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Basement membrane matrices in mouse embryogenesis, teratocarcinoma differentiation and in neuromuscular maturation

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ABSTRACT. In this paper we discuss studies on basement membrane and interstitial matrix molecules in early development and teratocarcinoma differentiation. In the early embryo a compartmentalization of newly formed cell types takes place immediately by formation of basement membranes. The stage-specific developmental appearance of extracellular matrix molecules such as type IV collagen, laminin, entactin, fibronectin and proteoglycans seems to reflect a diversified role of extracellular matrices already in the earliest stages of development. In teratocarcinoma cultures the appearance and composition of extracellular matrices during the differentiation of endoderm cells closely resembles that found in the early embryo. Also in this respect the teratocarcinoma system can be used as a model for studies on early development. In later developmental phenomena other matrix molecules can also be of importance. Merosin, a novel tissue-specific basement membrane-associated protein that appears during muscle and nerve maturation is an example of such molecules.

KEY WORDS: *Basement membrane, Extracellular matrix, Early embryo, Teratocarcinoma, Differentiation*

Introduction

In developmental phenomena such as embryonic induction and organ formation, cell interactions are of crucial importance. Despite more than half a century of research on inductive interactions (cf. Nakamura and Toivonen, 1978), the molecular mechanisms of these events are still largely unknown. However, recent findings suggest that molecules participating in many developmental events may be found on the cell surface and in the extracellular matrix (ECM) (Kemp and Hinchliffe, 1984; Trelstad, 1984; Ekblom *et al.*, 1986).

A number of developmental changes in ECM composition have been reported. In kidney tubule induction, the ECM changes from interstitial to basement membrane type during aggregation of the induced presumptive epithelial cells (cf. Saxén, 1987). In salivary gland development, the glycosaminoglycans of the basal lamina of the developing lobes are regulated by the adjacent mesenchyme (Bernfield and Banerjee, 1982). The distribution and amounts of collagens, fibronectin, laminin, and proteoglycans (e.g. syndecan) change with cell differentiation during limb bud chondrogenesis (Dessau *et al.*, 1980) and tooth development (Thesleff *et al.*, 1988). Moreover, immunocytochemical and ultrastructural studies suggest that the ECM components provide contact guidance for migrating embryonic cells such as neural crest cells (Newgreen and Thiery, 1980; Loring *et al.*, 1982). In general, it seems that assembly of the basal lamina is a requirement for terminal epithelial cell differentiation (Banerjee *et al.*, 1977).

In vitro studies with isolated ECM molecules have provided direct evidence for the functions of these molecules in development. Collagen promotes the fusion of myoblasts into striated muscle cells (Konigsberg and Hauschka, 1965), and laminin affects cell shape, motility and proliferation of skeletal myoblasts (Öcalan *et al.*, 1988) and promotes neurite outgrowth (Manthorpe *et al.*, 1983). In eye development, collagenous ECM secreted by lens capsule induces corneal epithelium to produce the corneal stroma (cf. Hay, 1980). Fibronectin and laminin increase blastocyst attachment and outgrowth *in vitro* (Armant *et al.*, 1986). Thus, direct and indirect evidence from studies of various systems points towards the significance of EMC components in development.

Basement membranes in development: structure and functions

Basement membranes are a specialized form of ECM interposed between epithelial and mesenchymal tissues (Martínez-Hernández and Amenta, 1983; Abrahamson, 1986). In electron microscopy, a dense continuous lamina, the basal lamina, parallels the epithelial cell membrane and together with adjacent mesenchymal anchoring fibrils corresponds to the light microscopic image of the basement membrane. The basal lamina *(lamina densa)* is resolved as a 20-80 nm thick dense network of fine fibrils separated from the plasma membrane by a 20- 30 nm thick lucent zone where only very few fibrils are detected -the *lamina rara (lucida)*. When stained with cationic dyes, basement membranes reveal a lattice of polyanionic particles connected to each other and to the cell surface by fine fibrils.

Basement membranes function (Table I) as semipermeable filters which control the passage of macromolecules by size-selective and charge-selective physico-chemical properties. Basement membranes are also involved in the maintenance of orderly tissue architecture, and they provide a substratum for cell migration, morphogenesis and tissue repair.

Basement membrane components (Table II)

Type IV collagen (M_r = 500,000 − 550,000) is a major structural component of all basement membranes and is synthesized as two chains, pro-α1(IV) and pro-α2(IV) (M_r = 185,000 − 170,000) (Tryggvason *et al.*, 1984; Kuehn *et al.*, 1985; Timpl *et al.*, 1985). In type IV collagen, the classical triple helical Gly-X-Y

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TABLE 1
BASEMENT MEMBRANE FUNCTIONS

Filter Function
Maintenance of Tissue Architecture
Substratum for cell Migration
— Embryonic development
— Nerve regeneration
— Tissue repair
Epithelial Cell Differentiation
— Polarized morphology
— Differentiated functions
— Signal mediation in organogenesis

structure is interrupted by non-triple helical globular domains which increase the flexibility of the molecule and its susceptibility to proteolytic enzymes. Deposition of type IV collagen into matrix form involves the formation of a three-dimensional network where four amino-terminal ends of type IV collagen are disulfide bonded to form the so-called 7-S collagen, and two carboxyterminal ends are linked by disulfide bonds.

