Int. J. Dev. Biol. 44: 815-818 (2000)

Short Communication

Differential expression of laminin genes in early chick embryo

NIKOLAS ZAGRIS*[1], ALBERT E. CHUNG[2] and VASSILIS STAVRIDIS[1]

[1]*Division of Genetics and Cell and Developmental Biology, Department of Biology, University of Patras, Greece and* [2]*Department of Biological Sciences, University of Pittsburgh, Pittsburgh, USA*

ABSTRACT The expression patterns of laminin α1 and β1 genes were examined by *In situ* hybridization of their mRNAs in the early chick embryo from the blastoderm at stage X (morula) to the 10-somite stage. The laminin α1 and β1 transcripts were found in abundance in the chick blastoderm at stage X before initiation of synthesis of the protein. Laminin polypeptides were detected shortly thereafter in embryos at stage XIII (blastula). The expression of the laminin transcripts was intense in the epiblast and in the hypoblast of embryos at stage XIII. During gastrulation (stage HH3-4), the laminin α1 and β1 cRNAs gave strong signals in the cells ingressing through the primitive streak, in the migrating mesenchymal cells and the cells of the lower layer. The expression of laminin α1 and β1 genes was restricted to specific cell populations later in development. At the neurula stage (stage HH5-6), the expression of laminin transcripts was low in epithelial ectoderm and strong in chordamesoderm - neural ectoderm and may implicate important developmental roles for laminin in the morphogenetic movements of neural plate bending during primary neurulation. At the 10-somite stage (stage HH10-11), the α1 and β1 cRNAs gave no signals in the neural tube, notochord, and ectoderm. The α1 and β1 cRNAs gave strong signals in neural crest cells and this may indicate that the neural crest cells can produce laminin. The α1 cRNAs gave strong signals in the dermamyotome and no signal in the sclerotome of somites, and intense signals in the pronephric tubules. The laminin expression pattern in somites may show transient expression of the α1 and/or expression of a distinct α1 isoform in the sclerotome. The selective expression of laminin α1 and β1 subunits which shows a developmentally regulated, tissue specific distribution suggests potential roles for different members of the same subfamily of genes in the developing chick embryo. .

KEY WORDS: *laminin, gene expression, extracellular matrix, morphogenesis, chick embryo.*

The laminins are large heterotrimeric glycoproteins that are major components of the extracellular matrix, including the basement membrane, and of embryonic tissues. The prototype of laminin consists of three distinct polypeptide chains designated as α1, β1 and γ1. Eleven distinct heterotrimeric isoforms assembled from five α, three β and two γ genetically distinct subunit chains have been identified to date with distinct tissue distributions and co-distributions, unique properties and developmentally regulated expression.

Immunohistochemistry studies using antibodies to mouse laminin reported scanty staining for laminin in quail or absence of laminin in chick (Reviewed by Zagris and Chung,1990) at stage X (morula). Massive presence of laminin coincided with the epithelization of the epiblast and was detected in the basement membrane on the ventral surface of the epiblast and in the extracellular matrix in the blastocoel in embryos at stage XIII (blastula) (Zagris and Chung,1990). In our present work, [^{35}S] methionine labeled embryos were extracted with EDTA-containing buffer and were subjected to molecular sieve chromatography on a column of sepharose CL-4B. [^{35}S] methionine labeled homogenates immunoprecipitated with rabbit polyclonal antiserum against mouse laminin GP-2 (binds to the 220 kDa β1 and γ1 chains of mouse laminin, Chung *et al.*, 1979), recognized a major chick polypeptide at approximately 210 kDa which is therefore likely to correspond to the mouse laminin β1 and/or γ1 chains in embryos at stage XIII. In contrast, the band was absent in embryos at stage X on fluorograms from SDS PAGE electrophoresis (Fig. 1). Thus, the immunoprecipitation results (Fig. 1) detected the first expression of laminin polypeptides at stage XIII in concert with the immunohistochemical findings (Zagris and Chung, 1990).

