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Embryonal carcinoma and the basement membrane glycoproteins laminin and entactin

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ABSTRACT **The mouse embryonal carcinoma lines PCC4-F and F9 have played important roles in the isolation and characterization of the two ubiquitous basement membrane proteins, laminin and entactin. The contributions of these cells to our work on extracellular matrices are briefly summarized. The *in vitro* differentiation of PCC4-F gives rise to a multiplicity of cell types. Two of these cell types have been propagated as cell lines. One of these, M1536-B3, synthesizes and deposits copious quantities of extracellular matrix glycoproteins, which led to the initial discovery and characterization of laminin and entactin. In addition, M1536-B3 provides a model system for analyzing the assembly of laminin and the laminin-entactin complex and for manipulating extracellular matrix structure and composition. The other cell line, 4CQ, synthesizes a matrix consisting of fibronectin and entactin. F9 cells differentiate to endodermal cells in response to retinoic acid and dibutyryl cyclic AMP (Strickland and Mahdavi, *Cell 15:* 393-402, 1978). The differentiated cells synthesize basement membrane components and provided the probes for the cDNA cloning of entactin and the three chains of laminin. The F9 cells have been widely employed to examine the regulation of expression of the laminin and entactin genes.**

KEY WORDS: *basement membranes, embryonal carcinoma, laminin, entactin*

Introduction

The importance of laminin in influencing cell behavior is now firmly established and has been extensively reviewed (Martin and Timpl, 1987; Beck *et al.*, 1990). Laminin as an integral component of the basement membrane influences neurite outgrowth, cell movement and adhesiveness, neuromuscular connectivity, maintenance and modulation of differentiated functions and the integrity of diverse structures. The mouse embryonal carcinoma played a central role in the discovery and the subsequent characterization of the major glycoproteins laminin and entactin in our laboratory. In malignant teratocarcinomas, embryonal carcinoma are the stem cells that can develop either spontaneously or from extrauterine grafting of early mouse embryos (Stevens, 1958, 1970). Kleinsmith and Pierce (1964) unequivocally demonstrated that single embryonal carcinoma cells were pluripotent, and thereby set the stage for their use in exploring problems of embryogenesis and cell differentiation. Furthermore, histochemical and morphological studies indicated that embryonal carcinoma cells have the characteristics of embryonic cells (Damjanov and Solter, 1975). Incorporation of these cells into mouse tissues after transplantation into blastocysts (Mintz and Illmensee, 1975; Papaioannou *et al.*, 1975) confirmed their validity as a model for embryonic development. Although teratocarcinomas could be propagated in mouse ascites, the demonstration of the retention of pluripotency by long-term cultures of clones of embryonal carcinoma cells by Finch and Ephrussi (1967) led to the subsequent establishment of several useful lines (Kahan and Ephrussi, 1970; Rosenthal *et al.*, 1970; Evans, 1972; Jakob *et al.*, 1973).

The line PCC3, derived from the transplantable 129/Sv testicular-derived teratocarcinoma OTT 6050 of Stevens (1970), was shown by Nicolas *et al.* (1975) to be capable of reproducibly differentiating in cell culture to yield derivatives of all three germ layers. Several laboratories confirmed these observations with independent lines (Martin and Evans, 1975; McBurney, 1976). The differentiation of PCC3 cells was induced by prolonged culture in monolayers. However, other clones such as PCC4 were apparently unable to differentiate under similar conditions. The *in vitro* differentiation of PCC4 was dependent on the prior formation of clusters of cells or "embryoid bodies" similar to those found in intraperitoneal embryonal carcinoma tumors (Pierce and Dixon, 1959). These embryoid bodies consist of an outer layer of endodermal cells that

Abbreviations used in this paper: RGD, arginine-glycine aspartic acid; EGF, epidermal growth factor; HPLC, high performance liquid chromatography.

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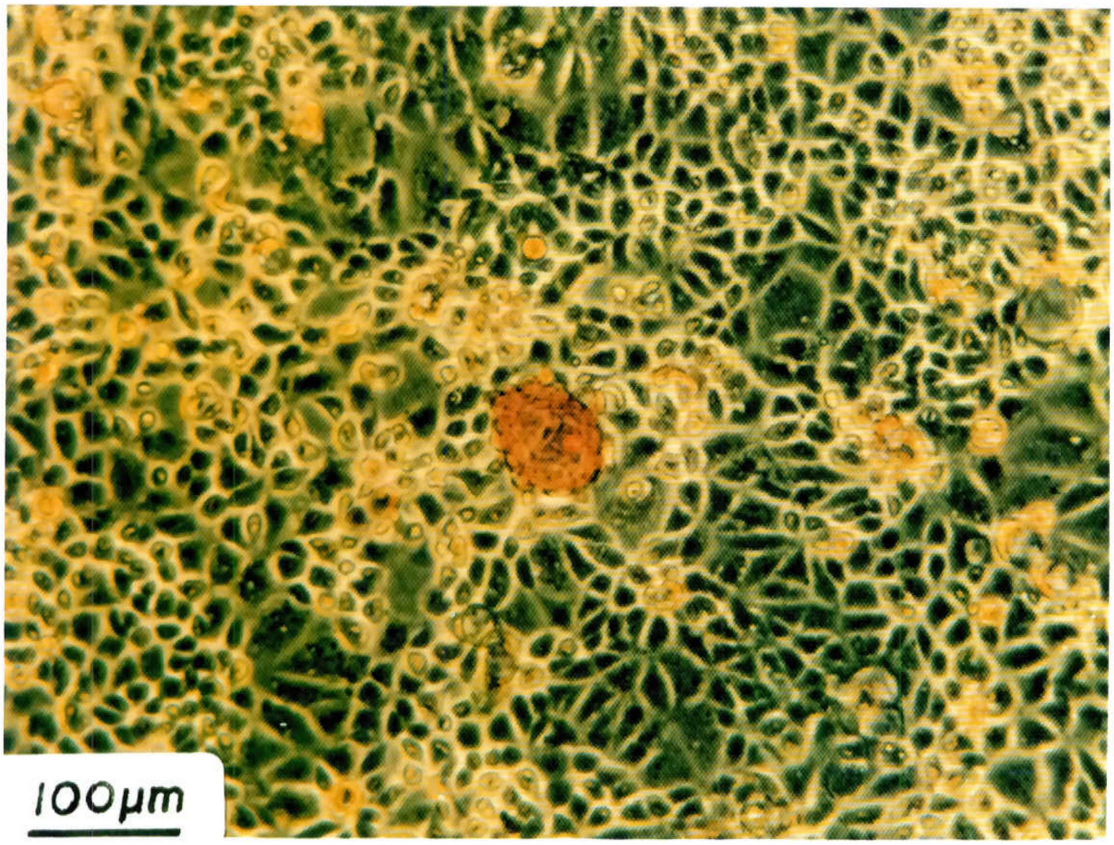