Laminin (M_r = 800,000 − 1,000,000) is the principal non-collagenous basement membrane glycoprotein and a ubiquitous component of all basement membranes (Kleinman *et al.*, 1985; Liotta *et al.*, 1986; Martin and Timpl, 1987). Laminin contains three disulfide-bonded polypeptide chains B1, B2 and A (M_r = 215,000, 205,000 and 400,000). In rotary shadowing electron microscopy, laminin is seen as an asymmetric cross with three short (37 nm) arms and one long (77 nm) arm with distinct globular domains at the ends of the arms. Laminin interacts with other basement membrane proteins, with heparin and heparan sulfate and with cell surfaces.

Entactin/nidogen (M_r = 150,000) is a sulfated glycoprotein found in various basement membranes and in teratocarcinoma cultures (Carlin *et al.*, 1981; Martin and Timpl, 1987). It has a dumbbell shape in rotary shadowing electron microscopy and forms a stable complex with laminin binding to the center of the laminin cross. The biological function of this complexing is presently unknown.

TABLE 2
BASEMENT MEMBRANE COMPONENTS

UNIVERSAL
Type IV collagen
Laminin
Entactin/Nidogen
Heparan sulfate proteoglycans
TISSUE-SPECIFIC
Goodpasture Antigen (Kidney, lung)
Epidermolysis Bullosa Acquisita Antigen (Skin)
Merosin (Placenta, Striated muscle, Peripheral nerve)
BASEMENT MEMBRANE-ZONE ASSOCIATED TISSUE SPECIFIC
Acetylcholinesterase (Neuromuscular junctions)
Type III Collagen (Skin, Amnion, Cornea)
Type VIII Collagen (Vessels, Cornea)
Fibronectin (Embryonic Tissues, Placenta, Kidney)
Complement Component C3d (Placenta, Glomerulus)

Proteoglycans exist in basement membranes possibly in three different forms (Dziadek *et al.*, 1985; Hassell *et al.*, 1986; Paulsson *et al.*, 1986). The large (M_r = 400,000 − 750,000) heparan sulfate proteoglycan (BM-1) contains a core protein of M_r = 270 − 420,000 and 2 to 5 heparan sulfate side chains. A small heparan sulfate proteoglycan (M_r = 130,000 − 350,000) and possibly a chondroitin sulfate proteoglycan of similar size have been described in basement membranes recently.

Antibodies reacting with the core proteins of basement membrane proteoglycans stain basement membranes of all tissues. A regular pattern of polyanionic particles seen in the glomerular basement membrane can be removed by heparitinase of heparinase, and this results in increased permeability of the glomerulus.

Merosin is a recently discovered basement membrane-associated protein of very restricted tissue distribution (Leivo and Engvall, 1988). The protein has been found in basement membranes of trophoblast, Schwann cells and striated muscle. In placental extracts, merosin contains an 80 kDa polypeptide chain, and a 65 kDa fragment has been isolated from proteolytic digests of placenta. The protein has not been detected in tumor cell cultures, and its function and interactions with other matrix molecules are not yet known.

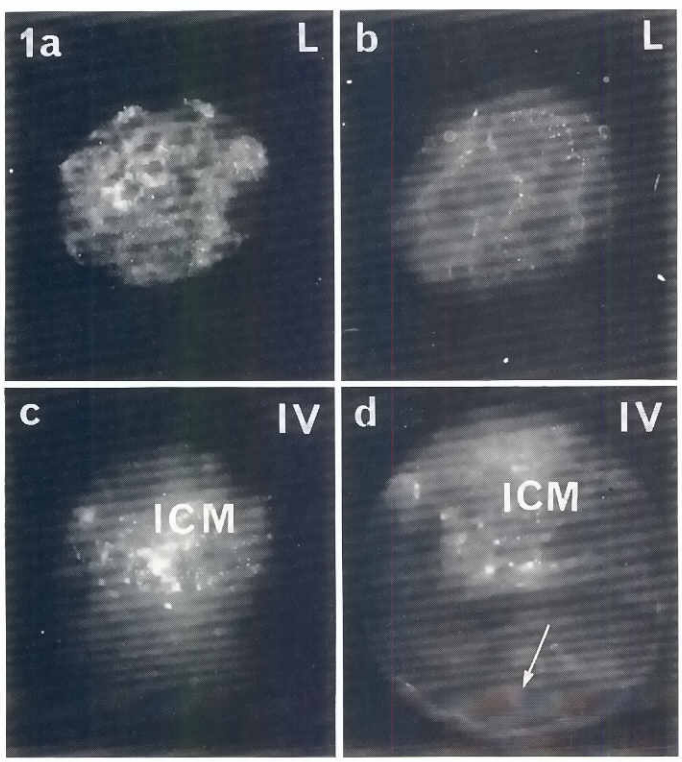


Fig. 1. *Preimplantation embryos stained for extracellular matrix proteins. Fixation with paraformaldehyde, followed by non-ionic detergent for the study of intracellular structures, except in (b). (a) In early morula, 16-32 cells, distinct cytoplasmic laminin (L) fluorescence is seen. x 650. (b) A 16-32 cell morula without zona stained for cell surface laminin. Granular fluorescence outlines several intercellular contours. x 650. (c) In a 3-day whole-mount blastocyst a granular pattern of fluorescence for type IV collagen (IV) is seen in the ICM mainly adjacent to the blastocoel cavity. x 650. (d) A 4-day blastocyst. In addition to granular type IV collagen fluorescence in the ICM, fluorescent patches are seen on the inner aspect of the trophectoderm where the Reichert's membrane is being deposited (arrow). (From Leivo* et al., *1980).*

A number of tissue-specific components of the basal lamina and the mesenchymal matrix subjacent to the basal lamina are listed in Table I (see also Martínez-Hernández and Amenta, 1983; Abrahamson, 1986). In addition, tissue-specific antigenic heterogeneity has been described in various basement membranes (Jaffe *et al.*, 1984; Wan *et al.*, 1984; Wewer *et al.*, 1987).

