Primer

Basement membranes

Ranjay Jayadev[1]
and David R. Sherwood[2,*]

Basement membranes (BMs) are thin, dense sheets of specialized, self-assembled extracellular matrix that surround most animal tissues (Figure 1, top). The emergence of BMs coincided with the origin of multicellularity in animals, suggesting that they were essential for the formation of tissues. Their sheet-like structure derives from two independent polymeric networks — one of laminin and one of type IV collagen (Figure 1, bottom). These independent collagen and laminin networks are thought to be linked by several additional extracellular matrix proteins, including nidogen and perlecan (Figure 1, bottom). BMs are usually associated with cells and are anchored to cell surfaces through interactions with adhesion receptors and sulfated glycolipids (Figure 1, bottom). Various combinations of other proteins, glycoproteins, and proteoglycans — including fibulin, hemicentin, SPARC, agrin, and type XVIII collagen — are present in BMs, creating biochemically and biophysically distinct structures that serve a wide variety of functions. BMs have traditionally been viewed as static protein assemblies that provide structural support to tissues. However, recent studies have begun to uncover dynamic, active roles for BMs in many developmental processes. Here, we discuss established and emerging roles of BMs in development, tissue construction, and tissue homeostasis. We also explore how cells traverse BM barriers, the roles of BMs in human diseases, and future directions for the field.

Basement membrane composition and structure

Studies in *Drosophila*, *Caenorhabditis elegans*, and mice support the idea that laminin is the foundational building block for the initial formation of BMs. Laminin is a secreted heterotrimeric protein made up of an α, a β, and a γ
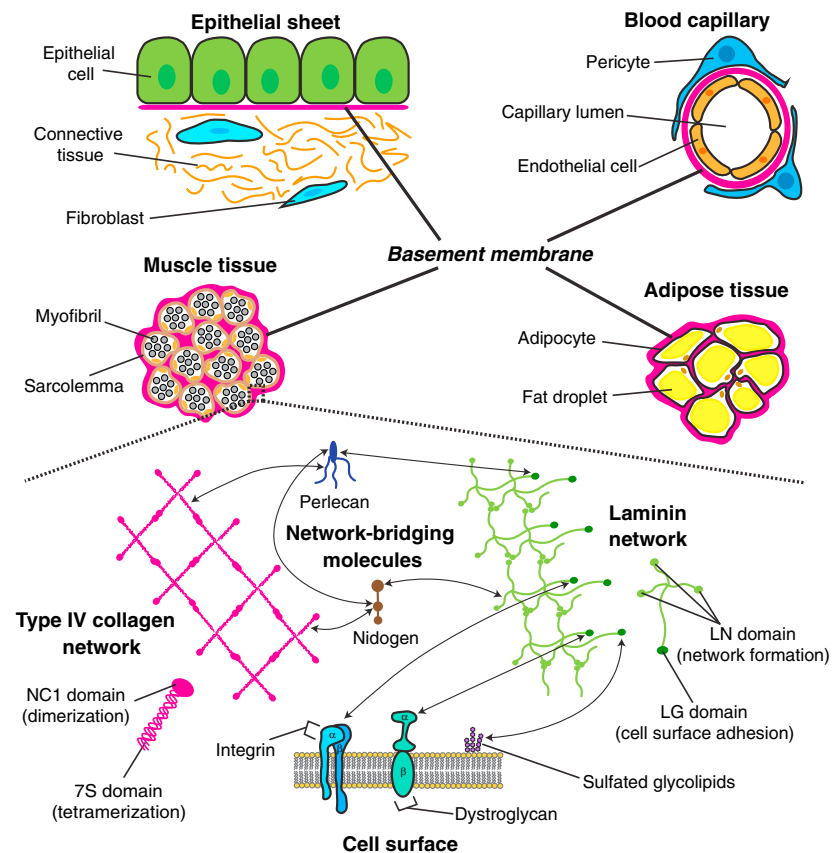