Fig. 1. PAS staining of extracellular material produced by by M1536-B3 cells in monolayer culture.

synthesize the extracellular Reichert's membrane and an inner core of embryonal carcinoma cells. Martin and Evans (1975) reported that floating embryoid bodies obtained from monolayer cultures of one of their lines, when replated in fresh culture medium, attached and underwent further differentiation.

During attempts to reproduce the observations on PCC3 cells with the PCC4 line in the laboratory of the late Professor Boris Ephrussi at CNRS at Gif-Sur-Yvette in 1974-75, we isolated the differentiated line PFHR-9 (Chung *et al.*, 1977a). The morphology of this cell was strongly reminiscent of a differentiated line obtained from teratocarcinomas by Pierce and co-workers (Lehman *et al.*, 1974). These cells exhibited a typical pavement-like pattern with interspersed rosettes of extracellular material that stained strongly positive with periodic acid-Schiff reagent (PAS) indicating the presence of glycoproteins (Fig. 1). This initial observation subsequently led to the identification and characterization of the two ubiquitous basal lamina glycoproteins laminin and entactin (Chung *et al.*, 1977b, 1979; Carlin *et al.*, 1981).

In addition to the production of laminin and entactin, the embryonal carcinoma played an important role in the determination of the molecular structures of both laminin and entactin. In this regard, a major contribution was made by Strickland and Mahdavi (1978) who demonstrated that the previously presumed nullipotent F9 embryonal carcinoma characterized by Ephrussi and co-workers (Bernstine *et al.*, 1973) could be induced to differentiate in response to retinoic acid and dibutyryl cyclic AMP. The differentiated cells produced copious amounts of Reichert's membrane components including laminin and type IV collagen (Strickland *et al.*, 1980). The F9 cells provided a source of differential molecular probes for the isolation of laminin and entactin cDNA clones and a model for exploring the regulation of their genes.

In this communication, I will summarize work that was critically dependent on the embryonal carcinoma system that was pioneered by Barry Pierce and his colleagues.

Laminin

Isolation from M1536-B3 cells

The embryonal carcinoma PCC4-F cell can be maintained in the undifferentiated state when passaged at frequent intervals in monolayer culture. Plating of these cells in bacteriological Petri dishes results in the formation of floating cell aggregates which increase in size over several days and which eventually become necrotic. The aggregates, after 24 h in suspension culture, reattach when replated on normal tissue culture dishes. The cells proliferate and in approximately 10 days, with frequent changes of medium, a variety of differentiated cell morphologies can be detected, some of which are shown in Fig. 2. Among these cells are fibroblasts, neuronal, fat, epithelial, muscle and endoderm-like cells. From these differentiated cultures we established two cell lines, one of which produced copious quantities of fibronectin (Chung *et al.*, 1979) and the other large quantities of extracellular matrix material that stained positively with PAS. This latter cell line was designated M1536-B3.

M1536-B3 cells can grow either in monolayer or suspension culture (Fig. 3). In suspension culture the cells form a monolayer on a spherical extracellular matrix raft which is elaborated and secreted from the basal surface of the cells. The cells in this aggregate are polarized, rich in rough endoplasmic reticulum, and contain large membrane-bound vesicles which enclose the extracellular matrix components (Fig. 4). Treatment of the aggregates with a solution of cytochalasin B at 10 µg/ml of phosphate buffered saline containing magnesium and calcium ions causes the cells to detach. The detached cells are easily separated from the extracellular matrix spheres by several cycles of gentle centrifugation and resuspension in fresh phosphate buffered saline (Fig. 3). After reduction with mercaptoethanol, the purified extracellular matrix display a remarkably simple protein pattern (Fig. 5) when examined on SDS polyacrylamide gels. The two major high molecular protein bands and the two quantitatively less significant bands of lower molecular weights were designated GP-1, GP-2, GP-3A and GP-3B in ascending order of mobility on the gel. The relationship of these bands to previously characterized molecules and to each other was not known then. The electrophoretic mobilities of the bands were distinct from plasma fibronectin (Fig. 5, lane c) and erythrocyte spectrin (Fig. 5, lane b). Amino acid and carbohydrate analyses (Chung *et al.*, 1977b, 1979) further indicated that these molecules were distinct from either spectrin or fibronectin. The carbohydrate composition was reminiscent of that previously reported by Pierce and colleagues for the basement membrane-like material produced by the mouse endodermal-like cells derived from embryonal carcinoma (Lehman *et al.*, 1974).

