The Laminins and their Receptors

BY

MARIA FERLETTA
Basement membranes are thin extracellular sheets that surround muscle, fat and peripheral nerve cells and underlay epithelial and endothelial cells. Laminins are one of the main protein families of these matrices. Integrins and dystroglycan are receptors for laminins, connecting cells to basement membranes. Each laminin consists of three different chains, (α, β, γ). Laminin-1 (α1β1γ1) was the first laminin to be found and is the most frequently studied. Despite this, it was unclear where its α1 chain was expressed. A restricted distribution of the α1 chain in the adult epithelial basement membranes was demonstrated in the present study. In contrast, dystroglycan was found to have a much broader distribution. Dystroglycan is an important receptor for α2-laminins in muscle, but binds also α1-laminins. The more ubiquitous α5-laminins were also shown to bind dystroglycan, but with distinctly lower affinity than α1- and α2-laminins.

The biological roles of different laminin isoforms have been investigated. Differences were found in the capacity of various tested laminins to promote epithelial cell adhesion. The α5-laminins were potent adhesive substrates, a property shown to be dependent on α3 and α6 integrins. Each receptor alone could promote efficient epithelial cell adhesion to α5-laminins. Yet, only α6 integrin and in particular the α6A cytoplasmic splice variant could be linked to laminin-mediated activation of a mitogen-activated protein kinase (MAP kinase) pathway. Attachment to either α1- or α5-laminins activated extracellular-signal regulated kinase (ERK) in cells expressing the integrin α6A variant, but not in cells expressing α6B. A new role for dystroglycan as a suppressor of this activation was demonstrated. Dystroglycan antibodies, or recombinant fragments with high affinity for dystroglycan, decreased ERK activation induced by integrin α6 antibodies. Integrin αvβ3 was identified as a novel co-receptor for α5-laminin trimers. Cell attachment to α5-laminins was found to facilitate growth factor induced cell proliferation. This proliferation could be blocked by antibodies against integrin αvβ3 or by an inhibitor of the MEK/ERK pathway. Therefore, integrin αvβ3 binding to α5-laminins could be of biological significance.

Keywords: Basement membrane, laminin, integrin, dystroglycan, epithelial cells, signal transduction

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Broad distribution of dystroglycan in adult mouse tissues (II)
Laminin-10/11 is a strong adhesive substrate for epithelial cells and is mediating ERK activation through integrin α6β1 (III, IV)
Laminin α5 containing laminins are ligands for integrin αvβ3 and are involved in FGF and VEGF stimulated proliferation of ECV304 cells (V)

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POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA
Cellers samspel med omgivande proteiner

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ABBREVIATIONS

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BM</td>
<td>Basement membrane</td>
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<tr>
<td>CCN</td>
<td>Cyr61, CTGF, Nov</td>
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<tr>
<td>DGC</td>
<td>Dystrophin-glycoprotein complex</td>
</tr>
<tr>
<td>E</td>
<td>Embryonic day</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
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<tr>
<td>ERK</td>
<td>Extracellular signal regulated protein kinase</td>
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<tr>
<td>ES</td>
<td>Embryonic stem cell</td>
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<tr>
<td>FAK</td>
<td>Focal adhesion kinase</td>
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<tr>
<td>FGF</td>
<td>Fibroblast growth factor</td>
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<tr>
<td>Grb2</td>
<td>Growth-factor-receptor-bound protein 2</td>
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<tr>
<td>JEB</td>
<td>Junctional epidermolysis bullosa</td>
</tr>
<tr>
<td>LCMV</td>
<td>Lymphocytic choriomeningitis</td>
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<td>LE</td>
<td>Laminin EGF-like repeats</td>
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<td>LFV</td>
<td>Lassa fever virus</td>
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<td>LG</td>
<td>Laminin globular domain</td>
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<tr>
<td>MAP kinase</td>
<td>Mitogen-activated protein kinase</td>
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<tr>
<td>MEK</td>
<td>MAPK/ERK kinase</td>
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<tr>
<td>NMJ</td>
<td>Neuromuscular junction</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>PKB</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>PMA</td>
<td>Phorbol 12-myristate 13-acetate</td>
</tr>
<tr>
<td>RGD</td>
<td>Arg-Gly-Asp</td>
</tr>
<tr>
<td>RTK</td>
<td>Receptor tyrosine kinases</td>
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<tr>
<td>SAPK/JNK</td>
<td>Stress activated protein kinase/Jun N-terminal kinase</td>
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<td>SOS</td>
<td>Son of sevenless</td>
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<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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INTRODUCTION

Cells continuously send and receive information, either from cell-cell contacts, from the surrounding extracellular matrix (ECM) or from circulating soluble hormones and growth factors. The ECM is a complex network surrounding cells, serving as a structural element in tissues. The ECM also provides the cells with information about their environment and thus influences tissue development. The ECM is composed of glycoproteins such as laminins, collagens and proteoglycans, which are secreted by the cells and assembled into networks to which cells can adhere. The ECM is present from the two-cell stage in mammalian embryos. The composition of the ECM varies and depends on the tissue and the stage of development. The matrix can be calcified and form hard teeth and bone structure, or it can be very tensile. In this thesis I will focus on the protein family laminin and its cell receptors. The laminin family is a component of the basement membrane, a specialized form of ECM. The adhesion of cells to basement membranes influences the tissue formation in many ways. In addition to stabilizing the tissue, basement membranes regulate cell behavior through different signaling cascades, which can start differentiation, movements, proliferation, growth and cell survival. It is known that disturbances or mutations in basement membrane proteins can lead to diseases such as muscular dystrophies, epidermolysis bullosa, or cancer. Thickened basement membranes are seen in later stages of diabetes. Further, some bacteria and viruses use the basement membrane as a link to infect cells. The basement membrane components are well conserved throughout the evolution, indicating the importance of correct basement membranes for development of a multicellular organism.

BASEMENT MEMBRANES

Basement membranes are thin extracellular sheets that surround muscle, fat, and peripheral nerve cells, and underlay all epithelial and endothelial cells. Basement membranes also provide tissue compartmentalization in many tissues, acting as a barrier for cells. Different basement membranes are quite heterogeneous molecularly and vary from tissue to tissue, as well as in the same tissue at different developmental stages (Durbeej et al., 1996). A few protein families are the main components of basement membranes. These include laminins and
collagen IVs, nidogens and the proteoglycans agrin and perlecan. In addition, there are several associated proteins including collagen XV, fibulins, and collagen XVIII (Erickson and Couchman, 2000; Timpl, 1996).

The basement membrane contains two networks that can self assemble independently of each other. Laminin isoforms form one of the networks by interacting with their N-terminal modules of the three short arms (Timpl and Brown, 1996). The second network consists of different type IV collagens. The collagen network is considered to stand for the mechanical stability and the laminin network for the dynamic flexibility in the basement membrane (Timpl and Brown, 1996; Yurchenco and O’Rear, 1994). The two networks are believed to be connected to each other by nidogens (Timpl, 1996; Timpl and Brown, 1996; Yurchenco and O’Rear, 1994).

THE LAMININ FAMILY

Structure

Laminins are large cross- or T-shaped glycoproteins. Each laminin is composed of one α, one β and one γ chain. So far 5 α, 3 β, and 3 γ chains are known, giving rise to at least 15 laminin heterotrimers (Fig. 1.) (Colognato and Yurchenco, 2000; Libby et al., 2000; Brunken, presentation at the 10th ISBM meeting). All chains are different gene products and a few of the individual polypeptide chains can be alternatively spliced (Miner et al., 1997), while some undergo extracellular proteolytic processing (Miner et al., 1997; Talts et al., 1998; Talts et al., 2000). Laminin trimers are assembled intracellularly, mediated by interactions of domain I of the C-terminal part of the chains (Beck et al., 1993) (Nomizu et al., 1994). All laminins share the same domain structure (fig. 2.). The α chains have five homologous laminin globular (LG) domains, named LG1-5, at the C-terminal end. Many cell surface receptors such as dystroglycan and integrins bind to the LG domains (Hohenester et al., 1999; Talts et al., 1999; Talts and Timpl, 1999). Domains I and II make up the triple α-helical coiled-coil structure forming the rod-like part of the long arm. Domain III and V in all three short arms of the laminin molecule consist of three to eight laminin EGF-like
Fig. 1. The laminin family. Chain composition and schematic structure of each laminin isoform.
Fig. 2. Structural model for laminin-1 composed of the α1, β1 and γ1 chains. Domains are indicated by roman numbers. The E3 and E8 (elastase) fragments are indicated by dashed boxes, the P1 (pepsin) fragment is indicated with a box. Binding sites for cell receptors and other matrix proteins are indicated with arrows.
(LE) repeats. They are rod-like and separate the globular domains IVa, IVb and VI.

The globular domain VI, also called the LN module, terminates the short arms and is the most conserved part of the laminins. It has been shown that laminin isoforms can self-assemble into a network when the three short arms are interacting via the N-terminal of the LN modules. In agreement with this only laminins with three full length chains, such as laminin-1, -2 and -4, have been reported to stabilize such a network (Cheng et al., 1997; Yurchenco and O’Rear, 1994). The α5 containing laminin-10 and -11 can probably also self-assemble.

Elastase and pepsin digestion of laminin-1 give some characteristic laminin fragments called E3, E8 and P1 (fig. 2), which all are recognized by integrin receptors (Paulsson et al., 1985; Rohde et al., 1980; Sonnenberg et al., 1990). The E3 fragment corresponds to the globular domains LG4 and LG5 of the C-terminal end of the α1 chain to which dystroglycan and heparin bind (Andac et al., 1999).

The laminins based on their α chains

At present 15 laminins are known and it is still possible that new chain combinations will be discovered (Cologna to and Yurchenco, 2000; Libby et al., 2000; Brunken, presentation at the 10th ISBM meeting). They are here grouped based on their α subunit, and some characteristics of each laminin will be discussed. The phenotypes of mice lacking laminin chains have been summarized in table 1.