Figure 1. BM localization and composition.
(Top) BMs underlie or surround most tissues, including epithelial, endothelial, muscle, and adipose tissues. (Bottom) The self-assembling polymeric networks of type IV collagen and laminin provide BMs with their core structure and these networks associate with each other through interactions (indicated by arrows) with bridging adaptor proteins, such as perlecan and nidogen. The laminin network is closely associated with cell surfaces through interactions (arrows) with integrins and dystroglycan receptors as well as sulfated glycolipids.

subunit. Laminin trimers self-assemble into a polymeric sheet-like lattice that is tightly associated with the cell surface. In *Drosophila* and *C. elegans*, two genes encode α subunits and the β and γ subunits are each encoded by a single gene: these subunits combine to form two heterotrimers. In mammals, five α, four β, and three γ chains have been identified; these assemble into at least 16 heterotrimeric complexes that are often expressed in a tissue-specific manner. In addition to being secreted locally, laminin can be secreted from distant sites and incorporated into BMs from the interstitial fluid. For example, *C. elegans* sublateral nerves are covered by a laminin α_2-containing BM, even though the neurons do not express laminin subunits. Similarly,

the mouse neural tube BM contains the laminin α_5 subunit, which is not expressed by the neural tube cells.

Following laminin assembly, an independent self-assembling, covalently crosslinked type IV collagen network is laid onto BMs. *Drosophila* and *C. elegans* possess two type IV collagen genes and produce a single collagen heterotrimer made up of two $\alpha1$ chains and one $\alpha2$ chain. Vertebrate genomes, however, encode six collagen chains — $\alpha1$ to $\alpha6$ — that assemble into three distinct heterotrimers. Similar to laminin, type IV collagen may be produced and incorporated into BMs locally or from distant sources. The collagen network in BMs is covalently linked together by multiple chemical bonds, including disulfide and sulfilimine bonds, that are
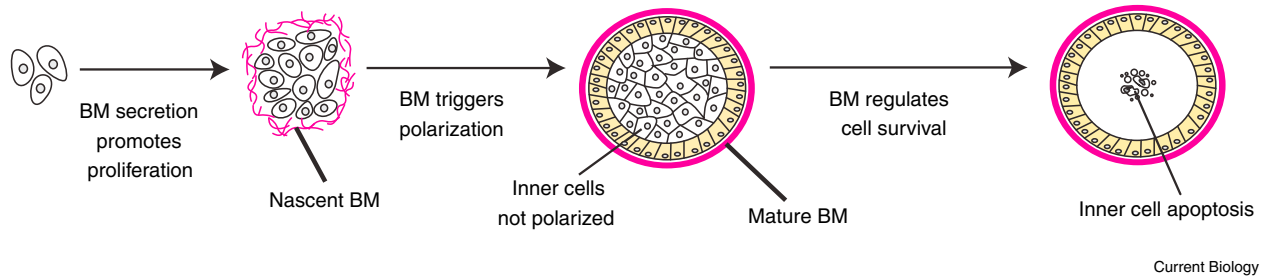
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Figure 2. BM function in polarity and signaling.
BMs regulate many cell signaling events. BM molecules such as laminin, type IV collagen, and BM-associated growth factors induce cell proliferation. The interactions of BM molecules and cell surface receptors facilitate cell and tissue polarization. Cues from the BM regulate cell survival and cell death.

thought to confer the tensile strength of the BM.

Various other proteins associate with the laminin and type IV collagen networks in BMs. The glycoprotein nidogen and the heparan sulfate proteoglycan perlecan are thought to link the two networks together, due to their high affinity for both laminin and collagen. The proteoglycan collagen XVIII and the glycoproteins SPARC and fibulin-1 are also often found within BMs. Recent proteomic analyses of isolated BMs have identified over 100 BM-associated proteins — many of which are involved in tissue-specific interactions — highlighting the complexity and diversity of BMs.