Our present work employing *In situ* hybridization showed a comparable pattern of strong signals for laminin α1 and β1 chain mRNAs from stage X to the early gastrula and differential expression of these mRNAs in the forming embryonic tissues and organs in the developing chick embryo. We used specific chain anti-sense RNA probes produced from mouse cDNA for the laminin α1 and β1 chains to study tissue specific and temporal patterns of laminin mRNA in the early chick embryo. The chick homologue of mouse laminin β1 has been identified as B1-1 which has 91% identity over the entire length of the corresponding mouse cDNA (O'Rear, 1992). The amino acid sequence identity of the deduced polypeptide of a chicken laminin chain α-like cDNA fragment shows a very high conservation (88%

***Address correspondence to:** Nikolas Zagris. Department of Biology, University of Patras, Patras, Greece. FAX: +30-61-997650. e-mail: zagris@upatras.gr

0214-6282/2000/$20.00

Printed in Spain
www.ehu.es/ijdb

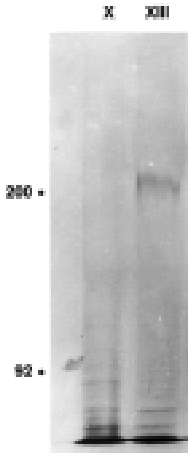

Fig. 1. Immunodetection of laminin β1/ γ1 chain(s) isolated from early chick embryo. *Embryos at stages X (morula) and XIII (blastula) were labeled with ^{35}S-methionine, lysed, precipitated with PYS mouse polyclonal antibodies against laminin GP-2 (β1/γ1 chains), analyzed in 5% SDS-PAGE under reducing conditions and visualized by fluorography. Chick polypeptides at approximately 210 kDa are likely to correspond to the mouse laminin β1/γ1 chain(s) in embryos at stage XIII while the band is absent in embryos at stage X. Molecular mass of identified polypeptides was determined according to electrophoretic migration of standards (Rainbow markers, Amersham).*

identity) with mouse α1 chain (Frade *et al.*, 1996). For any given hybridization assay using an anti-sense cRNA probe, a parallel assay was performed using cRNA sense strands to serve as controls.

Laminin α1 (Figs. 2 A,a) and β1 (Figs. 3 A,a) cRNAs detected a similar pattern of strong signals of mRNA expression in the chick embryo at stage X. At stage XIII, the laminin α1 (Figs. 2 B,b) and β1 (Figs. 3 B,b) cRNAs gave strong signals of mRNA expression in the epiblast and in the hypoblast. The *In situ* hybridization results (Figs. 2A, 3A) in concert with the immunoprecipitation (Fig. 1) and immunohistochemistry findings (Zagris and Chung, 1990) showed that strong signals of laminin mRNAs were detected at stage X but massive expression of the protein was detected shortly thereafter in the extracellular matrix in embryos at stage XIII. An interesting question is whether the laminin mRNA detected at stage X is oogenetic and/or zygotic. Zygotic gene expression was detectable at stage X but showed marked activation at stage XIII with gradual increase thereafter in the chick embryo (Zagris *et al.*, 1998). In amphibian embryos at pre-gastrula stages (Darribere *et al.*, 1986), experiments with the transcription inhibitor α-amanitin indicated that laminin-related polypeptides are translation products of a maternal mRNA (Riou *et al.*, 1987).

In embryos at stage HH3-4 (primitive streak/gastrula stage), the laminin α1 (Fig. 2 C,c) and β1 (Fig. 3 C,c) cRNAs gave strong signals in the cells ingressing through the primitive streak, in the migrating mesenchymal cells, in the cells of the lower layer, in the ectodermal cells neighboring the embryonic junction while more lateral ectoderm showed mild expression of transcripts. At the neurula stage (HH5-6), the laminin β1 cRNA (Figs. 3 D,d) revealed punctate groups of cells expressing laminin mRNA in chordamesoderm and in neural ectoderm and the signal was more intense at the site the chordamesoderm apposes the neural ectoderm during their interaction. This punctate form of laminin expression has been identified in many embryonic tissues such as the embryonic kidney mesenchyme (reviewed by Ekblom *et al.*, 1990). The intense expression of laminin transcripts in chordamesoderm and neural ectoderm may not be necessary for neural determination but implicates important developmental roles for laminin such as its involvement in the morphogenetic movements of the neural plate bending during primary neurulation (Duprat and Gualandris, 1984; Huang *et al.*, 1990). In the same embryo more anteriorly,

