Distinct roles for dystroglycan, β1 integrin and perlecan in cell surface laminin organization

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Accepted 9 January 2001
Journal of Cell Science 114, 1137-1144 © The Company of Biologists Ltd

SUMMARY

Dystroglycan (DG) is a cell surface receptor for several extracellular matrix (ECM) molecules including laminins, agrin and perlecan. Recent data indicate that DG function is required for the formation of basement membranes in early development and the organization of laminin on the cell surface. Here we show that DG-mediated laminin clustering on mouse embryonic stem (ES) cells is a dynamic process in which clusters are consolidated over time into increasingly more complex structures. Utilizing various null-mutant ES cell lines, we define roles for other molecules in this process. In β1 integrin-deficient ES cells, laminin-1 binds to the cell surface, but fails to organize into more morphologically complex structures. This result indicates that β1 integrin function is required after DG function in the cell surface-mediated laminin assembly process. In perlecan-deficient ES cells, the formation of complex laminin-1 structures is defective, implicating perlecan in the laminin matrix assembly process. Moreover, laminin and perlecan reciprocally modulate the organization of the other on the cell surface. Taken together, the data support a model whereby DG serves as a receptor essential for the initial binding of laminin on the cell surface, whereas β1 integrins and perlecan are required for laminin matrix assembly processes after it binds to the cell.

Key words: Dystroglycan, β1 integrin, Perlecan, Extracellular matrix assembly, Embryonic stem cell

INTRODUCTION

ECM molecules are integral to tissue form and function. They are composed of a set of glycoproteins and proteoglycans that are capable of many intermolecular interactions with one another (Timpl and Brown, 1996). Despite extensive cataloging of many ECM proteins and their associations over the past years, we still have a very incomplete understanding of how ECMs form in vivo. This is particularly true for basement membranes, the specialized ECM that forms adjacent to epithelial, neural, muscle, adipose and endothelial cell types. The most abundant components of basement membranes are the laminins and type IV collagens. In vitro, each of these molecules form the polymeric networks that are thought to comprise basic structural elements of basement membranes (Yurchenco and Furthmayer, 1984; Yurchenco et al., 1985; Cheng et al., 1997). Although these networks can be induced to self-assemble in vitro, the extent to which spontaneous organization of laminin and type IV collagen networks contribute to basement membrane assembly in vivo is uncertain and other mechanisms may be involved. For instance, in some developing tissues the sites of laminin protein synthesis and basement membrane assembly are spatially distinct, occurring in the mesenchymal and epithelial cell compartments, respectively (Thomas and Dziadek, 1993; Ekblom et al., 1994). This latter finding argues in favor of cell-directed mechanisms that can specify the sites of ECM assembly. Indeed, emerging evidence indicates that receptors on the cell surface can orchestrate the assembly of the ECM (Schwarzbauer, 1999).

Perhaps the best-known ECM receptors are the integrins (Hynes, 1992). The large family of integrin receptors interacts with many ECM proteins and these interactions have been shown to mediate a wide variety of biological events, including assembly of ECM proteins. In one of the most interesting examples, members of the β1 integrin family are thought to bind fibronectin monomers to expose cryptic self-assembly sites normally buried in the fibronectin molecule (Wu et al., 1995; Zhong et al., 1998). In this way integrins may catalyze the local assembly of fibronectin fibrils at the necessary times and places. Integrins have also been implicated in the assembly of laminin-based basement membranes, but their precise role(s) in this process remains unclear (Gardner et al., 1996; Bloch et al., 1997; DiPersio et al., 1997; Fleischmajer et al., 1998; Sasaki et al., 1998; Colognato et al., 1999).

Recently, we have shown that another cell surface receptor, dystroglycan (DG), plays a critical role in the formation of a basement membrane during early development. DG-null mutant embryos fail to progress beyond the early egg cylinder stage of development, and present with specific disruptions in Reichert's membrane-one of the earliest basement membranes that form during murine development (Williamson et al., 1997). DG is a heterodimeric integral membrane glycoprotein

that is expressed in many epithelial, muscle and neural cell types (Durbeej et al., 1998). Its extracellular α subunit binds to several ECM proteins including members of the laminin family, agrin and perlecan, through conserved laminin-G domains present in those molecules (Henry and Campbell, 1999). In several cell types, DG is required for the organization of laminin on the cell surface (Cohen et al., 1997; Henry and Campbell, 1998; Colognato et al., 1999). It is perhaps this ability of DG to bind to and organize laminin on the cell surface that underlies the disruption of Reichert's membrane formation in the DG-null mutant embryos. Indeed, DG-null ES cells that are deficient in cell surface laminin organization give rise to embryoid bodies that lack organized sub-endodermal basement membranes (Henry and Campbell, 1998).