Fibronectin (M_r = 500,000–550,000) is a dimer of two polypeptide chains and is found in some basement membranes, e.g. of the glomerulus and the placenta. However, fibronectin is not restricted to basement membranes, but is also a major non-collagenous glycoprotein of interstitial connective tissue stromata such as muscle sheaths, organ capsules, dermis and loose connective tissue (Yamada, 1983; Hynes, 1985; Ruoslahti, 1988). Functionally, fibronectin contains domains for binding to collagen, heparin and heparan sulfate, cell surfaces, fibrinogen and fibrin, staphylococci, actin and for fibronectin-fibronectin interaction. Fibronectin interacts with cell surfaces through the GRGDS-binding site and the molecule serves as an adhesive ligand between other matrix molecules and cell surfaces. The inability of transformed cells to retain a surface-associated fibronectin-containing matrix *in vitro* may associate with the tumorigenic and metastatic potential of malignant cells *in vivo*. Functions ascribed to fibronectin include influences on cell adhesion and morphology, cell migration and locomotion, phagocytosis and opsonization, proliferation and differentiation.

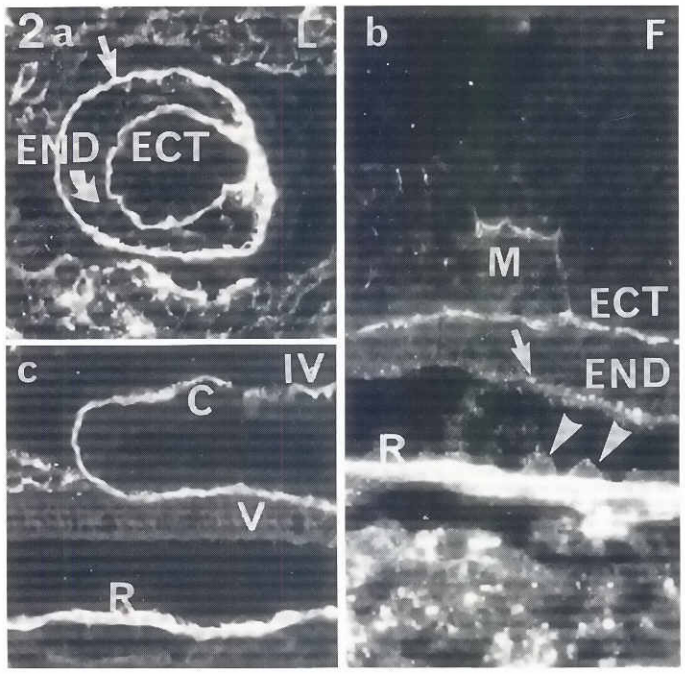


Fig. 2. *Sagittal sections of implanted early embryos. Ethanol fixation. (a) Laminin fluorescence in a 5-day egg cylinder stage embryo is seen in the basement membrane between the ectoderm (ECT) and the endoderm (END) and in the nascent Reichert's membrane (arrow). x 650. (b) Enlargement of the primitive streak area in a 7-day embryo. Brilliant fibronectin (F) fluorescence is seen in the Reichert's membrane (R) and as bands between endoderm (END) and the ectoderm (ECT), and around the nascent mesoderm (M) of the primitive streak. Granular fluorescence presumably of endocytozed fibronectin is present in the apical part of visceral endoderm cells (arrow). Some fluorescence is also associated with the parietal endoderm cells (arrowheads). x 1000 (a and c from Leivo* et al., *1980; b from Wartiovaara* et al., *1979).*

Basement membrane matrices in early development

Preimplantation development

In the early mouse embryo, laminin immunoreactivity was first detected by metabolic labeling and immunofluorescence in the 2-4 cell embryo (Cooper and McQueen, 1983; Wu *et al.*, 1983; Dziadek and Timpl, 1985). Intracellular and intercellular accumulation of laminin was apparent by the 8-16 cell embryo (Figs. 1a, b) (Leivo *et al.*, 1980; Wu *et al.*, 1983). By metabolic labeling, the synthesis of laminin B1 and B2 chains was detected already in the 4-8 cell embryos while synthesis of the A-chains was first shown in the 16-cell embryo (Cooper and MacQueen, 1983) coinciding with the intercellular accumulation of laminin at this stage (Leivo *et al.*, 1980; Wu *et al.*, 1983). Some large heparan sulfate proteoglycan of basement membranes could also be detected on cell surfaces of 2-4 cell embryos (Dziadek *et al.*, 1985). Entactin/nidogen was first detected on compacted 8-16 morulae (Wu *et al.*, 1983; Dziadek and Timpl, 1985).

At the blastocyst stage the embryo contains three distinct cell types: the ectoderm and the primitive endoderm form the inner cell mass (ICM) and the outer surface of the embryo is invested by trophectoderm cells. In 3-4 day blastocysts, laminin, entactin, type IV collagen and basement membrane heparan sulfate proteoglycan were found in the ICM cells and in their extracellular matrix during the assembly of the first embryonic basement membrane (Fig. 1c, d) (Leivo *et al.*, 1980; Wu *et al.*, 1983; Dziadek and Timpl, 1985). Type IV collagen and fibronectin did not accumulate in the morulae and were first detected in the extracellular matrix of blastocysts (Wartiovaara *et al.*, 1979).

