

REVIEW

Atypical basement membranes and basement membrane diversity – what is normal anyway?

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ABSTRACT

The evolution of basement membranes (BMs) played an essential role in the organization of animal cells into tissues and diversification of body plans. The archetypal BM is a compact extracellular matrix polymer containing laminin, nidogen, collagen IV and perlecan (LNCP matrix) tightly packed into a homogeneously thin planar layer. Contrasting this clear-cut morphological and compositional definition, there are numerous examples of LNCP matrices with unusual characteristics that deviate from this planar organization. Furthermore, BM components are found in non-planar matrices that are difficult to categorize as BMs at all. In this Review, I discuss examples of atypical BM organization. First, I highlight atypical BM structures in human tissues before describing the functional dissection of a plethora of BMs and BM-related structures in their tissue contexts in the fruit fly *Drosophila melanogaster*. To conclude, I summarize our incipient understanding of the mechanisms that provide morphological, compositional and functional diversity to BMs. It is becoming increasingly clear that atypical BMs are quite prevalent, and that even typical planar BMs harbor a lot of diversity that we do not yet comprehend.

KEY WORDS: Collagen IV, *Drosophila*, Extracellular matrix, Fibrosis, Integrin, Tissue morphogenesis

INTRODUCTION

Multicellular organisms consist not just of cells but also of an abundant extracellular matrix (ECM) produced by cells to ensure their physical coupling, social interactions and tissue organization. In animals, ECMs differ wildly in physical density, mechanical properties, molecular composition and cellular content (Mouw et al., 2014). Among these different ECMs, basement membranes (BMs) stand out as a seemingly well-defined and clear category (Jayadev and Sherwood, 2017; Yurchenco, 2011), defined by two main criteria – morphology and composition.

Morphologically, BMs are compact ECM assemblages. Their components are tightly packed into a dense planar layer of homogeneous thickness of ~100 nm when visualized through transmission electron microscopy (TEM) (Fig. 1A,C). However, this is an underestimation owing to the dehydration procedure that TEM samples must undergo, as measurements undertaken with atomic force microscopy and super-resolution light microscopy consistently produce thickness values that are twice as high as those obtained by TEM (Halfter et al., 2015; Howard et al., 2019; Suleiman et al., 2013). It was thought for a long time that BMs consisted of two layers: a dense external layer, the lamina densa, and

a clearer layer closer to the attached cells, the lamina lucida. However, this was also revealed as being a dehydration artifact, since samples prepared by freeze-substitution show a homogeneous high density (Chan et al., 1993) despite internal zonification of components (Suleiman et al., 2013). BMs are not an exclusive feature of epithelia; however, the basal presence of a BM is one of their defining features (Rodríguez-Boulán and Macara, 2014). 2D apicobasally polarized sheets of tightly adhered epithelial cells are the quintessential architectural motif in the construction of animal body plans. The evolution of BMs, therefore, played an essential role in enabling tissue-level organization of animal cells, driving the successful radiation of the metazoan kingdom.

The main components of BMs are collagen IV, laminin, nidogen and perlecan (Fig. 1B,C), all widely conserved proteins (Adams, 2013; Özbek et al., 2010) (see Box 1). Biochemical and genetic characterizations in humans and model organisms have yielded a basic model for how these components function (Isabella and Horne-Badovinac, 2015a; Jayadev and Sherwood, 2017; Kalluri, 2003; Miosge, 2001; Timpl, 1989; Yurchenco and Schittny, 1990). Both collagen IV and laminin are trimeric proteins that self-assemble into independent networks. Laminin is regarded as the initiator of BM assembly, whereas collagen IV, the most abundant BM component, is responsible for its tensile strength. The collagen IV and laminin networks interact with each other and this link is reinforced by nidogen. Finally, perlecan may determine hydration and turgency of the BM through the negative charges of its heparan sulfate sugar chains.

In contrast to this simple definition of BMs based on planar morphology and LNCP composition, many mysteries remain. For instance, it is not clear whether secretion of collagens and other large ECM proteins requires specific mechanisms for ER-to-Golgi transport (Liu et al., 2017; Malhotra and Erlmann, 2015). It is also unclear how BM components avoid premature polymerization inside secreting cells or at their plasma membrane. Given that BM components are abundant, we do not understand yet how their self-assembly results in regularly thin sheets instead of thicker ones or amorphous aggregates. Many physical interactions among BM components have been mapped, but we do not know which of these are relevant for BM assembly. Furthermore, the extent of the link between the collagen IV and laminin networks is unknown, and a definitive assembly hierarchy has eluded researchers. Finally, we have little understanding of the mechanisms through which pathological and age-dependent changes in BM thickness or composition occur and affect tissue physiology and mechanical properties.

In addition, typical BM components are present in atypical BMs and other structures that defy categorization as BMs and instead would be better described as aggregates, fibrils or loose matrices. In recent years, many atypical BMs have been described in the fruit fly *Drosophila melanogaster*, where transgenic labeling of BM proteins with fluorescent tags has brought unprecedented

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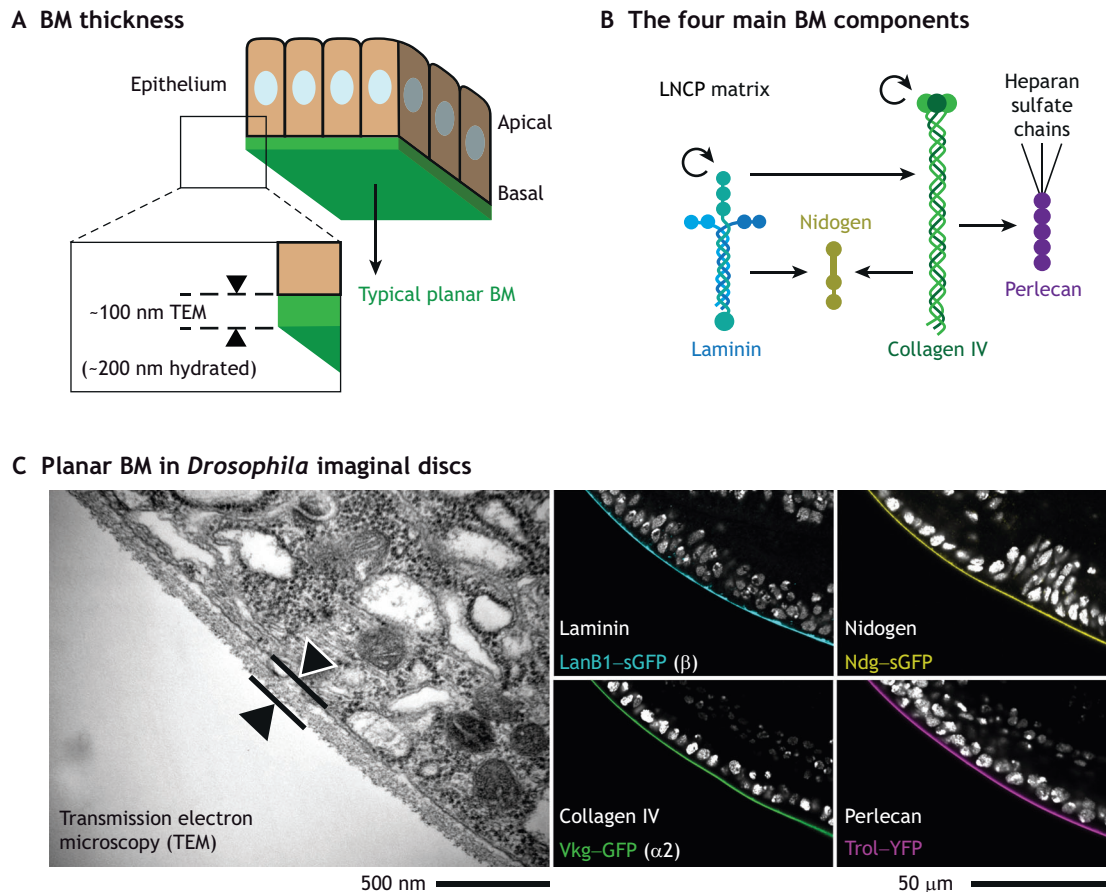