In this report, we have examined the process of cell surface laminin organization on ES cells in more detail. We find that clustering of laminin-1 on the surface of ES cells is a dynamic process whereby morphologically simple structures are consolidated into increasingly more complex arrays. Using null mutant ES cells, we examined the contributions of β1 integrin and perlecan, two molecules that have previously been implicated ECM assembly processes. We found that β1 integrin is required for the organization of laminin-1 into morphologically complex structures, but not for laminin binding per se. Perlecan and laminin-1 reciprocally modulate the organization of the other at the cell surface and in perlecan-deficient ES cells, laminin organization is defective. In sum, the data define critical molecular functions required for cell surface ECM assembly and organization.

MATERIALS AND METHODS

Cell lines and antibodies

In this study, we used the following ES cell lines: $DG^{+/-}$ and $DG^{-/-}$ R1 ES cells (Henry and Campbell, 1998); β1 $integrin^{+/-}$ and β1 $integrin^{-/-}$ (Fässler and Mayer, 1995) R1 ES cells and $perlecan^{-/-}$ R1 ES cells (Costell et al., 1999).

Rabbit polyclonal antibodies against mouse laminin-α1 chain (Durbeej et al., 1996) and fibronectin (Wennerberg et al., 1996) were previously described. A rat anti-heparin sulfate proteoglycan monoclonal antibody (Chemicon) and a mouse anti-agrin monoclonal antibody (Chemicon) were used to specifically detect perlecan and agrin, respectively. The following primary antibodies were used for FACS analysis to specifically detect integrin subunits: α1, hamster anti-rat (CD49a, Ha31/8, Pharmingen); α2, hamster anti-rat CD49b (Ha1/29, Pharmingen); α4, rat anti-mouse CD49d (R1-2, Pharmingen); α5, biotin rat anti-mouse CD49e (5H10-27, Pharmingen); α6, FITC rat anti-human CD49f (GoH3, Pharmingen); α7, mouse anti-mouse α7 integrin (3C12, kindly provided by Klaus von der Mark, Erlangen, Germany); β1, FITC hamster anti-rat CD29 (Ha2/5, Pharmingen); β3, biotin anti-mouse CD61 (2C9.G2, Pharmingen); β4, rat anti-mouse CD104 (346-11A, Pharmingen); β7, biotin rat anti-mouse integrin β7 (M293, Pharmingen).

Cell culture and immunofluorescence analysis

Wild-type and mutant ES cells were cultured as previously described (Henry and Campbell, 1998). For the laminin-1 clustering assay, ES cells were first plated onto coverslips coated with 5 $\mu g/cm^2$ human fibronectin (Collaborative Biomedical Products), which promoted cell adhesion and spreading. After plating and overnight incubation, the culture medium was replaced with fresh medium containing 7.5 nM laminin-1 (Collaborative Biomedical Products), and incubated them for various times. After incubation, coverslips were fixed in 4% paraformaldehyde, and processed for immunofluorescence with the primary antibodies mentioned above. Confocal microscopy was performed as described previously (Henry and Campbell, 1998). For quantification of morphologically distinct laminin-1 clusters on ES cells, cells were double labeled with an antibody specific for laminin-α1 chain and 4′,6-diamidino-2-phenylindole (Sigma) to stain the cell nucleus facilitating identification of individual cells.

FACS analysis

ES cells (wild-type R1 and β1 integrin-null (G110)) were briefly trypsinized, washed with growth medium and resuspended in PBS+1% BSA. 100,000 cells were incubated for 10 minutes on ice with 50 μl primary antibody and then washed with 200 μl PBS+1% BSA. After incubation with secondary antibody (if necessary) for 10 minutes on ice, the cells were washed again, resuspended in 200 μl PBS+1% BSA and analyzed by FACS (FACSort, Becton Dickinson). Secondary antibody only and autofluorescence measurements were performed as controls.

