Differential expression of mRNAs encoding laminin chain variants during *in vitro* development of mouse blastocysts *

Mojgan Azimi¹, Tomoaki Niimi², Naoko Yoshida¹ & Yasuo Kitagawa^{1,2,*}

¹ Graduate program of Biochemical Regulation, Graduate School of Agricultural Sciences, Nagoya University Chikusa-ku, Nagoya-shi 464-8601, Japan

² Nagoya University BioScience Center, Chikusa, Nagoya 464-8601, Japan

(* Author for all correspondence, e-mail: i45073a@nucc.cc.nagoya-u.ac.jp)

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Abstract

Expression of mRNAs encoding ten laminin chain variants was studied by reverse transcription-polymerase chain reaction (RT-PCR) of mRNA extracted from cultured mouse blastocysts. $\alpha 1$, $\beta 1$ and $\gamma 1$ mRNAs, of which products form laminin-1, were the only chains expressed in blastocysts before hatching. $\alpha 4$ and $\alpha 5$ mRNAs were additionally expressed after attachment of blastocysts to culture dishes. $\alpha 2$ and $\beta 2$ mRNAs started to be expressed at the stage of allantois formation. Among ten laminin chains, only the mRNA encoding components of laminin-5 ($\alpha 3$, $\beta 3$ and $\gamma 2$) was missing in embryos up to the stage of allantois formation.

Abbreviations: ICM, inner cell mass; RT-PCR, reverse transcription-polymerase chain reaction

Introduction

Laminins constitute a family of basement membrane glycoproteins which have important role in tissue morphogenesis by influencing attachment, migration, proliferation and differentiation of various types of cells (Martin et al., 1988; Timpl, 1989; Beck et al., 1990; Engel, 1992). Laminins are composed of α , β and γ chains which assemble into a disulfide-bonded, cross-shaped structure with three short arms and one rod-like long arm (Engel et al., 1981; Engel, 1993; Burgeson et al., 1994). The long arm is the site of chain assembly (Paulsson et al., 1985) and three chains form the coiled-coil driven by heptad-repeats abundant in this region. Many variants of laminin chains are identified and their cDNAs have been cloned. From mouse, $\alpha 1$ (Sasaki et al., 1988), $\alpha 2$ (Bernier et al., 1995), $\alpha 3$ (Galliano et al., 1995), $\alpha 4$ (Liu and Mayne, 1996; Niimi et al., 1997), $\alpha 5$ (Miner et al., 1995), $\beta 1$ (Sasaki et al., 1987), $\beta 2$ (Durkin et al., 1996), $\beta 3$ (Utani et al., 1995), $\gamma 1$ (Sasaki and Yamada, 1987)

and $\gamma 2$ (Sugiyama et al., 1995) have been cloned. Equivalent chains are cloned from human as well. Thus, mammal laminin family has members of five α chains, three β chains and two γ chains. Among them, $\alpha 1$, $\alpha 2$, $\alpha 5$, $\beta 1$, $\beta 2$ and $\gamma 1$ have a similar domain organization whereas $\alpha 3$, $\alpha 4$, $\beta 3$ and $\gamma 2$ are truncated at the short arm region. α chains form the largest subfamily and are importance because of the carboxyl-terminal globular G domain interacting with various extracellular matrix proteins and cell surface receptors. $\alpha 1$ and $\alpha 2$ have a similar domain organization while $\alpha 3$ and $\alpha 4$ are truncated at the short arm. $\alpha 5$ is the newest member and seems to be the most ancestral α chain because of its similarity to the only known α chain of *Drosophila* (Miner et al., 1995). $\alpha 3$ gene is the only laminin gene producing two polypeptides of $\alpha 3A$ and $\alpha 3B$ by alternative splicing of the transcript (Ferrigno et al., 1997).