BMs adhere to cell surfaces by interacting with various adhesion receptors and lipids. Laminin interacts with integrin and dystroglycan through laminin globular-like (LG) domains present in α subunits (Figure 1, bottom). Laminin also directly binds to sulfated glycolipids on the cell surface. Comparatively little is known about whether type IV collagen is directly anchored to cell surfaces. Discoidin domain receptors (DDRs) are unique collagen-binding receptor tyrosine kinases. These receptors require collagen binding for their kinase activity and downstream signaling, but their genetic loss does not seem to affect type IV collagen localization to BMs, suggesting that they do not anchor collagen to cell surfaces. *In vitro* assays have identified an RGD tripeptide in type IV collagen that binds with high affinity to integrin. While this interaction has been reported to have cell signaling functions, whether it promotes collagen association with cell surfaces has not been clearly established.

Recent ultrastructural and immunolocalization analyses of inner limiting membranes of vertebrate eyes have revealed new aspects of BM structure. While conventional transmission electron microscopy measurements made with desiccated tissues have estimated the thickness of most BMs to be less than 100 nm, new atomic force microscopy measurements made on hydrated chick, mouse, and human BMs estimate that they are at least two-fold thicker. This increased thickness would allow the large type IV collagen molecules (400 nm in length) and laminin molecules (80 nm in length) to adopt more diverse orientations in the BM than the flat chicken-wire configurations envisioned in most schematic models. In addition to being thicker than previous measurements, new evidence indicates that BMs exhibit sidedness. Laminin is enriched on the inner epithelial face of human adult ocular BMs, and this face is also two to four times stiffer than the outer stromal facing side. In contrast, type IV collagen is enriched on the stromal face and is oriented asymmetrically, with the 7S domain facing the stromal side and the NC1 domain facing the epithelial side. Further, epithelial cells and axons adhere more strongly to the epithelial rather than the stromal side of BMs isolated from numerous animals. Together, these observations suggest that BMs have unique sidedness and may actively organize tissues through distinct interactions on each BM surface.

BM functions in polarity, differentiation, signaling, and tissue maintenance
BMs perform many functions throughout development and in

adult tissues and organs. Laminin is deposited extracellularly very early in development. Mutations in laminin result in early embryonic lethality in *Drosophila*, mice, and *C. elegans*, with dramatic defects in tissue formation and adhesion, cell fate specification, cell migration, and polarity. Type IV collagen networks, however, appear later in development. BM integrity is heavily compromised in the absence of collagen, resulting in lethality during embryonic tissue movement and rearrangement. In collagen-deficient *C. elegans* and *Drosophila* embryos, muscle tissues detach from the body wall or epidermis as the muscle BM is unable to resist the stresses of muscle contraction. Mice lacking type IV collagen die by E10.5–E11.5, following thinning and fracture of vascular and extra-embryonic BMs. Together, these observations support the idea that laminin initiates BM formation and mediates early cell differentiation and tissue formation, while type IV collagen stabilizes and protects the BM from mechanical stresses.

During early morphogenesis, BMs coordinate cell and tissue polarity (Figure 2). *In vitro* studies using embryonic stem cells have highlighted a requirement for laminin in polarizing the epiblast (the primordial outer embryonic layer). During *C. elegans* pharyngeal (foregut) development, laminin signaling establishes the apical–basal polarity of pharyngeal cells. Similarly, laminin directs polarity in mammalian cell culture. For example, a single Madin-Darby canine kidney epithelial cell will divide and organize into a ball-like cyst only when it is embedded in a laminin-rich matrix.