RESULTS

Formation of laminin-1structures on the surface of ES cells

Earlier studies indicated that DG was colocalized with, and required for, the formation of discrete clusters of laminin-1 on the surfaces of individual ES cells (Henry and Campbell, 1998). Here we describe the formation of these clusters in more detail. We classified laminin-1 clusters on the basis of three distinct morphologies: dots: puncta less than 1 μm in diameter, clearly distinguishable in form from particulate staining present on the surface of the culture dish (Fig. 1A-C); lines: linear arrays, either continuous or strings of dots, approx. 2-15 μm long (Fig. 1D-F); and plaques: reticular, ovalesque forms approx. 5-10 μm in diameter (Fig. 1G-I). Individual cells exhibited predominantly one of these three morphologies, but there was occasional mixing of two or more of these types of clusters. For example, Fig. 1E shows a cell exhibiting both dots and lines and Fig. 1G shows a cell with linear arrays that are assuming an ovalesque form. These images suggest that a kinetic relationship exists among the different cluster morphologies. Therefore, we analyzed the formation of laminin-1 clusters during a time course of 0-24 hours (Fig. 2). Prior to addition of laminin-1, most ES cells do not possess laminin-1 clusters on their surfaces; however, some laminin-1 clusters are detected, probably due to laminin secreted by spontaneously differentiated endodermal cells contaminating the ES cell cultures at a low level. Within 1 hour of treatment with 7.5 nM laminin-1, there is a rapid increase in ES cells bearing dot-like laminin clusters. Over the next 11 hours there is an increase first in the number of linear arrays and then in plaque-like clusters and a corresponding decrease in the number of dot-like clusters. By 12 hours, plaques become the predominant cluster type and the distribution of cells bearing dot, line or plaque-like clusters remains relatively stable over the next 12 hours until 24 hours, when the experiment was terminated. These results indicate that DG-mediated laminin clustering is a dynamic process whereby simple dot-like structures are consolidated first into linear arrays and finally into complex plaques.

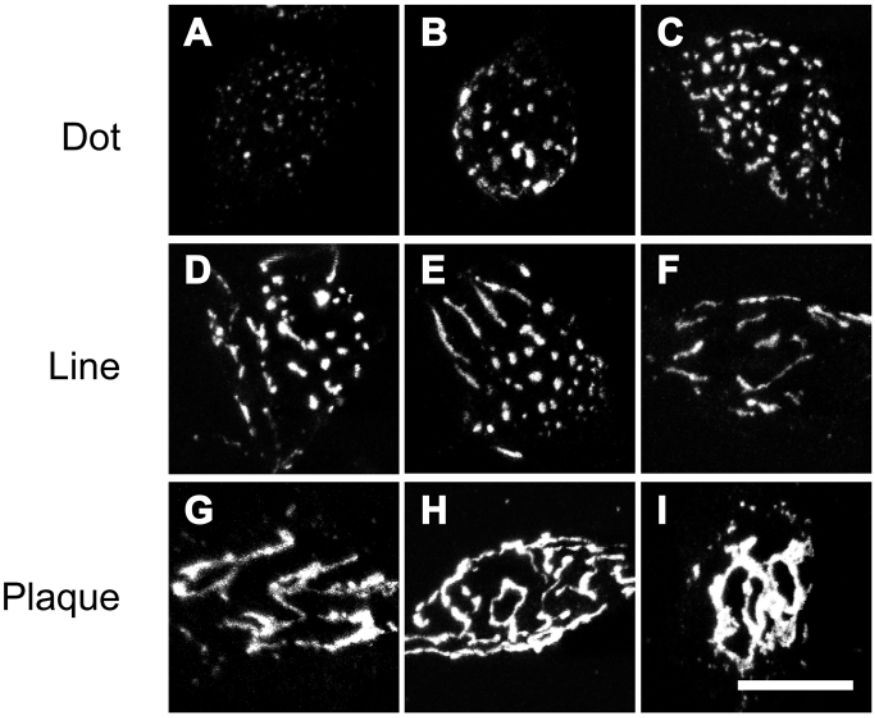

Fig. 1. Laminin-1 cluster morphologies on the surface of ES cells. The laminin-clustering assay was performed on wild-type R1 ES cells as described in Materials and Methods. 6 hours after addition of 7.5 nM laminin-1, the cells were fixed, stained with an anti-laminin antibody and examined by confocal microscopy. Single cells are shown. (A-C) Examples of dot-like morphologies; (D-F) examples of linear arrays. The cells in D and E show a mixture of dot-like and linear arrays. (G-I) Examples of plaque-like morphologies. Bar, 10 μm.