Fig. 1. Morphology, main components and evolution of BMs. (A) A typical planar BM on the basal side of an epithelium. The thickness in section is typically ~100 nm when visualized through transmission electron microscopy (TEM), but this value doubles when analyzed in native, hydrated conditions. (B) The four main components of BMs are laminin, nidogen, collagen IV and perlecan (LNCP matrix). Arrows indicate the general hierarchy of assembly as elucidated in *Drosophila*, but tissue-specific differences exist (see Fig. 4E). (C) TEM and confocal microscopy images of the planar BM surrounding *Drosophila* imaginal discs, larval precursors of the adult epidermis. Fusions to fluorescent protein were used to show the localization of the β Laminin chain Laminin B1 (LanB1), Nidogen (Ndg), α 2-like Collagen IV chain Viking (Vkg) and the perlecan ortholog Terribly reduced optic lobes (Trol).

resolution to ECM studies. As a result, it is increasingly clear that atypical organization of BM components is more prevalent than previously thought. Furthermore, functional genetic analyses in *Drosophila* and the nematode *Caenorhabditis elegans* have repeatedly shown that even typical planar BMs of different tissues differ in critical aspects.

In this Review, I describe examples of atypical BMs, starting with human tissues and followed by discoveries in *Drosophila*. I also summarize our growing understanding of the mechanisms that provide morphological and compositional diversity to BMs and related matrices by highlighting the influence of component deposition mode, receptors and matrix regulators on BM diversity. These mechanisms ensure the assembly of matrices with specific characteristics and physiological functions, as required by their different tissue contexts.

Atypical basement membranes and related structures

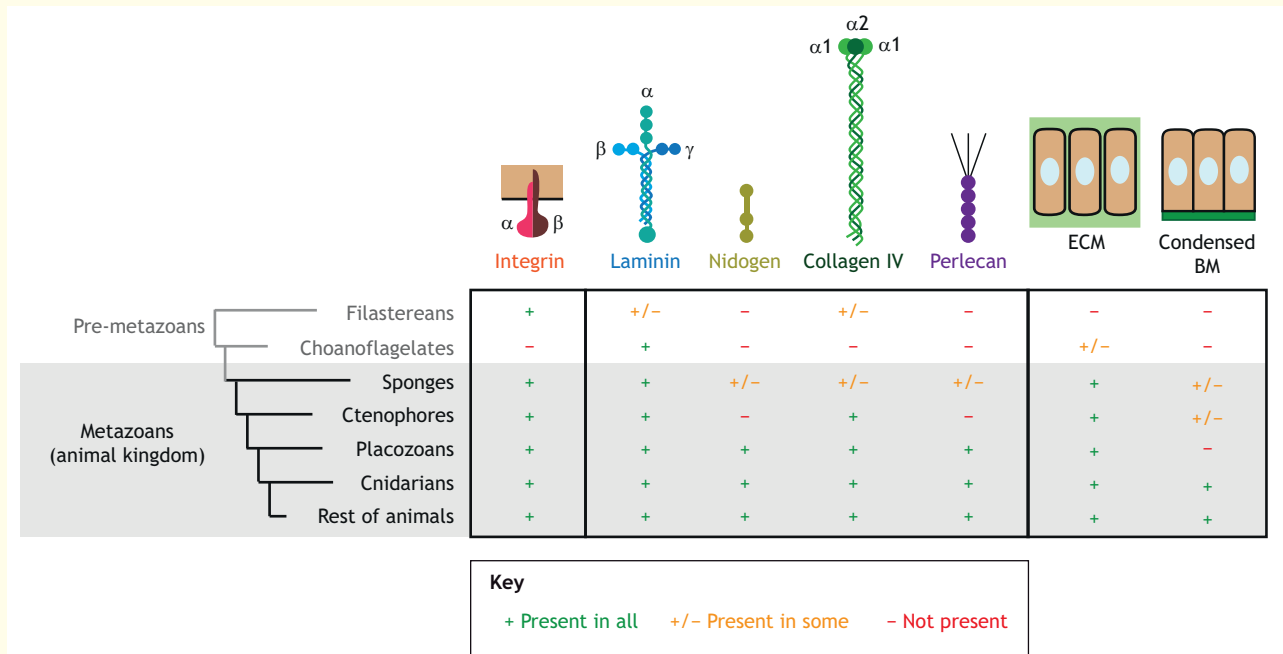
BM components localize to matrices that do not easily fit into the typical BM concept. One such class of atypical BMs are double BMs, which join two tissues through the linkage or fusion of two BMs through their basal sides (Keeley and Sherwood, 2019). The glomerular basement membrane (GBM), lying between the epithelial podocytes and endothelial cells in the kidney glomerulus, is a notorious example; it is extensively studied because of its

filtering role separating blood and urine (Fig. 2A). Aiding filtration is a system of slit diaphragms in the podocytes. The relative contributions of podocytes and GBM to filtration are controversial (Lawrence et al., 2017). It is nonetheless clear that GBM alterations lead to impaired filtration during diabetic nephropathy and several congenital syndromes, sometimes with fatal consequences (Miner, 2012). The GBM is ~400 nm thick and contains two laminin networks that meet at its collagen IV-rich center (Suleiman et al., 2013). Another well-studied double BM is formed by linkage of the uterine and vulval BMs in *C. elegans*. This linkage precedes invasion of the vulva by the anchor cell through this double BM (Sherwood, 2006). BM linkage (B-LINK) is mediated by hemicentin, a fibulin family member (Morrissey et al., 2014).

In addition to double BMs, other unusual types of LNCP matrices are known in humans. In cartilage, all four main BM components are expressed by chondrocytes and localize to their pericellular matrix (Fig. 2B) (Kvist et al., 2008). This pericellular matrix, however, does not resemble a BM, as it does not appear to be distinct in TEM from the dense cartilage matrix, formed by collagen II and aggrecan. Concentration of BM components is maximal within a 1 μ m distance of chondrocytes, but their diffuse presence can be detected further away (Kvist et al., 2008) (Fig. 2B). Another example of atypical BM is the fetus–mother placental interface, where cells of the embryonic trophoblast are organized into villous

Box 1. BM evolution

Matrices that contain laminin, nidogen, collagen IV and perlecan together (LNCP matrices) have existed since the Ediacaran period, 600 million years ago, when the first animals originated, and became a stable feature of animal tissues through the Cambrian explosion, which took place 500 million years ago and gave rise to all extant animal phyla (Adams, 2013; Nielsen, 2019; Özbek et al., 2010) (see figure). Laminin-related genes have been found in choanoflagellates, facultative colonial flagellates from which animals evolved (Fahey and Degnan, 2012), and in the even more ancient filasterean amoebas (Sebé-Pedrós et al., 2013). Collagen IV seems to have been lost in choanoflagellates, but is present in filastereans (Fidler et al., 2018), supporting the idea that collagen IV is the ancestral type of collagen from which fibrillar and other types of collagen evolved (Grau-Bové et al., 2017). Similarly lost in choanoflagellates but present in more ancient groups are the integrins, the main animal ECM receptors (Sebé-Pedrós et al., 2010). Nidogen and perlecan are less ancient and probably originated in sponges (Riesgo et al., 2014), likely the most basal animal phylum, despite some studies positioning ctenophores as a sister group to all other animals (Nielsen, 2019; Simion et al., 2017). Nidogen and perlecan are absent in ctenophores (Draper et al., 2019), and were therefore lost if the sponges-first evolutionary scenario holds. All other animal phyla possess genes encoding the four main BM components (see figure).



projections that invade the decidual tissue of the mother (Fig. 2C). The core of these invading villi contains all four main BM components, and do not form a planar sheet, but a loose 3D network (Oefner et al., 2015; Rukosuev, 1992). Furthermore, mesenchymal cells at the leading front of invading villi are surrounded by another amorphous matrix containing collagen IV and laminin (Oefner et al., 2015). Finally, another instance of atypical LNCP matrix is found in the eye cornea, where a thick stroma separates the epithelial and endothelial layers (Fig. 2D). In this stroma, which consists of collagen I and collagen V fibers, irregular elongated LNCP plaques are observed (Fitch et al., 1990; Pratt and Madri, 1985; Schittny et al., 1988). As is the case for the perichondrocyte matrix and the fetus–mother placental interface, the genesis and significance of these plaques is unknown. The existence of atypical BMs in the human body, therefore, is well documented. This is despite the fact that the ability of researchers to recognize and functionally analyze them has been limited. Ample evidence of the pervasiveness and functional importance of atypical BM structures exists, however, in *Drosophila*, as discussed in the following section.