Prompted by recent expansion of the laminin chain family (especially α chains), their distribution and function in mammalian tissues is now extensively studied. As a component of laminin-2 and 4, $\alpha 2$

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is abundant in mesodermally derived tissues such as skeletal muscle and cardiac muscle (Schuler and Sorokin, 1995). Northern blot and ribonuclease protection analysis revealed the expression of $\alpha 2$ mRNA in heart, kidney, liver, skin and lung as well as skeletal muscle (Miner et al., 1997). Mutation in $\alpha 2$ gene shows muscular dystrophy in human and mouse (Sunada et al., 1994; Xu et al., 1994). $\alpha 3$ is the component of laminin-5, 6 and 7, concentrated in epithelial basal lamina and its mRNA is highly expressed in skin and lung. *In situ* hybridization of mouse embryo revealed a focal localization of $\alpha 3$ mRNA in the central nervous system (Galliano et al., 1995). Altered expression of laminin-5 is associated with Herlitz junctional epidermolysis bullosa, a severe epidermal blistering disorder, which is inherited as an autosomal recessive disease (Vidal et al., 1995). $\alpha 4$ is the component of laminin-8 and 10 and strongly expressed in heart, lung and skeletal muscle (Iivanainen et al., 1997). Its expression depending on the adipose-conversion of 3T3-L1 cells (Niimi et al., 1997) suggested the role in microvascularization in connective tissues. $\alpha 5$ was expressed in a pattern similar to $\alpha 3$ except for weak expression in liver (Miner et al., 1995). Overall expression of $\alpha 4$ and $\alpha 5$ in various tissues is the broadest while that of $\alpha 1$ is more restricted.

Since laminin appeared to be the first demonstrable extracellular matrix protein during the embryogenesis, its expression in early embryos was studied immediately after the discovery of laminin, when A ($\alpha 1$), B1 ($\beta 1$) and B2 ($\gamma 1$) were the only known components. It is observed as a punctated distribution over the cell surface of blastomers at 2-cell stage following the first cleavage (Dziadek and Timpl, 1985). As the development proceeds, laminin is detectable in both the cell surface and the cytoplasm at 16-cell morula stage (Leivo et al., 1980; Wu et al., 1983). Many studies have shown that oocytes, unfertilized egg and fertilized embryo can synthesize only $\beta 1$. Embryos with 4–8 cells are able to synthesize $\beta 1$ and $\gamma 1$ before compaction, whereas the 16-cell stage embryos synthesize $\alpha 1$, $\beta 1$ and $\gamma 1$ (Cooper and McQueen, 1983). Concomitant with the expression of a set of three chains for assembly, extracellular accumulation of laminin occurs and its function as a component of basement membrane initiates. It has been well known that laminin plays crucial role in the development of blastocyst thereafter by supporting trophoblast, by separating primitive endoderm and the primitive ectoderm, by guiding the migration of parietal endoderm cells and by forming Reichert's membrane (Poelmann

and Mentink, 1982a,b; Nadijecka and Hillman, 1974). Despite important role of laminin in early embryogenesis, the component chains are not specified after expansion of the family.

Taking advantage of the cultured mouse blastocysts which mimic sequential development up to the allantois formation stage (Hsu, 1979; Libbus and Hsu, 1980), we have identified laminin chain mRNAs expressed during early postimplantation period. Reversed transcription polymerase chain reaction (RT-PCR) of mRNA extracted from the embryos at different stage showed that only $\alpha 1$, $\beta 1$ and $\gamma 1$ mRNAs were expressed in the blastocysts before hatching, $\alpha 4$ and $\alpha 5$ mRNAs appeared after attachment of the blastocysts, and $\alpha 2$ and $\beta 2$ mRNAs started to be expressed at the stage of allantois formation.

Materials and methods

Collection and in vitro culture of mouse blastocysts

ICR female mice (6–7 weeks old) were superovulated by administration of 5 IU human chorionic gonadotrophin 48 h after injection of 5 IU pregnant male serum gonadotrophin and then mated with male mice of the same strain. The vaginal plug was checked next morning and the age of embryos was assumed to be 0.5 day postcoitum (day p.c.). Blastocysts of 3.5 days p.c. were collected by flushing the uterine horns with 1 ml of CMRL 1066 medium (Gibco), washed with the medium and cultured at the density of 5 blastocysts per 35-mm plastic culture dish (Falcon) containing 2 ml of the medium supplemented with 1 mM L-glutamin, 1 mM sodium pyruvate and 10% fetal calf serum (Dainippon pharmaceutical Co.). No antibiotics were used at any stage of embryo culture. The dishes were constantly flushed with 5% CO₂ and 95% air and the cultures were maintained at 37 °C in a humidified incubator. After 2 days in culture, when the blastocysts hatched and attached to the dishes, the medium was changed to CMRL 1066 containing 20% fetal calf serum and the medium was renewed daily thereafter. The morphology of embryos was followed daily and the embryos 2, 4 and 6 days after culture were detached by trypsin treatment from dishes for mRNA extraction.