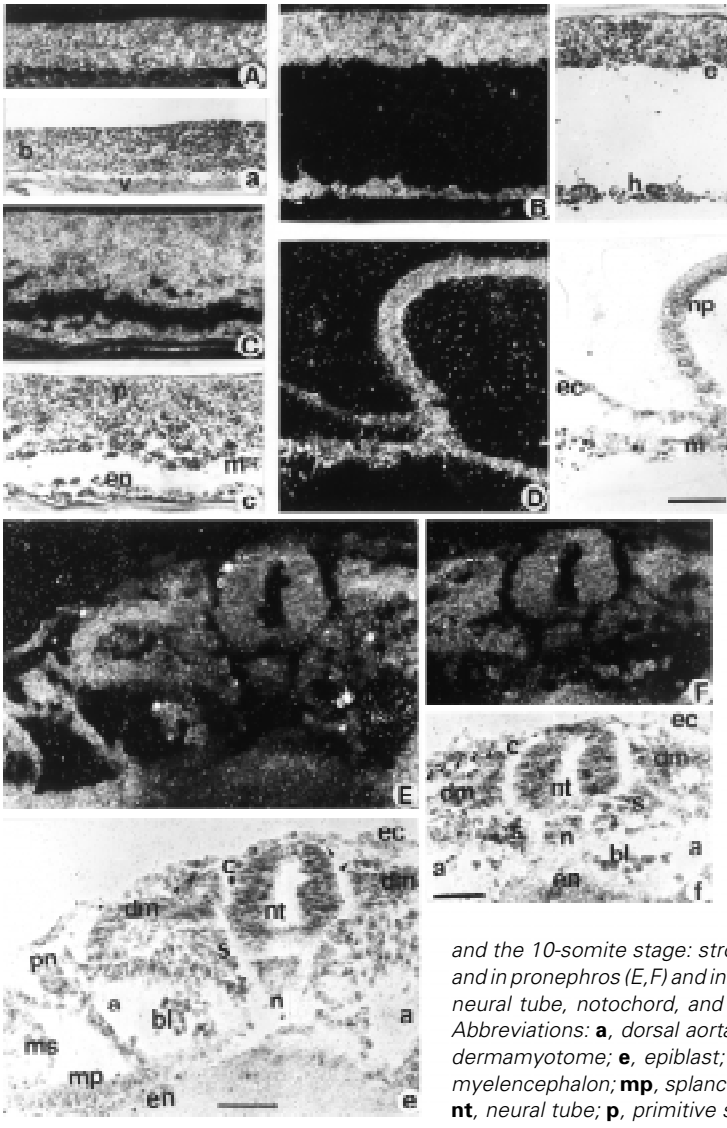

Fig. 2. Localization of transcripts detected by laminin α1 chain cRNA at various stages of chick embryonic development. *Consecutive transverse sections were processed for In situ hybridization as described in Experimental Procedures. A-F are the dark field pictures of transverse sections from embryos at stages X (morula) (A), XIII (blastula) (B), HH3-4 (primitive streak) (C), HH5-6 (neurula) (D) and HH10-11 (10 to 13 somites) (E,F); a-f are the matched bright field pictures.* **(A-C)** *The α1 cRNA detected strong expression of transcripts in embryos from morula to the early gastrula stages.* **(D-F)** *Differential expression of laminin transcripts is seen in embryos at early neurula and the 10-somite stage: strong signals are present in neural plate (D), in dermamyotome of somite and in pronephros (E,F) and in neural crest cells (indicated by arrowheads in e and f) but not in ectoderm, neural tube, notochord, and in sclerotome of somite (E,F). Bar, 50 μm (A,a - E,e) and 50 μm (F,f). Abbreviations:* **a**, *dorsal aorta;* **b**, *blastoderm;* **bl**, *blood cells;* **c**, *neural crest;* **cb**, *bulbus cordis;* **dm**, *dermamyotome;* **e**, *epiblast;* **ec**, *ectoderm;* **en**, *endoderm;* **g**, *gut;* **h**, *hypoblast;* **m**, *mesoderm;* **ml**, *myelencephalon;* **mp**, *splanchnic mesoderm;* **ms**, *somatic mesoderm;* **n**, *notochord;* **np**, *neural plate;* **nt**, *neural tube;* **p**, *primitive streak;* **pn**, *pronephros;* **s**, *sclerotome;* **v**, *vitelline membrane.*