The intercellular appearance of laminin in early embryos prior to the assembly of the first basement membrane coincides with a period of cell contact changes. This appearance may reflect cell adhesion-mediating functions for laminin in early embryos, as in other systems (Yamada, 1983; Martín and Timpl, 1987). Thus, the expression of basement membrane components in embryogenesis starts before the differentiation into ectoderm and primitive endoderm has taken place and the first basement membrane has been assembled. The asynchronous appearence of the various basement membrane components suggests that different combinations of basement membrane molecules on the early embryonic cell surface could serve as specific developmental signals. The results also indicate that the first basement membranes with typical protein composition are assembled between dissimilar cell populations during the emergence of the germ layers.

Postimplantation development

At the time of implantation of the mouse embryo on day 5, the ICM expands into the enlarging blastocoel cavity. At this egg cylinder stage, laminin, entactin, type IV collagen, heparan sulfate proteoglycan and fibronectin were detected in the basement membrane between the ectoderm and the primitive endoderm as well as in the Reichert's membrane (Adamson and Ayers, 1979; Wartiovaara *et al.*, 1979; Leivo *et al.*, 1980; Wartiovaara and Leivo, 1982; Leivo, 1983b; Wu *et al.*, 1983; Dziadek and Timpl, 1985) (Fig. 2a).

Immunofluorescence for fibronectin showed brilliant staining of the Reichert's membrane (Fig. 2b) (Wartiovaara *et al.*, 1979) but metabolic labeling studies indicated that fibronectin is not secreted by the parietal endoderm cells responsible for the synthesis of the membrane (Jetten *et al.*, 1979). It is then possible that the fibronectin in the Reichert's membrane is trapped to the membrane similarly to the binding of fibronectin to the basement membrane matrix of PYS-2 cells (see later).

Later, in the developing embryo, immunofluorescence shows the deposition of laminin, entactin, type IV collagen, heparan sulfate proteoglycan and fibronectin at sites of generation of new basement membranes (Wartiovaara *et al.*, 1979; Leivo *et al.*, 1980; Wu *et al.*, 1983) (Fig. 2c). The first accumulation of the interstitial collagens type I and type III was detected in the head and heart mesenchyme as well as in fetal membranes of the early 8-day embryo suggesting the acquisition of true connective tissue (mesenchymal) characteristics at this stage (Leivo *et al.*, 1980).

Basement membrane matrices in teratocarcinoma cultures

Mouse teratocarcinoma can be used as a model system for the study of early mammalian development (Silver *et al.*, 1983; Hogan *et al.*, 1984; Grover and Adamson, 1986). Similarities between teratocarcinoma cells and early embryonic cells include the expression of stage-specific cell surface antigens, isoenzyme profiles, and secretory glycoprotein patterns. Teratocarcinoma cells closely mimic early morphogenesis by forming cell aggregates termed embryoid bodies which are analogous to the inner cell mass of the 5-day blastocyst by morphology (Fig. 3) and by patterns of protein synthesis (Hogan *et al.*, 1983).

Matrix deposition during differentiation

When pluripotent mouse teratocarcinoma stem cells are induced to differentiate by the use of retinoic acid and cAMP, stem cells (embryonal carcinoma or EC cells) differentiate into endoderm-like or END cells (Hogan *et al.*, 1983; Silver *et al.*, 1983). This differentiation step results in the synthesis and extracellular deposition of laminin, entactin/nidogen, type IV collagen and fibronectin (Wartiovaara *et al.*, 1978; Hogan, 1980; Dziadek and Timpl, 1985; Grover and Adamson, 1985). Thus, these *in vitro* events parallel the deposition of the first embryonic basement membrane in the blastocyst. Interestingly,

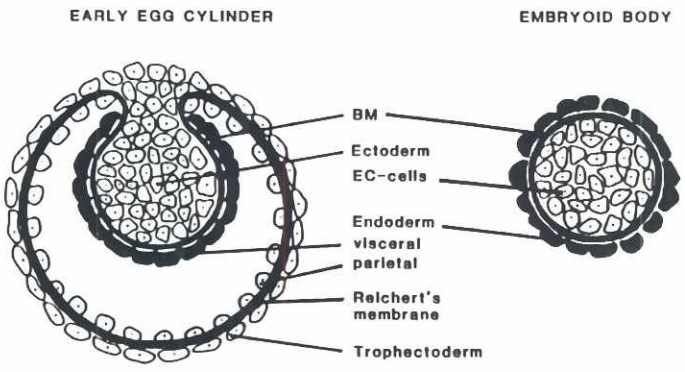


Fig. 3. *Analogy between structures in mouse egg cylinder and in teratocarcinoma embryoid body. BM, basement membrane material; EC-cells, embryonal carcinoma cells. (From Leivo, 1983b).*

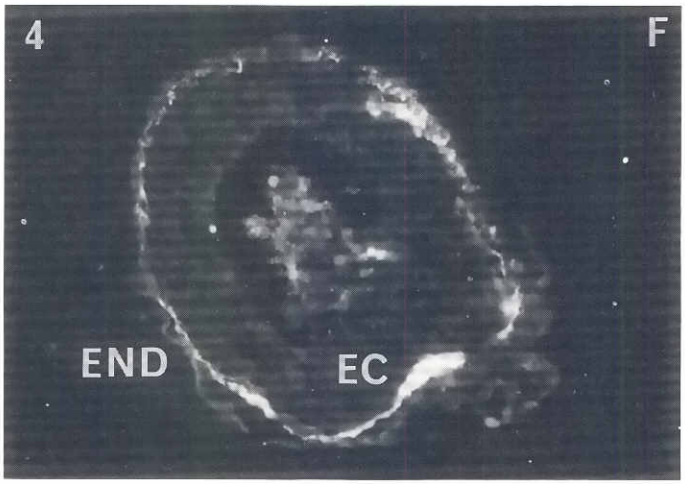


Fig. 4. *Section of a cystic teratocarcinoma embryoid body of the line OC15S1 grown in suspension culture for 8 days. Ethanol fixation. Fibronectin fluorescence is seen under the peripheral endoderm cells (END). No staining of the EC cells is present. Some non-specific staining of necrotic cells inside the cyst is seen. x 700.*

although fibronectin is not deposited by the EC cells in monolayer cultures, metabolic labeling studies (Hogan, 1980; Hogan *et al.*, 1983) indicate that the EC cells synthesize fibronectin.