mRNA preparation

A RNA purification kit (Pharmacia Biotech) was used for mRNA preparation. Briefly, embryos were ho-

Figure 1. Postimplantational development of mouse blastocysts *in vitro*. Mouse blastocyst collected at 3.5 days p.c. in *zona pellucida* and (a) hatched within 24 h of culture and attached to the surface of culture dishes (b). The blastocysts grew rapidly after the attachment and the egg cylinder having began to project above the surface of dishes at 2.5 days in culture (c). A well developed egg cylinder initiated to have posterior amniotic folds at 4 days in culture (d). Three distinct compartments of ectoplacental cavity, exocoelomic and amniotic cavity were developed and allantois became visible at 6 days in culture (e and f). The scale bars correspond to 100 μm .

mogenized in the extraction buffer containing guanidinium thiocyanate and N-lauroyl, diluted with the elution buffer containing 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. The homogenate was added to 1 ml of oligo(dT)-cellulose (25 mg/ml) and gently mixed for 3 min. The pellet was washed with the low-salt buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA and 0.1 M NaCl and then with the high-salt buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA and 0.5 M NaCl. The pellet was transferred to

microspin column and mRNA was eluted with 500 μl of pre-warmed elution buffer. The concentration of eluted mRNA was adjusted to 2.4 $\mu\text{g}/\text{ml}$. mRNAs were precipitated by adding 10 μl of glycogen solution and 40 μl of 2.5 M potassium acetate to the 500 μl aliquot of mRNA solution. After addition of 1000 μl of 95% ethanol, the solution was kept at -20°C for 30 min and the pellet was collected by centrifuging at $16000 \times g$ for 10 min.

Figure 2. Amplification of laminin chain mRNAs extracted from the cultured blastocysts by RT-PCR. mRNA extracted from blastocysts before hatching from *zona pellucida* (A), cultured for 2 days (B), 4 days (C) or 6 days (D) was amplified by 40 cycles of RT-PCR using ten paired primers designed based on reported cDNA sequences of mouse $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\beta 1$, $\beta 2$, $\beta 3$, $\gamma 1$, $\gamma 2$ and β -actin. The products were electrophoresed on a 1% agarose gel with λ -DNA fragments digested with *HindIII* and *EcoRI* as size maker (M).

Figure 3. Semi-quantification of α chain mRNAs by interrupted PCR. mRNA extracted from blastocysts before cultured for 2 days (A) or 6 days (B) was amplified by 30, 35 or 40 cycles of RT-PCR using the paired primers for mouse $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$ and $\alpha 5$, $\beta 1$, $\beta 2$, $\beta 3$, $\gamma 1$, $\gamma 2$. Other details were the same as Figure 2.

RT-PCR analysis

The mRNA pellet was dissolved in 9 μ l of 0.1% diethyl pyrocarbonate-treated water and incubated for 10 min at 70 °C, chilled on ice and then added to a master mixture containing 5 μ l of 5x first strand synthesis buffer, 2 μ l of 0.1 M dithiothreitol, 4 μ l of 2.5 mM mixture of four deoxynucleotide triphosphate, 0.5 μ l of ribonuclease inhibitor and 100 ng of random primers. After incubating the reaction mixture at 37 °C for 2 min, 200 units of Super Script II reverse transcriptase (GIBCO BRL) was added and incubation was continued for 2 h at 37 °C. Single stranded cDNA in 0.7 μ l of the reaction mixture were amplified by 40 cycles of PCR (94 °C for 1 min; 60 °C

for 1 min and 72 °C for 1 min) using Tth DNA polymerase (Boehringer Mannheim) after pre-incubating at 94 °C for 2 min. PCR products (8 μ l out of 50 μ l of the reaction mixture) were separated on 1% agarose gels and sized using a λ -DNA digested with *Hind*III and *Eco*R I. For PCR of mouse $\alpha 1$ cDNA, a paired primers of 5'-GCGCGTAAAGATTTCAGCC-3' and 5'-TCTGTCCAAAGCTCCTG-3' (nucleotides 5113-5736, Sasaki et al., 1988) were used expecting a 626-bp product. For mouse $\alpha 2$ cDNA, a paired primers of 5'-GGGTCGGATCAATCATGCTG-3' and 5'-CGTGGAAACATAAGCTTCTCG-3' (nucleotides 8457-9043, Bernier et al., 1995) were used expecting a 589-bp product. For mouse $\alpha 3$ cDNA, a paired primers of 5'-AAAGGTGCACCTGGTGGTGG-3'