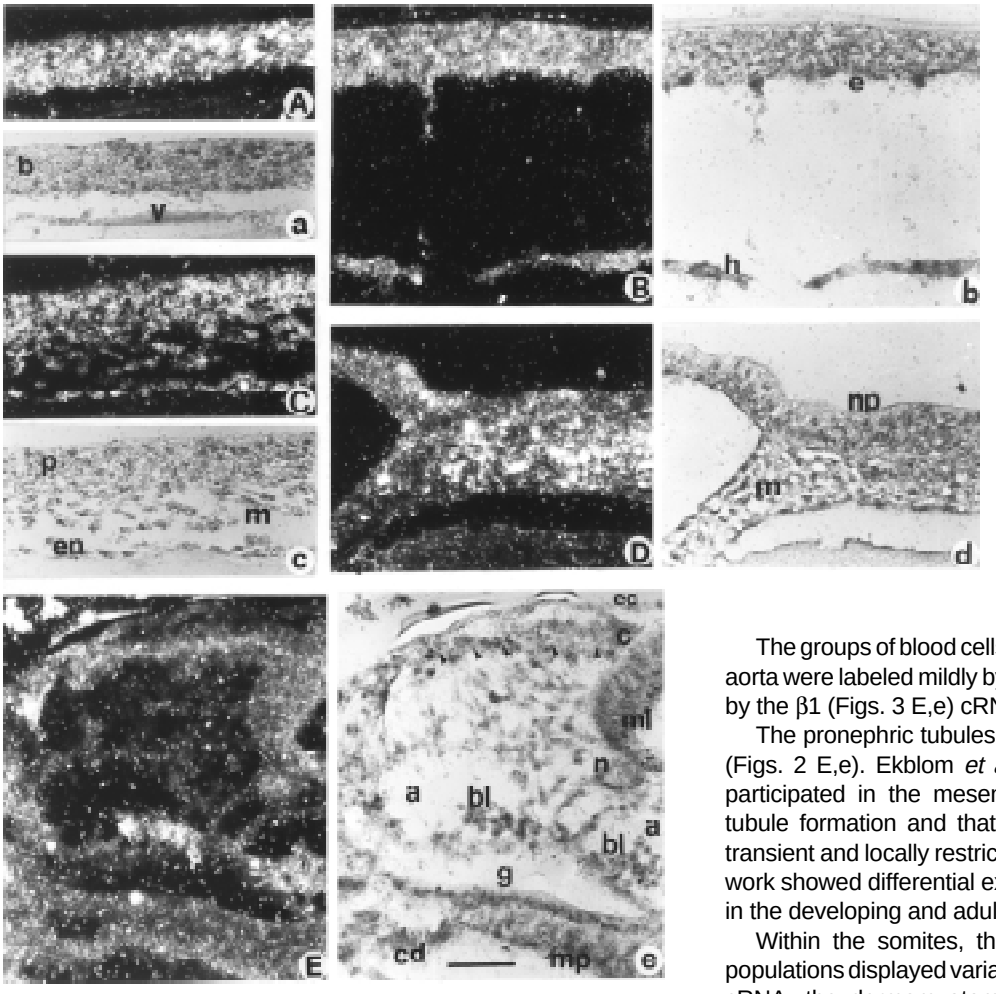

Fig. 3. Localization of transcripts detected by laminin β1 chain cRNA at various stages of chick embryo. *Details and legends are as in Fig. 2 except that the laminin β1 probe was used.* **A-E** *are the dark field pictures from embryos at stages X (morula)* **(A)**, *XIII (blastula)* **(B)**, *HH3-4 (primitive streak)* **(C)**, *HH5-6 (neurula)* **(D)** *and HH10-11 (10 to 13 somites)* **(E)**; **a-e** *are the matched bright field pictures.* **(A-C)** *The β1 cRNA probe detected strong expression of transcripts in embryos from morula to early gastrula stages.* **(D,E)** *Differential expression of laminin transcripts is seen in embryos at early neurula and the 10-somite stage: strong signals are present in chordamesoderm-ectoderm at the site they interact during early neurula stage* **(D)**, *in neural crest cells migrating dorsolaterally (arrowheads* **E**,**e***) and in blood cells in dorsal aorta* **(E)** *but not in ectoderm, myelencephalon, notochord* **(E)**. *Bar, 50 μm. Abbreviations as in Fig. 2.*

the laminin α1 cRNA (Figs. 2 D,d) gave strong signals in the neural ectoderm, in axial mesoderm which continued to show a punctate pattern of laminin expression, in lateral mesoderm and in ectoderm at the ecto- neuroectoderm junction but low expression of transcripts were observed in lateral ectoderm and in endoderm.

At the 10-somite stage (HH10-11) of a single embryo, the laminin α1 cRNA (Figs. 2 E,e and F,f) gave no signals in neural tube, notochord, and ectoderm, and the β1 cRNA (Figs. 3 E,e) gave no signals in the myelencephalon, notochord and ectoderm. The α1 (Figs. 2 E,e and F,f) and β1 (Figs. 3 E,e) cRNAs gave strong signals in neural crest cells. Neural crest cells migrate extensively along well-characterized pathways through cell-free space rich in laminin/other matrix molecules and laminin is enriched in basement membranes of the neural tube, ectoderm, and somite (Duband and Thiery, 1987; Perris *et al.