Laminins containing the α1 chain

Laminin-1 (α1β1γ1), the first found laminin (Timpl et al., 1979) has been the most studied so far. It seems to be a major laminin during early embryogenesis. Laminin β1 and γ1 chains are expressed already at the two-four-cell stage intracellularly, and the α1 chain is expressed at the 16-cell stage. β1 and γ1 chains are expressed in almost all basement membranes and can assemble with most known α chains. The α1 chain is largely limited to the epithelial basement membrane during embryogenesis (Ekblom et al., 1990; Sorokin et al., 1997; Thomas and Dziadek, 1993), but there has been conflicting data about its
<table>
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<tr>
<th>Laminin subunit</th>
<th>Laminin deficient phenotypes</th>
<th>References</th>
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<tr>
<td>α1</td>
<td>Deletion of the E3 fragment of the α1 chain is embryonic lethal, the mouse dies shortly after implantation.</td>
<td>(Schéele et al., 2001)</td>
</tr>
<tr>
<td>α2</td>
<td>Deficient mice display growth retardation and severe muscular dystrophic symptom and die at 5 weeks of age. Spontaneous mutant mice dy21 and dy suffer from merosin-deficient congenital muscular dystrophy.</td>
<td>(Miyagoe et al., 1997; Sunada et al., 1994; Xu et al., 1994)</td>
</tr>
<tr>
<td>α3</td>
<td>The mouse dies 2-3 days after birth. It develops progressive blistering of forepaws, limbs and mucosa.</td>
<td>(Ryan et al., 1999)</td>
</tr>
<tr>
<td>α4</td>
<td>The deficient mouse develops normally and is fertile but have uncoordinated movements and changes in NMJ.</td>
<td>(Patton et al., 2001)</td>
</tr>
<tr>
<td>α5</td>
<td>The deletion is embryonic lethal at E13.5-16.5. The mouse has defects in limb and placenta development, exencephaly, and defects on internal organs, lung, heart, intestine and kidney.</td>
<td>(Miner et al., 1998; Miner and Li, 2000)</td>
</tr>
<tr>
<td>β1</td>
<td>N.D.</td>
<td></td>
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<tr>
<td>β2</td>
<td>The mutant has postnatal lethal defects in glomerula filtration and in NMJ.</td>
<td>(Noakes et al., 1995a; Noakes et al., 1995b)</td>
</tr>
<tr>
<td>β3</td>
<td>Mutations are found in the LAMB3 gene in patients suffering from JEB.</td>
<td>(Kivirikko et al., 1996)</td>
</tr>
<tr>
<td>γ1</td>
<td>The deficient mice die before E5.5. Complete lack of basement membranes.</td>
<td>(Smyth et al., 1999)</td>
</tr>
<tr>
<td>γ2</td>
<td>Mutations in the LAMC2 gene are found in patients with the disease JEB.</td>
<td>(Pulkkinen et al., 1994)</td>
</tr>
<tr>
<td>γ3</td>
<td>N.D.</td>
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Table 1. Summary of the phenotypes of deleted laminin chains. N.D., no data.
distribution in the adult body. Some data suggested a broad expression pattern for the laminin α1 chain at adult stage (Engvall et al., 1990; Sanes et al., 1990; Virtanen et al., 1995), but this was based on studies with the monoclonal antibody 4C7 (Engvall et al., 1986), now known to recognize the laminin α5 chain (Tiger et al., 1997).

Mouse laminin-1 can strongly stimulate cell attachment, migration and differentiation (Colognato and Yurchenco, 2000; Ekblom, 1996). In vitro studies suggest that laminin α1 chain plays a role in branching epithelial morphogenesis and in neurite outgrowth (Edgar, 1996; Kadoya et al., 1995). Tumor growth in nude mice was significantly increased by transfecting the α1 chain in colon cancer cells, suggesting that laminin α1 chain is a potent activator of cell growth (De Arcangelis et al., 2001). Mice with targeted deletion of the laminin γ1 chain gene die before day E5.5, and no covalently bound laminin subunits were found, which was coupled with complete lack of basement membrane structures (Smyth et al., 1999). Mice with a targeted disruption of the C-terminal domain E3 of the laminin α1 chain die shortly after implantation (Schéele et al., 2001). Hence, binding of the cell receptor dystroglycan for the E3 fragment of laminin α1 could be important for cell survival. These few examples from various approaches performed during the past 20 years suggest a significant role for laminin-1 in epithelial cell differentiation and survival.

The α1 chain is also found in laminin-3 (α1β2γ1), which can be found in human placenta (Champliaud et al., 2000), and possibly also in developing human muscle in myotendinous junctions, since laminin α1 chain is co-distributed with the β2 chain there (Pedrosa-Domellöf et al., 2000). The distribution of the β2 chain could be broader than often assumed, so it could be of interest to study laminin-3 in more detail.

**Laminins containing the α2 chain**

The laminin α2 chain appears in three different laminins, laminin-2 (α2β1γ1), laminin-4 (α2β2γ1) and laminin-12 (α2β1γ3). The laminin α2 chain is presumably not expressed before day 11 of mouse development, but then it becomes predominantly expressed in skeletal and heart muscle, as well as in peripheral nerves. Further it has been identified in lung, intestinal crypts, testis, kidney, liver, skin, and brain vasculature (Leivo et al., 1988 Lefebvre et al., 1999; Miner et al., 1997; Sasaki et al., 2002). The laminin-12 heterotrimer has been identified in human placenta and was the first laminin not to be localized
within the basement membrane. The γ3 mRNA expression was very broad, with high levels in spleen, testis, lung and placenta. The γ3 chain was also found on the apical surface of ciliated epithelial cells of lung, oviducts, and epididymis. In human skin, γ3 was found along the dermal-epidermal junction (Iivanainen et al., 1999; Koch et al., 1999).

Mutations in the laminin α2 chain in humans have been found in patients suffering from congenital muscular dystrophies (McGowan and Marinkovich, 2000). There are several mouse models with muscular dystrophies with defective laminin α2 chain. The dyW and dy3 mice have engineered mutations, while dy and dy23 mice have spontaneous mutations in the α2 chain (Kuang et al., 1998b; Miyagoe et al., 1997; Sunada et al., 1994; Xu et al., 1994). Disruption of the LAMA2 gene in embryonic stem cells did not abrogate myotube development, but the myotubes failed to contract and underwent degradation and apoptosis, suggesting that laminin α2 ensures stable attachment of muscle fibers during contraction (Kuang et al., 1998a). To experimentally study this issue further, the mutant dyW mouse with complete lack of laminin α2 was mated with transgenic mice carrying the human LAMA2 gene, regulated by a muscle specific promoter. The human laminin α2 protein became localized in skeletal and heart muscle, and the life span increased in these mice. However, from 3 weeks of age, these mice became increasingly paralyzed and developed muscle contractures. Most likely, this is a neurological problem because the human laminin α2 chain was not re-expressed in nerves (Kuang et al., 1998b).

Based on immunofluorescence data with a monoclonal antibody, the β2 chain has been suggested to have a fairly restricted expression as compared with the β1 chain. It was reported to be expressed in skeletal neuromuscular junctions, basement membrane surrounding Schwann cells, glomerular basement membrane, and in some arterioles and vessels (Durbeej et al., 1996; Miner and Patton, 1999). However, this apparent restricted expression could be due to masking of epitopes (Durbeej et al., 1996), and the β2- laminins could thus be common laminins in many locations. Mice missing the laminin β2 chain die 2-4 weeks after birth possibly because of defects in the renal glomerulus, causing filtration problems. In addition, they have defects in the neuromuscular junction (Noakes et al., 1995a; Noakes et al., 1995b).
Laminins containing the $\alpha 3$ chain

In laminin-5 ($\alpha 3\beta 3\gamma 2$) all three chains are truncated. Laminin-5 is expressed in the basement membrane of stratified epithelia as one of the molecules making up the hemidesmosomal complex. Laminin-5 is the main form of laminin in skin and bladder, and is also found in oesophagus and large intestinal epithelium (Miner et al., 1997; Tunggal et al., 2000). Mutations in laminin $\alpha 3$, $\beta 3$, or $\gamma 2$ chains have been found in patients suffering from junctional epidermolysis bullosa (JEB), characterized by skin fragility. Blisters can also occur on mucous membranes and may affect internal organs (Christiano and Uitto, 1996; Kivirikko et al., 1996; Pulkkinen et al., 1994). Laminin $\alpha 3$ deficient mice die 2-3 days after birth and develop progressive blistering of the forepaws, limbs and oral mucosa (Ryan et al., 1999). The epidermis of these mice contained clusters of cells with an undifferentiated morphology. A similar pathology was seen in $\beta 4$-integrin null mice (Dowling et al., 1996; van der Neut et al., 1996). The skin phenotype in mouse is comparable with the human disease JEB. The same blistering phenotype was also seen in mice with a spontaneous disruption in the laminin $\beta 3$ subunit (Kuster et al., 1997).

Laminin-6 ($\alpha 3\beta 1\gamma 1$) is expressed at the same places as laminin-5 (Miner et al., 1997; Tunggal et al., 2000), and laminin-7 ($\alpha 3\beta 2\gamma 1$) is found in the amnion (Champliaud et al., 1996). Laminins, which lack one or more LN domains can not self-aggregate into a network, but both laminin-6 and -7 can associate with laminin-5 and co-polymerize to a stable basement membrane formation (Champliaud et al., 1996).

Laminins containing the $\alpha 4$ chain

Laminin-8 ($\alpha 4\beta 1\gamma 1$), laminin-9 ($\alpha 4\beta 2\gamma 1$) and laminin-14 ($\alpha 4\beta 2\gamma 3$) are the laminins containing the $\alpha 4$ chain. The laminin $\alpha 4$ chain is widely expressed in brain, intestine, heart, kidney, liver, lung, muscle and skin (Miner et al., 1997; Talts et al., 2000). Libby et al recently found the combination for the laminin-14 heterotrimer in the retinal matrix (Libby et al., 2000).

Laminin $\alpha 4$ chain deficient mice develop normally and are fertile, but have uncoordinated movements of the hindlimbs. Analyses at ultra structural levels showed that the active zones in the nerve terminal of the neuromuscular junction (NMJ) were not positioned directly opposite each other. Studies of the laminin $\alpha 4$ -/- mouse suggest that the $\alpha 4$ chain regulates the localization of the
active zones to each other in the nerve terminals and that something else controls
the formation (Patton et al., 2001). In addition, β2 deficient mice have defects in
the NMJ (Noakes et al., 1995a; Noakes et al., 1995b).