Characterization

In order to demonstrate the relationship of these extracellular matrix molecules to authentic basement membranes, the protein bands were purified by electrophoresis as shown in Fig. 6. The individual bands were used to prepare polyclonal and monoclonal antibodies. The most extensive studies were initially carried out with antibodies against GP-2 since this species was highly antigenic. The antibodies which stained the extracellular matrix spheres (Fig. 7) were shown to stain basement membranes of the kidney and subsequently all the basement membranes that were examined (Chung *et al.*, 1979; Bender *et al.*, 1981; Martínez-Hernández and Chung, 1984). This clearly established that the molecule was an integral component of the basement membrane. The GP-2 band was shown to consist of two polypeptide chains of slightly different molecular weights which, because of glycosylation, had virtually identical electrophoretic mobilities on SDS gels. The two chains could be distinguished in M1536-B3 cells (Durkin, 1985) or the parietal endoderm cell line PYS-2 (Cooper *et al.*, 1981; Howe and Dietzschold, 1983), by treatment with tunicamycin, which blocks N-glycosylation. In addition, carboxymethylation differentially decreased the electrophoretic mobilities of the two chains allowing them to be readily separated (Chakravarti, 1989). The larger polypeptide is now known as the B1 chain and the smaller B2 (Hogan *et al.*, 1980). The glycosylated B1 and B2 chains, each approximately 220 kDa, were shown to be linked to each other and to the larger A chain of 400 kDa by disulfide bonds since the three chains ran as a single band of high molecular weight under non-reducing conditions. The name laminin for this heterotrimeric molecule was coined by Timpl and co-workers for an identical molecule isolated from the mouse EHS tumor (Timpl *et al.*, 1979). The organization of the three chains into a cross-shaped structure was established by rotary shadowing (Engel *et al.*, 1981).

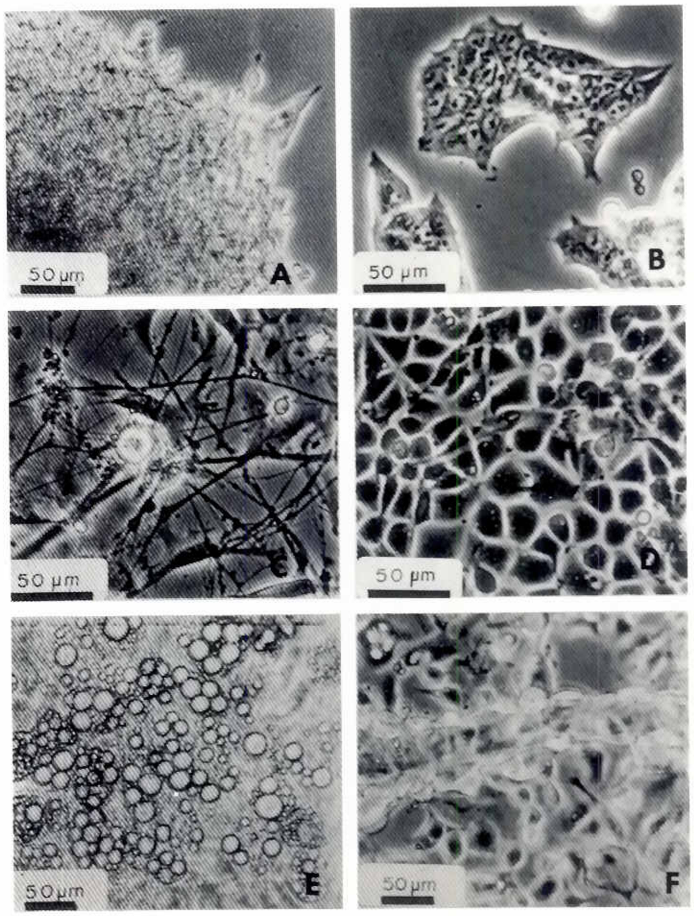

Fig. 2. Examples of differentiated cell types produced in differentiated cultures of the embryonal carcinoma PCC4-F. *Undifferentiated cells* **(A, B)**, *neuronal type cells,* **(C)**; *parietal endoderm,* **(D)**; *fat cells,* **(E)**; *tube-like structures* **(F)**.

Primary structure of laminin

The complete primary structures of the three chains of mouse (Sasaki and Yamada, 1987; Sasaki *et al.*, 1987, 1988; Durkin *et al.*, 1988a) and human laminin (Pikkarainen *et al.*, 1987, 1988; Nissinen *et al.*, 1991) as well as the B1 and B2 chains of *Drosophila* laminin have been determined by cDNA cloning (Montell and Goodman, 1988, 1989; Chi and Hui, 1989). In addition, the complete amino acid sequence of S-laminin (Hunter *et al.*, 1989), an analog of the B1 chain, and partial sequences of human merosin, an analog of the A chain (Ehrig *et al.*, 1990), and that of the A chain of *Drosophila* laminin (Garrison *et al.*, 1991) have been reported. Our own efforts were focused on the cloning of the mouse cDNA from differentiated F9 as well as M1536-B3 cells which constitutively synthesized large quantities of laminin. cDNA libraries were constructed from mRNA derived from the differentiated F9 embryonal carcinoma and M1536-B3 cells. Since neither amino acid, cDNA sequences nor expression libraries were available it was necessary to employ a differential screening approach. cDNA probes were synthesized with mRNAs derived from F9 embryonal carcinoma and M1536-B3 cells (Durkin *et al.*, 1986). This screening procedure

