CHAPTER NINE

Building from the Ground up: Basement Membranes in *Drosophila* Development

Adam J. Isabella[1] **and Sally Horne-Badovinac**[1,2,*]

[1]Committee on Development, Regeneration, and Stem Cell Biology, The University of Chicago, Chicago, IL, USA
[2]Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL, USA
*Corresponding author: E-mail: shorne@uchicago.edu

Contents

Abstract

Basement membranes (BMs) are sheetlike extracellular matrices found at the basal surfaces of epithelial tissues. The structural and functional diversity of these matrices within the body endows them with the ability to affect multiple aspects of cell behavior and communication; for this reason, BMs are integral to many developmental processes. The power of *Drosophila* genetics, as applied to the BM, has yielded substantial insight into how these matrices influence development. Here, we explore three facets of BM biology to which *Drosophila* research has made particularly important contributions. First, we discuss how newly synthesized BM proteins are secreted to and assembled exclusively on basal epithelial surfaces. Next, we examine how regulation of the structural properties of the BM mechanically supports and guides tissue morphogenesis.

Current Topics in Membranes, Volume 76
ISSN 1063-5823
http://dx.doi.org/10.1016/bs.ctm.2015.07.001

Finally, we explore how BMs influence development through the modulation of several major signaling pathways.

1. INTRODUCTION

Extracellular matrices (ECMs) are proteinaceous networks that accumulate nearly ubiquitously in the spaces between cells. ECMs link and coordinate cells both within and between tissues; their existence therefore likely contributed greatly to the rise and success of multicellular life, especially in the metazoan lineage (Ozbek, Balasubramanian, Chiquet-Ehrismann, Tucker, & Adams, 2010). Among the most ancient ECMs, the basement membrane (BM) is a specialized matrix that associates with the basal surfaces of epithelial tissues, as well as endothelial, fat, muscle, and Schwann cells. This chapter will focus predominantly on epithelial BMs. By electron microscopy, BMs appear as thin sheets (generally ~100 nm thick). They are composed primarily of two independent weblike networks of laminin and type IV collagen (collagen IV), which are heavily interlinked by proteins such as nidogen and the heparan sulfate proteoglycan (HSPG) perlecan (Yurchenco, 2011) (Figure 1). Adhesion of the BM to cells is achieved via interactions with transmembrane receptors, such as integrins and dystroglycan. Beyond the four core BM constituents, a large number of

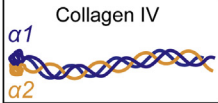
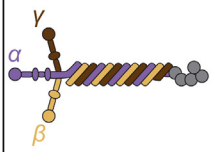
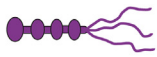
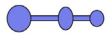
Protein	*Drosophila* genes	Human homologs
Collagen IV 	α1: *Collagen gene at 25C (Cg25C)*	*COL4A1, COL4A3, COL4A5*
	α2: *viking (vkg)*	*COL4A2, COL4A4, COL4A6*
Laminin 	α1,2: *wing blister (wb)*	*LAMA1, LAMA2*
	α3,5: *Laminin A (LanA)*	*LAMA3, LAMA4, LAMA5*
	β: *Laminin B1 (LanB1)*	*LAMB1, LAMB2, LAMB3, LAMB4*
	γ: *Laminin B2 (LanB2)*	*LAMC1, LAMC2, LAMC3*
Perlecan 	*terribly reduced optic lobes (trol)*	*HSPG2*
Nidogen 	*Nidogen/entactin (Ndg)*	*NID1, NID2*

Figure 1 Overview of the core basement membrane proteins in *Drosophila*. (See color plate)

accessory proteins have been found to contribute to the network (Hynes & Naba, 2012). Differential incorporation of minor components, as well as varying isoforms and posttranslational modifications of the core proteins, lends great structural and functional diversity to the many BMs found throughout the body.