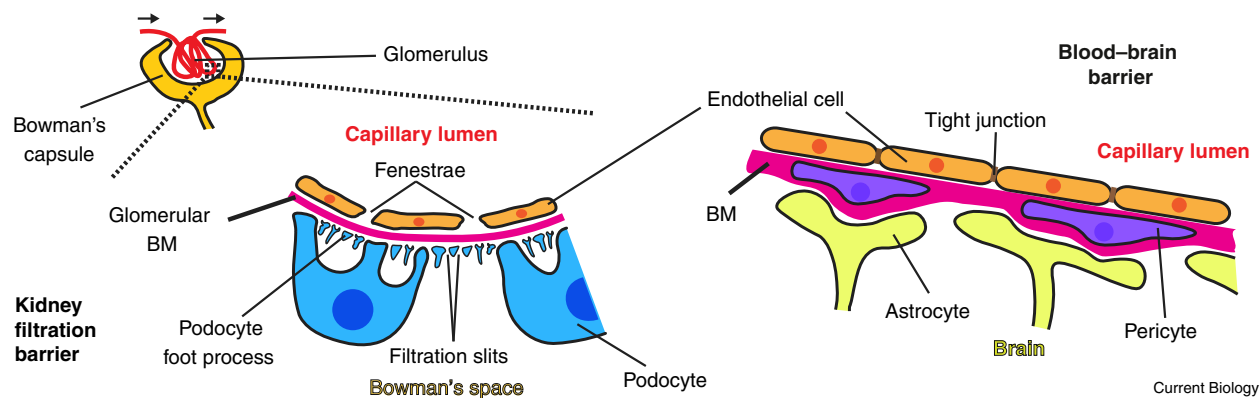BMs can function as signaling platforms during development and in

Figure 3. BMs form barriers between tissues.
(Left) The glomerular BM regulates the flow of molecules from the blood into the urine. (Right) At the blood–brain barrier, the BMs of the vasculature and the brain merge together and organize the endothelial cells that regulate the flow of solutes into the brain.

mature tissues by sequestering many growth factors and other ligands (Figure 2). The BM proteoglycans perlecan, type XVIII collagen, and agrin tether growth factors, such as vascular endothelial growth factor, transforming growth factor-β, and fibroblast growth factor, through binding interactions with their heparan sulfate glycosaminoglycan chains. These growth factors regulate cell survival, stem cell divisions, migration, and proliferation by binding and signaling through cell surface receptors. BM laminin and type IV collagen can directly activate cell signaling pathways. Laminin promotes not only polarity, but also cell survival, migration, and differentiation by binding to several integrin family receptors as well as the dystroglycan receptor. Several cell surface receptors interact with type IV collagen to regulate proliferation, migration, and polarity. These include type IV collagen-binding integrins and the discoidin domain receptor 1 (DDR1). BMs harbor matrix metalloproteases (MMPs), a large family of broad-spectrum proteases that function in degradation and remodeling of the BM. Importantly, by degrading BM scaffolds, MMPs can release cryptic fragments with signaling functions. For example, cleavage of collagen IV by MMP9 exposes a cryptic site involved in angiogenesis, while cleavage of laminin $\alpha_1\beta_1\gamma_1$ by MMP2 releases a laminin fragment that regulates the epithelial-to-mesenchymal transition in embryonic stem cells.

BMs also provide essential barrier functions. The glomerular BM is a critical component of the kidney filtration barrier that prevents the leakage of plasma proteins into the urine. The BM is sandwiched between the endothelial cells of the glomerular capillaries and the podocytes that wrap around the glomerulus (Figure 3). The laminin and collagen networks of the glomerular BM are thought to maintain the selective permeability of the glomerular filter by controlling the porosity of the BM to plasma proteins, selecting for both size and charge. BM barrier function is also observed in the blood–brain barrier — the interface between the vascular system and the brain that controls the movement of solutes between the two tissues (Figure 3). The capillary endothelial BM and the parenchymal BM of brain astrocytes merge at the blood–brain barrier. Tight junctions that form between the endothelial cells prevent the paracellular transport of most molecules, especially small ions. BM laminin and type IV collagen are essential for the formation of these tight junctions and maintenance of transendothelial electrical resistance, indicating that the BM is a crucial component of the blood–brain barrier.