Atypical basement membranes in *Drosophila*

The four main BM components are conserved in *Drosophila* (Table 1). A single variety of the collagen IV heterotrimer is formed by the products of *Collagen at 25C* (*Cg25C*, now officially named *Col4a1*) and *viking* (*vkg*), encoding $\alpha 1$ - and $\alpha 2$ -like chains,

respectively (Le Parco et al., 1986; Natzle et al., 1982; Pastor-Pareja and Xu, 2011; Yasothornsrikul et al., 1997). Two different laminin heterotrimers can form with alternative α chains encoded by genes *Laminin A* (*LanA*) and *wing blister* (*wb*), whereas unique β and γ chains are encoded by the *Laminin B1* (*LanB1*) and *Laminin B2* (*LanB2*) genes (Fessler et al., 1987; Kusche-Gullberg et al., 1992; Martin et al., 1999; Montell and Goodman, 1988, 1989). One perlecan protein encoded by *terribly reduced optic lobes* (*trtl*) (Park et al., 2003; Voigt et al., 2002) and the product of *Nidogen* (*Ndg*) (Dai et al., 2018; Wolfstetter et al., 2019; Wolfstetter et al., 2009) complete the *Drosophila* LNCP set. For all four, transgenically expressed tagged versions, mutants and tissue-specific RNAi tools are available and have been used in multiple studies. Based on these, a lot of evidence shows that BM components localize and function in multiple structures that are different from typical BMs.

Atypical BMs in the embryo

Typical condensed BMs are not formed until stage 14 of *Drosophila* embryogenesis (Tepass and Hartenstein, 1994). Despite this, earlier requirements for Collagen IV and Laminin have been described. During stage 5, a loose matrix of maternally transcribed Collagen IV surrounds cells of the blastoderm (Fig. 3A), which is already a polarized epithelium. This matrix, although immature, accomplishes an essential function already by regulating diffusion of transforming growth factor β (TGF- β) family ligand

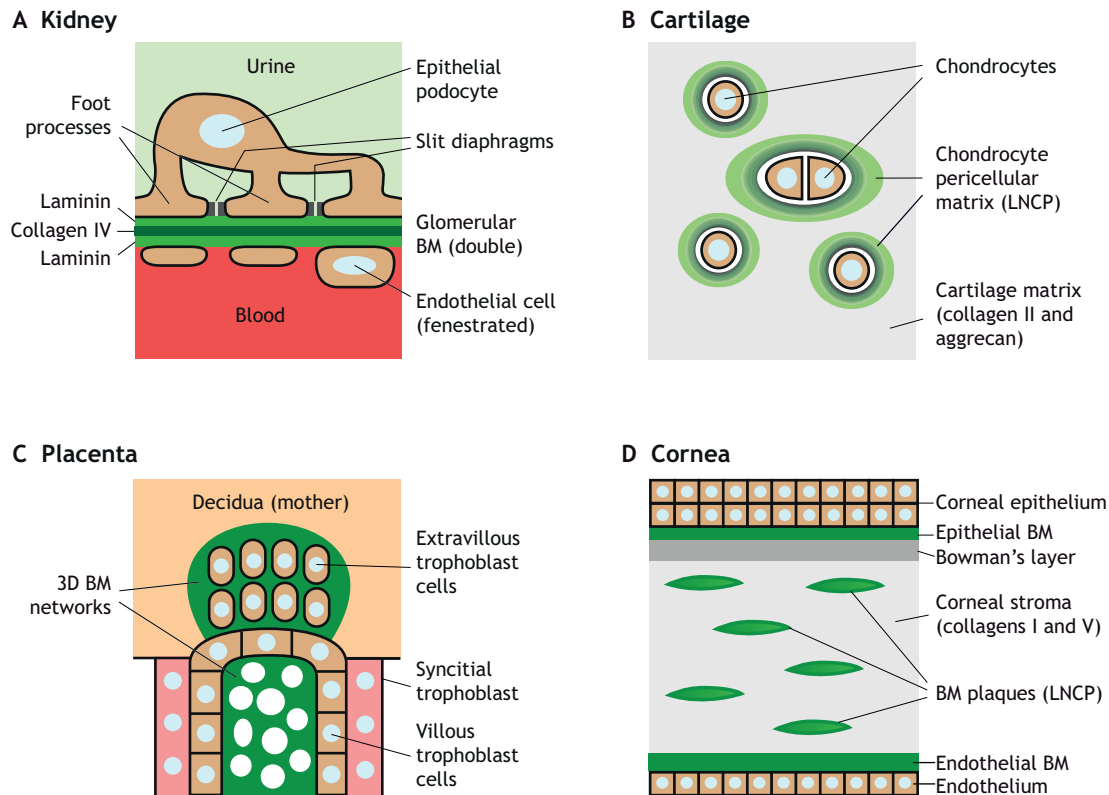


Fig. 2. Atypical BMs in humans. (A) The glomerular basement membrane (GBM), a double BM formed by podocytes and endothelial cells, plays an essential filtering role separating blood and urine in kidney glomeruli. The GBM is 400 nm thick and is made of two laminin networks that meet at its Collagen IV rich center. Slit diaphragms in between podocyte foot processes also contribute to filtration. (B) A diffuse pericellular LNCP matrix surrounds chondrocytes in cartilage, which appears to be indistinct from the dense cartilage matrix that is rich in collagen II and aggrecan. (C) Trophoblast cells organize into column-shaped villi that invade the decidua of the mother in the placenta during early pregnancy; they display amorphous, meshwork-like LNCP matrices inside their core and surround the mesenchymal extravillous cells at the invading front. (D) In the eye cornea, a stroma containing collagen I and collagen V fibers is sandwiched between two BMs – the epithelial BM, in contact with a dense region of the stroma called Bowman's layer, and the endothelial BM, also known as Descemet's membrane. Elongated LNCP plaques of unknown function are found in the corneal stroma. See main text for references.

Decapentaplegic (Dpp), which Collagen IV can bind to (Wang et al., 2008). In the absence of Collagen IV, Dpp, which is produced in dorsal cells, diffuses in excess, preventing those cells from activating maximal levels of downstream signaling and thereby impairing dorsoventral patterning. During later development, Collagen IV also hinders Dpp diffusion in typical planar BMs, such as those in the Malpighian tubules (the fly kidney-like organ) (Bunt et al., 2010) and imaginal discs (Ma et al., 2017), indicating a sustained relationship between Collagen IV and TGF- β signaling.