**Laminins containing the α5 chain**

Laminin α5 chain has a broad distribution in the embryo, but it is absent from
most embryonic blood vessels (Durbeej et al., 1996; Sorokin et al., 1997). The
α5 chain is found in developing epithelial sheets known to produce very small
amounts of the α1 chain, as shown for the embryonic kidney (Durbeej et al.,
1996). In the adult stage the laminin α5 chain is a major α chain of most
basement membranes, where it forms the laminin-10 and -11 trimers (Miner et
al., 1995; Miner et al., 1997; Sorokin et al., 1997). Laminin-11 (α5β2γ1) is
presumably present in the glomerular basement membrane, neuromuscular
synaptic cleft in skeletal muscle, in placenta and lung. Motorneurons and
Schwann cells recognize laminin-11 and stop growing upon contact (Miner and
Patton, 1999; Patton et al., 1997). Laminin-15 (α5β2γ3) was recently found in the
retinal matrix (Libby et al., 2000). The laminin-10/11 complex has been shown
to stimulate cell adhesion for various cell types (Ferletta and Ekblom, 1999; Gu
et al., 1999; Kikkawa et al., 1998; see results).

The β2 deficient mice, which have defective glomerular filtration and
defects in the NMJ also show a loss of the α5 chain in the synaptic basement
membrane (Noakes et al., 1995a; Patton et al., 1997). Mouse embryos lacking
the laminin α5 chain die late during embryogenesis between E13.5 and 16.5.
Defects were seen in many different tissues, for example the limbs failed to form
separate digits (syndactyly) and the brain was enlarged, misshaped and not
covered by skin in 60% of the mice. Also, the placental labyrinth was
malformed. Additional defects were seen in some internal organs such as lung,
heart, intestine, and kidney (Miner et al., 1998; Miner and Li, 2000).

**BASEMENT MEMBRANE COLLAGENS**

The collagen super family consists of at least 24 protein members found in the
ECM, and the super family is divided into different families depending on their
assembly and on other features (Myllyharju and Kivirikko, 2001). Collagens are
formed from three α chains, arranged in a triple-helix. The type IV collagen
family is a very common component of the basement membrane. There are six known \( \alpha \) chains of the collagen type IV family: the most common collagen type IV isoform consists of two \( \alpha_1 \) chains and one \( \alpha_2 \) chain (Brown and Timpl, 1995; Kuhn, 1995). Collagen type IV triple helices forms a covalently stabilized polymer network through amino-terminal and carboxy-terminal bonds, and interacts laterally with their triple-helical domains. Collagen IV belongs to the non-fibrillar, network-forming collagens (Kuhn, 1995; Yurchenco and O’Rear, 1994). Collagen type I and IV bind to the globular domain 2 of nidogen-1, and nidogen binds closely to the C-terminal end of collagen type IV (Aumailley et al., 1989; Kohfeldt et al., 1998). Interestingly, collagen IV does not bind laminin, but supramolecular complexes are formed in solutions containing laminin-nidogen and collagen type IV (Aumailley et al., 1989). Collagen type IV is involved in interactions with cells and this is mediated among others by the integrins \( \alpha_1 \beta_1 \), \( \alpha_2 \beta_1 \) and \( \alpha_{11} \beta_1 \) (Kuhn, 1995; Tiger et al., 2001). Integrin \( \alpha_1 \beta_1 \) and \( \alpha_2 \beta_1 \) bind to distinct epitopes of collagen within the triple helix (Kuhn, 1995).

Collagen XVIII is the second most abundant basement membrane collagen. Three different forms of collagen XVIII with various tissue distributions have been found in mouse. Among other places, expression was seen in the vascular system. Collagen type XV is a structurally homologous to type XVIII collagen and has a widespread tissue expression. Collagen XVIII has been shown to be a heparan sulfate proteoglycan and collagen XV is a disulfide-bound chondroitin sulfate proteoglycan (Erickson and Couchman, 2000; Marneros and Olsen, 2001). Both collagens bind the laminin-nidogen complex, but collagen XVIII binds with stronger affinity (Sasaki et al., 2000). Recombinant proteins of the C-terminal part of collagen XVIII, called endostatin, bind to \( \alpha_5 \)- and \( \alpha_v \)- integrins on the surface of endothelial cells (Marneros and Olsen, 2001; Rehn et al., 2001). Endostatin has also been shown to have tumor-suppressing activity and to inhibit endothelial proliferation (Erickson and Couchman, 2000; Marneros and Olsen, 2001).
NIDOGENS

Nidogens, also called entactins, are thought to connect the laminin and the collagen type IV network. So far two nidogens have been identified (Durkin et al., 1988; Kimura et al., 1998; Kohfeldt et al., 1998; Mann et al., 1989). The two nidogens have a similar shape and consist of three globular domains connected with a flexible link and a rod-like domain (Fox et al., 1991; Kohfeldt et al., 1998; Mayer et al., 1995). Nidogen-1 has been shown to bind several basement membrane proteins such as laminins, collagen type I, IV, XV and XVIII, perlecan and fibulins (Kohfeldt et al., 1998; Mayer et al., 1993; Sasaki et al., 2000). The nidogen-1 binding site on laminin has been mapped to the laminin γ1 chain and more precisely to the LE module γ1III4 (fig. 2) (Mayer et al., 1993). Since nidogen-1 binds to the γ-chain of laminin, it can bind 10 out of the known 15 laminin trimers. Human nidogen-2 binds collagen I, IV and perlecan, but the binding to laminin-1 and the γ1III4 fragment is only moderate compared to that of nidogen-1 (Kohfeldt et al., 1998).

Nidogen-2 may be more cell adhesive than nidogen-1, but the responsible receptors for nidogen-2 are not yet known. An RGD site has been identified in the rod-like region of nidogen-1, indicating that the binding could be mediated by integrins (Kohfeldt et al., 1998; Mann et al., 1989). Based on organ cultures studies, it has been suggested that the laminin-nidogen interactions are important for epithelial morphogenesis. Antibodies against the nidogen-1 binding site on laminin inhibited branching epithelial morphogenesis of embryonic kidney, lung and submandibular gland in vitro. Epidermal growth factor (EGF) increased expression of nidogen-1, and also counteracted the effect of the blocking antibodies (Ekblom et al., 1994; Kadoya et al., 1997). Surprisingly, the nidogen-1 deficient mice are viable and fertile. This could be due to a compensatory effect of nidogen-2, since the expression patterns of the two nidogens are very similar in mice (Murshed et al., 2000; Salmivirta et al., 1999). In mice lacking the nidogen binding site on laminin γ1 chain, laminin heterotrimers are formed but nidogen-1 is absent from basement membranes. The mice die postnatally due to severe kidney and lung defects (Halfter et al., 2001). This confirms that the laminin γ1 nidogen-binding site is crucial for development of some epithelia and that proper binding of nidogen to laminin γ1 is required.
LAMININ RECEPTORS

Interactions between ECM and cells are mediated by transmembrane cell surface receptors, which link the ECM to intracellular signaling pathways. Dystroglycan and integrins have been shown to be main receptors for laminins. Figure 3 shows a schematic model of laminin and the receptors.

Dystroglycan

Structure of dystroglycan

The protein complex dystroglycan is derived from one single transcript that is divided into two subunits, α- and β-dystroglycan, as a result of post-translational modifications of the precursor protein (Ibraghimov-Beskrovnaya et al., 1992). α-Dystroglycan is noncovalently attached to β-dystroglycan. β-Dystroglycan in turn spans through the plasma membrane and binds to cytoskeletal proteins at the inside of the cell (Ervasti and Campbell, 1991). Dystroglycan was first found in skeletal muscle as a component of the dystrophin-glycoprotein complex (DGC) (Ervasti et al., 1990) but has later been shown to have a much broader distribution and is found in many non muscle cells (Ibraghimov-Beskrovnaya et al., 1992). In particular, dystroglycan is prominent on the basal side of epithelial cells (Durbeej et al., 1998a; Durbeej et al., 1998b).

In muscle, dystroglycan associates with the sarcoglycan-sarcospan complex (α-, β-, γ- and δ-sarcoglycan, and sarcospan). α-Dystroglycan binds to the laminin α2 chain and β-dystroglycan links the complex to dystrophin (Ervasti et al., 1990; Sunada et al., 1994). In epithelial cells, the dystroglycan complex is not associated with any of the known sarcoglycans or sarcospan and it seems that dystroglycan forms distinct complexes depending on the tissue (Durbeej and Campbell, 1999).

Function and ligand binding of dystroglycan

α-Dystroglycan binds to ECM proteins such as laminin-1 and -2, perlecan and agrin. For laminin-1 it has been shown that the E3 fragment, or more precisely the α1LG4 domain, is a major binding site for α-dystroglycan. Also for laminin-2 and perlecan, the binding sites for α-dystroglycan are within the LG domains.
Fig. 3. A schematic model showing the interaction between laminins and their main receptors integrins and dystroglycan, linking epithelial cells to the basement membrane.
(Andac et al., 1999; Talts et al., 1999). Laminin-1, -2 and perlecan all share overlapping binding sites on α-dystroglycan (Talts et al., 1999). The binding of α-dystroglycan to the E3 fragment is calcium-dependent and can be inhibited by heparin (Gee et al., 1993; Pall et al., 1996). A recombinant fragment α5LG1-5 of the laminin α5 chain has been shown to interact with dystroglycan, but this fragment was made in bacteria and could lack important post-translational modifications (Shimizu et al., 1999). In the present study, dystroglycan-laminin-10/11 interactions were therefore studied more in detail.

Muscle β-dystroglycan binds dystrophin, but it has also been shown to bind the growth factor receptor binding protein 2 (Grb2), an adapter protein that can mediate signal transduction through the Ras/Raf pathway (Yang et al., 1995a). This suggests that dystroglycan could be involved in signal transduction as well.