β1 integrin function is required for assembly of laminin-1 on the cell surface

Since members of the β1-integrin family and α6β4 integrin are well-documented laminin receptors (Ekblom, 1996), we wanted to test whether these receptors were involved in the laminin clustering process. To examine the role of the β1-integrin family of receptors in laminin-1 clustering on ES cells, we utilized a previously described β1 integrin-null mutant ES cell line (Fässler and Mayer, 1995). We compared the abilities of DG-null, β1 integrin-null and their heterozygous counterparts to cluster laminin on their surfaces. Fig. 3 shows that laminin clusters form similarly on both DG (Fig. 3A) and β1 integrin (Fig. 3C) control heterozygous cell lines. As expected, laminin clusters fail to form on DG-null ES cells (Fig. 3B). In contrast, although laminin-1 appeared to associate with the surfaces of β1 integrin-null ES cells (Fig. 3D), it was not organized in the same way as the heterozygous controls. Specifically, we did not detect linear and plaque-like arrays of laminin-1 on the β1 integrin-null ES cells. Instead, even after a 24-hour incubation, the laminin remained in dots or larger patches of staining. We also examined the status of the fibronectin matrix on DG-null and β1 integrin-null ES cells. Consistent with earlier findings, the fibronectin matrix formed normally on the DG-null ES cells (Fig. 3E). On the β1 integrin-null ES cells, we did not detect any fibronectin fibrils (Fig. 3F), which was a phenotype apparently more severe than previously reported for a mesenchymal cell line derived from the same β1 integrin-null ES cell clone used here. Taken together, these results indicate that β1 integrin function remains intact in DG-null ES cells, supporting FN assembly, but is insufficient to support LM assembly.

In a previous study, fibronectin fibrillogenesis was perturbed on the GD25 β1 integrin-null cells, but some fibrils were still evident and attributed to compensatory integrin expression (Wennerberg et al., 1996). To examine which integrins are expressed on parental R1 ES cells and the β1 integrin-null mutant cells, we probed these cells with integrin-specific antibodies and subjected them to FACS analysis. The results, shown in Table 1, indicate that α6β1 is the predominant, if not exclusive, laminin-binding integrin on R1 ES cells. Importantly no β4 integrin was detected, indicating that the α6β4 laminin-binding integrin is not expressed on wild-type R1 ES cells. Furthermore, in the absence of β1 integrin there is no apparent upregulation of other known laminin-binding integrins in R1 ES cells. Our results indicate that both DG and β1 integrin, probably the α6β1 integrin, are required for the appropriate

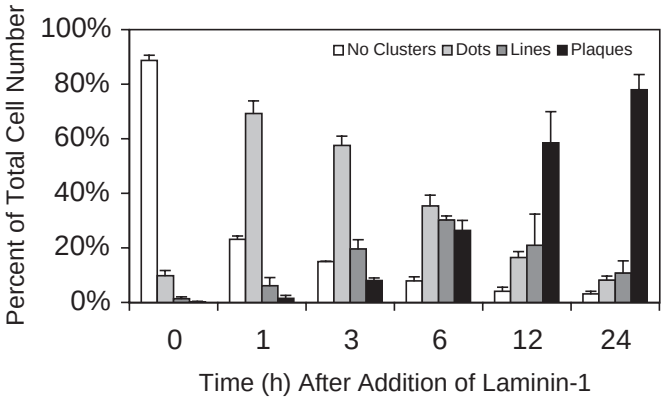

Fig. 2. Kinetic analysis of laminin-1 cluster formation. Clusters were morphologically scored as dots, lines or plaques, counted and expressed as a percentage of the total number of cells counted at each time point. Approx. 1000 cells were scored for each time point. Data was averaged from three independent time points and error bars are indicated.

Table 1. Specific expression of integrin subunits on normal and β1 integrin-null R1 ES cells

Integrin	$\beta1^{+/+}$	$\beta1^{-/-}$
α1	0	0
α2	0	0
α4	0	0
α5	108.5	0
α6	29.8	0
α7	0	0
β1	243.1	0
β3	22.4	16.6
β4	0	0
β7	0	0

Integrin subunit expression was determined by FACS analysis. Data presented are the specific median fluorescence in arbitrary units. In controls, values of less than 3.5 were non-specific and are indicated as 0.

formation of laminin clusters on ES cells, but the phenotypes of the two mutant ES cell lines suggest divergent functions for these two molecules in the process of laminin clustering.