Several major roles for BMs have emerged, which will be discussed briefly here and in greater depth throughout this chapter. First, the mechanical properties of the network establish it as a physical scaffold. This property allows BMs to maintain tissue shape and integrity in the face of deformation forces and act as a substrate against which forces can be generated for cellular contraction and migration. Because of their small pore size, BMs can also provide a barrier function that helps to limit the movement of cells and large macromolecular complexes between body compartments. The ability to bind several secreted signaling molecules further allows these matrices to facilitate cell–cell communication both within and between tissues.

The functional capabilities of the BM described above make it well suited to facilitate the specification, compartmentalization, growth, and morphogenesis of distinct tissue and organ systems. Thus, it is not surprising that BMs are essential for embryonic development. The fruit fly *Drosophila melanogaster* has provided a particularly powerful system in which to dissect the specific contributions that BMs make to these processes. Nearly all developing tissue and organ systems have been well characterized and are visually and experimentally accessible. The powerful genetic techniques available, especially the ability to precisely manipulate gene expression in time and space, are also advantageous, particularly when studying a structure that plays such diverse roles in development. Moreover, the creation of functional GFP protein trap alleles of the collagen IV α2 gene *viking* and the perlecan gene *terribly reduced optic lobes* (*trol*) have transformed BM research in *Drosophila* by allowing unprecedented visual resolution of the native proteins in both fixed and living tissues (Buszczak et al., 2007; Morin, Daneman, Zavortink, & Chia, 2001).

While the core BM proteins and their receptors are well conserved between flies and humans, the fly BM can be viewed as a simplified version of its mammalian counterpart. Flies produce only two distinct laminin trimers compared to sixteen in humans, one collagen IV trimer versus three in humans, and two β and five α integrin subunits versus eight β and eighteen α subunits in humans. Although this simplicity means that flies cannot recapitulate the diversity of human BMs and ECM receptors, it increases the power to dissect protein function by limiting problems associated with redundancy.

In this chapter, we highlight important contributions that *Drosophila* research has made to our understanding of BM assembly and function during development. Because the literature on this topic is extensive, we have not attempted to provide a comprehensive summary of the data. Instead, we focus on three topic areas that exemplify the breadth and depth of BM research in this organism. First, we address the longstanding question of how BM proteins are precisely targeted to basal epithelial surfaces. We discuss how proteins produced from a variety of cellular sources achieve this goal, with a special focus on the intracellular trafficking pathway that operates within epithelial cells to transport newly synthesized BM proteins to basal regions of the plasma membrane for secretion. Second, we address the process of morphogenesis during development. We explore mechanical contributions of BMs to this process, specifically how regulated remodeling of BM structure can help to shape a tissue. In this section, we offer an in-depth discussion of the complex contributions of the BM to egg chamber elongation. Third, we address molecular signals that mediate cell—cell communication during development. We discuss contributions that BMs make to this process through the modulation of several major signaling pathways.

2. SYNTHESIS, SECRETION, AND ASSEMBLY OF BMS ON BASAL CELL SURFACES

Epithelial cells exhibit a highly polarized architecture with four distinct membrane domains—apical, junctional, lateral, and basal. To build and maintain a BM, newly synthesized components must be assembled exclusively on the basal epithelial surface. Here, we explore several ways in which BM proteins are targeted to this membrane domain, including a mechanism for the polarized secretion of BM proteins by an epithelium.