In organ cultures, antibodies that block dystroglycan binding to laminin-1 perturb epithelial development (Durbeej et al., 1995; Durbeej et al., 2001). The phenotype of dystroglycan null mice also indicates that dystroglycan is important for basement membranes during early embryogenesis. These null mice die already at E6.5, due to disruption of Reichert’s membrane (Williamson et al., 1997). Targeted deletion of the E3 domain of laminin α1 chain, containing the binding site of dystroglycan, also display an early phenotype (Schéele et al., 2001). Further, embryoid bodies that lack dystroglycan cannot organize a basement membrane (Henry and Campbell, 1998). Recently it was shown that α-dystroglycan is the cell receptor that arenaviruses use for infecting cells (Cao et al., 1998). Mycobacterium Leprae binds both α-dystroglycan and laminin-2, which together form a bridge for Mycobacterium Leprae to invade Schwann cells and thereby cause damage to peripheral nerves (Rambukkana et al., 1998). Mutations in dystrophin, sarcoglycans or laminin α2 chain all give rise to muscular dystrophies, but no dystrophy has been linked directly to dystroglycan, even though decreased expression of dystroglycan has been noticed. However, chimaeric mice lacking dystroglycan in skeletal muscle do develop muscular dystrophy (Cote et al., 1999).
The Integrin family

The large family of integrins connects cells to the surrounding ECM, and these interactions initiate cell signaling, which mediate a wide variety of biological effects such as cell growth, death, differentiation and movements.

Integrins are integral membrane glycoproteins and they are heterodimers composed of one α and one β chain, which are non-covalently linked to each other. To date, 18 α chains and 8 β chains are known and they associate into 24 different integrins (Hynes, 1999). Many α chains associate with only one β chain, but a few α chains bind to different β chains. For example, α6 can associate with both the β1 and the β4 chains. The integrins are often divided into subfamilies depending on the β subunit. The β1 subunit can combine with 12 different α chains. It is widely expressed and binds many extracellular matrix proteins, such as different laminins. In addition, laminins also bind α6β4 and to some extent three of the integrins containing the αv subunit (αvβ3, αvβ5 and αvβ8) (Hynes, 1992; Hynes, 1999).

Many cells express multiple integrins, each capable to interact with specific extracellular proteins. The α/β pairing specifies the ligand binding abilities of the integrin. Differences in adhesiveness are dependent not only on the affinity to the ligand, but also on cell surface clustering and cytoskeletal associations. Binding to the ligands also requires divalent cations such as Mg^{2+} and Ca^{2+}, and alterations in the divalent cation composition can influence cell adhesion (Humphries, 2000; Plow et al., 2000). Most integrins bind more than one ECM protein, and one ligand can recognize several integrins.

Integrin structure

The N-terminal parts of both the α and the β subunits are important for ligand binding. The α integrin subunit contains a large N-terminal extracellular domain with 7 conserved repeats, of which 3 repeats has putative divalent cation-binding sites (Loftus et al., 1994; Corbi et al., 1987). These seven repeats are folded into a seven-bladed β-propeller, each containing 4 β-sheets (Springer, 1997). Some integrins contain an I-domain of 200 amino acids (also known as the A-domain), which is inserted between the second and the third N-terminal repeat. This I-domain has been shown to contain a metal ion-dependent adhesive site and recognizes binding ligands to the integrin (Kern et al., 1994; Lee et al., 1995). The transmembrane part of the integrin subunits is highly conserved and the...
transmembrane and cytoplasmic domains are not necessary for ligand binding (Dana et al., 1991). The extracellular parts have been suggested to be involved in the assembly of the heterodimer (Humphries, 2000), but in one study deletions in the integrin α6A cytoplasmic domain prevented association with the β1 subunit (De Melker et al., 1997). A number of integrin subunits (α3, α5, α6, α7, α8, αv and αIIb) are posttranslationally cleaved in the extracellular part close to the membrane spanning region, resulting in two chains (one N-terminal heavy chain and one C-terminal light chain) which are held together by a disulfide bridge (Corbi et al., 1990; Hynes, 1992). Uncleaved α6 integrin can still dimerize with the β4 subunit (Lehmann et al., 1996) as well as with the β1 subunit (Delwel et al., 1996). Further, the cells could attach to laminin-1 after stimulation with antibodies against the β1 subunit and thus outside/in signaling was not affected. On the contrary, PMA-induced inside/out signaling was disturbed, since the cells could not bind to laminin-1 after PMA stimulation (Delwel et al., 1996). This suggests that the proteolytic cleavage is important for integrin activation.

The integrin cytoplasmic domain connects the extracellular environment with the intracellular structures and interacts directly or indirectly with the cytoskeletal-signaling network. Both the α and the β cytoplasmic domains have been shown to be involved in signaling. Several cytoplasmic proteins such as talin, α-actinin and focal adhesion kinase (FAK) bind directly to the integrin β1 cytoplasmic domain (Burridge and Chrzanowska-Wodnicka, 1996; Yamada and Geiger, 1997). The cytoplasmic domain is usually very short and does not contain more than 50 amino acids, except for the β4 subunit, which has a larger cytoplasmic domain with more than 1000 amino acids (Hynes, 1992).

**Alternative splice variants**

Some integrin subunits undergo alternative splicing of their extracellular or cytoplasmic domain in a tissue-type specific manner. The alternative splicing increases the integrin diversity even more. Alternative splicing of the cytoplasmic domains suggests specific intracellular functions as well as ability to mediate different signals. A few of the laminin binding integrins including α3β1, α6β1 and α7β1 undergo alternative splicing. Both the α3 and the α6 subunit are spliced into A and B variants. This results in a frameshift, which leads to two different cytoplasmic domains (Hogervorst et al., 1991; Tamura et
The α7 subunit undergoes alternative splicing in the extracellular as well as in the cytoplasmic domain. In addition to A and B splice variants, α7 also has a C cytoplasmic variant and two extracellular variants named X1 and X2 (Ziober et al., 1993).

Five different splice variants of the β1 subunit have been found. The β1A variant is the most widely expressed and found in most tissues and cells, but in cardiac and skeletal muscle, for example, the β1A subunit is displaced by the β1D variant (de Melker and Sonnenberg, 1999).

**Integrin interactions and functions**

There is a lot of information available about integrin expression, function and their different binding partners. Here I will concentrate on a few integrins, which are main receptors for laminins. Some of these integrins bind only one specific ligand but other integrins as for example integrin α6β1 is a receptor for many different ligands. Phenotypes of mice deficient in different integrin subunits are summarized in table 2.

Integrin α6β1 is a main receptor for laminin-1 and the E8 fragment has been shown to be the binding domain, but integrin α6β1 also binds laminin-2, -4, -5, -8 and -10/11 (Colognato and Yurchenco, 2000; Gu et al., 1999; Kortesmaa et al., 2000). Further, integrin α6β1 binds other types of ligands such as the angiogenic factor Cyr61 and CTGF (Chen et al., 2001). Surprisingly, integrin α6 deficient mice survive to birth, but die shortly thereafter with severe blistering of the skin and of other epithelia (Georges-Labouesse et al., 1996). The integrin α6 subunit also dimerizes with the β4 subunit, and the α6β4 integrin has been shown to be a major receptor for laminin-5 in hemidesmosomes (Marchisio et al., 1993). The β4 null mice also die shortly after birth with detachment of the epidermis and no hemidesmosomes were found (van der Neut et al., 1996). Patients with mutations in either of the laminin-5 chains suffer from epidermolysis bullosa. A very similar phenotype is seen in laminin α3 deficient mice, which develop blisters and die at 2-3 days after birth (Kivirikko et al., 1996; Pulkkinen et al., 1994; Ryan et al., 1999). This supports the idea that integrin α6β4 is a receptor for laminin-5 and that they are important components of the hemidesmosomes. Another integrin subunit that binds laminin-5 is the integrin α3 subunit. Integrin α3 deficient mice show less severe blisters, but in addition they have abnormal epithelia in kidneys and lungs (DiPersio et al., 1997; Kreidberg et al., 1996). The sequence similarity of the integrin α3
<table>
<thead>
<tr>
<th>Integrin subunit</th>
<th>Integrin deficient phenotypes in mice</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1</td>
<td>The mice are viable, but have proliferation defects and a disturbed regulation of collagen synthesis in skin fibroblasts.</td>
<td>(Gardner et al., 1999; Gardner et al., 1996; Pozzi et al., 1998)</td>
</tr>
<tr>
<td>α2</td>
<td>The mutant is viable and fertile, but has defects in mammary gland branching, and platelet adhesion to collagen is defected.</td>
<td>(Holtkotter et al., 2002)</td>
</tr>
<tr>
<td>α3</td>
<td>The mutant mice survive birth but die neonatally. The kidneys and lung developed abnormally, there was reduced branching morphogenesis and disorganized basement membrane in the skin.</td>
<td>(DiPersio et al., 1997; Kreidberg et al., 1996)</td>
</tr>
<tr>
<td>α4</td>
<td>Embryonic lethal, the mutant had defects in the placenta and the heart.</td>
<td>(Yang et al., 1995b)</td>
</tr>
<tr>
<td>α5</td>
<td>The mutant embryo dies around E10-11, the embryo had defects in the posterior trunk and the yolk sac mesodermal structures.</td>
<td>(Yang et al., 1993)</td>
</tr>
<tr>
<td>α6</td>
<td>The mice developed normally before birth but die shortly thereafter with severe blisters of the skin and other epithelia. The phenotype was similar to epidermolysis bullosa and hemidesmosomes were absent from the mutant.</td>
<td>(Georges-Labouesse et al., 1996)</td>
</tr>
<tr>
<td>α7</td>
<td>The mutant is viable and fertile, but histological analysis shows a progressive muscular dystrophy starting soon after birth.</td>
<td>(Mayer et al., 1997)</td>
</tr>
<tr>
<td>α8</td>
<td>Most mice die soon after birth due to kidney defects. Many of the surviving mice have difficulty balancing, consistent with the structural defects of the inner ear.</td>
<td>(Muller et al., 1997) (Littlewood Evans and Muller, 2000)</td>
</tr>
<tr>
<td>α9</td>
<td>The mutant dies 6-12 days after birth with respiratory failure.</td>
<td>(Huang et al., 2000)</td>
</tr>
<tr>
<td>α10</td>
<td>No apparent phenotype.</td>
<td>(Bouvard et al., 2001)</td>
</tr>
<tr>
<td>α11</td>
<td>To be further characterized.</td>
<td>C. Tiger and D. Gullberg unpublished data.</td>
</tr>
<tr>
<td>αv</td>
<td>80% of the mutants die in midgestaion, probably because of placental defects. 20% are born alive with intracellebral hemorrhages.</td>
<td>(Bader et al., 1998)</td>
</tr>
<tr>
<td>β1</td>
<td>Preimplantational lethal did not gastrulate.</td>
<td>(Fassler and Meyer, 1995)</td>
</tr>
<tr>
<td>β4</td>
<td>The homozygous mice die shortly after birth with detachment of the epidermis and other squamous epithelia. No hemidesmosomes were found.</td>
<td>(van der Neut et al., 1996) (Dowling et al., 1996)</td>
</tr>
<tr>
<td>Dystroglycan</td>
<td>Embryonic lethal dies around E6.5 with a disrupted Reichert’s membrane.</td>
<td>(Williamson et al., 1997)</td>
</tr>
</tbody>
</table>