Identification of secreted and deposited matrix proteins

PYS-2 (parietal yolk sac carcinoma) cells are differentiated endoderm-like cells isolated from teratocarcinoma cultures (Pierce *et al.*, 1962), and they resemble the parietal endoderm cells of the early embryo (Leivo, 1983b; Hogan *et al.*, 1984).

Electrophoretic analysis of the secreted ^{3}H-proline labeled proteins of PYS-2 cultures indicated that the cells secrete type

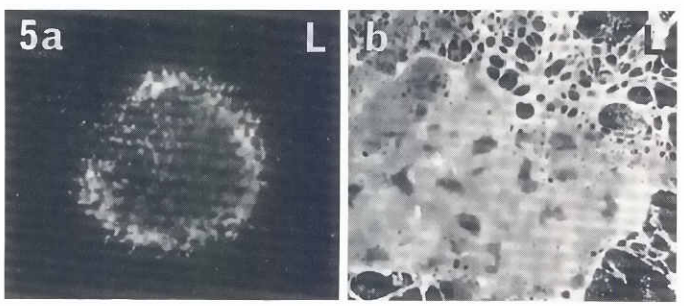


Fig. 5. (a) *Intact attached PYS-2 cell 60 min after seeding on the dish stained for extracellular laminin. Paraformaldehyde fixation. A multipunctate matrix deposition is seen under the cell. x 2000.* (b) *Extracellular matrix preparation of a 5-day PYS-2 culture isolated with the deoxycholate procedure. Fluorescence for laminin indicates distribution of the protein throughout the lamellar matrix structure. x 600. (From Leivo* et al., *1982).*

IV collagen and laminin but not fibronectin (Leivo *et al.*, 1982). Extracellular matrices of labeled PYS-2 cultures prepared by detergent treatment contained laminin and type IV collagen. In radioimmunoassays the weight ratio between laminin and type IV collagen in the extracellular matrix was 3:1. Entactin was not found in significant quantities in PYS-2 cultures.

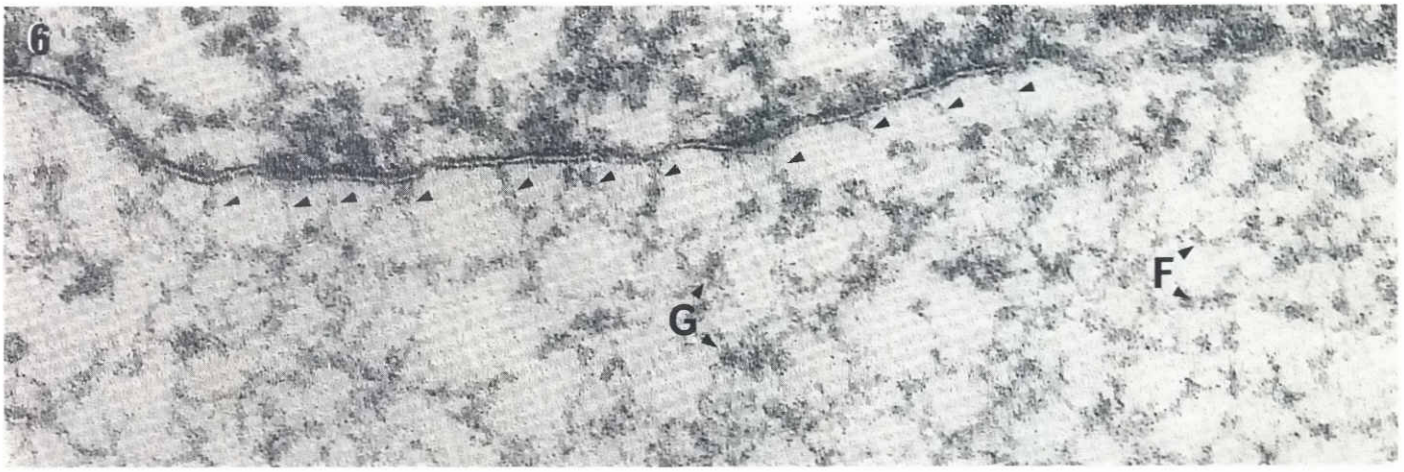


Fig. 6. *Transmission electron micrograph of a subconfluent PYS-2 culture. The matrix network is composed of 1-4 nm fibrils (F) and 8-12 nm grains (G) distributed along the fibrils, often at crossing points. Perpendicular thin 60-80 nm long fibrils (arrowheads) connect the matrix network to the plasma membrane. Tannic acid stain. x 100,000.*

Matrix formation and structure

When suspended PYS-2 cells were allowed to attach, an extracellular deposit of laminin was found within 30 minutes under the attached cells (Fig. 5a), suggesting that laminin may function in the adhesion of PYS-2 cells (Leivo *et al.*, 1982). Type IV collagen was also deposited extracellularly but appeared first 3 hours later.