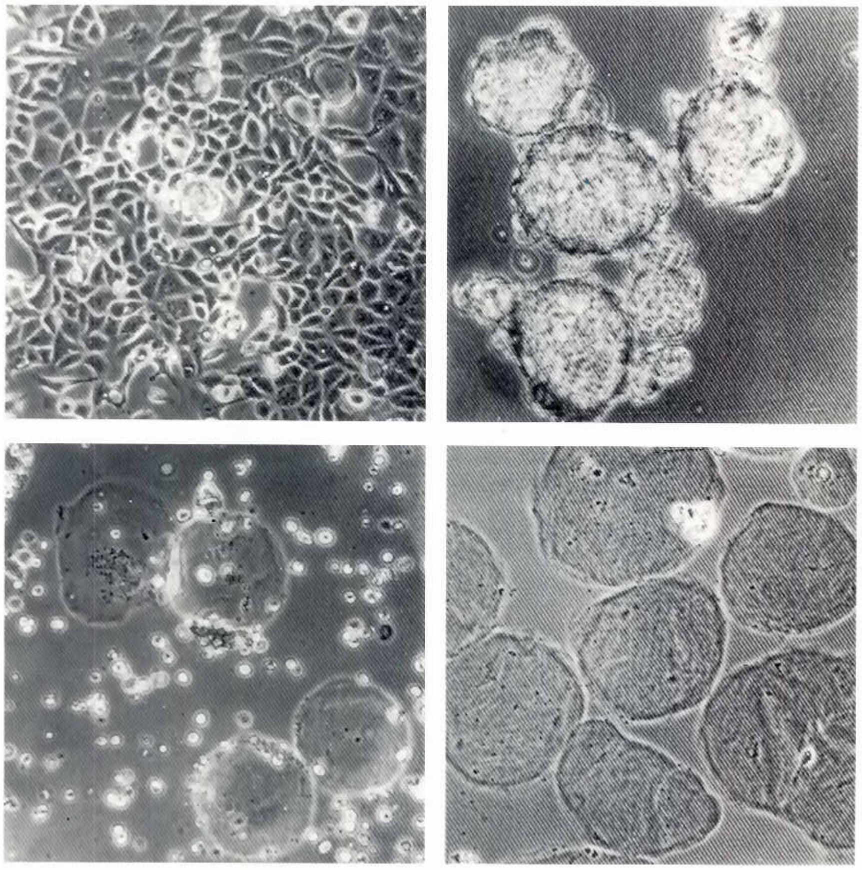

Fig. 3. Isolation of extracellular matrix from M1536-B3 cells. *Upper left panel, cells in monolayer cultures; upper right panel, spheres of cells in Petri dishes; lower left, spheres treated with cytochalasin B; lower right, purified extracellular matrix spherical rafts.*

yielded several clones that hybridized to the probes derived from the M1536-B3 but not the undifferentiated cells. An example of the results are shown in Fig. 8. The identity of each clone was established by hybrid selection of mRNA from M1536-B3 cells and translation of the selected mRNA *in vitro*. The translation products were immunoprecipitated with antibodies derived from the A and B chains of laminin (Durkin *et al.*, 1986). DNA sequencing of the clones later confirmed their identity. The clone p16 contained sequences for the B2 chain and both p51 and p59 for the A chain. In addition to these, clone p2 contained sequences for the B1 chain. The clones p16, p2 and p59 hybridized to mRNA species of 8, 6 and 9.8 kilobases respectively. The primary structures of the various laminin chains deduced from sequencing of overlapping cDNA clones have revealed a high degree of structural conservation across species. Each chain is comprised of distinct structural and functional domains that are important in modulating cell behavior in development and metastasis and in the assembly of the extracellular matrix (Beck *et al.*, 1990).

Regulation of laminin synthesis and assembly

The mouse embryonal carcinoma F9 cell has been extensively used by several laboratories including our own to explore the regulation of expression of the laminin and entactin genes (Strickland *et al.*, 1980; Carlin *et al.*, 1983; Cooper *et al.*, 1983; Wang *et al.*, 1985; Durkin *et al.*, 1986, 1987; Kleinman *et al.*, 1987). The cells can be induced to differentiate in the presence of retinoic acid or a combination of retinoic acid and dibutyryl cyclic AMP (Strickland and Mahdavi, 1978). Retinoic acid alone promotes the differentiation of F9 cells to distal endoderm and in conjunction with dibutyryl cyclic AMP to parietal endoderm (Hogan *et al.*, 1983). The dramatic morphological changes which transform the closely packed embryonal carcinoma cells to the pavement-like epithelial morphology are accompanied by equally dramatic changes in the biosynthesis of extracellular matrix components. The steady state levels of the three chains of laminin increase coordinately as the cells differentiate (Durkin *et al.*, 1986; Kleinman *et al.*, 1987). The molecular details of the regulation mechanism of the genes are actively being pursued. Regulatory elements in the 5'-flanking region of the genes for the B1 (Vasios *et al.*, 1989) and B2 (Ogawa *et al.*, 1988) genes have been identified, although their role in the regulation of the genes *in vivo* remains to be determined.