BMs separate epithelia from stromal connective tissues by facilitating stable cell-to-matrix adhesions through hemidesmosomes. BM laminin is a key component of hemidesmosomes, interacting with both integrin on cell surfaces, and type VII collagen of stromal anchoring fibrils. This forms a stable adhesion between epithelia and connective tissues, thus protecting tissues from destabilizing shear forces.

Active and dynamic roles for BMs in shaping and connecting tissues

Recent studies have uncovered new roles for BMs as dynamic structures that help to sculpt tissues. One clear example is during *Drosophila* egg chamber elongation. The egg chamber is made up of germ cells surrounded by a follicular epithelium. At the time of elongation, the initially spherical egg chamber rotates within its surrounding BM, which remains static. As the egg chamber rotates, the follicular epithelium secretes and deposits BM fibrils perpendicular to the axis of elongation. These polarized BM fibrils constrict the egg chamber, restricting circumferential growth, causing the developing egg to grow elliptically. Similarly, during mouse mammary gland duct development, BM accumulates at the base of duct buds, thereby constricting and elongating them.

The composition of the BM shapes tissues. Type IV collagen networks apply constrictive forces on growing tissues. In *Drosophila* egg chamber development, the onset of elongation is tightly correlated with an increase in collagen production by follicle cells, and collagen mutations result in rounded eggs. Furthermore, loss of these constrictive forces by post-embryonic reduction of collagen in *Drosophila* results in the dilation of many tissues, including the wing disc (Figure 4). In contrast, perlecan maintains the elasticity of BMs as tissues grow. Reduction of perlecan results in the aberrant compaction of the wing disc, suggesting an increase in BM tension (Figure 4). Because perlecan
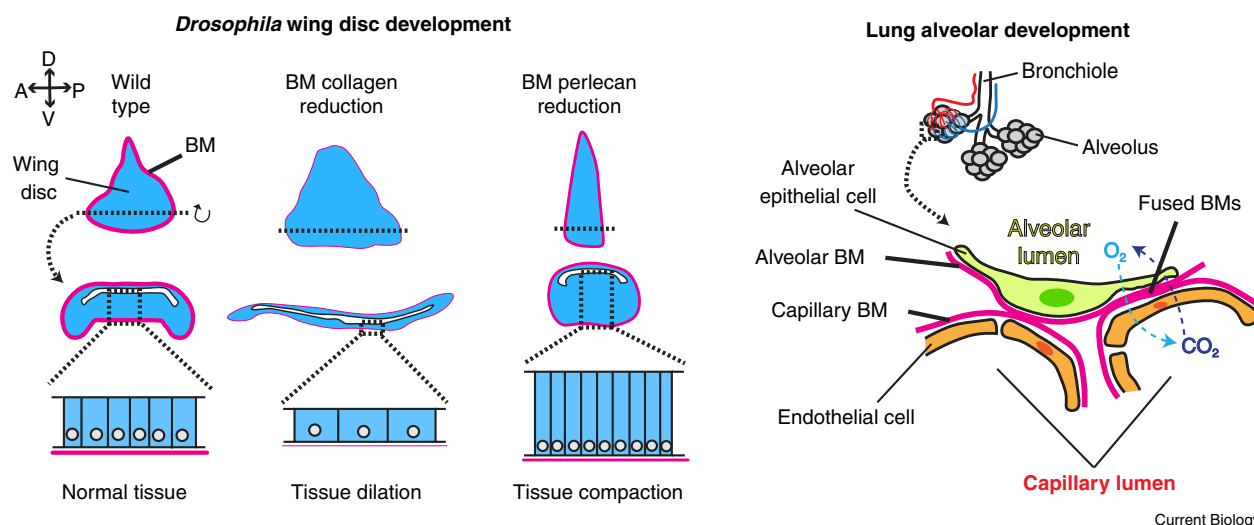
Figure 4. BMs shape and connect tissues and provide mechanical support.
(Left) BM collagen and perlecan act in opposition to shape the developing *Drosophila* wing disc. (Right) Fusion of the lung alveolar and capillary endothelial BMs brings the two tissues together to facilitate gas exchange.

does not affect the incorporation or levels of collagen in the BM, these observations suggest that perlecan directly counteracts the mechanical constriction provided by collagen in BMs to regulate tissue shaping.