Table 2. Summary of the phenotypes of the laminin receptors.
and α6 subunits suggests that they could compensate for each other. Indeed, mice lacking both integrin α3 and α6 died at E16.5 with more severe abnormalities, they seem to have synergistic activities in limbs, lungs and the urogenital system (De Arcangelis et al., 1999). The preferred ligands for integrin α3β1 seems to be laminin -5, -10, and -11, however integrin α3 is also seen in focal contacts on other matrix components such as laminin-1, fibronectin and collagen IV. In the latter cases it could be that integrin α3β1 is not the main cell adhesion receptor but is recruited to mediate secondary responses to various extracellular matrices (DiPersio et al., 1995).

Integrin αvβ3 is known to bind ligands such as vitronectin, fibronectin, Von Willebrand factor, Cyr61 and denatured collagens in a RGD dependent manner (Chen et al., 2001; Plow et al., 2000). Recently it was shown that integrin αvβ3 also binds recombinant fragment of laminin α5 in a RGD dependent fashion (Sasaki and Timpl, 2001). This suggests that α5 containing laminin trimers might be ligands for integrin αvβ3 (see results). Integrin αvβ3 is suggested to be involved in angiogenesis and tumor growth (Eliceiri and Cheresch, 2001). Deletion of the αv gene is lethal, and the mice exhibit intracerebral and intestinal hemorrhages (Bader et al., 1998). In contrast, mice deficient in integrin β3 subunit develop apparently normal blood vessels (Hodivala-Dilke et al., 1999). There is some evidence for cross talk between growth factor receptors and integrin αvβ3, and integrin αvβ3 co-precipitates with VEGF receptor 2 as well as with PDGF receptor (Eliceiri and Cheresch, 2001).

**CELL SIGNALING**

The integrin-dependent anchorage of cells to the ECM can activate intracellular signaling pathways. The composition of the ECM together with the receptors expressed on the cell surface decides whether a cell will survive, proliferate or exit the cell cycle and differentiate. Integrins can recruit a number of intracellular proteins to specialized focal adhesion complexes. Some proteins in these complexes are structural elements while others mediate signals. There are many known signaling pathways such as MAP kinase, PI3 kinase, Akt, p38MAP kinase, FAK, SAPK/JNK, and Cytokine/JAK/Stat (Aplin et al., 1998). Here I will concentrate on the highly conserved mitogen-activated protein kinase (MAP kinase) cascade and especially on the MEK/ERK pathway.
The MAP kinase family

MAP kinases are a family of serine/threonine kinases found in all eukaryotic organisms. They are involved in many cell programs such as cell proliferation, differentiation, cell movements and death. MAP kinase cascades can be divided into three classical subfamilies (ERK, p38MAPK, SAPK/JNK). The ERK (extracellular signal regulated protein kinase) family plays a critical role in regulation of cell growth and differentiation. The p38MAPK family is activated by different types of environmental stress such as ionizing radiation, heat shock, oxidative stress, osmotic shock, UV-light, inflammatory cytokines and growth factors. p38MAPK regulates proliferation, apoptosis, and cell differentiation. SAPK/JNK (stress activated protein kinase/Jun terminal kinase) family is also activated through environmental stress including UV and γ radiation, inflammatory cytokines and in some instance growth factors. SAPK/JNK pathway has been shown to regulate transcription (Cobb, 1999; Robinson and Cobb, 1997).

Each MAP kinase cascade consists of at least three enzymes that are activated in series (Fig. 4): the MAP kinase or ERK is activated by MAPK/ERK kinase or MEK, which in turn is activated by a MEK kinase or MEKK. The MEK kinase is activated by interactions with small GTPases and/or other protein kinases connecting the MAP kinase module to the cell surface receptor or external stimuli (Cobb, 1999; Robinson and Cobb, 1997).

Basic regulation of the MEK/ERK pathway

The incoming signal, which can be a hormone, growth factor, differentiation factor or a matrix protein, stimulates integrins or receptor tyrosine kinases (RTK). Integrins have no intrinsic kinase activity, whereas RTK become autophosphorylated when activated. The archetypal Ras exchange factor, Sos (son of sevenless), is brought to the cell membrane by the Grb2 adaptor protein. Grb2 recognizes tyrosine phosphate sites on the receptor or on the receptor substrate proteins. Sos enhances GDP release and GTP binding to Ras, which converts Ras into its active form (Li et al., 1993; McCormick, 1993). The GTP activation of Ras functions as an adaptor that binds to Raf and brings it to the plasma membrane where its protein kinase activity is activated and the kinase cascade can start (fig. 5) (Morrison and Cutler, 1997). MEK is activated by phosphorylation of two serine residues. Phosphorylated MEK1/2 in turn
phosphorylates MAP kinases ERK1 and ERK2 (also called p42 and p44 MAP kinase). ERK1 and 2 must be phosphorylated on both their threonine and tyrosine residues for full activation. (Cobb and Goldsmith, 1995). In unstimulated cells ERK1 and 2 are located in the cytoplasm. Phosphorylation of ERK1 and 2 induces dimerization, which seems to be required for translocation to the nucleus (Khokhlatchev et al., 1998). After the nuclear translocation, ERK is thought to phosphorylate a number of transcription factors such as Elk, c-Fos and c-Myc, which in turn serve as important regulators of transcription (Aplin et al., 1998). Phosphorylated ERK has also been found in newly formed focal contacts, suggesting a role for activated ERK in cell adhesion/cytoskeletal network (Fincham et al., 2000). It is also possible that ERKs are involved in negative feedback mechanisms for upstream components by disassembly of the Sos complex and termination of Ras activity (Kolch, 2000).

Fig. 4. Schematic picture over the ERK/MAPK pathway.
The scheme outlined above is simplified, since a large number of components can regulate the ERK pathway at many levels of the cascade. This fine-tuning is probably crucial for proper signaling, but is still poorly understood. A further complication is the accumulating evidence of extensive cross talk between different pathways. For example, PI3K can also mediate MAP kinase activation. PI3Ks have been found to have both lipid kinase activity and serine/threonine kinase activity. When domains responsible for PI3K lipid kinase activity were mutated, the mutant could still activate MAP kinase but not Akt/PKB (protein kinase B) (Bondeva et al., 1998).

Integrins and MAP kinase signaling

Although both integrins and RTK can activate ERK, there are some notable differences. Signals from growth factors usually lead to an immediate strong but short response lasting for a few minutes. The signals produced by integrins are as a rule of lower amplitude, and activated after 30-60 minutes and then sustained for a few hours (Aplin et al., 1998). Interestingly, a third type of ERK activation was recently demonstrated for the CCN growth factor family. Two members of this family were shown to bind integrins α6β1 or αvβ3, which lead to a sustained activation of ERK lasting for up to 12 hours (Chen et al., 2001). At present, it is unclear why ECM components do not stimulate a similar long lasting activation through integrins. One possibility is that some domains of the large ECM components bind other receptors that act as inhibitors of ERK activation. Another possibility is a cross talk between integrins and growth factor receptors.

Activation of the MAP kinase pathway by integrins differs at some points from the basic regulation of the MEK/ERK pathway. Integrins transduce signals by associating with adaptor proteins that connect the integrins to the cytoskeleton, cytoplasmic kinases or transmembrane growth factor receptors. Integrin signaling and cytoskeleton assemblies are tightly linked. When integrins bind to an ECM protein, they become clustered in the plane of the cell membrane and associate with a cytoskeletal and signaling complex that promotes the assembly of actin filaments (Giancotti and Ruoslahti, 1999).

Some integrin-transduced signals are mediated by activation of FAK. FAK might interact directly with the cytoplasmic part of the integrin β subunit or indirectly by cytoskeletal proteins such as talin and paxillin (Akiyama et al., 1994; Aplin et al., 1998; Miyamoto et al., 1995). When FAK is phosphorylated,
a binding site for Src or Fyn is creating a stable complex (Schaller et al., 1994; Schlaepfer and Hunter, 1997). Src then phosphorylates a number of focal adhesion components. Besides this, Src can phosphorylate FAK, which creates a binding site for the Grb2-Sos complex, linking FAK to the MAP kinase pathway (Calalb et al., 1995; Schlaepfer and Hunter, 1997). FAK has also been shown to activate the MAP kinase pathway via Shc, which associates with Grb2 (Schlaepfer and Hunter, 1997; Schlaepfer et al., 1998). In addition, integrins can also mediate MAP kinase activation without FAK involvement. In this case the integrin \( \alpha \) subunit is apparently involved. Ligation of the integrin activates Fyn, which associates with caveolin-1, leading to phosphorylation of Shc and formation of the Shc-Grb2 complex, which finally activates the MAP kinase pathway (fig. 6.) (Wary et al., 1996; Wary et al., 1998; Mainiero et al., 1997).

What is known about laminins and MAP kinase signaling?

Laminin-integrin interactions can in some settings activate ERK, but due to the large number of ligands and receptors, information is only available for some interactions. Further, the cell types used differ and that makes it even more difficult to draw any clear conclusions, and the studies some times contradict each other.