Not only Collagen IV, but also Laminin is required before typical BMs are formed. Laminin mutants show defects by stage 12 during

germband retraction (Schock and Perrimon, 2003), although the specific nature of this requirement is not known. Another early Laminin requirement takes place at stage 14 for adhesion of the yolk membrane to the amnioserosa, an epithelium covering the dorsal part of the embryo (Fig. 3A). This is at a time when typical LNCP BMs have formed. However, the Laminin matrix that joins the yolk and the amnioserosa contains no Collagen IV (Narasimha and Brown, 2004). BMs lacking Collagen IV are few in *Drosophila*, but numerous in *C. elegans* (Graham et al., 1997). Furthermore, loose matrices containing certain BM components, such as the diffuse Collagen IV blastoderm matrix and the amnioserosa–yolk Laminin

Table 1. Genes encoding the four main BM components and integrins in *Drosophila*, *C. elegans* and humans

	<i>Drosophila</i>	<i>C. elegans</i>	Humans
Laminin α	<i>LanA</i> , <i>wb</i>	<i>lam-3</i> , <i>epi-1</i>	<i>LAMA1</i> , <i>LAMA2</i> , <i>LAMA3</i> , <i>LAMA4</i> , <i>LAMA5</i>
Laminin β	<i>LanB1</i>	<i>lam-1</i>	<i>LAMB1</i> , <i>LAMB2</i> , <i>LAMB3</i> , <i>LAMB4</i>
Laminin γ	<i>LanB2</i>	<i>lam-2</i>	<i>LAMC1</i> , <i>LAMC2</i> , <i>LAMC3</i>
Nidogen	<i>Ndg</i>	<i>nid-1</i>	<i>NID1</i> , <i>NID2</i>
Collagen IV $\alpha 1$	<i>Cg25C</i> (<i>Col4a1</i>)	<i>emb-9</i>	<i>COL4A1</i> , <i>COL4A3</i> , <i>COL4A5</i>
Collagen IV $\alpha 2$	<i>vkq</i>	<i>let-2</i>	<i>COL4A2</i> , <i>COL4A4</i> , <i>COL4A6</i>
Perlecan	<i>trol</i>	<i>unc-52</i>	<i>HSPG2</i>
Integrin α	<i>mew</i> ($\alpha PS1$), <i>if</i> ($\alpha PS2$), <i>scb</i> ($\alpha PS3$), <i>ItgaPS4</i> , <i>ItgaPS5</i>	<i>ina-1</i> , <i>pat-2</i>	<i>ITGAD</i> , <i>ITGAE</i> , <i>ITGAL</i> , <i>ITGAM</i> , <i>ITGAV</i> , <i>ITGAX</i> , <i>ITGA1</i> , <i>ITGA2</i> , <i>ITGA2B</i> , <i>ITGA3</i> , <i>ITGA4</i> , <i>ITGA5</i> , <i>ITGA6</i> , <i>ITGA7</i> , <i>ITGA8</i> , <i>ITGA9</i> , <i>ITGA10</i> , <i>ITGA11</i>
Integrin β	<i>mys</i> (βPS), <i>Itgbv</i>	<i>pat-3</i>	<i>ITGB1</i> , <i>ITGB2</i> , <i>ITGB3</i> , <i>ITGB4</i> , <i>ITGB5</i> , <i>ITGB6</i> , <i>ITGB7</i> , <i>ITGB8</i>