BMs shape tissues by connecting them together. In the kidney, BMs of the vascular endothelium and podocyte epithelium fuse together to form the glomerular BM. During lung alveolar development, fusion of the alveolar and capillary BMs into a single sheet brings the alveolus and capillaries into close contact, which likely facilitates efficient gas exchange (Figure 4). Studies in *C. elegans* have revealed a new BM adhesion system, the B-LINK, that connects the uterus to the vulva through attachments between the uterine and vulval BMs. These attachments are mediated by the conserved extracellular matrix component hemicentin. The cytolinker plakin and adhesion receptor integrin are also components of the B-LINK complex. It is likely that this adhesion system functions broadly to connect tissues through neighboring BMs. For example, hemicentin promotes attachment of the epidermal and somite BMs in zebrafish embryos and may function as part of an as yet undescribed B-LINK complex.

BM transmigration
BMs have tiny pores of approximately 10–130 nm in diameter due to the compact meshwork of interconnected laminin and type IV collagen lattices. Consequently, cells cannot traverse this barrier without removing it. Yet cells routinely move through BMs and enter tissues during development and normal physiological functions. For example, cells often transmigrate BMs when undergoing epithelial-to-mesenchymal transitions during organogenesis, and leukocytes move across multiple vascular BMs as they traffic through the circulatory system during immune surveillance. Moreover, misregulated BM transmigration is causally associated with the progression of many human diseases, including the pregnancy disorder pre-eclampsia, inflammatory immune diseases, and cancer metastasis.

Some BMs, such as those of vascular and lymph system, contain pre-formed portals or gaps in specific locations, allowing circulating immune cells to enter or leave the vasculature. In most cases, however, *de novo* openings must be created to allow cells to traverse them. Many invasive cells extend invadosomes — specialized membrane-associated actin-rich structures — into the BM to degrade it locally during transmigration. For example, tumor cells are thought to use invadosomes to metastasize; embryonic trophoblast cells degrade the uterine wall BM using invadosomes for successful implantation; and growth cones extend invadosomes that locally degrade BM during axon guidance through tissues.

Localized loss of the adhesion receptor dystroglycan is another mechanism that facilitates BM breaching. During chick gastrulation, dystroglycan localization to the basal surfaces of epiblast cells contacting the embryonic BM is lost, resulting in BM breakdown and migration of epiblast cells into the interior of the developing embryo. In other cases, mechanical forces might break open the BM to facilitate transmigration. In mouse embryogenesis, spatial restriction of embryonic growth by surrounding maternal uterine tissues causes mechanical stresses that appear to rupture the embryonic BM, allowing epiblast cells to cross through these gaps and initiate the establishment of the anterior–posterior axis.

BMs in disease
Defects in BM assembly or composition result in a multitude of human diseases. Many arise from mutations in one or more BM proteins and can affect a variety of tissue and organ systems. Mutations in laminin disrupt hemidesmosomes, leading to skin blistering diseases. Mutations in the $\alpha3$, $\alpha4$, and $\alpha5$ chains of type IV collagen result in the kidney disease Alport's syndrome, which is primarily characterized by leakage of plasma proteins into the urine (proteinuria)

due to disruptions in BM pore size. BM protein mutations underlie many diseases of the muscle. Agrin mutations cause congenital myasthenic syndrome, where patients suffer from progressive muscle weakness and fatigue, due to defects at the neuromuscular junction. Mutations in laminin α_2 cause congenital muscular dystrophy type 1A, characterized by the early onset of muscle fiber degeneration.