When the cell layer of confluent cultures was removed, an abundant lamellar matrix containing laminin and type IV collagen was observed on the substratum (Fig. 5b). If the cells had been grown in fibronectin-free fetal calf serum, fibronectin was not present in the matrix. However, if the cells had been grown in ordinary fetal calf serum containing fibronectin, the PYS-2 matrix had bound fibronectin. Also COOH-terminal M_r = 120,000 − 140,000 fragments of fibronectin (which contain heparin-binding domains, but no collagen-binding domains) bound to fibronectin-free PYS-2 matrices (Leivo *et al.*, 1986). Binding of exogenous fibronectin to the basement membrane-like PYS-2 matrix is presumably due to interactions of fibronectin with matrix collagen or glycosaminoglycans, or both (Yamada, 1983; Ruoslahti, 1988).

In transmission electron microscopy, the PYS-2 matrix consisted of a loose network of fine 4 nm fibrils and 8 to 20 nm grains along the fibrils (Fig. 6). The fibril network was connected to plasma membranes by perpendicular 4 nm fibrils 60-80 nm in length.

In immunoelectron microscopy, a dense ferritin labeling for laminin was present at the junction of the matrix network and the attachment fibrils, and few ferritin particles were seen in the interior of the matrix network (Leivo, 1983a) (Fig. 7a). Ferritin was found along the attachment fibrils (Fig. 7a) also close to the plasma membrane (Fig. 7a, inset). Thus, the attachment fibrils of the PYS-2 matrix may correspond to parts of the laminin molecule.

Immunostaining for the large heparan sulfate proteogyclan of basement membranes (BM-1) showed ferritin in small clusters associated with fibrils throughout the matrix network (Fig. 7b) (Leivo, 1983a). Such clusters often appeared at the junction of the attachment fibrils and the matrix network. Negatively-charged sites in the matrix were seen in a similar distribution using the cationic probe ruthenium red (RR) (Fig. 7c). Digestion with heparinase removed most of the RR particles while digestion with chondroitinase ABC, leech hyaluronidase or neuraminidase did not affect the particles.

To characterize the glycosaminoglycan component of the PYS-2 matrix, cell cultures were metabolically labeled with both ^{35}S sulfate and ^{3}H glucosamine, and glycosaminoglycans of isolated matrices were fractionated by ion exchange chromatography (Leivo *et al.*, 1986). The radiolabels eluted in a single peak between the elution positions of hyaluronic acid and heparin. In molecular sieve chromatography the above sulfated macromolecules eluted in a single peak at $K_{av} = 0.17$ which indicates that a large heparan sulfate proteoglycan with approximate M_r = 500,000 − 1,000,000 was the major glycosaminoglycan in the PYS-2 matrix (Oohira *et al.*, 1982; Leivo *et al.*, 1986).

In immunostaining for type IV collagen, ferritin was seen throughout the matrix network but not at the attachment fibrils suggesting that type IV collagen is not a constituent of these fibrils. Thus the PYS-2 matrix consists of a network of type IV collagen fibrils in which heparan sulphate proteoglycan molecules are attached. This resembles the network of type IV collagen in basement membranes *in vivo* (Kuehn *et al.*, 1985). However, the presence of heparan sulfate proteoglycan within the PYS-2 matrix network differentiates this *in vitro* matrix from most basement membranes where polyanionic sites are not found in the *laminae densae*. It appears that laminin and heparan sulphate proteoglycan are present at the cell attachment-mediating fibrils of the PYS-2 matrix. This parallels findings that laminin promotes epithelial cell adhesion (Liotta *et al.*, 1986).

A novel BM matrix component in neuromuscular maturation

A tissue-specific basement membrane antigen

Two monoclonal antibodies prepared by immunization with placental extracts have been shown to have a highly tissue-specific basement membrane-associated reactivity (Leivo and Eng-