2.1 Sources of BM proteins and implications for polarized assembly

In *Drosophila*, the sources of BM proteins are complex and, in many cases, are still not clear. Some epithelia synthesize all of their own BM components, while others rely on production by other tissues. Some epithelia combine these approaches, producing a subset of their own proteins and relying on external sources for others (Figure 2). The major nonepithelial source of BM proteins also varies by developmental stage. In the embryo, BM proteins

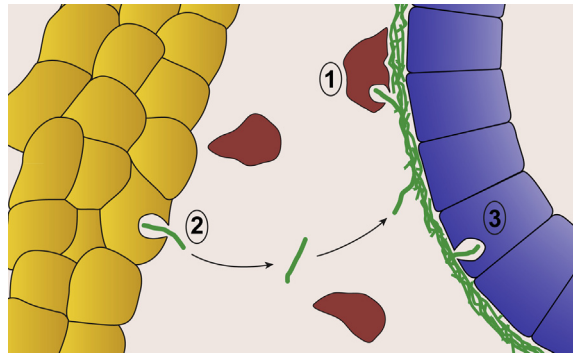


Figure 2 ***Cellular sources of basement membrane proteins in*** **Drosophila.** BM proteins are synthesized and secreted by three primary cell types. 1: Synthesis and local deposition by hemocytes. 2: Synthesis by and long range diffusion from the fat body. 3: Synthesis and secretion by the epithelium itself. (See color plate)

are primarily produced by hemocytes—circulating immune cells that migrate throughout the body. Hemocytes display the primary signal for transcripts encoding collagen IV and laminin by in situ hybridization (Kusche-Gullberg, Garrison, MacKrell, Fessler, & Fessler, 1992; Le Parco, Knibiehler, Cecchini, & Mirre, 1986; Mirre, Cecchini, Parco, & Knibiehler, 1988; Yasothornsrikul, Davis, Cramer, Kimbrell, & Dearolf, 1997) and, when cultured, produce large volumes of BM proteins (Fessler, Nelson, & Fessler, 1994). It should be noted, however, that some embryonic and larval epithelia appear to produce their own laminin and/or perlecan (Denef, Chen, Weeks, Barcelo, & Schüpbach, 2008; Martin et al., 1999; Sorrosal, Pérez, Herranz, & Milán, 2010).

How do proteins secreted by hemocytes assemble specifically on basal epithelial surfaces? This process must require establishment of the basal epithelial membrane as a competent surface to bind soluble BM proteins, likely by expression of cell surface BM receptors. Hemocytes also tend to cluster around BM-containing tissues, probably for the purpose of BM deposition (Kusche-Gullberg et al., 1992). This phenomenon is analogous to ECM deposition by fibroblasts in vertebrates. In this case, epithelia likely recruit hemocytes to their basal surfaces.

While hemocytes continue to produce BM proteins throughout development, during late embryogenesis and larval stages collagen IV and laminin production is also observed strongly in the fat body—a major metabolic organ in insects (Kusche-Gullberg et al., 1992; Le Parco et al., 1986; Mirre

et al., 1988; Yasothornsrikul et al., 1997). The fat body appears to take over as the major production center, at least for collagen IV, in larvae. Blocking collagen IV production in the fat body results in drastic loss of this protein from BMs throughout the body, including full loss from the BM surrounding the imaginal wing disc epithelium—the pouchlike precursor to the adult wing (Pastor-Pareja & Xu, 2011).

Because the fat body is fixed in place, proteins secreted from this organ must diffuse long distances through the extracellular space to their target tissues. Yet they are still incorporated efficiently into distant BMs. As was discussed with hemocytes, this is most likely achieved by ensuring that basal epithelial surfaces have the necessary adhesive properties to capture diffusing proteins. In this case, it is equally important to prevent premature protein aggregation and promiscuous adhesion to the wrong tissues. The task of escaping the fat body is particularly onerous, as this tissue is itself surrounded by a BM through which secreted proteins bound for other tissues must pass without adhering. SPARC (secreted protein acidic and rich in cysteine) appears to promote collagen IV diffusion away from the fat body to distant epithelia, as loss of SPARC from this tissue leads to an aberrant accumulation of collagen IV between fat body cells (Pastor-Pareja & Xu, 2011; Shahab et al., 2015).