and 5'-TTCGGTGGGAAGGAAAGCTG-3' (nucleotides 6944-7528, Galliano et al., 1995) were used expecting a 587-bp product. For mouse $\alpha 4$ cDNA, a paired primers of 5'-CATGGGATCCTATTGGCCTG-3' and 5'-CACATAGCCGCCTTCTGTGG-3' (Liu and Mayne 1996; Niimi et al., 1997) were used expecting a 664-bp product. For mouse $\alpha 5$ cDNA, a paired primers of 5'-ACGGCTCAGAAGGTTTCCCG-3' and 5'-CTTCAGCGGGTATGACTTCC-3' (nucleotides 9673-10221, Miner et al., 1995) were used expecting a 551-bp product. For mouse $\beta 1$ cDNA, paired primers of 5'-GCTGGATCCGCTTGACAGCAGAGTGCAGCTGA-3' and 5'-CGCGAATTCGCTAAGCAGGTGCTGTAAACCG-3' (nucleotides 4858-5535, Sasaki et al., 1987) were used expecting a 702-bp product. For mouse $\beta 2$ cDNA, a paired primers of 5'-CTGCAGCGGGTATGACTTCC-3' and 5'-GGCTTGTGCAGCCAGAACAC-3' (nucleotides 4475-5022, Durkin et al., 1996) were used expecting a 550-bp product. For mouse $\beta 3$ cDNA, a paired primers of 5'-CAGGTAGATGATGTGGTCGG-3' and 5'-ACTGCGGATCTGCTCCACAC-3' (nucleotides 3079-3621, Utani et al., 1995) were used expecting a 545-bp product. For $\gamma 1$ cDNA, a paired primers of 5'-GCGGGATCCCCAATGACATTCTCAACAAC-3' and 5'-GCAGATATCGGGCTTCTCGATAGACGGGG-3' (nucleotides 4331-5067, Sasaki and Yamada, 1987) were used expecting a 760-bp product. For mouse $\gamma 2$ cDNA, a paired primers of 5'-TATTAGCCAGAAGGTTGCGG-3' and 5'-TAGTCTCCAGCAGATGGAGG-3' (nucleotides 2973-3520, Sugiyama et al., 1995) were used expecting a 549-bp product.

Results

Postimplantational development of mouse blastocysts in vitro

To follow the expression of laminin chain variants during early postimplantation period, we took advantage of *in vitro* culture of mouse blastocysts (Hsu, 1979; Libbus and Hsu, 1980). The egg cylinder development from blastocysts *in vitro* is closely resembles the fourth to seventh days of mouse gestation *in vivo* and this system allowed us to eliminate the contamination with mRNAs from maternal tissues. The micrographs in Figure 1 show that we could reproduce the morphological changes of the blastocysts in culture.

Mouse blastocysts collected at 3.5 days p.c. were still enclosed in *zona pellucida* and consisted of a

layer of trophoblast cells surrounding the blastocole cavity and inner cell mass (ICM) (Figure 1a). Within 24 h of culture, the blastocysts hatched and increase in the size. Following the loss of *zona pellucida*, the blastocysts attached to the surface of culture dishes at 2 days of culture (Figure 1b). After the attachment, mural and polar trophoblast cells became distinguishable because the former cells have large nuclei and vacuolated cytoplasm whereas the latter cells have smaller nuclei. Attachment accelerated the growth and the organization of blastocysts. ICM became surrounded by endoderm cells except in a small area where ICM maintained contact with the polar trophoblast. At 2.5 days in culture (Figure 1c), the embryos acquired a distinct proamniotic cavity and began to project above the surface of dishes. At 4 days in culture (Figure 1d), squamous and cubical endoderm cells had differentiated and a posterior amniotic fold could be observed. Posterior and anterior amniotic folds came in contact, giving rise to exocoelom by 6 day in culture (Figure 1e). At this stage, the embryos exhibited three distinct compartments; ectoplacental cavity, exocoelom and amniotic cavity separated by chorion and amnion (Figure 1f). The allantois became visible at this stage.