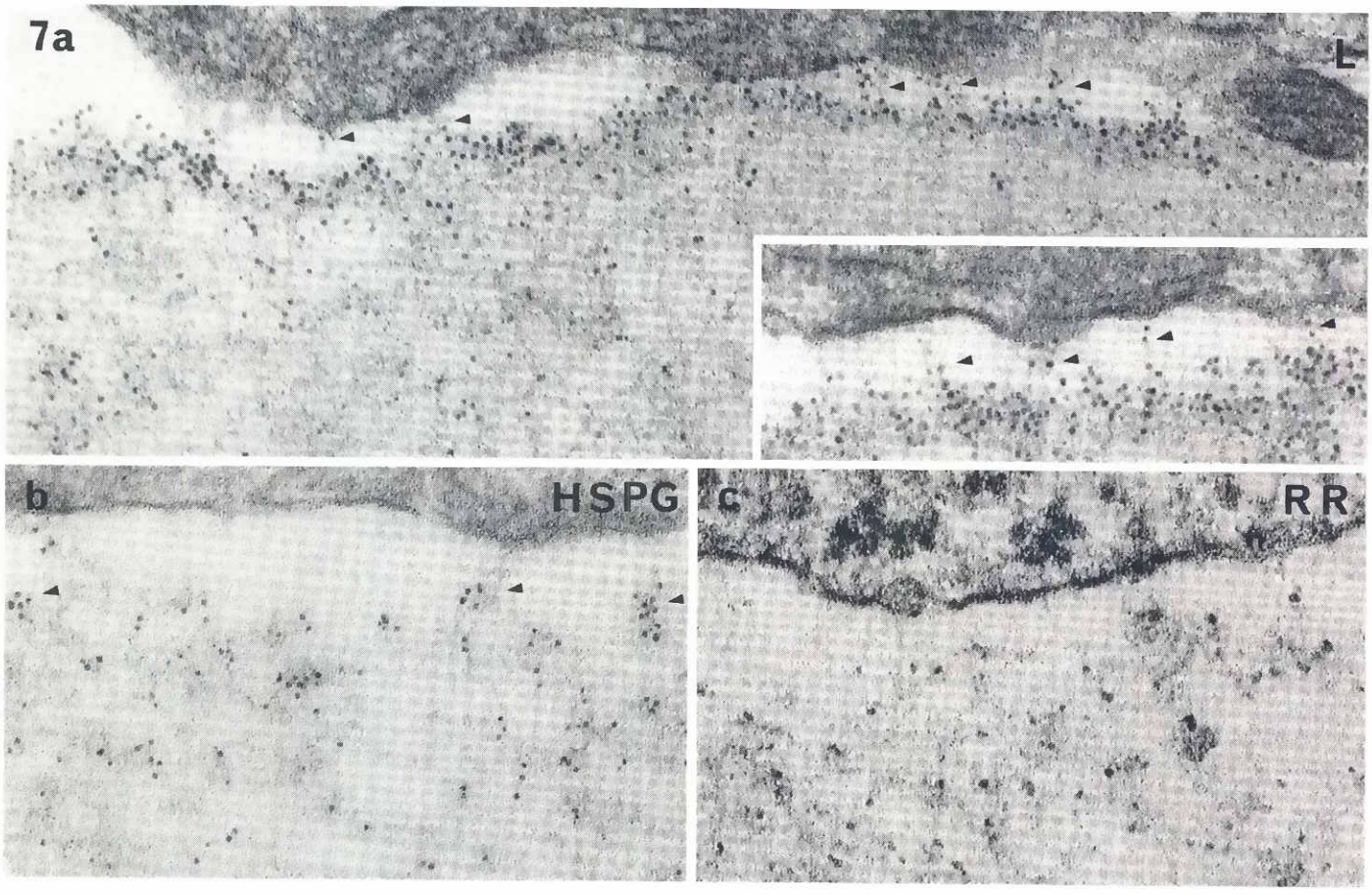
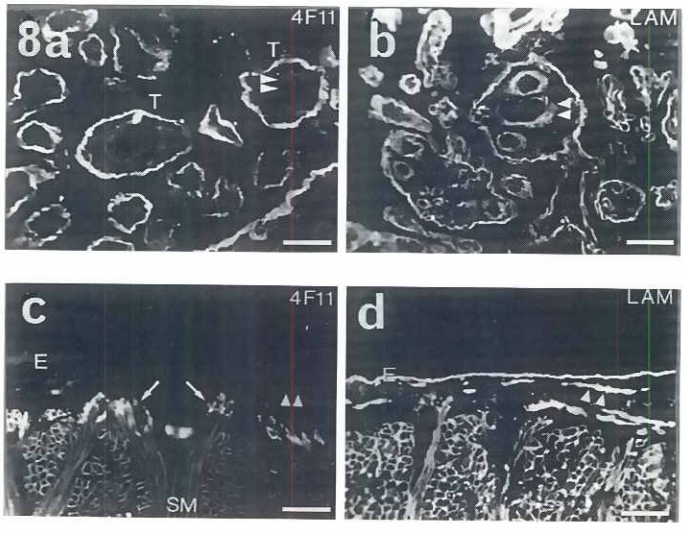


Fig. 7. (a) *Indirect immunoferritin staining for laminin in the PYS-2 matrix. A band of ferritin particles is seen at the junction of the attachment fibrils and the matrix network whereas only few ferritin particles are present in the interior of the matrix network. Ferritin decorates the protruding attachment fibrils (arrowheads). x 85,000; inset x 120,000.* (b) *Staining for the large basement membrane heparan sulfate proteoglycan (HSPG). Ferritin is seen throughout the matrix and a cluster of ferritin is often found at the junction of the attachment fibrils and the matrix network (arrowheads). x 100,000.* (c) *Staining with ruthenium red (RR) for negative-charged sites. 10-20 nm RR- positive particles are present in the matrix network and at the junction of the attachment fibrils and the matrix network. x 140,000. (From Leivo, 1983a).*



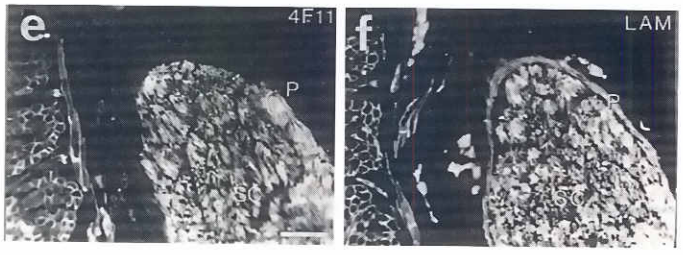


Fig. 8. *Comparison of the tissue distribution of merosin (4F11 antigen) and laminin (LAM) by immunofluorescence in term placenta (a, b), tongue (c, d) and peripheral nerve (e, f). In placenta (a) merosin is seen in trophoblast (T) basement membranes. In tongue (c) and peripheral nerve (e), merosin is seen in skeletal muscle (SM) basement membranes and Schwann cells (SC) basement membranes but not in the epithelial (E) basement membrane of the tongue or in the perineurium (P). Note the particularly intense merosin fluorescence in the pointed ends of muscle fibers that insert into dermis (c; arrows). Merosin is absent from vascular walls (arrowheads in a, c), whereas laminin is present at these sites (arrowheads in b, d) and in all basement membranes in all tissues. Bars = 25 um. (From Leivo and Egnvall, 1988).*