The assembly of the laminin molecule and its insertion into the extracellular matrix requires the synthesis of the three polypeptide chains, their correct folding and association to form the heterotrimeric molecule, glycosylation, disulfide cross-linking, and transport from the endoplasmic reticulum to the extracellular compartment. Each of these steps must be regulated to ensure that a functionally appropriate matrix is synthesized. The complexity of the assembly process is further emphasized by the discovery of S-laminin and merosin which, in some tissues, can replace the B1 and A chains

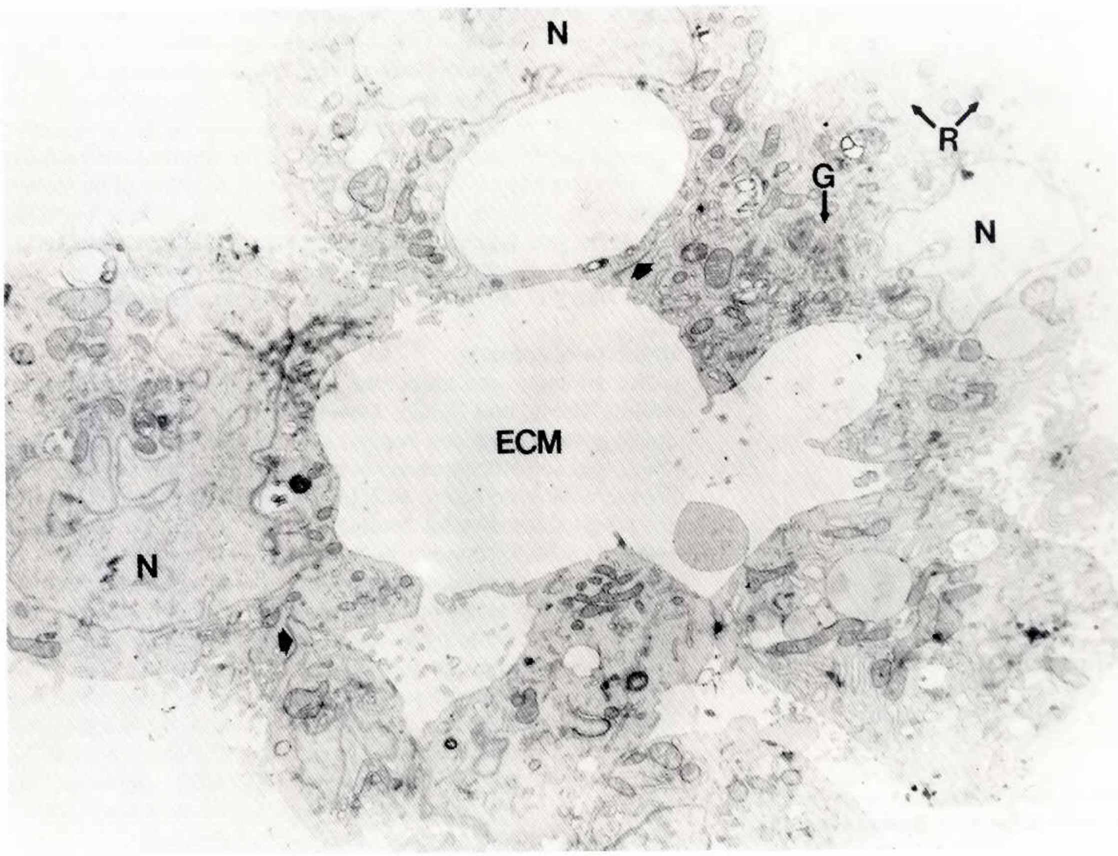

Fig. 4. Electron micrograph of thin section through a small spherical aggregate of M1536-B3 cells. *N, nucleus; G, Golgi; R, rough endoplasmic reticulum, ECM, extracellular matrix.*

respectively (Engvall *et al.,* 1990). It remains to be determined whether matrix biosynthesis and deposition are regulated at the level of gene transcription, translation of the messages or in the assembly and processing of the three chains.

We have examined the early stages in the assembly of the three chains of laminin in M1536-B3 cells (Wu *et al.,* 1988). These cells provide an especially attractive model since they synthesize laminin in great abundance. Early intermediates in the assembly pathway were identified by radiolabeling and immunoprecipitation with chain-specific polyclonal and monoclonal antibodies. Our major conclusions were (a) the assembly occurred in the endoplasmic reticulum cisternae; (b) the assembly or the three chains did not follow an obligatory path requiring the prior formation of a dimer of the two B chains. This latter conclusion is not in agreement with those derived from *in vitro* studies on the reassembly of laminin fragments (Hunter *et al.,* 1990) and those in the human choriocarcinoma JAR cells (Peters *et al.,* 1985), which synthesize much smaller quantities of laminin. The various results are not necessarily incompatible since different systems and reagents were used in the studies. It does appear, however, that like other heteropolymers, proper folding and assembly are required for the molecule to progress through the intracellular compartments. Although the rate of synthesis of the two B chains appears to be faster than that of the A chain, the only species observed in the matrix in M1536-B3 cells is the heterotrimeric molecule. Mechanisms are likely to be present in the endoplasmic reticulum for the degradation of excess incorrectly assembled molecules.