Differential expression of mRNAs encoding laminin chain variants during postimplantational development of mouse blastocysts in vitro

To explore the laminin genes expressed during postimplantation period of mouse gestation, mRNAs extracted from the cultured blastocysts were amplified by RT-PCR using ten pairs of primers designed based on reported cDNA sequences of mouse laminin chains. Since the efficiency of amplification depends on many factors such as G/C content of primers, RT-PCR may not reflect the quantity of mRNAs. However, sensitive amplification of mRNA having specified sequence by RT-PCR allowed us to identify the expressed laminin gene in limited amount of blastocysts available. The identity of amplified fragments was confirmed based on the length and partial sequence determined after subcloning them into pBluescript.

RT-PCR of mRNAs extracted from blastocysts at 3.5 days p.c. gave strong bands for $\beta 1$ and $\gamma 1$, and weak band for $\alpha 1$ (Figure 2A). The primer for other laminin chains did not give any signal even after 40 cycles of the amplification. Consistent with the observation by Cooper et al. (1983), laminin-1 ($\alpha 1\beta 1\gamma 1$) is the only trimer to be synthesized in the blastocysts

before implantation. If we can assume the relative intensity of signals to reflect the quantity of mRNAs, expression of $\alpha 1$ gene may limit the laminin-1 assembly at this stage.

mRNAs extracted from the blastocysts at 2 days in culture, when blastocysts attached to the surface of dishes, additionally gave strong signal for $\alpha 4$ and weak signal for $\alpha 5$ (Figure 2B). This suggests that laminin-8 ($\alpha 4\beta 1\gamma 1$) and laminin-10 ($\alpha 5\beta 1\gamma 1$) can be assembled after implantation. The signal for $\alpha 1$ became as strong as those for $\beta 1$ and $\gamma 1$, suggesting that $\alpha 1$ gene expression is no longer the limit for laminin-1 assembly. Despite the dramatic morphogenesis resulting in projection of egg cylinder above the surface of dishes and formation of posterior amniotic fold, mRNAs extracted from the blastocysts 4 days in culture gave strikingly similar pattern of signals (Figure 2C).

Further development of blastocyst to the embryo having three distinct compartments and allantois was accompanied with additional expression of $\alpha 2$ and $\beta 2$ mRNAs. This implies an expansion of laminin family synthesized because laminin-2 ($\alpha 2\beta 1\gamma 1$), laminin-3 ($\alpha 1\beta 2\gamma 1$), laminin-4 ($\alpha 2\beta 2\gamma 1$), laminin-9 ($\alpha 4\beta 2\gamma 1$) and laminin-11 ($\alpha 5\beta 2\gamma 1$) became the potential laminin trimers to be assembled. The only laminin mRNAs missing at this stage were $\alpha 3$, $\beta 3$ and $\gamma 2$, the components of laminin-5 which is concentrated at the binding site of epidermal basal cells via hemidesmosomes (Masunaga et al., 1996; Tamura et al., 1997). RT-PCR on RNA extracted from mouse skin showed amplification of these mRNAs by the primers designed in this experiment (result not shown).

For a semi-quantification of α mRNAs, RT-PCR on mRNAs extracted from the blastocysts cultured for 2 days (Figure 3A) and 6 days (Figure 3B) was interrupted at 30, 35 and 40 cycles and amplified fragments were visualized after separation on agarose gels (Figure 3). Logarithmic amplification of cDNA depending on the cycle numbers suggests a kinetics that the smallest cycle number giving detectable fragment of the interest is an index of mRNA quantity and the intensity increases to a saturating level where the substrate nucleotides are depleted. Since the efficiency of PCR is expected to be identical for a same mRNA species amplified with a same pair of primers, this kinetics may be valid even if the mRNA is extracted from different tissues.

The amplified fragment of $\alpha 1$ mRNA was detectable at 30 cycles of PCR on mRNA from the 2 days

culture (Figure 3A) whereas that for 6 day culture was already saturated at 30 cycles (Figure 3B). This indicates that $\alpha 1$ mRNA in the blastocysts at 2 day of culture increased markedly during additional two days of culture. The same was true for $\alpha 4$ and $\alpha 5$ mRNAs since the cycle numbers giving detectable fragments were smaller for mRNA from the 6 days culture. These imply that the early stage of egg cylinder formation (from Figure 1b through Figure 1d) is important period of laminin gene expression from quantitative aspect.