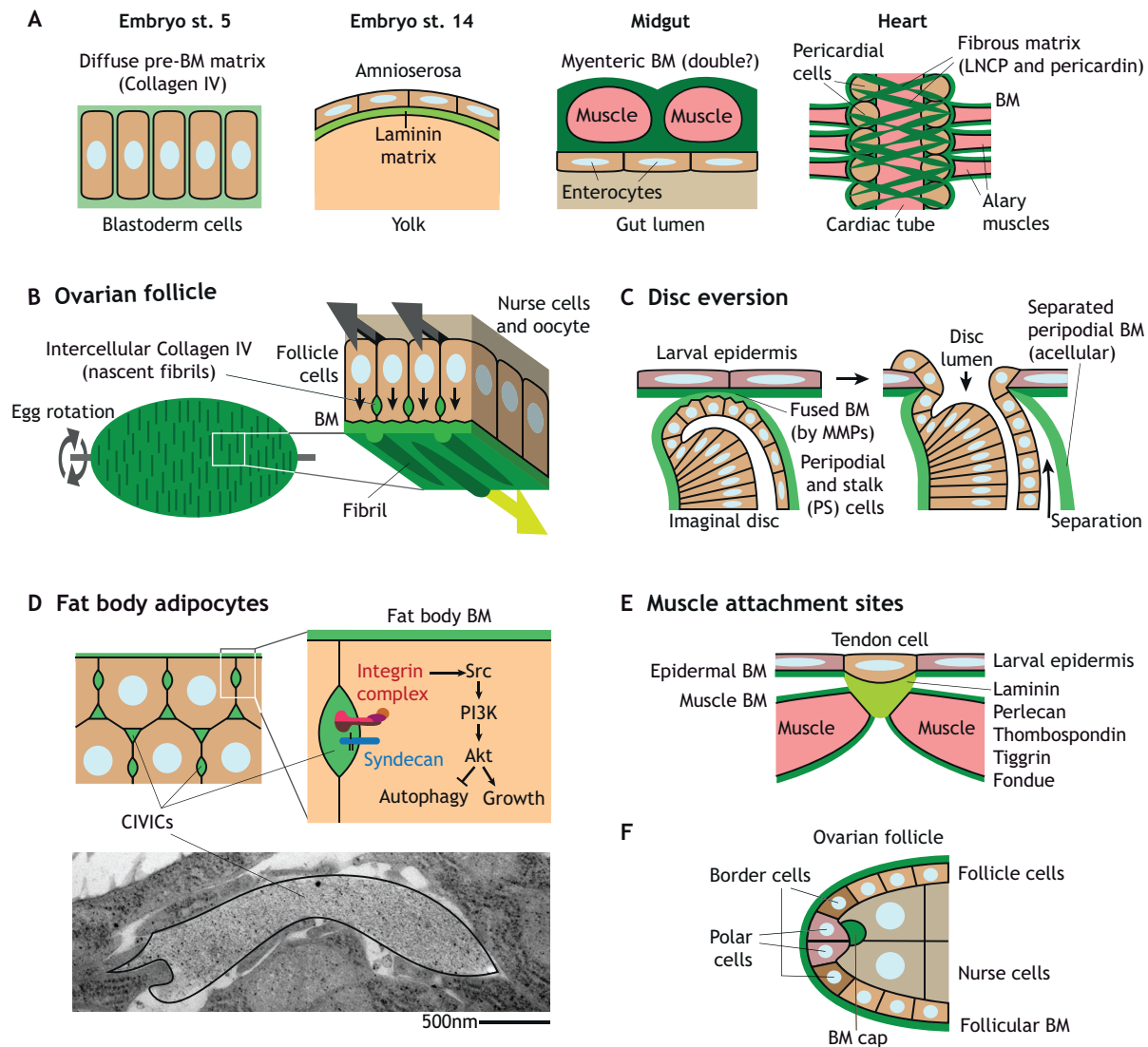


Fig. 3. Atypical BMs in *Drosophila*. (A) BM diversity in *Drosophila*. At embryonic stage (st.) 5, an uncondensed Collagen IV matrix surrounds cells of the cellularized blastoderm 5. In the stage 14 embryo, a Laminin matrix lacking Collagen IV joins the amnioserosa epithelium and the yolk membrane on the dorsal side of embryo. In the larval midgut, a continuous LNCP matrix surrounds muscles and midgut. In the heart, a fibrous LNCP matrix surrounds the cardiac tube, which is contiguous with the BMs around pericardial cells and alary muscles, and contains the heart-specific component Pericardin, a Collagen family protein with similarities to Collagen IV. (B) Ovarian follicles are surrounded by a fibril-containing BM produced by the follicle cells, an epithelium of mesodermal origin that encapsulates the oocyte and nurse cells. Follicles gradually elongate along the AP axis; this is coupled to polarized fibril deposition and rotational migration. The fibrils that are produced in the intercellular spaces of follicle cells descend and become incorporated into the BM oriented along the direction of migration. (C) During disc eversion, the BMs of the PS cells of the disc and the larval epidermis fuse, in a process mediated by fibril metalloproteinases, preceding invasion of the larval epidermis by the disc. Subsequently, the peripodial BM separates to allow contraction of the peripodial cells; this contributes to the final step of eversion by pushing the disc outside. (D) CIVICs in the fat body mediate inter-adipocyte adhesion, allowing organization of the mesenchymal adipocytes into a compact tissue layer. CIVICs are thick, irregularly shaped LNCP aggregates that are discontinuous with the planar BM that surrounds the fat body. Integrins and syndecan are required for CIVIC formation. CIVICs also signal through an integrin–Src–PI3K–Akt pathway to promote growth and prevent autophagy. A TEM image of a fat body CIVIC (outlined) is shown below. (E) The matrix that joins the muscles and tendon cells at muscle attachment sites contains Laminin and Perlecan (but not Collagen IV or Nidogen), as well as specific matrix components such as Thrombospondin, Tiggrin and Foundue. (F) Anterior polar cells in ovarian follicles form an apical BM cap of unknown function. See main text for references.

matrix, could be precursors to condensed BMs in both development and evolution. Indeed, some groups of sponges (Leys et al., 2009) and some ctenophores (Fidler et al., 2017), as well as all placozoans (Srivastava et al., 2008), lack condensed BMs despite possessing some or all of the four main BM components (see Box 1).

Loose BM-related matrices in midgut and heart

Other examples of loose ECMs that contain BM components exist in the midgut and heart. The midgut epithelium is surrounded by visceral muscles and harbors a continuous spongy LNCP matrix

sandwiched in between two layers of denser material, instead of separate BMs for epithelium and muscles (Howard et al., 2019) (Fig. 3A). It is not known whether this ECM results from fusion of BMs or is a single structure from the beginning. In the heart, a fibrous LNCP matrix is required for correct morphogenesis and homing of blood cells (Cevik et al., 2019; Rotstein and Paululat, 2016). The heart matrix contains an additional specific component called Pericardin, a collagen family protein with similarities to Collagen IV (Chartier et al., 2002) (Fig. 3A). Some evidence exists for prolyl-4-hydroxylase-dependent formation of Pericardin

multimers (Wilmes et al., 2018), which are produced in the larval fat body but specifically deposited in the heart (Drechsler et al., 2013). The correct deposition of Pericardin is dependent on Laminin (Urbano et al., 2009) and, to a lesser extent, on Collagen IV (Hollfelder et al., 2014). Together with the loose matrices of the embryo, the midgut and heart matrices highlight the importance of less-compacted LNCP matrices, of which more examples may remain unrecognized thus far.

Fibrils in the BM of egg follicles

A peculiar mix of fibers and planar BM is present in ovarian egg follicles (Fig. 3B), each consisting of one oocyte, 15 nurse cells and a surrounding follicular epithelium of somatic origin (Isabella and Horne-Badovinac, 2015a). Egg follicles are initially spherical, but gradually elongate along the anterior–posterior (AP) axis. AP elongation of egg follicles is coupled to their rotation around that axis, which is powered by migration of the follicular epithelium on the BM that encloses it (Haigo and Bilder, 2011). Although in appearance planar, this BM, produced entirely by the epithelial follicle cells (Isabella and Horne-Badovinac, 2015b), contains long fibril-shaped aggregates (Gutzeit et al., 1991; Haigo and Bilder, 2011). The elongated aggregates originate in the intercellular spaces of follicle cells, travel basally and deposit into the BM along the direction of migration (Isabella and Horne-Badovinac, 2016). The function of these fibrils is not entirely clear. Fibrils are proposed to act as a mechanical corset that directionally constrains follicle growth. Fibrils, indeed, locally increase BM stiffness (Chlasta et al., 2017). An alternative proposal is that polarized fibrils do not generate the directional strain for elongation themselves, but act as a cue for the orientation of actin stress fibers inside follicle cells, which are connected to them by integrin and dystroglycan receptors (Cerqueira Campos et al., 2020; Isabella and Horne-Badovinac, 2015a). A definitive dissection of the different aspects that contribute to egg shape may prove difficult, as rotatory migration, cytoskeleton polarization and fibril orientation could be linked to reinforce each other. Furthermore, other BM heterogeneities (see below) have also been ascribed a role in elongation (Chen et al., 2019; Crest et al., 2017).

Atypical BMs during metamorphosis

Unusual BMs play essential roles at two points during imaginal disc eversion (Fig. 3C). The imaginal discs – precursors of the adult wings, legs and other organs – are invaginations of the larval epidermis to which they are connected by narrow stalks (Cohen, 1993). Discs develop inside the animal until the start of metamorphosis, when they evert (Agnes et al., 1999; Martin-Blanco et al., 2000; Usui and Simpson, 2000; Zeitlinger and Bohmann, 1999). Disc eversion is not an orderly opening of the stalk, which is too narrow for allowing the disc through, but an outright invasion of the larval epidermis. The first step of the eversion process involves formation of a transient double BM joining the larval epidermis and the disc peripodial and stalk (PS) cells (Pastor-Pareja et al., 2004). BM fusion and the ensuing invasion of the larval epidermis by PS cells is mediated by matrix metalloproteinases (Srivastava et al., 2007). After this, PS cells on the surface of the animal undergo a partial epithelial-to-mesenchymal transition (Pastor-Pareja et al., 2004). As a result, several holes open and quickly coalesce into a single hole that widens through intercalation, connecting the lumen of the disc with the outside. A second involvement of an atypical BM occurs at this point, when a portion of the peripodial epithelium that envelops the disc contracts, pushing the disc outside through the growing hole (Aldaz et al., 2013; Milner et al., 1984). During this contraction, peripodial cells detach from their BM (Proag et al., 2019).

Later during metamorphosis, after the wings have emerged, they flatten through mutual adhesion of their dorsal and ventral surfaces. This is a complex process in which dorsal and ventral surfaces first adhere, then separate and finally adhere again (Waddington, 1940). Adhesion involves integrins (Brower and Jaffe, 1989; Wilcox et al., 1989; Zusman et al., 1990), Laminin (Henchcliffe et al., 1993; Martin et al., 1999) and the microtubule cytoskeleton (Gregory and Brown, 1998), as mutants in all these factors lead to separation of the two surfaces (blisters). Recently, it has been shown that the first adhesion requires BM degradation, since failure of degradation results in blisters (De Las Heras et al., 2018; Diaz-de-la-Loza et al., 2018). It is therefore clear that dorsal and ventral BMs need to be removed early on for the process to proceed. However, the identity of the matrix structure that finally links the two surfaces is not known, because a planar BM is not apparent in TEM studies (Fristrom et al., 1993; Milner and Muir, 1987; Mogensen and Tucker, 1987). The purpose of this mysterious adhesion–separation–adhesion cycle is also not known. Together with disc eversion, wing adhesion points to the metamorphic development as a time of particularly fluid behavior of the matrix.

CIVICs and other BM-related aggregates

Collagen IV intercellular concentrations (CIVICs) were recently identified in fat body adipocytes and represent an additional unusual matrix structure (Fig. 3D) (Dai et al., 2017). Unlike the BM surrounding the same tissue, CIVICs are thick irregularly shaped LNCP aggregates. CIVICs mediate intercellular adhesion, allowing the otherwise mesenchymal adipocytes to organize into compact tissue layers (Dai et al., 2017; Zajac and Horne-Badovinac, 2017). This adhesive role of CIVICs is similar to that of the double BMs, in this case coupling individual cells rather than whole tissues. In addition to their adhesion role, CIVICs signal through an integrin–Src–phosphoinositide 3-kinase (PI3K)–Akt pathway to promote adipocyte growth and prevent autophagy (Dai et al., 2017). It has been shown that insulin signaling in these cells is regulated by movement downstream of integrins and upstream of PI3K–Akt (Kim et al., 2018), suggesting that CIVICs may be involved in mechanotransduction. During stromal-to-decidual transition after human fertilization, the mesenchyma-derived decidual cells form discontinuous BMs in their pericellular spaces reminiscent of CIVICs (Jones-Paris et al., 2017). CIVICs and similar BM-related matrices, therefore, may have a general role in conferring tissue organization to non-epithelial cells.