Although it is likely that the hemocytes and fat body continue to produce BM proteins throughout the life of the fly, there is one epithelium, found in adult females, that is known to synthesize and secrete all of its own major BM proteins—the follicular epithelium that surrounds the developing germ cells within the ovary. For the rest of this section, we will describe recent studies, primarily performed within this tissue, that have begun to elucidate how BM proteins synthesized within the epithelium itself are targeted exclusively to basal regions of the plasma membrane for secretion (Figure 3).

2.2 Basal localization of BM protein synthesis

In the follicular epithelium, newly synthesized BM proteins exhibit a polarized localization within the cell from the moment of translation. The mRNAs encoding both collagen IV chains (*viking and Cg25c*) and the laminin β chain (*LanB1*) show a 70% enrichment in the basal half of the cell (Lerner et al., 2013). Because the endoplasmic reticulum (ER) stretches throughout the cytoplasmic volume, this observation suggests that these transcripts are primarily translated into a specific subregion of this organelle.

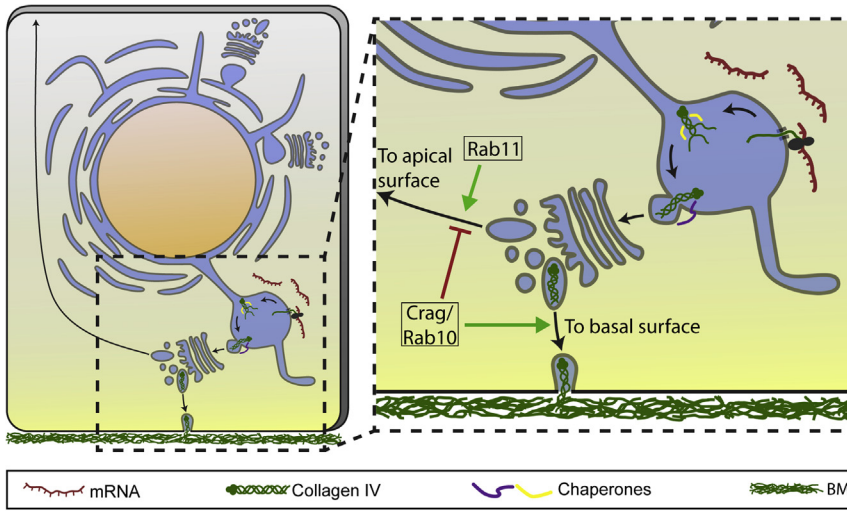


Figure 3 ***Local synthesis and polarized secretion of collagen IV in the follicular epithelium.*** Within the follicular epithelium, *collagen IV* transcripts accumulate basally and are translated and Translocated into a basal region of the endoplasmic reticulum (ER). ER-resident proteins assist in the folding and packaging of collagen IV for transport to the Golgi. After collagen IV transits through the Golgi, Crag and Rab10 promote delivery of collagen IV-containing exocytic vesicles to basal regions of the plasma membrane and prevent Rab11-dependent targeting of collagen IV-containing vesicles to the apical surface. Inset: blow-up of indicated region. (See color plate)

Thus, mRNA localization may help to establish a distinct ER compartment specialized for BM protein production.

Why might such a distinct ER compartment exist? Collagen IV places a notorious burden on the ER's protein production and transport machinery. Each collagen IV protomer is assembled from three polypeptides that wind into a triple helical structure nearly 400 nm long (Khoshnoodi, Pedchenko, & Hudson, 2008). This complex folding reaction requires a suite of ER-resident chaperones, several of which are collagen-specific. For instance, procollagen lysyl hydroxylase (Plod) and prolyl-4-hydroxylase-alpha EFB (PH4αEFB) catalyze hydroxylation of lysines and prolines, respectively, primarily within the triple helical domain; and both enzymes are required for trimer assembly and stability (Myllyharju & Kivirikko, 2004). Collagen IV's large size also prevents it from being packaged into standard CopII-coated vesicles for transport to the Golgi. The transmembrane protein Tango1 is required at ER exit sites (ERESs) to help load collagens into enlarged Golgi-bound vesicles (Pastor-Pareja & Xu, 2011; Saito et al., 2009; Venditti et al., 2012; Wilson et al., 2011). Compartmentalization of collagen IV

production could, therefore, increase biosynthetic efficiency while limiting any potential negative impact on other ER processes.