The B1 chain of laminin and its analog may be especially important in the assembly and exocytosis of laminin. Recent experiments by Sanes, Merlie and their collaborators have shown that S-laminin can replace the B1 chain in laminin. In all of the laminin variants examined, the B2 chain was invariably present (Green *et al.,* 1991). We have shown that rat pheochromocytoma PC12 cells synthesize the B2 message but not the B1 message (Reing *et al.* unpublished). These cells do not deposit laminin (Fig. 9A) but instead deposit fibronectin (Fig. 9B) in the extracellular matrix. However, transfection of these cells with the full length cDNA for the B1 chain of laminin results in the deposition of laminin (Fig. 9C) and in this cell line a decreased amount of fibronectin (Fig. 9D). These data suggest that laminin molecules cannot be properly assembled with two B2 chains and that the B1, and in some cells, S-laminin is necessary either for the proper assembly or to provide the correct signals for exocytosis and deposition of laminin.

The coordination of the synthesis and processing of laminin and its variants presents an important problem of special importance in development, wound healing, neovascularization, cell polarization and a variety of pathological conditions. To obtain insight into the regulatory pathways several groups of investigators including our own have examined the temporal and spatial expression of the genes in mouse embryos. The major conclusions indicate that the three chains of laminin are not necessarily coordinately expressed. The B chains are expressed as early as the 2-4 cell embryonic stage with the A chain appearing at a later stage (Cooper and MacQueen, 1983; Wu *et al.,* 1983; Dziadek and Timpl, 1985). In the developing

Fig. 5. Comparison of extracellular matrix proteins from M1536-B3 cells with spectrin and fibronectin on sodium dodecyl sulfate gel. *Extracellular matrix proteins,* **(a)**; *erythrocyte spectrin,* **(b)**; *plasma fibronectin,* **(c)**; *mixture of extracellular matrix proteins and fibronectin,* **(d)**; *extracellular matrix proteins,* **(e)**.

kidney, A chain synthesis appears to be coincidental with epithelial polarization (Ekblom *et al.*, 1990). Recent experiments in the developing mouse eye have shown that the expression of the three chains of laminin are not coordinately regulated, and *in situ* hybridization suggests that the B1 (Sarthy and Fu, 1990) and B2 chains, but not the A chain messenger RNA, are synthesized by the ganglion cell layer of the retina (Dong and Chung, 1991). In other structures of the eye the expression of the three genes is closely regulated as development progresses (Dong and Chung, 1991). The temporal relationship between the synthesis of laminin and its cell membrane receptors is crucial for the transduction of regulatory signals that influence the behavior and fate of the cell. The rapid progress in identification and characterization of laminin receptors (Mecham, 1991) will greatly facilitate our understanding of the molecular mechanisms of signal transduction.

Entactin

Isolation from M1536-B3 cells

The extracellular matrix from M1536-B3 cells as shown in Fig. 6 contained the laminin chains as well as two protein bands with molecular weights of approximately 150,000 and 130,000. The two polypeptides were shown to be sulfated by labeling with $^{35}SO_4$ (Carlin *et al.*, 1981). Subsequent experiments with polyclonal antibodies, peptide mapping by HPLC and microsequencing revealed that the smaller molecule was a proteolytic cleavage product of the larger. This sulfated glycoprotein was named entactin (Carlin *et al.*, 1981) because of its intimate association with the endodermal cells from which it was derived. The protein was isolated by preparative electrophoresis, as previously described for laminin, and antibody preparations against it were found to stain a variety of basement membranes (Bender *et al.*, 1981; Carlin *et al.*, 1981; Martínez-Hernández and Chung, 1984). A similar protein was identified in mouse parietal endoderm (Hogan *et al.*, 1982) and later in the EHS tumor (Timpl *et al.*, 1983). This latter protein was called nidogen.

Structure of entactin

The structure and properties of entactin have been reviewed recently (Chung and Durkin, 1990). The amino acid sequence of mouse entactin deduced from a combination of cDNA cloning and amino acid sequencing consists of 1217 amino acids divided into three structural domains, an N-terminal globule consisting of two smaller globules of total estimated mass of 85 kDa (Domain I) linked by a stalk (Domain II) to a smaller C-terminal globule of estimated mass of 38 kDa (Domain III) (Durkin *et al.*, 1988b). Domain II consists of 5 cysteine-rich repeats linked in tandem, the first four of which are similar to those found in epidermal growth factor and the fifth reminiscent of that found in thyroglobulin. Two other cysteine-rich EGF-like repeats are present, one of which is located between the two globules at the amino terminus end and the other near the C-terminus. The first cysteine-rich EGF-like repeat in the Domain II contains an integrin-recognition RGD sequence. The molecule contains two potential N-glycosylation sites, calcium binding sequences and potential O-glycosylation sites.

Biological properties of entactin

The biological functions of entactin are only now being studied in detail. These studies will be greatly accelerated with the recent construction of full length cDNA molecules that have been expressed in the baculovirus system (Tsao *et al.*, 1990) and in

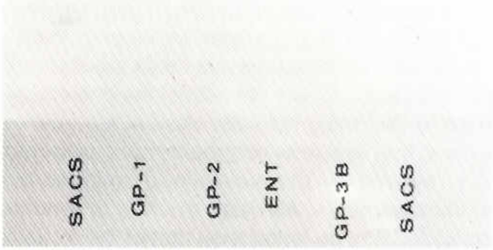

Fig. 6. Purification of extracellular matrix proteins. *Extracellular matrix proteins, SACS; laminin A chain, GP-1; laminin B chains, GP-2; entactin, ENT; entactin 130kDa fragment, GP-3B.*