Discussion

Our study on early postimplantational development of mouse blastocysts *in vitro* gave novel pieces of information about laminin α chain expression. We confirmed that $\alpha 1$ is the first α chain expressed during the embryogenesis and its expression keeps increasing up to the late egg cylinder stage. Expression of $\alpha 4$ and $\alpha 5$ became detectable after the attachment of blastocysts and increased slowly thereafter. In adult bodies, $\alpha 4$ and $\alpha 5$ distribute overall the tissues while that of $\alpha 1$ is restricted. Whereas $\alpha 5$ is regarded as the most ancestral α chain from evolutionary aspect, our result showed that $\alpha 1$ is the main α chain functioning during early stages of the mammalian development. Expression of $\alpha 2$ was detectable at the latest stage of egg cylinder formation when allantois developed rapidly. Considering the fact that allantois is a typical extraembryonic mesoderm, $\alpha 2$ can be regarded as a mesoderm-specific α chain. Together with $\beta 3$ and $\gamma 2$, $\alpha 3$ was missing during mouse embryogenesis up to the late stage of egg cylinder formation. This observation is consistent with its restricted distribution in skin of adult body as a component of the anchoring fibril for basal cells epidermis.

Differential expression of β chains is another interesting observation in this study. Expression of $\beta 1$ have been demonstrated even in unfertilized egg and fertilized embryo (Cooper and MacQueen, 1983) and it remained to be as only β chain expressed during the early embryogenesis. Additional expression of $\beta 2$ was detected at the latest stage of egg cylinder formation when the variety of tissues expanded explosively. An attractive hypothesis concerning this differential expression of β chains is 'step-wise expansion of laminin family' during the embryogenesis. At the early stages where only $\beta 1$ is expressed, the expression of $\alpha 1$, $\alpha 2$, $\alpha 4$ and $\alpha 5$ together with $\gamma 1$ may create laminin-1 ($\alpha 1\beta 1\gamma 1$), laminin-2 ($\alpha 2\beta 1\gamma 1$),

laminin-8 ($\alpha 4\beta 1\gamma 1$) and laminin-10 ($\alpha 5\beta 1\gamma 1$). Additional expression of $\beta 2$ at the later stages may expand the laminin family so as to include laminin-3 ($\alpha 1\beta 2\gamma 1$), laminin-4 ($\alpha 2\beta \alpha \gamma 1$), laminin-9 ($\alpha 4\beta 2\gamma 1$) and laminin-11 ($\alpha 5\beta 2\gamma 1$) additionally. However, this hypothesis is based on the replaceable assembly of laminin chains in a same intracellular space (Tokida et al., 1980). For further discussion of the hypothesis, we need to demonstrate at least the simultaneous expression of $\beta 1$ and $\beta 2$ mRNAs in individual cell of the *in vitro* cultured blastocysts.

Important question to be addressed in this study is to what extent do the *in vitro* cultured blastocysts mimic the process of implantation *in vivo*. Implantation is the initial step of active and complicated interaction between maternal and fetal systems for the organization of placenta. It is obvious that the attachment of blastocysts to the surface of culture dish is too simplified model to reflect the *in vivo* process. However, the expression of $\alpha 4$ and $\alpha 5$ mRNAs sharply dependent on the attachment attract our interest of further analysis. Since the attachment also accelerates the differentiation of primitive endoderm to form visceral and parietal endoderm, we need be aware that expression of $\alpha 4$ and $\alpha 5$ after the attachment can not always be attribute to trophoblast cells. Our preliminary study of the laminin gene expression in embryonal carcinoma F9 cells, which mimic the differentiation of these embryonic endoderm, showed the expression of $\alpha 4$, $\alpha 5$ mRNA in addition to $\alpha 1$ ¹. To explore the novel role of these α chains during the implantation process, we first have to identify the cells expressing $\alpha 4$ and $\alpha 5$ mRNA in the cultured blastocysts.