Laminin-5 was reported to activate the Ras-ERK and Rac-Jnk signaling pathways in keratinocytes and the signaling was mediated through integrin \( \alpha 6\beta 4 \) via Shc and Grb2. It was proposed that the major laminin receptors integrin \( \alpha 6\beta 1 \) and \( \alpha 3\beta 1 \) do not belong to the integrins coupled to the ERK pathway (Mainiero et al., 1997; Wary et al., 1996). In addition, Wary et al reported that endothelial cells attached to laminin-1 or laminin-4 were not able to stimulate ERK, through the presumed adhesion receptor, integrin \( \alpha 2\beta 1 \) (Wary et al., 1996).

The results above are in contradiction to what other investigator report. It has been shown that laminin-5 activates ERK phosphorylation in epithelial cells through integrin \( \alpha 3\beta 1 \) leading to cell proliferation, however laminin-1 was not able to activate ERK in these cells (Gonzales et al., 1999). In macrophages, laminin-1 did not activate ERK, but a shorter laminin \( \alpha 1 \) peptide did. The involved receptor has in this case not yet been identified (Khan and Falcone, 2000). Further, it has been shown that laminin-1 can activate ERK in fibroblasts (Chen et al 2001; Fincham et al 2000). Interestingly, macrophages expressing either integrin \( \alpha 6A \) or \( \alpha 6B \) variant did attach to laminin-1, but only cells
Fig. 5. Schematic outline of some of the signaling molecules activated by integrins involved in the MEK/ERK signaling pathway. Arrows indicate activation. See text for details.
expressing the integrin α6A were able to phosphorylate MEK and ERK (Shaw et al., 1995; Wei et al., 1998). However, both α6Aβ1 and α6Bβ1 expressing macrophages were able to induce ERK activation when attached to fibronectin (Wei et al., 1998). Dystroglycan has been reported to have an interplay with integrin α6 on β-cells attached to laminin-1, where integrin α6 promotes proliferation through MEK/ERK signaling and dystroglycan cell differentiation (Jiang et al., 2001). In another report, β-casein expression was inhibited when integrin α6 or β1 subunit was blocked on mammary epithelial cells adhering to laminin-1. When E3 fragments and heparin were added, both cell shape changes and β-casein expression induced by laminin-1 were inhibited. This indicates that the heparin-binding region within the laminin E3 fragment could be one of the participating parts in the interaction of laminin-1 with mammary epithelial cells (Muschler et al., 1999). The receptor involved could be dystroglycan since it binds to the E3 fragment of laminin-1 (Andac et al., 1999; Gee et al., 1993). How dystroglycan could be involved in regulation of ERK is unknown, but it is known that β-dystroglycan interacts with the Grb2 adaptor protein (Russo et al., 2000; Yang et al., 1995a). These conflicting data with laminins involved in ERK signaling are suggesting that ERK activation in response to laminins could be regulated at many levels.
PRESENT INVESTIGATIONS

Specific aims of the thesis

- To compare the distribution of the laminin α1 chain and dystroglycan in adult mouse tissues.
- To study epithelial cell binding to different laminin isoforms
- To identify adhesion receptors for laminin-10/11
- To study signal transduction mediated by laminin receptors

RESULTS AND DISCUSSION

The laminin α1 chain has a restricted distribution in adult mouse tissues (I)

Even though laminin-1 was the first discovered laminin, there have been conflicting data about the expression of its α1 chain. To study the distribution of the laminin α1 chain, many investigators have used the monoclonal antibody 4C7 (Engvall et al., 1986; Engvall et al., 1990; Virtanen et al., 1995). However, it was shown that 4C7 instead reacted with the laminin α5 chain (Kikkawa et al., 1998; Tiger et al., 1997). This was in agreement with reports that showed a broad expression of the laminin α5 chain at adult stage (Miner et al., 1995; Miner et al., 1997). The aim of this study was to clarify the expression pattern of the α1 chain in adult mouse tissues. We used a well-characterized rat monoclonal antibody (mAb 200) against the E3 fragment of the laminin α1 chain in immunofluorescence of frozen sections (Sorokin et al., 1992). We found strong reactivity for the α1 chain in several but not all organs. The reactivity was restricted to epithelial basement membranes.

The reproductive system both in male and female mice stained positive for the α1 chain. Prominent staining was seen in testis, epididymis and prostate. In the female reproductive organs the ovary, placenta and the mammary glands expressed laminin α1. As previously reported, a prominent expression of the laminin α1 chain was seen in the proximal tubes of the kidney (Ekblom et al., 1990; Sorokin et al., 1992) and a faint staining of the urinary bladder. In the central nervous system, larger blood vessel walls expressed the laminin α1 chain. Astrocytes or invaginated epithelial cells rather than endothelial cells are the probable sources of laminin α1 in these tissues (Sixt et al., 2001). Laminin
α1 expression was also seen in the pia mater covering the brain. In sensory organs the vitreous chamber and the lens of the eye expressed the α1 chain.

In the gastrointestinal tract the α1 chain was detected at the basal side of the dentine of unerupted tooth. Yet, no staining was detected with a polyclonal antibody against all three laminin-1 chains (α1β1γ1). This could indicate the presence of a novel laminin isoform, masking of the epitope for β1 and γ1 chains, or unspecific reactivity of mAb 200 in this location. In addition to tooth, Brunner’s gland was the only tissue expressing the α1 chain in the gastrointestinal tract. In endocrine organs, the α1 chain was expressed in the cortex of the adrenal gland. No staining was seen in muscle, heart, cartilage, fat, or Schwann cells.

The study established that the distribution of the laminin α1 chain is restricted to some epithelial basement membranes in the adult mouse body. A very similar distribution of the α1 chain was recently demonstrated for adult human tissues by novel monoclonal antibodies against the human laminin α1 chain (Virtanen et al., 2000). Our findings are also strongly supported by recent quantitative measurements, which demonstrate substantial amounts of laminin α1 chain in those adult mouse tissues that stain positively with mAb 200. However, a few other adult mouse tissues that show no reactivity with mAb 200 (esophagus, intestine, liver, spleen and stomach) nevertheless express the α1 chain. This could be demonstrated by using a polyclonal antibody against the N-terminal part of the α1 chain. Apparently, the C-terminal part of the laminin α1 chain is masked by other ECM components in these tissues (Sasaki et al., 2002). Taken together, current data provide strong support for the view that laminin α1 chain is almost exclusively expressed in epithelial basement membranes both in embryonic and adult tissues. This distribution is conserved between mice and man, suggesting conserved functions for epithelial cells. In these locations, several β and γ chains might associate with the α1 chain, and the trimer composition should be experimentally proven for each case.

**Broad distribution of dystroglycan in adult mouse tissues (II)**

Integrins and dystroglycan are main laminin receptors. Dystroglycan is encoded by a single RNA transcript but is later posttranslationally cleaved into two parts resulting in an extracellular part, α-dystroglycan (156kDa), and a transmembrane subunit, β-dystroglycan (43kDa) (Ervasti and Campbell, 1991; Ibraghimov-Beskrovnaya et al., 1992). Dystroglycan was first shown to bind
laminin-2 in muscle, connecting the cells to the ECM (Ervasti et al., 1990), but northern blot analyses showed that dystroglycan is expressed in non-muscle tissues as well (Ibraghimov-Beskrovnaya et al., 1992; Ibraghimov-Beskrovnaya et al., 1993). Further, dystroglycan was shown to bind laminin-1 (Ibraghimov-Beskrovnaya et al., 1992). Immunofluorescence and in situ hybridization of embryonic tissues identified epithelial cells as major sources of non-muscle dystroglycan. In many embryonic tissues, laminin α1 chain and dystroglycan are coexpressed (Durbeej et al., 1995), suggesting that the binding of the α1LG4 module to dystroglycan is involved in epithelial development (Andac et al., 1999). This is supported by data showing that branching epithelial morphogenesis can be perturbed in vitro by antibodies that block binding of dystroglycan to α1LG4 (Durbeej et al., 1995; Durbeej et al., 2001; Kadoya et al., 1995). The early lethality of dystroglycan null mice at E6.5 also points to this possibility (Williamson et al., 1997). All these data suggest an important role for dystroglycan in basement membrane assembly and mouse development. It was therefore of interest to analyze dystroglycan expression in various adult mouse tissues.

Western blot analysis of the β-dystroglycan subunit showed a broad tissue distribution of the 43kDa protein. Expression was seen in all tested tissues such as in kidney, liver, stomach and uterus. Two different antibodies were used for immunofluorescence; FP-B detecting α/β-dystroglycan and AP83 recognizing only the β subunit. Both antibodies stained the sarcolemmal membrane in skeletal muscle as expected and previously shown (Ibraghimov-Beskrovnaya et al., 1992). At the sarcolemmal membrane dystroglycan was co-localized with the laminin α2 chain (Schuler and Sorokin, 1995). Dystroglycan was broadly expressed in the digestive tract. Expression was seen at the surface epithelium and in the smooth muscles of the following tissues, salivary gland, pancreas, intestinal glands, villi, liver, trachea and kidney. Laminin α2 chain was co-localized in salivary gland, pancreas, intestinal glands, and trachea. In liver, a polyclonal antibody against laminin-1 stained the sinusoidal linings and larger blood vessels but no staining was seen with antibodies against the laminin α1 or α2 chains.

Dystroglycan was also expressed in the reproductive organs at the epithelial cells lining the basement membranes. Expression was detected in the mammary glands, uterus and testis. In mammary gland and testis both laminin α1 and α2 chain co-localized with dystroglycan. In addition, α/β-dystroglycan was expressed in all epidermal layers of the skin. In a few locations such as in
the intestinal glands, spermatogenic cells and Sertoli cells in the testis and in all epidermal layers of the skin dystroglycan staining was seen over the entire cell surface. Dystroglycan has previously been detected at cell-cell contacts by Belkin and Smalheiser (Belkin and Smalheiser, 1996). In a few locations dystroglycan was only detected by one of the antibodies, such as in the tubular basement membrane of the kidney and at the surface epithelial basement membrane in the villi of the small intestine. Here, only the FP-B antibody was reactive, indicating that α-dystroglycan is expressed in the absence of β-dystroglycan, or that the AP83 epitope is masked.