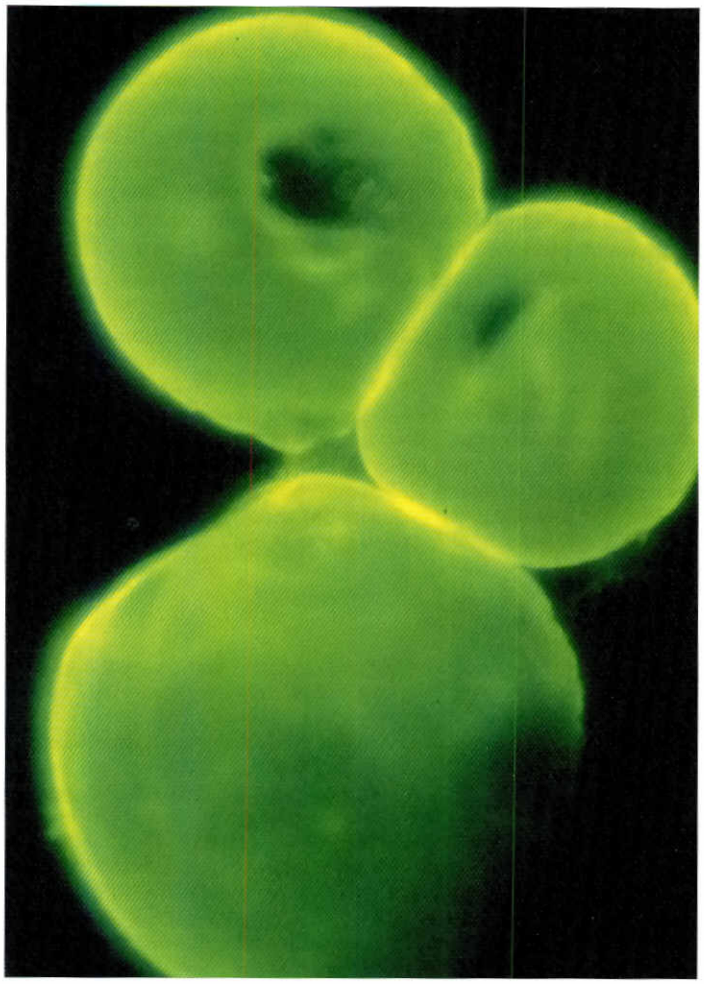

Fig. 7. Extracellular matrix spheres labeled by indirect immunofluorescence with anti-GP-2 (laminin B chains) rabbit polyclonal antibodies.

mammalian cells (Fox *et al.,* 1991). It has long been known that entactin forms a very stable stoichiometric complex with laminin (Chung *et al.,* 1977; Carlin *et al.,* 1983; Kurkinen *et al.,* 1983; Timpl *et al.,* 1983). This interaction has been reported to occur between the C-terminus globule of entactin and the short arm of the B2 chain of laminin close to the intersection of the arms of laminin (Paulsson *et al.,* 1987; Fox *et al.,* 1991). Entactin binds to collagen IV most likely through its N-terminus globular domain (Fox *et al.,* 1991). These binding activities suggest that entactin can serve as an organizer of the extracellular matrix. In addition, we have recently shown that the embryonal carcinoma-derived 4CQ cell forms an extracellular matrix in which fibronectin and entactin are co-localized (Wu *et al.,* 1991). Direct binding of labeled entactin to immobilized fibronectin was also demonstrated. The biological implications of these binding activities remain to be established but it is reasonable to assume that entactin can modulate not only the structure of the extracellular matrix but also its biological activities.

The biological activities of entactin extend beyond its role in matrix organization. Experiments *in vitro* with recombinant entactin as well as the molecule isolated from M1536-B3 extracellular matrix suggest alternate functions. These include promotion of attachment of several types of cells including neutrophils (Alstadt *et al.,* 1987; Chakravarti *et al.,* 1990; Senior *et al.,* 1991), promotion of neutrophil chemotaxis and phagocytosis (Senior *et al.,* 1991), binding of calcium ions (Chakravarti *et al.,* 1990; Tsao *et al.,* 1990) and binding to the Aα and Bβ chains of fibrinogen (Wu and Chung, 1991). Several of these properties are not unlike those of fibronectin and suggest that entactin may be involved in inflammatory responses, wound healing and hemostasis. Although the cell membrane receptors for entactin have not been fully characterized, inhibition of function by anti-integrin antibodies suggests that the leukocyte response integrin (LRI) mediates some of the interactions with neutrophils (Senior *et al.,* 1992), and a preliminary report suggests that the $\alpha 3\beta 1$ integrin may also be a receptor (Jewell *et al.,* 1991).