*, 1989). In our results, it is intriguing neural crest cells express laminin genes strongly and even single cells within a stream of migrating cells (arrowheads) can be seen labeled (Figs. 2 E,F and 3E). This may indicate that neural crest cells can produce laminin which may influence their migratory behavior but it is more likely it be required for their aggregation and/or interactions with other cells. Pertinent to this is the finding of the appearance of laminin in the vicinity of neural crest cells at the time of their aggregation into cranial and spinal sensory ganglia of the peripheral nervous system which indicates that laminin participates in the aggregation process (Duband and Thiery, 1987).

The groups of blood cells seen inside or attached to the wall of the aorta were labeled mildly by the α1 (Figs. 2 E,e and F,f) and strongly by the β1 (Figs. 3 E,e) cRNAs but the aorta itself gave no signal.

The pronephric tubules were labeled intensely by the α1 cRNA (Figs. 2 E,e). Ekblom *et al.,* (review, 1990) showed that laminin participated in the mesenchymal aggregation phase leading to tubule formation and that the expression of laminin α1 chain is transient and locally restricted during kidney organogenesis; recent work showed differential expression of five laminin α (α1–5) chains in the developing and adult mouse kidney (Sorokin *et al.*, 1997).

Within the somites, the dermamyotome and sclerotome cell populations displayed variable hybridization signals of the laminin α1 cRNA; the dermamyotome produced a strong signal while the sclerotome was not labeled by the α1 cRNAs (Figs. 2 E,e and F,f). Laminin has been detected in basement membranes of the dermamyotome and it is known that also the sclerotome cells synthesize laminin in somites (Perris *et al.*, 1996). One can infer from our results transient expression of the laminin α1 gene and/or expression of a distinct laminin α1 isoform in the sclerotome in somites. These regional variations are important because they point to the synthesis of variant chains and they imply distinct functions for laminin isoforms during development in chick embryo. It was shown recently that laminin α1 has a restricted distribution in adult mouse tissues including smooth muscle, myocardium or striated muscle (Falk *et al.*, 1999). Laminin α1 has been shown to be important for the development of the polarized state of various epithelial cells and its gradual replacement by laminin α5 during maturation of epithelium may reflect a role for the variant laminin form in maintenance of the fully differentiated or polarized phenotype (Sorokin *et al.*, 1997; Tiger *et al.*, 1997).