Beside CIVICs, other matrix aggregates containing BM components exist at muscle attachment sites and anterior pole cells. Laminin and Perlecan, but not Collagen IV or Nidogen, are components of the specialized matrix joining muscles and tendon cells (Maartens and Brown, 2015), similar to the cell adhesion role of CIVICs (Fig. 3E). However, embryos lacking Laminin only show weak muscle detachment defects (Martin et al., 1999; Prokop et al., 1998; Urbano et al., 2009), and such defects are not reported at all for Perlecan mutants. This contrasts with the strong requirements for integrins and specific components such as Thrombospondin (Chanana et al., 2007; Subramanian et al., 2007), Tigrin (Bunch et al., 1998) and Fondue (Green et al., 2016), suggesting that the role of BM components is minor here. Finally, anterior polar cells, a pair of cells at the anterior end of the follicular epithelium, accumulate BM material in an apical cap as they begin an epithelial-to-mesenchymal transition (Fig. 3F) (Medioni and Noselli, 2005). Epithelial-to-mesenchymal transition by anterior polar cells precedes border cell migration, a collective migration process in which these two cells recruit six other cells into a border cell cluster

that will travel through the nurse cells towards the oocyte (Montell et al., 2012). The function of this apical cap, formed by basal-to-apical transcytosis of BM material, is unexplored, but its existence suggests, together with CIVICs and muscle attachment sites, that it has an important role of LNCP aggregates in multiple tissues and developmental contexts.

Mechanisms underlying atypical organization and BM diversity

ECMs are adapted to perform specific functions and physiological roles depending on their tissue context. Our understanding of how BM components can assemble into BMs or BM-related matrices with specific properties in different tissues is very preliminary. Nonetheless, some principles and mechanisms can be inferred from the growing number of studies addressing this question.

Cellular origin and deposition mode

One aspect that may critically influence matrix assembly is the mode of component deposition. Depending on their origins, components can assemble at short or long distance from their cellular sources, the latter favoring a more homogeneous incorporation. Studies in *Drosophila* show variability in the origin of BM components for different tissues and matrices (Fig. 4A), but also two general tendencies. First, mesodermal tissues are more capable of secreting matrix, as is the case with blood cells and fat body, the major sources of BM components for the embryo and larva, respectively (Dai et al., 2018; Fessler and Fessler, 1989; Matsubayashi et al., 2017; Pastor-Pareja and Xu, 2011). The follicular epithelium – a rare example of an epithelium of mesodermal origin (Brookman et al., 1992; King et al., 1968) – is indeed able to secrete all four main BM components (Isabella and Horne-Badovinac, 2015b). A second tendency in the expression of *Drosophila* BM components is that Laminin is more often produced in a tissue-intrinsic way. This is the case for imaginal discs (Fig. 2F), which secrete their own Laminin, but receive Collagen IV, Nidogen and Perlecan from the fat body through the hemolymph (Dai et al., 2018; Henchcliffe et al., 1993; Urbano et al., 2009). In some cases, a certain BM component may have a mixed origin, as it is in the case of the BM surrounding the larval central nervous system, which receives its Collagen IV from both intrinsic (perineural glia) and extrinsic (fat body) sources (Dai et al., 2018; Petley-Ragan et al., 2016).

Evidence in *Drosophila* indicates that short-range deposition in tissues that produce their own matrix favors aggregate formation due to an increased local concentration. Examples of this are fat body and follicle cells, where short and long-range modes of deposition coexist, each mode producing a different type of matrix. Fat body adipocytes produce the four main components for their BM and CIVICs (Dai et al., 2018; Kim and Choe, 2014; Pastor-Pareja and Xu, 2011; Zang et al., 2015). In CIVICs, Collagen IV is deposited at short range by contacting cells, whereas Collagen IV in the surrounding BM comes from the common soluble pool in the hemolymph (Dai et al., 2017). In follicle cells, the two modes of deposition cooperate to generate planar BMs decorated with fibril-shaped aggregates. Aggregates are formed between the lateral membranes of follicle cells in a manner dependent on the GTPase Rab10 and laid down on the BM as cells migrate, whereas the bulk of the BM is incorporated at a more constant rate from a soluble pool, also emanating from the follicle cells (Isabella and Horne-Badovinac, 2016). An interesting example of extrinsically produced matrix deposited at short range is found in the embryo, where BM components produced by blood cells are incorporated mostly

from the hemolymph, but also as streak-like deposits ‘painted’ by migrating blood cells (Matsubayashi et al., 2017; Sánchez-Sánchez et al., 2017). CIVICs, follicle fibrils and streak-like embryonic BM deposits all form in reduced intercellular spaces, suggesting that limited diffusion and high local concentration are factors favoring aggregation. Consistent with this notion is the finding of fibrotic LNCP deposits on the surface of fat body adipocytes upon reducing endocytosis (Fig. 4B) (Ke et al., 2018; Zang et al., 2015). Reducing endocytic membrane internalization in these highly secretory cells leads to striking overgrowth of their plasma membranes, which become highly convoluted. In such conditions of limited diffusion, first Collagen IV and then other BM components form dense fibrotic aggregates that trigger a tissue damage immune response through unknown mechanisms (Zang et al., 2015). All these examples highlight the influence of cellular origin and local conditions on the deposition of BM components to generate diversified matrices.

Receptor variability

Integrins are heterodimeric receptors formed by an α and a β subunit that are capable of binding numerous ECM ligands, among them laminin and collagen IV. In mammals, 18 α -subunits and eight β -subunits combine to form 24 distinct heterodimers with varied ligand-binding preferences (Hynes, 2002). The *Drosophila* genome encodes five α -subunits and two β -subunits (Narasimha and Brown, 2000) (Table 1). Our understanding of their differential ligand preferences is incomplete, but evidence exists that α PS2– β PS dimers are receptors for Arg-Gly-Asp (RGD) tripartite motif-containing ligands Tigrin and the Wb laminin α chain, whereas α PS1– β PS dimers bind the alternative laminin α -chain LanA (Fogerty et al., 1994; Gotwals et al., 1994; Zavortink et al., 1993). In *C. elegans*, there are two α -subunits, α INA-1 and α PAT-2, and a single β PAT-3. Recent work has shown that the two different heterodimers these subunits can form, α INA-1– β PAT-3 and α PAT-2– β PAT-3, dictate distinct collagen IV recruitment mechanisms, which translate into different collagen IV contents in two different BMs (Jayadev et al., 2019). In the gonad, α INA-1– β PAT-3 integrin, a laminin receptor, directs laminin recruitment as well as moderate laminin-dependent recruitment of collagen IV. In contrast, the RGD receptor α PAT-2– β PAT-3 acts in the pharynx to recruit large amounts of collagen IV in a laminin-independent manner (Fig. 4C). Interestingly, this study identified the Rap GTPase RAP-3 as a specific α PAT-2– β PAT-3 activator in the pharynx (Jayadev et al., 2019), showing that regulation of which integrin dimers act in which tissue is not exclusively at the level of their expression, but also a result of their activation. Besides integrins, other receptors may provide specific characteristics to certain matrices, as is the case with Syndecan, which is required in *Drosophila* adipocytes for formation of CIVICs, but not the surrounding BM, indicating specific assembly mechanisms (Dai et al., 2017).

