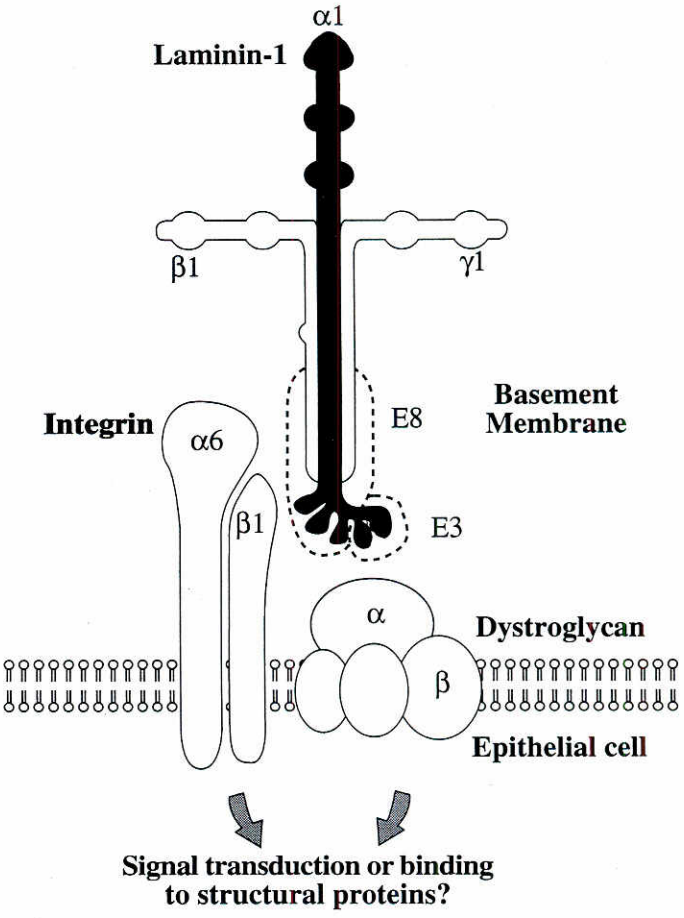

Fig. 3. Scheme of interactions between the laminin-1 complex with its 2 receptor complexes during epithelial morphogenesis. *The interactions between laminin γ1 chain and fragment E8 and integrin α6β1 are fairly well established. It is still not very well known how α-dystroglycan binds to laminin-1, but current evidence suggests that laminin-1 fragment E3 binds to α-dystroglycan.*

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 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 β1, 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.,* 1990). 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.,* 1988) 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 β1 and γ1 chains do not contribute to E3 fragment (Sasaki *et al.,* 1988). The E3 fragment of laminin-α1 chain should thus have a receptor on the epithelial cell surface. The dystroglycan complex could be such 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 nidogen, 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 (Salmivirta *et al.,* 1994) and proteoglycans including syndecan-1 are expressed in embryonic kidney (Vainio *et al.,* 1992; Lash *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 kidney 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, 1993; 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.,* 1993; 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-

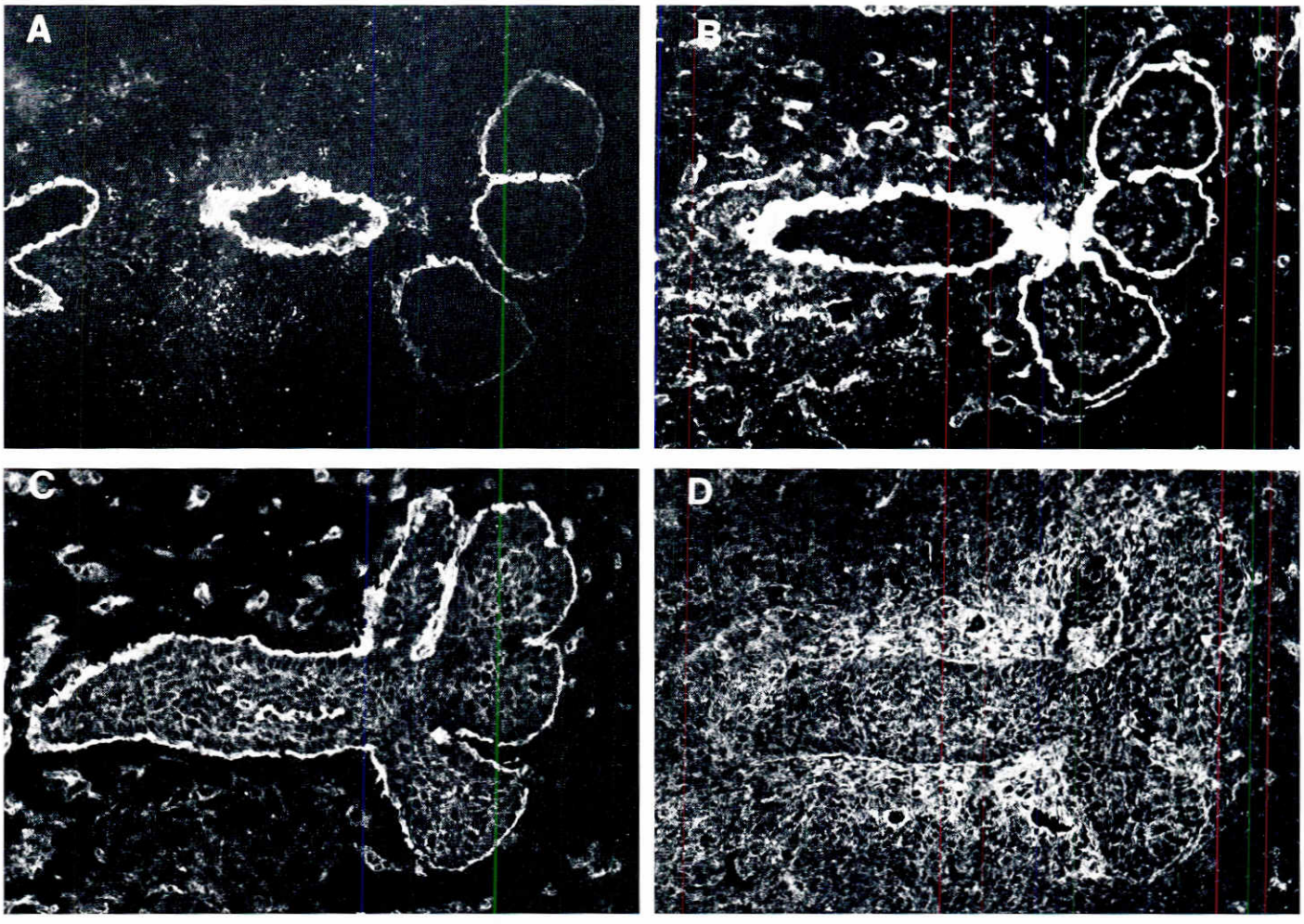

Fig. 4. Expression of laminin chains and integrin chains α6 and β1 during branching epithelial morphogenesis of submandibular gland. *Monoclonal antibodies against E3 fragment of laminin α1 chain stains only basement membranes of epithelia* **(A),** *whereas polyclonal antibodies detecting laminin chain 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).*

onic 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 one 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 epimorphin 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 (Chiquet-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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