Compositional and ultrastructural changes in BM are linked to various human disorders. In Goodpasture's syndrome, autoantibodies are produced against the α_3 chain of type IV collagen. The consequent disruption of BM collagen networks results in acute kidney failure and lung hemorrhage that can be fatal. In diabetes mellitus patients, the glomerular and tubular BMs of the kidney are abnormally thickened, causing reduced glomerular filtration and proteinuria. Similarly, endothelial BM thickening has been observed in Alzheimer's disease. As a result, β-amyloid peptides that are typically removed from the brain via the endothelial BM accumulate in the walls of cerebral blood vessels, which is associated with a decline in cognition. In multiple sclerosis, an inflammatory disease of the central nervous system, extensive BM deposition and concomitant leukocyte accumulation is observed in active white matter lesions. These aberrations are associated with astroglial scarring and axon damage as the disease progresses.

BM dysregulation is a hallmark of many cancers. Overexpression of laminin is linked to cell hyperproliferation in breast, prostate, and colon tumors. Laminin and perlecan also stimulate the development of new blood vessels to provide oxygen to proliferating cancer cells. Recent proteomic analysis of the extracellular matrix signatures of metastatic tumors has revealed that several BM components — including laminin and type IV collagen — are secreted by both stromal and tumor cells in highly metastatic tumors, but are only secreted by the stroma in poorly metastatic tumors. These observations suggest that primary tumor cells actively alter the composition of BMs, and that the resulting changes in BM ultrastructure or signaling or both may drive metastasis.

Outlook

We are only just beginning to appreciate the complexity of BMs and many open questions about their biology remain. Recent proteomic studies have revealed that BMs have diverse compositions and can harbor around 100 to 200 distinct proteins, many with unknown roles in BM function. We do not yet know the targets of many MMPs and other BM proteases. By studying the activity of these proteases, we will likely identify additional cryptic binding sites in BM proteins, as well as novel signaling fragments from these proteins. The functional consequences of post-translational modifications of BM proteins is another understudied area. Comprehensive maps of glycosylation and hydroxylation sites in human type IV collagen generated by high-resolution mass spectrometry have identified over 200 modified amino acids. Many of these sites are also conserved in mouse collagen IV, suggesting that they may be *bona fide* modifications necessary for BM function. Mapping of these sites on other BM proteins and determining their functional significance represents an important area of future study.

While we understand BM construction in embryos, we know very little about how BMs grow post-embryonically. Type IV collagen cross-linking must be finely tuned to balance tissue support while maintaining pliability as tissues rapidly expand during development. How are crosslink formation and breakage coupled to allow a BM to grow? In bacterial cell wall expansion, as new peptidoglycan is added to the growing cell wall, existing crosslinks are hydrolyzed. Breaking these crosslinks is thought to redistribute mechanical stresses, thereby stretching the existing network and allowing further addition of peptidoglycan. Similar 'network-breaking' strategies may be utilized in BM growth, but whether particular hydrolases or reductases are required is unknown.

BMs also undergo profound changes in composition, structure, and physical properties with age. The age-associated thickening of BMs (by more than 100 fold in some instances) is often linked with tissue degeneration and disease, as we have discussed in this article. Interestingly, new evidence suggests that extracellular matrix remodeling promotes longevity in *C. elegans*, indicating that BM turnover might be a key and underappreciated component of aging.

Given that BMs originated at the dawn of animal multicellularity, it should come as no surprise that their functions are interlinked with nearly all processes important to multicellular life. Understanding the varied cellular interactions with this extracellular scaffold, and the mechanisms that organize BM construction and remodeling, remain exciting and largely unexplored areas of future research with important basic and clinical implications in development, physiology, and aging.

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[1]Department of Biology, Program in Cancer Biology, Duke University, Durham, NC 27705, USA. [2]Department of Biology, Regeneration Next, Duke University, Durham, NC 27705, USA.
*E-mail: david.sherwood@duke.edu