Chick laminin subunits have not been extensively characterized. Chick laminin seems to be composed of homologues to several mammalian laminin subunits which are likely to be assembled to various isoforms as indicated by immunodetection (reviewed in Brandenberger *et al.*, 1996) and by amino acid sequence comparison (O'Rear, 1992; Ybot-Gonzalez *et al.*, 1995; Frade *et al.*, 1996; Liu *et al.*, 1998) of mouse and chick laminin. In our present work, the comparable pattern of strong signals for laminin mRNA detected by

the laminin α1 and β1 (and γ1, unpublished results) cRNA probes in the early chick embryo from the morula stage (stage X) to early gastrula (stage HH2) is intriguing. Whether the laminin mRNA detectable during these stages is oogenetic and/or zygotic and whether laminin-1 be the only form of laminin synthesized in the early embryo remains to be determined. Laminin-1 may provide the proper substrate and cues for guiding migrating cells and for folding of epithelial tissues in the early embryo. As development progresses, the laminin α1 and β1 chains exhibited restricted distribution in the forming embryonic tissues and organs suggesting potential roles for different members of the same subfamily of genes in the chick embryo.

Experimental Procedures

Embryos

Embryos at stages X (morula), XIII (blastula), HH2-4 (primitive streak/gastrula), HH5-6 (neurula), and HH10-11 (10 to 13 somites) were removed from the eggs, were subsequently fixed in Carnoy fixative (formula B), dehydrated through graded ethanol solutions, embedded in paraffin and sectioned at 5 μm.

Radioactive labeling and laminin immunoprecipitation

Embryos at stages X and XIII (10 embryos per stage) were removed from the eggs, cleaned, and flattened epiblast-side against the surface of vitelline membrane «rafts». Embryos on rafts were placed on Ringer solution containing 335 μCi L-[^{35}S] methionine (1,134 Ci/mmol; New England Nuclear 009T) per milliliter for 5 h at 37°C.

The 10 embryos of each stage X and stage XIII, separately, were solubilized and laminin was purified by laminin GP-2 antibody-protein A-Sepharose CL-4B chromatography. Production and specificity of antibodies to mouse laminin GP-2 (β1/γ1 chains) were described previously (Chung *et al.*, 1979). Immunoprecipitates were analysed on 5% slab SDS-PAGE (Laemmli, 1970) and processed for fluorography.

Preparation of probes and in situ *hybridization*

The cDNA clones p51 (nucleotides 3975-4634) and p66 (nucleotides 7929-9059) (review Dong and Chung, 1991) for laminin β1 and α1 chains, respectively, were used to produce ^{35}S-labeled probes for *In situ* hybridization. The preparation of probes was performed as described previously (Dong and Chung, 1991).

In situ hybridization was performed essentially as described previously (Dong and Chung, 1991) with the following modifications. The paraffin sections were deparaffinized and rehydrated sequentially in alcohols of decreasing strength down to hydration. The sections were treated with 10 μg/ml protease K (Sigma) for 7 min at room temperature. The probes for *in situ* hybridization were diluted in the hybridization buffer to give a final concentration of 10000 cpm/μl; 30 μl of this solution was applied to each slide and hybridized for 16 h at 60°C.

Acknowledgments

This work was supported by grants from the General Secretariat for Research and Technology of Greece and the European Social Fund (99 EΔ 352), and from NATO (no. 890752).

References

BRANDENBERGER, R., KAMMERER, R.A., ENGEL, J. and CHIQUET, M. (1996). Native chick laminin-4 containing the β2 chain (s-laminin) promotes motor axon growth. *J. Cell Biol.* 135:1583-1592.

CHUNG, A.E., JAFFE, R., FREEMAN, I.L., VERGNES, J-P., BRAGINSKI, J.E. and CARLIN, B. (1979). Properties of a basement membrane-related glycoprotein synthesized in culture by a mouse embryonal carcinoma derived cell line. *Cell* 16: 277-287.