In conclusion, dystroglycan has a broader expression pattern than the laminin α1 or α2 chains in adult mouse. It is expressed at the basal side of most epithelial cells in several analyzed tissues and this suggests that dystroglycan is a cell receptor linking the epithelial cells to the basement membrane. A pericellular expression of dystroglycan was seen in epidermis, which could point to a function for dystroglycan even in hemidesmosomes, but this needs to be further investigated. It is also possible that α-dystroglycan is present without β-dystroglycan at some places, as in the villi of the small intestine and tubular basement membrane of the kidney. It has been suggested that α-dystroglycan is expressed in rat cerebellum without β-dystroglycan (Tian et al., 1996). However, it could be a problem of epitope masking. In most tissues dystroglycan is co-localized with laminin α1 or α2 chain, which are known dystroglycan ligands, but laminin α5 chain has an even broader distribution and could be a ligand as well (Miner et al., 1995; Miner et al., 1997). Recently it was shown that a recombinant fragment of the laminin α5 globular domain produced in bacteria was able to bind α-dystroglycan (Shimizu et al., 1999). In paper IV we therefore compared the binding affinity of laminins to α-dystroglycan.

**Laminin-10/11 is a strong adhesive substrate for epithelial cells and mediates ERK activation through integrin α6β1 (III, IV)**

Cells are probably differentially influenced by adhesion to different laminins, but this has not yet been extensively studied. The monoclonal antibody 4C7 detecting the laminin α5 chain (Tiger et al., 1997) has been used to isolate laminins from placenta. Such commercially available laminins have been widely used. In some reports this laminin is still incorrectly, referred to as human laminin-1.
To evaluate findings obtained with laminins isolated with the 4C7 antibody, it is useful to characterize them in further detail. We therefore identified the chain composition of human placental laminin purified with the 4C7 antibody. Western blot analysis showed that this preparation contained the α5, β1, β2 and the γ1 chains and no α1 chain. Based on these Western blots we assumed that this preparation was containing laminin-10 (α5β1γ1) and -11 (α5β2γ1). It will here be referred to as laminin-10/11. Under reduced conditions the α5 chain seemed to be slightly degraded, but posttranslational cleavage has been reported to give rise to bands sized 380, 350 and 210kD of the laminin α5 chain (Miner et al., 1997). To our knowledge, this is the only commercially available laminin-10/11. Our characterization of this laminin was important for the identification of integrin α6β1 (Gu et al., 1999) and Lutheran glycoprotein (Parsons et al., 2001) as receptors for laminins containing the α5 chain.

We compared the ability of two epithelial like cell lines to adhere to different laminin isoforms. The WI-26 VA4 lung epithelial cell line attached well to laminin-10/11 and to fibronectin. Adhesion to laminin-1 and a mixture of laminin-2 and -4 was slightly less effective. A similar binding profile was established for the WCCS-1 cell line, derived from Wilm’s tumor (Talts et al., 1993). Laminin-10/11 has also been shown to be adhesive for other cell types and was shown to be inhibited by antibodies against either integrin α3β1 (Gehlsen et al., 1989; Kikkawa et al., 1998), integrin α6β1 (Gu et al., 1999) or α6β4 (Kikkawa et al., 2000). Yet, in our initial attempts adhesion of WCCS-1 or WI-26 VA4 cells to laminin-10/11 could not be inhibited with antibodies against integrin β1, α3 or α6 in one-hour assays (study III). However, in subsequent assays carried out at 10 minutes after attachment, a combination of antibodies against integrin α3 and α6 completely inhibited WCCS-1 or WI-26 VA4 cell binding to laminin-10/11. In these assays, blocking with integrin β1 gave a total inhibition of WCCS-1 cell adhesion to laminin-10/11, whereas a residual adhesion of 10% was still seen with WI-26 VA4 cells. Antibodies against either α3 or α6 still failed to inhibit adhesion of these cell types, even in these short-term assays. This suggests that one of these receptors is enough to promote cell adhesion to laminin-10/11, as reported for other cell lines (Gehlsen et al., 1989; Gu et al., 1999; Kikkawa et al., 1998). The most likely reason why laminin-10/11 binding of certain carcinoma cells could be inhibited by antibodies against integrin α3β1 (Kikkawa et al., 1998) and to primitive hematopoietic cells by antibodies integrin α6β1 (Gu et al., 1999) is that these cell lines express only one of these receptors.
Laminins can in some cases activate ERK phosphorylation in cells. Since both laminin-1 and -10/11 promoted adhesion of WCCS-1 and WI-26 VA4 cells, it was of interest to compare the ability of laminin-1 and -10/11 to activate ERK in these cell lines. The laminin receptors expressed by WCCS-1 or WI-26 VA4 cell were analyzed in more detail by immunoprecipitation and FACS analyses. This revealed that both cell lines lacked integrin \( \alpha 6\beta 4 \) but expressed several \( \beta 1 \) integrins including integrin \( \alpha 6\beta 1 \) and \( \alpha 3\beta 1 \). Although laminin-10/11 was a slightly stronger adhesion complex than laminin-1 for both cell lines, both laminins activated ERK only in the WI-26 VA4 cell line. These findings clearly revealed that adhesion to a laminin does not invariably lead to activation of a signaling cascade, even though the same cellular receptors seem to be used for attachment. This suggested that activation of ERK could be critically dependent on the expression of a proper receptor, with capacity to initiate a signaling cascade.

Three different assays were used to identify the receptors involved in ERK activation in the responding WI-26 VA4 cell line. First we tested the ability of antibodies against integrin \( \alpha 3 \) and \( \alpha 6 \) to influence ERK activation in cells attached to laminins. In this assay antibodies against the integrin \( \alpha 6 \) subunit reduced laminin-induced ERK activation. To confirm this finding, we tested the ability of the different antibodies to influence ERK activation. Cells were allowed to attach to the antibodies, or were incubated with the antibodies in solution. In both these assays, antibodies against the \( \alpha 6 \) subunit activated ERK, whereas the other antibodies failed to do so. These findings are not in conflict with the results showing that the \( \alpha 6 \) antibody reduced ERK activation in cells allowed to attach to laminin substrates. A reasonable assumption, supported by our data, is that the natural ligands, the laminins, are more efficient activators of ERK than the integrin antibodies.

To clarify why adhesion to the laminins only activated ERK in one of the cell lines when both expressed integrin \( \alpha 6 \), we analyzed the expression of the integrin \( \alpha 6 \) splice variants (\( \alpha 6A \) and \( \alpha 6B \)) which have different cytoplasmic domains. The responding cell line expressed both splice variants but \( \alpha 6A \) more prominently, whereas the non-responding cell line expressed only \( \alpha 6B \). This is in complete agreement with a report suggesting that \( \alpha 6A \) but not \( \alpha 6B \) is involved in ERK activation (Wei et al., 1998). We conclude that laminin-1 and -10/11 share the ability to activate ERK via the \( \alpha 6\beta 1 \) integrin in epithelial cells, and that this response is regulated by the cytoplasmic domains of the \( \alpha 6 \) subunit.
Laminins could be more efficient activators of ERK than the α6 antibodies, possibly since the natural ligands stimulate several receptors. However, we found that dystroglycan antibodies reduced ERK activation induced by integrin α6 antibodies. This raised the interesting possibility that natural ligands for dystroglycan might act as physiological negative regulators of ERK activation. Cells attached to wells coated with integrin α6 antibodies were therefore incubated with recombinant laminin fragments known to bind α-dystroglycan with varying affinities (Andac et al., 1999; Talts et al., 1999). Only the fragments with high affinity to α-dystroglycan reduced ERK activation induced by the integrin α6 antibodies. Based on these observations, we suggest a novel role for dystroglycan as a suppressor of ERK activation. Different domains of the large laminins can apparently have opposing roles in signal transduction. In this context, it is notable that the CNN family of growth factors can promote a much more sustained integrin-mediated activation of ERK than the laminins. One possibility is that this is due to the opposing influence of discrete domains of the large extracellular matrix components. Our findings could also be relevant for the interpretation of organ culture studies carried out with function blocking antibodies. It has been clearly demonstrated that both integrin α6 or dystroglycan antibodies influence epithelial cell development in organ cultures of embryonic tissue in distinct ways (Durbeej et al., 1995; Falk et al., 1996).

Since binding of dystroglycan from an endothelial cell line to recombinant fragment α5LG1-5 has been reported, we compared binding of purified laminin-1 and -10/11 to purified dystroglycan from muscle and epithelium in overlay assays, and tested dystroglycan binding to these laminins in solid phase assays. In solid phase assays some binding to the α5 containing laminin-10/11 was noted, but compared with the much stronger binding to the α1 containing laminin-1, it was of modest affinity as also shown for LG domains of the laminin α4 chain (Talts et al., 2000). Overlay assays also demonstrated binding of laminin-10/11 to dystroglycan isolated both from muscle and a tissue rich in epithelium (kidney). Our quantitative binding studies show a clear hierarchy among laminin isoforms for α-dystroglycan binding. This is in reasonable agreement both with structural predictions (Hohenester et al., 1999; Timpl et al., 2000) and the report that α5LG1-5 fragment can interact with dystroglycan (Shimizu et al., 1999).

The biological significance of the differential binding of laminins to dystroglycan remains to be clarified. Recently, dystroglycan has been identified
as the cellular receptor for lymphocytic choriomeningitis virus (LCMV) and Lassa fever virus (LFV). LCMV and laminin-1 use, in part, an overlapping binding site on α-dystroglycan, and the ability of an LCMV isolate to compete with laminin-1 for receptor binding is determined by its binding affinity to α-dystroglycan. This competition of the virus with ECM molecules for receptor binding might explain the recently found correlation between the affinity of LCMV binding to α-dystroglycan, tissue tropism, and pathological potential (Kunz et al., 2001). Taken together, our current observations on the distribution of laminin-1 in adult tissues and the binding studies with dystroglycan raise the possibility that LCMV binding to dystroglycan could occur more efficiently in locations lacking laminin-1. Although this remains to be shown, it provides one example that mapping the expression patterns of the laminins and measurements of binding affinities to cellular receptors could be relevant for seemingly different issues in biology and medicine.