Perlecan binding to the cell surface is DG-dependent and is required for assembly of laminin-1 structures

In addition to laminin, perlecan and agrin have been identified as ECM ligands for α-dystroglycan (Campanelli et al., 1994; Gee et al., 1994; Peng et al., 1998; Talts et al., 1999). However, the functions of perlecan and agrin in laminin matrix assembly remain unclear. To examine the organization of agrin and perlecan on the ES cell surface, we stained $DG^{+/-}$ and DG-null ES cells with specific antibodies for perlecan and agrin. Wild-type and mutant ES cells stained with anti-agrin antibodies showed no signal, suggesting that R1 ES cells do not express appreciable amounts of agrin (data not shown). However, ES cells stained with anti-perlecan antibodies showed a pattern of labeling that was dependent on the status of DG expression and was modulated by the presence of laminin. 6 hours after the addition of 7.5 nM laminin-1, the perlecan signal precisely coaligns with the laminin-1 signal in the aforementioned cluster morphologies (Fig. 4A-C). Cells not supplied with exogenous laminin-1 still displayed perlecan clusters on their surfaces, but these structures were not present in complex linear and plaque-like morphologies seen in the presence of laminin-1 (Fig. 4D,E). Perlecan was not detected on the surface of DG-null ES cells, indicating that perlecan binding to the cell surface is dependent on DG (Fig. 4F). In a control experiment, no perlecan signal was detected with our anti-perlecan antibody on perlecan-null ES cells (Fig. 4I), demonstrating both to the specificity of the antibody reagent and that the perlecan detected is of cellular origin and not supplied by the serum-containing medium.

Perlecan binds to both laminin-1 and DG and therefore seems an excellent candidate to modulate laminin-1 organization (Battaglia et al., 1992; Talts et al., 1999). To study the involvement of perlecan in the organization of laminin-1 on the ES cell surface, we examined perlecan-null mutant ES cells. 6 hours after the addition of 7.5 nM laminin-1 to the cell cultures, the appearance of laminin clusters on the surface of perlecan-null ES cells was markedly different from controls (Fig. 4G,H). Although laminin was detected on the surface of perlecan-null ES cells, most cells only showed dot-like morphologies and very few exhibited linear or plaque-like arrays. The perturbation of laminin-1 clusters on perlecan-null ES cells suggests that perlecan may facilitate the organization of laminin into more complex linear and plaque-like arrays on the cell surface. In sum, we have shown that DG is necessary for cell surface binding of perlecan and that perlecan and laminin-1 have a reciprocal relationship, each modulating the organization of the other at the cell surface.

DISCUSSION

Assembly of the extracellular matrix remains a fundamental biological problem. We have undertaken a genetic approach to this problem. As a model system, we have examined the cell surface organization of laminin on ES cells. For the following reasons, we believe that this system provides an experimentally attractive cell-based assessment of certain aspects of basement