References

- Aberdam D, Aguzzi A, Baudoin C, Galliano MF, Ortonne JP and Meneguzzi G (1994) Developmental expression of nicein adhesion protein (laminin-5) subunits suggests multiple morphogenic roles. *Cell Adhes Commun J* 2: 115–129.
- Beck K, Hunter I and Engel J (1990) Structure and function of laminin: anatomy of a multidomain glycoprotein. *FASEB J* 4: 148–160.
- Bernier SM, Utani A, Sugiyama S, Doi T, Polistina C and Yamada Y (1994) Cloning and expression of laminin $\alpha 2$ chain (M-chain) in the mouse. *Matrix Biol* 14: 447–455.
- Burgeson RE, Chiquet M, Deutzmann R, Ekblom P, Engel J, Kleinman H, Martin GR, Meneguzzi G, Paulsson M, Sanes J, et al. (1994) A new nomenclature for the laminins. *Matrix Biol* 14: 209–211.
- Champlaud MF, Lunstrum GP, Rousselle P, Nishiyama T, Keene DR and Burgeson RE (1996) Human amnion contains a novel laminin variant, laminin 7, which like laminin 6, covalently associates with laminin 5 to promote stable epithelial-stromal attachment. *J Cell Biol* 132: 1189–1198.
- Cooper AR and MacQueen HA (1983) Subunits of laminin are differentially synthesized in mouse eggs and early embryos. *Dev Biol* 96: 467–471.
- Durkin ME, Gautam M, Loechel F, Sanes JR, Merlie JP, Albrechtsen R and Wewer UM (1996) Structural organization of the human and mouse laminin $\beta 2$ chain genes, and alternative splicing at the 5' end of the human transcript. *J Biol Chem* 271: 13407–13416.
- Dziadek M and Timpl R (1985) Expression of nidogen and laminin in basement membranes during mouse embryogenesis and in teratocarcinoma cells. *Dev Biol* 111: 372–382.
- Engel J (1993) in: *Molecular and cellular aspects of Basement Membrane*, Rohrbach DH and Timpl R (eds) pp. 147–176.
- Engel J (1992) Laminin and other strange proteins. *Biochemistry* 31: 10643–10651.
- Engel J, Odermatt E, Engel A, Madri JA, Furthmayr H, Rohde H and Timpl R (1981) Shapes, domain organizations and flexibility of laminin and fibronectin, two multifunctional proteins of the extracellular matrix. *J Mol Biol* 150: 97–120.
- Ferrigno O, Virolle T, Galliano MF, Chauvin N, Ortonne JP, Meneguzzi G and Aberdam D (1997) Murine laminin $\alpha 3A$ and $\alpha 3B$ isoform chains are generated by usage of two promoters and alternative splicing. *J Biol Chem* 272: 20502–20507.
- Galliano MF, Aberdam D, Aguzzi A, Ortonne JP and Meneguzzi G (1995) Cloning and complete primary structure of the mouse laminin $\alpha 3$ chain. Distinct expression pattern of the laminin $\alpha 3A$ and $\alpha 3B$ chain isoforms. *J Biol Chem* 270: 21820–21826.
- Hsu YC (1979) *In vitro* development of individually cultured whole mouse embryos from blastocyst to early somite stage. *Dev Biol* 68: 453–461.
- Iivanainen A, Kortessmaa J, Sahlberg C, Morita T, Bergmann U, Thesleff I and Tryggvason K (1997) Primary structure, developmental expression, and immunolocalization of the murine laminin $\alpha 4$ chain. *J Biol Chem* 272: 27862–27868.
- Leivo I, Vaheri A, Timpl R and Wartiovaara J (1980) Appearance and distribution of collagens and laminin in the early mouse embryo. *Dev Biol* 76: 100–114.
- Libbus BL and Hsu YC (1980) Sequential development and tissue organization in whole mouse embryos cultured from blastocyst to early somite stage. *Anat Rec* 197: 317–329.
- Liu J and Mayne R (1996) The complete cDNA coding sequence and tissue-specific expression of the mouse laminin $\alpha 4$ chain. *Matrix Biol* 15: 433–437.
- Martin GR, Timpl R and Kuhn K (1988) Basement membrane proteins: molecular structure and function. *Adv Protein Chem* 39: 1–50.
- Masunaga T, Shimizu H, Ishiko A, Tomita Y, Aberdam D, Ortonne JP and Nishikawa T (1996) Localization of laminin-5 in the epidermal basement membrane. *J Histochem Cytochem* 44: 1223–1230.
- Miner JH, Lewis RM and Sanes JR (1995) Molecular cloning of a novel laminin chain, $\alpha 5$, and widespread expression in adult mouse tissues. *J Biol Chem* 270: 28523–28526.
- Miner JH, Patton BL, Lentz SI, Gilbert DJ, Snider WD, Jenkins NA, Copeland NG and Sanes JR (1997) The laminin α chains: expression, developmental transitions, and chromosomal locations of $\alpha 1$ – $\alpha 5$, identification of heterotrimeric laminins 8–11, and cloning of a novel $\alpha 3$ isoform. *J Cell Biol* 137: 685–701.
- Nadijcka M and Hillman N (1974) Ultrastructure studies of the mouse blastocyst substages. *J Embryol Exp Morphol* 32: 675–695.
- Niimi T, Kumagai C, Okano M and Kitagawa Y (1997)