Compositional diversity

In mammals, the existence of alternative genes and transcripts for BM components is a known source of BM heterogeneity through their differential expression. This is evidenced not just by antibody staining, but also the specific phenotypes of knockout mice and the distinct pathologies in congenital syndromes where single genes are mutated (Hessle et al., 1984; Kruegel and Miosge, 2010). For collagen IV and laminin, one needs to add combinatorial heterotrimer diversity. Laminin genes in mammals encode five α -subunits, four β -subunits and three γ -subunits, which can combine into at least 16 confirmed heterotrimers (Yurchenco, 2011).

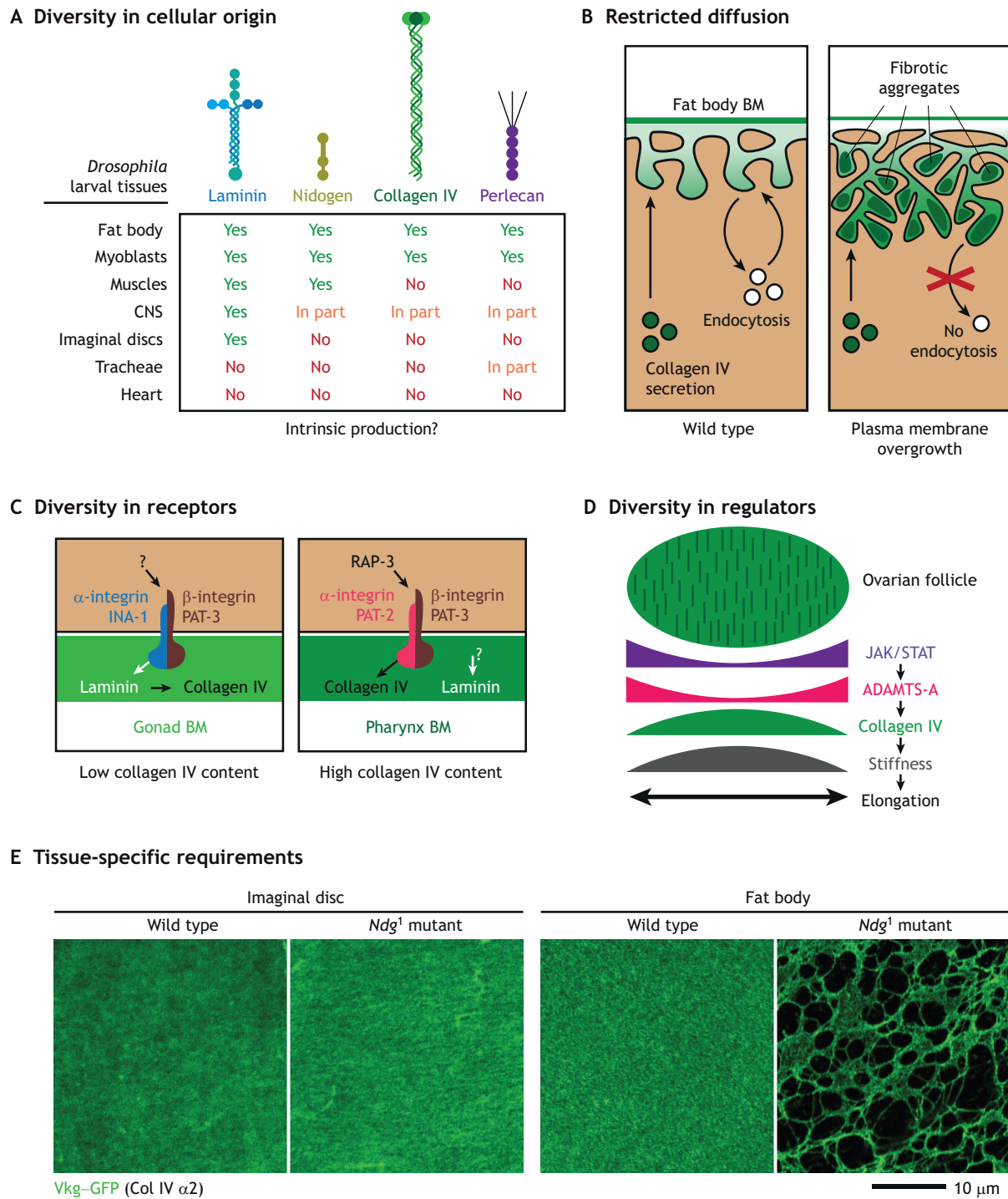


Fig. 4. Mechanisms underlying atypical organization and BM diversity. (A) Intrinsic production of the four main BM components in different tissues of the *Drosophila* larva. Mesodermal tissues such as blood cells in the embryo, follicle cells in the ovary, and fat body, myoblasts and muscles in the larva, typically have a higher ability to produce BM components. Intrinsic production of Laminin is frequent. (B) Restricted diffusion favors aggregate formation and hinders a homogeneous BM deposition in *Drosophila* fat body adipocytes. In conditions reducing endocytic membrane removal, the plasma membrane overgrows and becomes highly convoluted. On the surface of this convoluted plasma membrane, an increased local concentration of Collagen IV causes fibrotic aggregation. (C) Alternative α subunits of the integrin receptor differ in their affinity for collagen IV and thus determine the differential collagen IV content in *C. elegans* BMs. α INA-1 directs moderate laminin-dependent collagen IV recruitment to the gonad, whereas α PAT-2 recruits large amounts of collagen IV to the pharynx independently of laminin and downstream of GTPase RAP-3. (D) Differential expression of matrix regulators can produce BM heterogeneities. In *Drosophila* follicles, a gradient of JAK/STAT activity, which is higher at the poles, results in differential expression of the metalloproteinase ADAMTS-A, a matrix regulator. This creates an opposing Collagen IV content gradient, resulting in a stiffness gradient that, together with fibril orientation (see Fig. 3B), contributes to follicle elongation. Other possible JAK/STAT matrix regulator targets are SPARC and Mmp2. (E) Tissue-specific requirement of the BM components. *Drosophila* *Nidogen* (*Ndg*) mutants exhibit loss of BM integrity in the fat body, but not in imaginal discs or most other tissues. Confocal micrographs of disc and fat body BMs in the wild type and *Ndg*¹ mutant are shown. See main text for references.

Similarly, six mammalian genes encoding collagen IV α -chains and three heterotrimer combinations (denoted $\alpha 1$ - $\alpha 2$ - $\alpha 1$, $\alpha 3$ - $\alpha 4$ - $\alpha 5$ and $\alpha 5$ - $\alpha 6$ - $\alpha 5$, where the numbers refer to the α subunits present) are found (Hynes, 2012; Khoshnoodi et al., 2008). Temporal changes in expression as BMs mature are frequent. During kidney glomerulogenesis, the predominant laminin heterotrimer in the GBM is first LM-111, then LM-511 and finally LM-521 (where the numbers refer to the α -subunit, β -subunit and γ -subunit present, respectively) (Miner and Sanes, 1994). Similarly, collagen IV $\alpha 1$ - $\alpha 2$ - $\alpha 1$ is gradually replaced by $\alpha 3$ - $\alpha 4$ - $\alpha 5$ heterotrimers (Miner, 1998). However, our understanding of the functional implications and specific characteristics these alternative components confer to different BMs is very limited.