In conclusion, different laminin isoforms have varying capacities to mediate cell adhesion and different laminin receptors are involved. Both laminin-10/11 and laminin-1 have the ability to activate ERK signaling. Only one of the two epithelial cell lines were able to signal in contact with these laminins even though both cell lines used integrin α3β1 and α6β1 for adhesion. The signaling was mediated by integrin α6Aβ1 and the non signaling cell line, WCCS-1, only expressed the integrin α6B splice variant compared to WI-26 VA4, which expressed both splice variants. We also propose a novel role for dystroglycan as a suppressor of ERK activation and we show that α5 containing laminins are ligands for dystroglycan.

Laminin α5 containing laminins are ligands for integrin αvβ3, and are involved in FGF and VEGF stimulated proliferation of ECV304 cells (V)

The human ECV304 cell line is particularly useful for analyses of three-dimensional tube formation mediated by basement membrane components (Hughes, 1996). However, receptors involved in such processes have not been characterized, and may differ for distinct laminins. It was therefore of interest to analyze the attachment of these cells to distinct laminins, and to identify the involved receptors. ECV304 cells adhered to all tested laminin isoforms including laminin-1, -2/4 and -10/11. The potent adhesive capacity of laminin-10/11 (Ferletta and Ekblom, 1999) was confirmed. Unexpectedly, these cells did not adhere at all to fibronectin in our hands despite previous data that fibronectin
can influence signaling in these cells. Antibody inhibition studies showed that laminin-1 binds via integrin α6β1, and laminin-2/4 via the α6β1 and α3β1 combination, as could be expected from previous data. ECV304 attachment to laminin-10/11 was more complex. Antibodies against integrin α3 blocked cell adhesion by 80%. A total inhibition was seen with the integrin α6 and α3 antibody combination, but also by combining the α3 antibody either with antibodies against αvβ3 or RGD peptides. For the β-subunits a complete inhibition was only seen when antibodies against β1, β3 and β4 were combined. This raised the possibility that laminin-10/11 use integrin αvβ3 as a co-receptor, which was confirmed in solid phase assays. Both the commercial laminin-10/11 complex and a laminin-10 trimer bound αvβ3 in a cation-dependent fashion. This could be inhibited by certain RGD peptides. While our work was in progress, another study demonstrated that a recombinant fragment of mouse laminin α5 chain can bind integrin αvβ3 (Sasaki and Timpl, 2001). Our data clearly show that an intact laminin trimer can interact with integrin αvβ3. This is in contrast to intact laminin-1, which cannot bind integrin αvβ3, even tough the P1 fragment of laminin-1 can do so (Aumailley et al., 1990; Pfaff et al., 1994). Integrin αvβ3 has earlier been shown to bind in a RGD dependent manner to other ligands such as vitronectin and fibronectin (Plow et al., 2000) and in a RGD independent manner to matrix metalloproteinases (Eliceiri and Cheresch, 2001). Both mouse and human laminin α5 chain have been shown to contain two RGD sites each (Miner et al., 1995; Nagase et al., 1998).

It is known that growth factors together with laminins can enhance proliferation (Drago et al., 1991). To test this possibility, we analyzed proliferation of ECV304 cells attached to laminins in the presence or absence of VEGF or FGF. Both FGF and VEGF enhanced proliferation on cells attached to laminin-10/11, but not on the other substrates. This suggests a specific role for the adhesive receptors unique for laminin-10/11. To identify this receptor, proliferation assays were carried out in the presence of integrin antibodies. The antibody against integrin αvβ3 decreased the additional proliferation induced by the presence of FGF and VEGF, whereas the other tested antibodies failed to do so. In addition, proliferation also decreased when an inhibitor of MEK was added, suggesting that the MEK/ERK signaling pathway is necessary for the proliferation. The phosphorylation of MEK and ERK was very weak on gelatin and on laminin-1, but considerable phosphorylation occurred when cells were attached to laminin-2/4 and laminin-10/11. Phosphorylation was also seen when ECV304 cells adhered to antibodies against integrin α6 and αvβ3. We conclude
that the combined influence of growth factor receptors and integrin αvβ3 enhances proliferation of ECV304 cells. One necessary signaling pathway for this seems to be αvβ3-dependent ERK activation. Interestingly, integrin α6 antibodies were equally potent activators of ERK as integrin αvβ3 antibodies, and yet integrin α6 could not be linked to enhanced proliferation. This indicates the presence of biologically distinct signaling pathways that can be activated by the discrete integrins. The nature of these pathways remains to be explored.

It is well known that integrin αvβ3 can be associated with growth factor receptors. Such interactions are emerging as possible physiologically significant signaling platforms (Eliceiri and Cheresch, 2001). This association may initially occur in the extracellular space (Miyamoto et al., 1996), emphasizing the importance to define ligands for integrin αvβ3 in locations were this integrin plays a major role. αvβ3 is considered to be important for cancer progression, and has also received considerable attention in angiogenesis research. However, it is unclear whether it acts as an inhibitor or stimulator of angiogenesis (Reynolds et al., 2002). Further advances may require a better knowledge of the many physiological ligands expressed at sites of angiogenesis. Several basement membrane components can interact with αvβ3. This includes angiogenesis inhibitors such as endostatin (Rehn et al., 2001) and tumstatin (Maeshima et al., 2001), which are fragments of basement membrane collagens. Our data show that an intact laminin trimer can interact with αvβ3. It will therefore be of interest to analyze the role of α5-laminins and their integrin receptors in angiogenesis.
CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This thesis has mainly been focusing on the behaviors of epithelial cells in contact with laminins containing either the $\alpha_1$ or the $\alpha_5$ chain. The receptors involved in these interactions have also been investigated. Figure 6 summarizes the main conclusions and is based on the results from different types of cell adhesion assays and solid phase results.

Fig. 6. Schematic model over the interactions between laminin-10/11 and their integrins receptors and dystroglycan, linking laminin-10/11 to the epithelial cell and to the MAP kinase pathway.
Recently it was shown that LCMV and LCM, in order to enter the host cell, use the same binding domain on dystroglycan as laminin-1. The receptor binding affinity between viruses for α-dystroglycan vary, some can displace laminin-1 and others not (Kunz et al., 2001). Here we show that dystroglycan has a much broader tissue distribution compared to laminin α1 chain. This suggests that viruses are competing with other ligands than laminin-1 in most tissues. α5 containing laminins could be these ligands. The tissue distribution of the α5 chain is as broad as that of dystroglycan (Miner et al., 1995; Miner et al., 1997). We have also shown that dystroglycan binds laminin-10/11 with lower affinity than laminin-1. This suggests that it could be even easier for viruses to disturb the laminin α5-dystroglycan interaction. With this in mind, it would be very interesting to study which domains are involved in the laminin α5-dystroglycan interaction and if viruses use the same domains.

Integrin αvβ3 is suggested to be involved in angiogenesis and tumor growth (Eliceiri and Cheresch, 2001) and integrin αvβ3 interacts with angiogenic inhibitors such as endostatin and tumstatin (Maeshima et al., 2001; Rehn et al., 2001). We show that integrin αvβ3 bind α5 containing laminins. It would therefore be very interesting to investigate the role of laminin α5 fragments versus αvβ3 in angiogenesis.

Laminins are able to signal through the MAP kinase pathway and we have suggested integrin α6Aβ1 as the mediating receptor. In addition, we propose an opposing role for dystroglycan. Target deletion of the C-terminal domain, E3, of laminin α1 chain in mice are embryonic lethal (Schéele et al., 2001), which also indicate an important role of the E3 receptor dystroglycan. E3 null cells would not only show the importance of the E3 fragment, they could also serve as an excellent tool for investigating dystroglycans role in signaling in embryoid bodies and during early development. Integrin α6 has been targeting deleted in mice and these mice die at birth (Georges-Labouesse et al., 1996). To compare the ability of cells from these null mice to signal would also be interesting. In addition, the branching morphogenesis in kidney organ cultures is inhibited when integrin α6 or dystroglycan are blocked (Durbeej et al., 1995; Falk et al., 1996). It would therefore be of interest to analyze the MAP kinase activation in these cultures.
Cellers samspel med omgivande proteiner


I avhandlingens första del har jag bland annat studerat var laminin α1-kedjan är uttryckt i fullvuxna möss. Det visade sig att den endast fanns på ett fåtal ställen i kroppen medan den i musfoster var något mer uttryckt. I tidigare studier av laminin α1-kedjan har man använt en antikropp som istället kände
igen en annan lamininkedja, nämligen laminin $\alpha 5$-kedjan. Detta har gjort att man länge trodde att laminin $\alpha 1$-kedjan fanns i stort sett i alla basalmembran vilket vi nu visat att så är det inte. Laminin-1, som består av kedjorna $\alpha 1$, $\beta 1$ och $\gamma 1$, använder sig ofta av en mottagare eller receptor på cellen som kallas för dystroglycan. Vi har också studerats var dystroglycan finns i den vuxna muskroppen. Dystroglycan visade sig finnas i flera organ, i vissa organ fanns den tillsammans med laminin $\alpha 1$-kedjan och i andra organ tillsammans med $\alpha 2$-kedjan. Dystroglycan verkar vara en mycket viktig receptor som binder cellerna till basalmembranet.

I andra delen har vi studerat hur celler i odling reagerar när de träffar olika sorters laminin. Vi kunde se att cellerna fäste sig olika bra till de olika lamininerna och att de fick olika utseenden beroende på vilken lamininsort de fäste sig till. Vidare har vi studerat vilka receptorer cellerna använder för att fästa sig till respektive laminin och vi upptäckte att de använde olika receptorer beroende på vilket laminin de satte sig på.


Cellernas kommunikation med sin omgivning är förmodligen mycket mer komplex än vi kan tänka oss, men med en ökad förståelse för hur cellerna i kroppen fungerar och interagerar med sin omgivning kommer vi bättre kunna förstå sjukdomsmekanismer och förhoppningsvis då också finna nya botemedel.
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