- Differentiation-dependent expression of laminin-8 (alpha 4 beta 1 gamma 1) mRNAs in mouse 3T3-L1 adipocytes. *Matrix Biol* 16: 223–230.
- Paulsson M, Dziadek M, Suchanek C, Huttner WB and Timpl R (1985) Nature of sulphated macromolecules in mouse Reichert's membrane. Evidence for tyrosine O-sulphate in basement membrane proteins. *Biochem J* 231: 571–579.
- Poelmann RE and Mentink MM (1982a) Parietal yolk sac in early gestation mouse embryos: structure and function. *Bibl Anat* 22: 123–127.
- Poelmann RE and Mentink MM (1982b) The maternal-embryonic barrier in the early post-implantation mouse embryo: a morphological and functional study. *Scan Electron Microsc Pt 3*: 1237–1247.
- Sasaki M, Kato S, Kohno K, Martin GR and Yamada Y (1987) Sequence of the cDNA encoding the laminin B1 chain reveals a multidomain protein containing cysteine-rich repeats. *Proc Natl Acad Sci USA* 84: 935–939.
- Sasaki M, Kleinman HK, Huber H, Deutzmann R and Yamada Y (1988) Laminin, a multi-domain protein: the A chain has a unique domain and homology with the basement membrane proteoglycan and laminin B chains. *J Biol Chem* 263: 16536–16544.
- Sasaki M and Yamada Y (1987) The laminin B2 chain has a multidomain homologous to the B1 chain. *J Biol Chem* 262: 17111–17117.
- Schuler F and Sorokin L (1995) Expression of laminin isoforms in mouse myogenic cells *in vitro* and *in vivo*. *J Cell Sci* 108: 3795–3805.
- Sugiyama S, Utani A, Yamada S, Kozak CA and Yamada Y (1995) Cloning and expression of the mouse laminin $\gamma 2$ (B2t) chain, a subunit of epithelial cell laminin. *Eur J Biochem* 228: 120–128.
- Sunada Y, Bernier SM, Kozak CA, Yamada Y and Campbell KP (1994) Deficiency of merosin in dystrophic dy mice and genetic linkage of laminin M chain gene to dy locus. *J Biol Chem* 269: 13729–13732.
- Tamura RN, Oda D, Quaranta V, Plopper G, Lambert R, Glaser S and Jones JC (1997) Coating of titanium alloy with soluble laminin-5 promotes cell attachment and hemidesmosome assembly in gingival epithelial cells: potential application to dental implants. *J Periodontal Res* 32: 287–294.
- Tokida Y, Aratani Y, Morita A and Kitagawa Y (1990) Production of two variant laminin forms by endothelial cells and shift of their relative levels by angiostatic steroids. *J Biol Chem* 265: 18123–18129.
- Timpl R (1989) Structure and biological activity of basement membrane proteins. *Eur J Biochem* 180: 487–502.
- Utani A, Kopp JB, Kozak CA, Matsuki Y, Amizuka N, Sugiyama S and Yamada Y (1995) Mouse laminin B1 (laminin $\beta 3$ chain): cloning and tissue distribution. *Lab Invest* 72: 300–310.
- Vidal F, Baudoin C, Miquel C, Galliano MF, Christiano AM, Uitto J, Ortonne JP and Meneguzzi G (1995) Cloning of laminin alpha 3 chain gene (LAMA3) and identification of a homozygous deletion in a patient with Herlitz junctional epidermolysis bullosa. *Genomics* 30: 273–280.
- Wu TC, Wan YJ, Chung AE and Damjanov I (1983) Immunohistochemical localization of entactin and laminin in mouse embryo and fetuses. *Dev Biol* 100: 496–505.
- Xu H, Christmas P, Wu XR, Wewer UM and Engvall E (1994) Defective muscle basement membrane and lack of M-laminin in the dystrophic dy/dy mouse. *Proc Natl Acad Sci USA* 91: 5572–5576.