DARRIBERE, T., RIOU, J. F., SHI, D.L., DELARUE, M. and BOUCAUT, J.C. (1986). Synthesis and distribution of laminin-related polypeptides in early amphibian embryos. *Cell Tissue Res.* 246: 45-51.

DONG, L.-J. and CHUNG, A.E. (1991). The expression of the genes for entactin, laminin A, laminin B1 and laminin B2 in murine lens morphogenesis and eye development. *Differentiation* 48: 157-172.

DUBAND, J.-L. and THIERY, J.P. (1987). Distribution of laminin and collagens during avian neural crest development. *Development* 101: 461-478.

DUPRAT, A.-M. and GUALANDRIS, L. (1984). Extracellular matrix and neural determination during amphibian gastrulation. *Cell Differ.* 14: 105-112.

EKBLOM, M., KLEIN, G., MUGRAUER, G., FECKER, L., DEUTZMANN, R., TIMPL, R. and EKBLOM, P. (1990). Transient and locally restricted expression of laminin A chain mRNA by developing epithelial cells during kidney organogenesis. *Cell* 60: 337-346.

FALK, M., FERLETTA, M., FORSBERG, E. and EKBLOM, P. (1999). Restricted distribution of laminin α1 chain in normal adult mouse tissues. *Matrix Biology* 18: 557-568.

FRADE, J.M., MARTINEZ-MORALES, J.R. and RODRIGUEZ-TEBAR, A. (1996). Laminin-1 selectively stimulates neuron generation from cultured retinal neuroepithelial cells. *Exp. Cell Res.* 222: 140-149.

HUANG, S., SAINT-JEANNET, J.P., KAN, P. and DUPRAT, A.M. (1990). Extracellular matrix: an immunological and biochemical (CAT and TOH activity) survey of *in vitro* differentiation of isolated amphibian neuroblasts. *Cell Differ. Dev.* 30: 219-233.

LAEMMLI, U.K. (1970). Cleavage of structural proteins during assembly of the head bacteriophage T4. *Nature* 227: 680-685.

LIU, J., SWASDISON, S., XIE, W., BREWTON, R. G. and MAYNE, R. (1998). Primary structure and expression of a chicken laminin β chain: Evidence for four β chains in birds. *Matrix Biology* 16: 471-481.

O' REAR, J.J. (1992). A novel laminin B1 chain variant in avian eye. *J. Biol. Chem.* 267: 20555-20557.

PERRIS, R., PAULSSON, M. and BRONNER-FRASER, M. (1989). Molecular mechanisms of avian neural crest cell migration on fibronectin and laminin. *Dev. Biol.* 136: 222-238.

RIOU, J.F., DARRIBERE, T., SHI, D.L., RICHOUX, V. and BOUCAUT, J.C. (1987). Synthesis of laminin-related polypeptides in oocytes, eggs and early embryos of the amphibian *Pleurodeles waltlii. Roux Arch. Dev. Biol.* 196: 328-332.

SOROKIN, L. M., PAUSCH, F., DURBEEJ, M. and EKBLOM, P. (1997). Differential expression of five laminin α(1-5) chains in developing and adult mouse kidney. *Dev. Dyn.* 210:446-462.

TIGER, C-F., CHAMPLIAUD, M-F., PEDROSA-DOMELLOF, F., THORNELL, L-E., EKBLOM, P. and GULLBERG, D. (1997). Presence of laminin α5 chain and lack of laminin α1 chain during human muscle development and in muscular dystrophies. *J. Biol. Chem.* 272: 28590-28595.

YBOT-GONZALEZ, P., RUNSWICK, S., SMYTH, N. and EDGAR, D. (1995). Regulated expression of a novel laminin β subunit during the development of the chick embryo. *Differentiation* 59: 215-223.

ZAGRIS, N. and CHUNG, A.E. (1990). Distribution and functional role of laminin during induction of the embryonic axis in the chick embryo. *Differentiation* 43: 81-86.

ZAGRIS, N., KALANTZIS, K. and GUIALIS, A. (1998). Activation of embryonic genome in chick. *Zygote* 6: 227-231.

Received: February 2000
Accepted for publication: July 2000