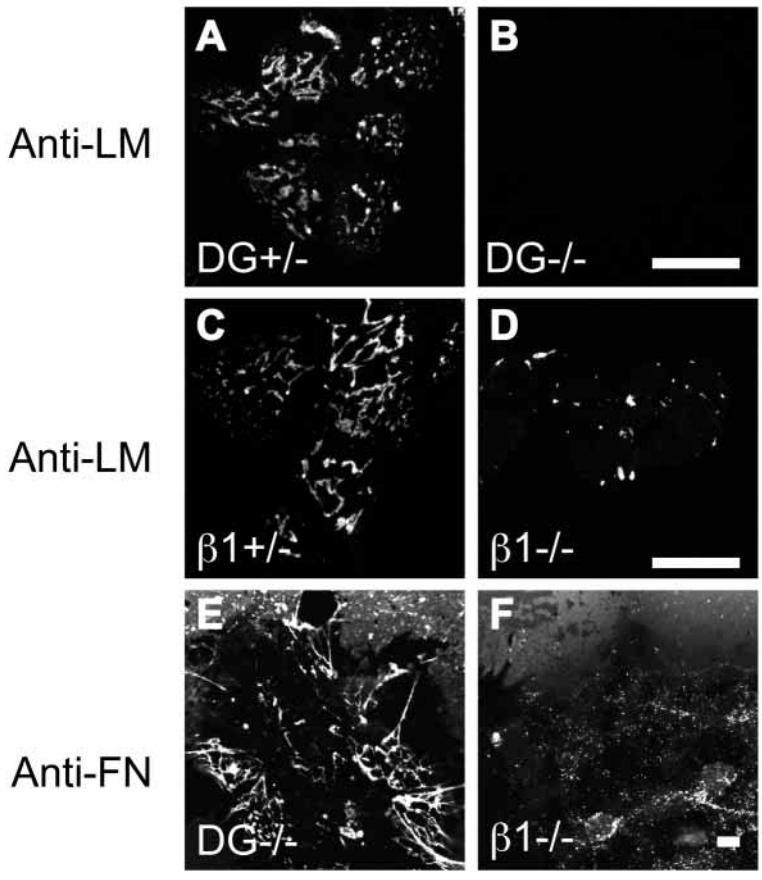

Fig. 3. β1 integrin function is required for laminin clustering. Laminin clustering assay was performed on heterozygous (+/–) control lines (A,C) and DG-null ($DG^{-/-}$) (B,E) and β1-integrin-null ($\beta1^{-/-}$) (D,F) ES cell lines. Cells were stained with anti-laminin (A-D) and anti-fibronectin (E,F). Each panel shows a representative single, small colony of ES cells. Fibronectin signal is also detected on the coverslips, which were precoated with fibronectin. Bars, 10 μm.

membrane assembly, a process that normally occurs in a tissue context. First, ES cells are amenable to unambiguous loss-of-function experiments through gene targeting (Mortensen et al., 1991). Second, as we show here and discuss below, mutations in several genes that yield basement membrane phenotypes in tissues also show phenotypes in our cell-based assay. Third, other null mutant ES cell lines exhibit normal cell surface laminin organization and this process is independent of the strain background of the ES cell lines (M.D.H., K.P.C., unpublished data). Fourth, ES cells are derived from, and in culture maintain properties similar to, the inner cell mass cells of the blastocyst which, during murine development, are among the first cells involved in de novo basement membrane formation (Evans and Kaufman, 1981; Martin, 1981; Dziadek and Timpl, 1985). And finally, the detailed morphologies of the laminin clusters on ES cells resemble laminin and fibronectin structures lining the blastocoel of amphibian and mouse embryos, suggesting that these structures are nascent ECM assemblies (Darribere et al., 1986; Thorsteinsdottir, 1992).

Previously, we showed that DG was required for the organization of laminin-1 on ES cells (Henry and Campbell, 1998), which is supported by other studies demonstrating a role for DG in cell surface laminin organization in cultured myotubes (Cohen et al., 1997; Colognato and Yurchenco, 1999; Montanaro et al., 1999). In this report, we describe the process of cell surface laminin organization on ES cells in more detail. We find that initial, simple dot-like laminin-1 deposits are assembled into linear arrays and finally into complex, plaque-like structures. Because no laminin appears bound to the surface of DG-null ES cells, we suggest that DG is required for the initial binding of laminin-1 on the cell surface, potentially an essential, early step in the process of cell mediated laminin assembly. The