In *Drosophila* and *C. elegans*, diversity in the main BM components is comparatively restricted (Table 1). *Drosophila* mutants in the two alternative laminin α chains LanA and Wb differentially affect some tissues (Hollfelder et al., 2014; Schock and Perrimon, 2003; Urbano et al., 2011), but whether differing expression patterns alone or also their binding properties account for these differential effects has not been elucidated. Of note is the strikingly large number of perlecan isoforms in both *Drosophila* and *C. elegans* (Mullen et al., 1999; Voigt et al., 2002), at 22 and 16 predicted alternative transcripts, respectively (<http://www.flybase.org>; <http://www.wormbase.org>). Again, the implications of such variability are not understood. Studies in *Drosophila* show that Perlecan is a terminal component that is not essential for the deposition of any other BM components, but has a large influence on BM thickness and mechanical properties. Indeed, the absence of Perlecan has been shown to make the BM thinner and less elastic in imaginal discs (Pastor-Pareja and Xu, 2011) and egg follicles (Chlsta et al., 2017). The opposite, however, occurs in the lymph gland, a larval hematopoietic organ. This organ contains abundant ECM in the form of compartmentalizing BM septa that surround groups of hemocytes (Grigorian et al., 2011). In the Perlecan mutant, BM septa become thicker, filling the lymph gland with amorphous BM material (Grigorian et al., 2013).

Besides the LNCP components, numerous additional BM components have been identified through proteomic studies (Chew and Lennon, 2018). Although the functions of many of them are unexplored, the examples of the *Drosophila* heart matrix and muscle attachment sites show that certain components can confer specific characteristics to BMs and determine assembly of atypical structures.

Diversity of BM regulatory proteins

Apart from BM components and their receptors, other matrix regulators may play important roles in generating diverse matrices, again exemplified in the *Drosophila* follicular BM. A Collagen IV content gradient exists in this BM, with higher Collagen IV incorporation in the center and less in the anterior and posterior poles (Fig. 4D) (Crest et al., 2017). This Collagen IV gradient forms downstream of an opposing gradient of Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling. The Collagen IV gradient creates a stiffness gradient, which contributes to orienting cell rearrangements and determining the elongated egg shape (Chen et al., 2019). JAK/STAT signaling does not seem to affect collagen transcription. However, a recent screen has identified among the targets of JAK/STAT signaling the protein a disintegrin and metalloproteinase with thrombospondin motifs-A (ADAMTS-A), whose loss produces rounder eggs (Wittes and Schüpbach, 2019). Overexpression of ADAMTS-A has been shown to reduce Collagen IV levels in BMs, although it is not clear

whether this is a direct proteolytic effect (Skeath et al., 2017). In addition to ADAMTS-A, this same screen uncovered the matrix metalloproteinase Mmp2 and collagen solubilizing factor secreted protein acidic and rich in cysteine (SPARC) as targets of JAK/STAT signaling, raising the possibility that multiple matrix regulators contribute to the gradient (Wittes and Schüpbach, 2019). *Drosophila* matrix metalloproteinases Mmp1 and Mmp2, which are capable of degrading collagen and other matrix proteins, can mediate not just an outright destruction of BMs, but also the formation of atypical structures, such as the transient double BM between PS cells and larval epidermis during disc eversion (Page-McCaw et al., 2003; Srivastava et al., 2007). SPARC in turn is a conserved protein that binds collagen, aiding its solubilization and long-range transport through unknown mechanisms. SPARC loss triggers formation of a thick fibrillar matrix on the surface of the fat body (Pastor-Pareja and Xu, 2011; Shahab et al., 2015), whereas its overexpression reduces BM thickness in follicle cells (Isabella and Horne-Badovinac, 2015b) and interferes with the formation of CIVICs (Dai et al., 2017). Similar effects have been observed for *C. elegans* SPARC (Morrissey et al., 2016). In summary, and as exemplified by the follicle BM, matrix regulators such as ADAMTS-A, MMPs and SPARC have the potential to impart not only specific physical properties to BMs, but also intra-BM heterogeneity.

Hidden diversity revealed by mutants

Finally, there is plenty of diversity among BMs that we do not understand; this is evidenced by the differential effects of BM component mutants when compared among different tissues and developmental contexts. Such differential effects on BM integrity and deposition of other components are well documented in *Drosophila*, where they cannot be attributed to compensation or genetic redundancy. In the *Drosophila* model, Perlecan deposition depends on Collagen IV in all tested contexts (Dai et al., 2017; Hollfelder et al., 2014; Isabella and Horne-Badovinac, 2015b; Pastor-Pareja and Xu, 2011), but mutual relations among other BM components appear more context-dependent, indicating that the BM assembly hierarchy might be tissue-specific. In the embryo, the order of assembly follows the temporal order of expression of the different components, with Laminin incorporated first; this is required for correct Collagen IV deposition, which, in turn, is a prerequisite for Perlecan deposition (Matsubayashi et al., 2017). It must be noted, however, that in the absence of Laminin, Collagen IV still deposits on embryonic surfaces, albeit in a disorganized way (Urbano et al., 2009). In contrast to what is seen in the embryo, Laminin absence did not affect Collagen IV deposition after wounding the larval epidermis (Ramos-Lewis et al., 2018). In the fat body BM, Laminin and Collagen IV are not completely required, but clearly reinforce mutual deposition (Dai et al., 2018). These divergent results indicate that the hierarchy of BM assembly might be tissue specific and dependent on factors that remain entirely unknown.

A striking example of tissue-specific effects is presented by *Drosophila* Nidogen (Ndg). *Ndg* mutant flies are viable, presenting mild fertility and behavioral defects (Dai et al., 2018; Wolfstetter et al., 2019). When BMs of *Ndg* mutant animals were examined, dramatic loss of BM integrity was observed only in the fat body and some adult muscles (Fig. 4E) (Dai et al., 2018). Structure–function analysis confirmed that Nidogen acts as a link between Collagen IV and Laminin, and defects in fat body BM result from the uncoupling of their networks (Dai et al., 2018). Why then do the BMs of *Ndg* mutants break only in certain tissues? One possibility is that BMs of fat body and muscles are subjected to high levels of mechanical stress that make the additional Laminin–Collagen IV

link that is provided by Nidogen essential in these tissues to preserve BM integrity. Indeed, genetic interaction experiments show that loss of Nidogen also enhances the defects seen in the absence of Laminin or Perlecan in other tissues (Dai et al., 2018). Fat body cells increase their size very quickly during larval stages through polyploidization, which could explain an enhanced requirement for Ndg. However, this is also true of other tissues, such as the salivary glands, in which BMs that lack Nidogen still preserve their integrity. In summary, while tissue-specific functions of Nidogen and other BM components are well documented, we lack a mechanistic understanding of this phenomenon.

Conclusions and perspectives

The multitude of studies and descriptions of atypical structures reviewed here challenge a strict definition of BMs as planar matrices. Also questionable is the clear distinction between the BM and other types of ECM, such as loose matrices, fibrils and aggregates. Finally, in terms of their composition, there are well-documented examples of laminin, nidogen, collagen IV and perlecan localizing and functioning outside of typical BMs, and of BMs that lack some of these four main BM components. Although these examples could be dismissed as limiting cases and exceptions to the rule, research in *Drosophila*, where the numbers of BM studies have exploded in the past decade, indicates that such atypical BM structures are actually quite prevalent. Even for typical planar BMs, studies repeatedly reveal that each of them is special in its own way, with large gaps in our understanding of their diversity, tissue-specific component requirements and differential properties.

Here, I have summarized our precarious knowledge of the mechanisms generating BM diversity and increased thickness or aggregate-like deposition in atypical structures. Importantly, changes in BM thickness are associated with diseases that affect the blood brain barrier, kidneys, cornea or retina (Chew and Lennon, 2018; Gatseva et al., 2019; Pozzi et al., 2017; Sekiguchi and Yamada, 2018). Furthermore, thick matrix deposits are seen in fibrotic conditions, where the expression of matrix proteins is upregulated, but the mechanisms that make that excess matrix form aberrant structures are unknown. Uncovering the mechanisms that generate the normal homogeneous thickness of planar BMs and the increased thickness of certain atypical BMs, therefore, may help researchers to understand not only the normal assembly and roles of atypical structures, but also the pathological assembly linked to human diseases.

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Competing interests

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