Conclusions

The mouse embryonal carcinoma has provided a remarkably valuable system for exploring mechanisms of cell differentiation and gene regulation. The *in vitro* differentiation of these stem cells to give rise to multiple cell types has provided cell lines enabling our laboratory to isolate and characterize in molecular detail two ubiquitous basement membrane proteins, entactin and laminin. The differentiated cell lines have provided an especially favorable model system for exploring the mechanism of assembly and processing of laminin and the laminin-entactin complex, as well as the interaction of entactin with fibronectin. The constitutive synthesis of the laminin-entactin complex by these cells will allow perturbation of the assembly pathway to yield further information on the regulation of complex formation and exocytosis. Furthermore, they have the potential to generate new assemblies of extracellular matrix by

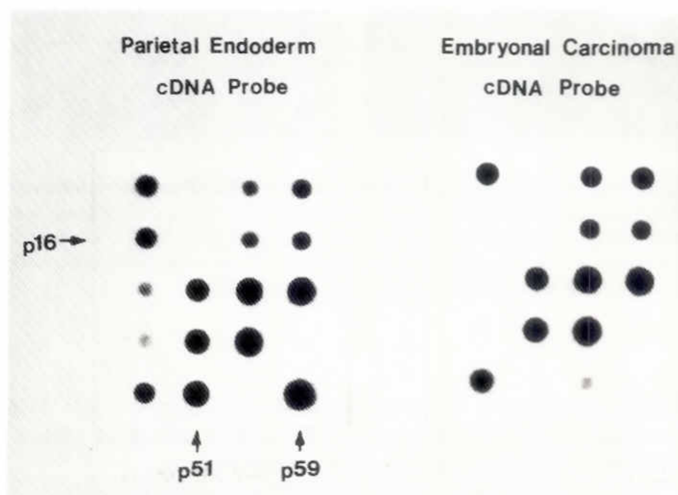

Fig. 8. Differential screening of cDNA clones derived from differentiated F9 cDNA library. *DNA from different clones were applied to nitrocellulose in duplicate and probed with cDNAs obtained from mRNAs isolated either from parietal endoderm M1536-B3 cells or from undifferentiated F9 embryonal carcinoma cells.*

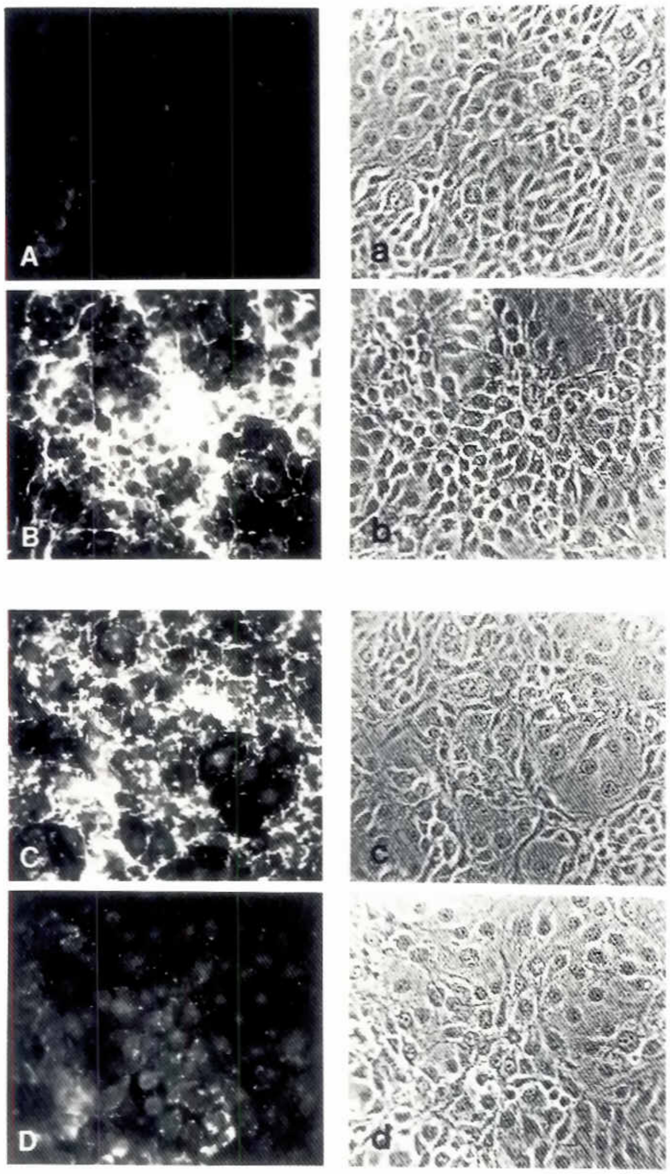

Fig. 9. Transfection of rat pheochromocytoma PC12 cells with laminin B1 full length cDNA. (A-D) *Cells stained by indirect immunofluorescence,* **(a-d)**, *corresponding phase contrast micrographs.* **(A and B)**, *PC12 cells;* **(C and D)**, *B1 transfected cells.* **(A)** *and* **(C)** *stained with anti-laminin antibodies and* **(B and D)** *with anti-fibronectin antibodies.*

genetic manipulation. These matrices will be invaluable in providing substrates to explore the specificity and physiological consequences of cell-extracellular matrix interactions.

The hormone-directed differentiation of embryonal carcinoma cells to parietal endoderm, which expresses a specific set of genes, has provided an excellent system to explore the detailed mechanisms of gene regulation. This system will be even more useful as the structures of the induced genes are further characterized. Among the advantages are the control that can be exercised and the reproducible behavior of the system. The differentiated F9 embryonal carcinoma cells produce a well-defined functional group of extracellular matrix proteins and hence it should be possible to examine the intricate interplay between the regulatory pathways for this set of genes and perhaps those of their receptors. The relative ease of manipulation of the system makes it possible to determine precisely the levels of gene regulation both at the transcriptional and post-transcriptional levels. The isolation of cell lines such as M1536-B3, which produces laminin and entactin constitutively, and 4CQ, which produces fibronectin and entactin, from the parental PCC4-F embryonal carcinoma provides an opportunity to analyze the factors which regulate the choice between different sets of extracellular matrix molecules during cell differentiation.

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