vall, 1988). These antibodies stained the trophoblast basement membranes of the placenta, basement membranes surrounding striated muscle fibers in skeletal and cardiac muscle, and the Schwann cell basement membranes of peripheral nerves (see Fig. 8a, c, e). In tongue, the staining was particularly strong at the ends of muscle fibers that attach to the dermis (Fig. 8c). No staining was seen in the epithelial basement membranes, vessel walls or other tissues. In contrast, the tissue distribution of laminin (Fig. 8b, d, f) included all basement membranes and smooth muscle of e.g. vessel walls, colon, and bladder, and the perineural lining of nerves.

The antigen recognized by these antibodies has been isolated by antibody-affinity chromatography from pepsin and chymotrypsin digests of placenta (Leivo and Engvall, 1988). The protein isolated from these digests migrated in SDS-PAGE under nonreducing conditions as a single polypeptide band at 55 kDa and at 65 kDa after reduction. In immunoblotting, monoclonal antibodies prepared against the denatured 65 kDa polypeptide detected an 80 kDa polypeptide in SDS-extracts of placenta. Unreduced, the protein had an apparent molecular mass of 70 kDa. This protein was named merosin (*meros,* Greek for «to separate into compartments»), since merosin compartmentalizes Schwann cells, muscle cells and trophoblast cells from the interstitial matrix.

Merosin in development

Pre- and postnatal stages of intercostal and hindlimb muscle development in the mouse were studied using antibodies to the 65 kDa polypeptide starting from day 15 of gestation when the skeletal muscle basement membranes first appear. We found that all prenatal stages, which include the appearance of primary, secondary, and tertiary myofibers, were negative for merosin (Fig. 9b) but expressed laminin (Fig. 9a). Not until the first postnatal days were basement membranes in mouse intercostal muscles significantly positive for merosin (Fig. 9c). Basement membranes in the sciatic nerve stained for merosin a few days later.

Results on the late developmental appearance of merosin suggest that the protein is expressed only in advanced stages of differentiation. In any case, the developmental appearance of merosin in mouse muscles and nerves several days after the deposition of laminin and type IV collagen into basement membranes indicates that merosin is not a basic structural component of these basement membranes, but perhaps relates to the functional maturation of muscles and peripheral nerves.

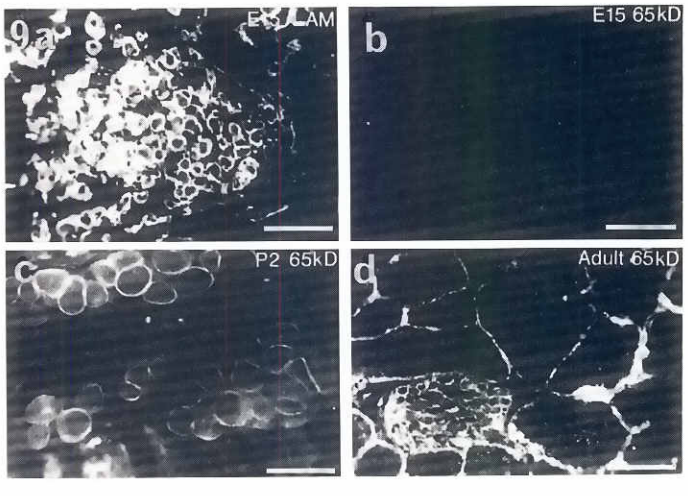


Fig. 9. *Immunofluorescence staining of developing mouse intercostal* (a, b, c) *and adult mouse hindlimb* (d) *muscles.* (a) *Staining for laminin outlines basement membranes around developing myoblasts.* (b) *No staining with antibodies to merosin (65-kD polypeptide) is seen at this stage.* (a and b) *embryonic day 15 (E15).* (c) *At postnatal day 2 (P2) staining for merosin (65-kD polypeptide) shows the appearance of the protein in basement membranes of intercostal muscle cells.* (d) *Distinct immunofluorescece for merosin is seen in basement membranes of adult muscle cells and intramuscular nerve Schwann cells. Bars* = 25 um. (From Leivo and Engvall, 1988).

Concluding remarks

Scientists are only beginning to understand the molecular mechanisms active in developmental phenomena. In this overview, we have outlined studies on basement membrane and interstitial matrix molecules in early development and teratocarcinoma differentiation. In the early mouse embryo, extracellular matrix molecules appear asynchronously. Thus, different combinations of these molecules exist on the embryonic cell surfaces at different time points, and consequently they could provide signals for the regulation of early development. The stage-specific developmental appearance of basement membrane and interstitial matrix molecules also reflects the diversified roles of these matrices already in the earliest stages of development. There seems to be a basic tendency towards immediate compartmentalization of dissimilar cell types through the assembly of interposed basement membranes. The appearance and composition of extracellular matrices during the differentiation of endoderm cells in teratocarcinoma cultures closely resembles events in the early embryo. The teratocarcinoma system can be used as a model for studies on the composition and assembly of these extracellular matrices.

Futher studies on the various factors influencing cell interactions in early development are needed before the role of basement membranes in these phenomena can be fully understood. In such research, effort should also be focused on the discovery of new molecules participating in developmental phenomena. Merosin, a novel tissue-specific basement membrane-associated protein, which appears late in muscle and nerve maturation, exemplifies efforts of such research.

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