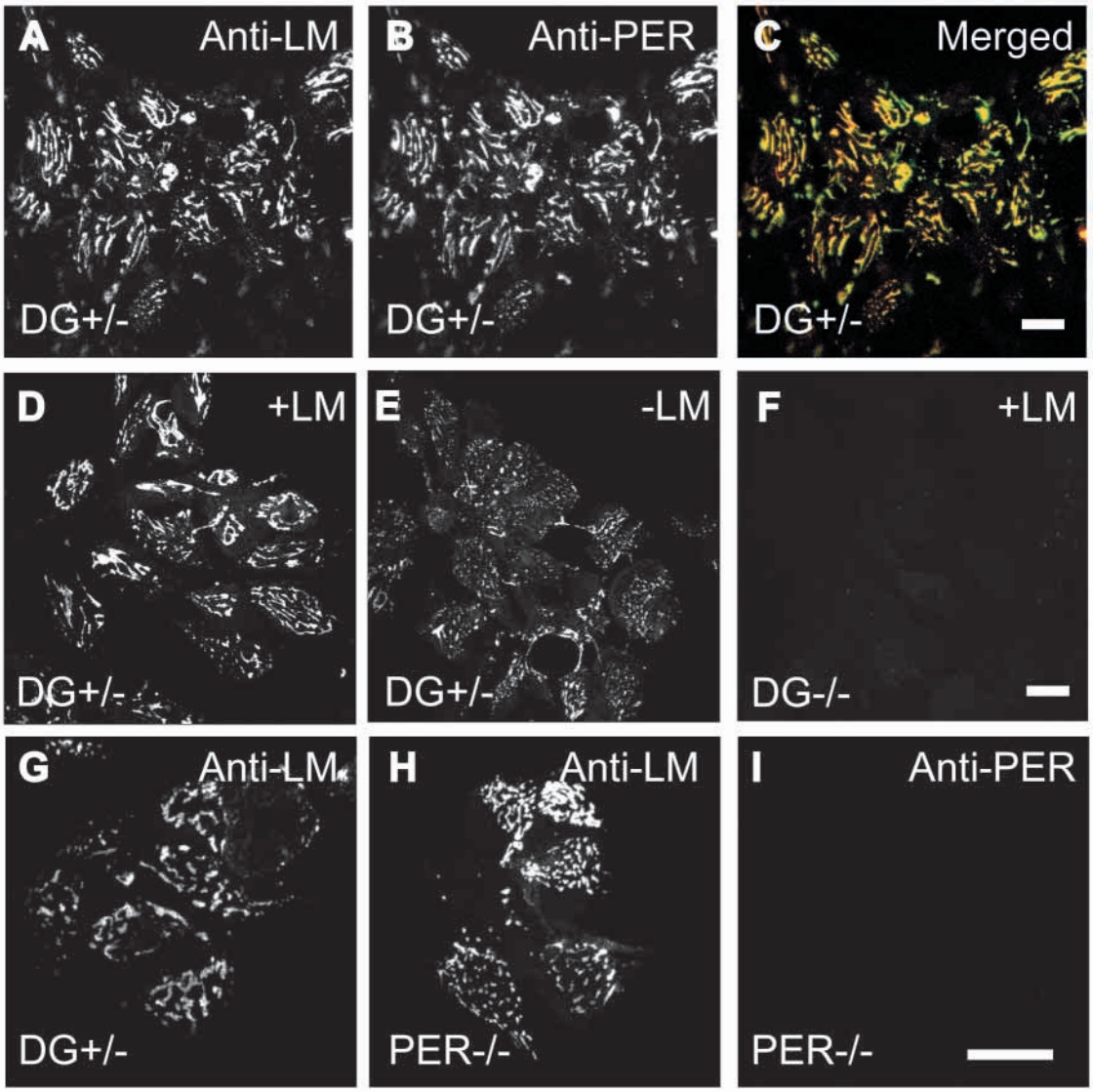

Fig. 4. Perlecan binds to DG and is involved in laminin clustering. The laminin clustering assay was performed on $DG^{+/-}$ ES cells and the cells were subsequently double-labeled with anti-laminin (A) and anti-perlecan (B). (C) is a merged image of A and B. (D,E) Perlecan localization in the presence (D) and absence (E) of exogenously applied laminin-1. In DG-null ES cells, there is no perlecan signal detectable (F). Laminin clustering is disrupted on perlecan-null ES cells (H) as compared to controls (G). (I) A specificity control for the anti-perlecan antibody used in this study. In all panels, the genotype of the ES cell line is indicated in the lower left hand corner and the antibody used for staining is shown in the upper right hand corner of the panel. In each panel a single small representative colony is shown. Bars, 10 μm.

requirement for DG in this dynamic cell-mediated process of ECM organization, is likely to explain its role at the tissue level of organization. DG-null mouse embryos and embryoid bodies exhibit failed basement membrane formation (Williamson et al., 1997), and antibody perturbation experiments of DG and its binding site on the laminin α chain perturb kidney epithelial morphogenesis and disrupt basement membrane development in organ cultures (Durbeej et al., 1995; Kadoya et al., 1995). Thus, these studies place DG in a critical role for basement membrane assembly in vivo and suggest that its ability to organize laminin on the cell surface is central to this role. DG is expressed on many cell types in apposition to basement membranes (Durbeej et al., 1998); however, it is not yet clear whether DG is required for the appropriate formation of all basement membranes. For example, one study of DG-null chimeric mice suggests that DG may not be required for the formation of skeletal muscle basement membrane (Cote et al., 1999). Although these studies are complicated by that fact that there is incomplete ablation of DG expression in the syncytial muscle cells of the DG-null chimeric mice, and more subtle perturbations of the muscle basement membrane were not ruled out, this result suggests the possibility that in some tissues other molecules or mechanisms may compensate for the lack of DG function.

One class of molecules that might be predicted to compensate for DG is the β1 integrin family of laminin receptors. Several genetic ablation experiments in the mouse have established roles for β1 integrins, in basement membrane assembly (Fässler et al., 1995). Disruption of β1 integrin itself results in peri-implantation lethality, at a similar time to the laminin-γ1 knockout, but a day earlier than the DG knockouts and Reichert's membrane development (Fässler and Mayer, 1995; Stephens et al., 1995; Smyth et al., 1999). Both the β1 integrin and laminin-γ1 knockouts exhibit disruption of the trophectodermal basement membrane, which suggests a more primal function for β1 integrins in basement membrane formation than DG. However, since β1 integrins also may mediate critical trophic cellular responses to laminin and other ligands in the developing embryo, it is possible that disruption of this basement membrane is secondary to disruption of cell signaling events in the early embryo. Indeed, teratomas and embryoid bodies derived from β1 integrin-null ES cells display reduced expression of laminin-1 in addition to disrupted basement membranes, suggesting that basement membrane disruption may be secondary to the reduction of laminin-1 expression (Sasaki et al., 1998; Aumailley et al., 2000).

The studies reported here also support a role for β1 integrin in basement membrane assembly, as indicated by its role in cell surface laminin organization. β1 integrin-deficient ES cells accumulate laminin on their surfaces, but fail to organize it into morphologically complex structures. This basic finding has several implications. First, it indicates that DG and β1 integrin functions are non-overlapping with respect to cell-surface laminin organization. It also suggests that β1 integrin function is required after DG function, in the process of cell-surface laminin organization. And finally, although we cannot assess the role of β1 integrin in regulating the expression of laminin since ES cells do not express laminin, our results indicate that β1 integrin's contribution to basement membrane assembly extends beyond its influence on laminin expression.

Perlecan is a ubiquitous basement membrane proteoglycan that has also been implicated in basement membrane assembly (Iozzo, 1998). Perlecan-null mice exhibit failed development between E9.5 and E17.5, far beyond the stages at which the laminin-γ1, β1-integrin and DG-null mice perish (Costell et al., 1999; Arikawa-Hirasawa et al., 1999). Although the data suggest that perlecan is not universally required for basement membrane assembly, perlecan-null mice exhibit basement membrane disruptions in some tissues such as the myocardium and expanding brain vesicles. Some authors suggest that perlecan may be required for the integrity of some basement membranes in tissue exposed to mechanical stress (Costell et al., 1999). The data presented here are consistent with a more limited role for perlecan in basement membrane assembly. Perlecan-deficient ES cells show defective formation of complex linear and plaque-like laminin arrays. Perhaps perlecan's promotion of the laminin assembly process is a critical modifier of basement membrane stability. Interestingly, laminin was also required for modulating perlecan localization on the cell surface. Whether this occurs directly through an interaction between laminin and perlecan or indirectly through interaction with DG is not yet known. However, since laminin-1 and perlecan compete for the same binding site on DG (Talts et al., 1999), the former idea seems more likely.

Based on the observations in this report and those of previous studies, we propose the following model for cell-surface laminin organization on ES cells. First, soluble laminin binds to αDG on the cell surface. DG's affinity for laminin in the low nanomolar range is well below that required for spontaneous assembly of laminin in vitro (Ibraghimov-Beskronaya et al., 1992; Gee et al., 1993; Yurchenco et al., 1985). After binding to DG, laminin engages β1 integrins, probably the α6β1 integrin on ES cells. An interesting question that arises is whether this occurs in a ternary complex including laminin, DG and β1 integrins. Such an arrangement seems possible since DG and β1 integrin bind to distinct sites on laminin-1 (Gee et al., 1993; Sonnenberg et al., 1990). Binding to β1 integrin may signal reorganization of the actin cytoskeleton, which may, in turn, influence the laminin assembly process outside the cell through interactions with cytoplasmic domains of receptors inside the cell (Colognato et al., 1999; M.D.H., K.P.C., unpublished results). Receptor-bound laminin-1 may also undergo a self-assembly process on ES cells, as it has been shown to do on the surface of myotubes (Cohen et al., 1997; Colognato et al., 1999). Together, cytoskeletal functions and laminin self-assembly may contribute to drive the organization of the more complex linear and plaque-like laminin arrays. Perlecan may facilitate this latter process in some as yet unknown way, and its influence on the assembly process may be critical for the development of the mechanical properties of basement membranes.

M.D.H. was supported by a National Research Service Award (DK09712). J.S.S. was supported by a National Institute of Health Predoctoral Grant. K.P.C. is an Investigator of the Howard Hughes Medical Institute. We thank Drs Lydia Sorokin (University of Erlangen) and Staffan Johansson (University of Uppsala) for their kind gifts of antibodies.

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