In support of this idea, the mRNAs encoding Plod, PH4αEFB, and Tango1 all display enrichment in the basal cytoplasm, similar to the collagen IV-encoding mRNAs, and the Tango1 protein primarily localizes to basal ERESs (Lerner et al., 2013). Importantly, knocking down expression of any of these three proteins causes collagen IV to become trapped in a discrete region of the ER near the basal cell surface. When this happens, collagen IV does not diffuse from this location, even over long time periods (Lerner et al., 2013). This observation suggests that a mechanism exists to prohibit diffusion of BM proteins away from their site of synthesis. Localized production of ECM proteins within subregions of the ER has also been observed in vertebrates, which suggests that this may be a conserved biosynthetic strategy (Vertel, Velasco, LaFrance, Walters, & Kaczman-Daniel, 1989).

Because the ER compartment where BM proteins are synthesized is in the basal region of the cell, it is intriguing to speculate that this localization may also act as an initial step to bias secretion to the basal plasma membrane. While the Golgi typically takes the form of a singular organelle in mammalian cells, called the Golgi ribbon, *Drosophila* cells contain many dispersed Golgi stacks that each associate with a single ERES (Kondylis, Pizette, & Rabouille, 2009; Kondylis & Rabouille, 2009). This organization has led to the hypothesis that individual ERES-Golgi units could function independently of one another to facilitate polarized protein secretion. Synthesis within a basal region of the ER could, therefore, promote protein transport through basally localized ERES-Golgi units and subsequent delivery to the adjacent basal plasma membrane. However, there are several reasons to question this assertion. First, mRNA localization does not appear to be an absolute requirement for high-fidelity polarized secretion—*perlecan* (*trol*) mRNA does not display a basal bias, but the protein is still faithfully secreted to the basal surface (Lerner et al., 2013). Nor is it sufficient, as disruption of post-Golgi BM protein trafficking causes aberrant secretion to the apical surface (see next subsection). Additionally, whether such a mechanism could function in mammalian cells is unclear. Trafficking through a centralized Golgi ribbon would erase the polarity induced by mRNA localization. However, not all mammalian cells have a Golgi ribbon. Golgi outposts—analogous to the independent Golgi units in *Drosophila*—have been shown to promote polarized protein secretion, possibly in conjunction with localized mRNAs, in vertebrate neurons (Bramham & Wells, 2007; Hanus &

Ehlers, 2008; Horton et al., 2005; Lowenstein et al., 1994; Pierce, Mayer, & McCarthy, 2001; Ramírez & Couve, 2011). A distributed Golgi system has also been described in gastric parietal cells (Gunn et al., 2011).

It is important to note that the two hypotheses as to why the BM proteins are preferentially produced in a basal region of the ER are not mutually exclusive and could simultaneously promote BM formation. These observations offer interesting insight into potential mechanisms regulating BM protein production and secretion, but further study is required to understand the implications of local BM protein production. Experimentally disturbing the basal bias of BM protein production in these cells will be especially useful in discerning the veracity of these hypotheses.

2.3 Post-Golgi trafficking of BM proteins to the basal surface

Apicobasal polarity depends on the polarized trafficking of newly synthesized transmembrane proteins to either the apical or combined basal and lateral (basolateral) membrane domains. Several sorting mechanisms have been identified that direct individual proteins to each of these locations (Rodriguez-Boulan, Kreitzer, & Müsch, 2005; Stoops & Caplan, 2014). Knowledge of these pathways, however, has failed to provide insight into the polarized trafficking pathway that transports BM proteins exclusively to the basal surface. Indeed, several classic studies performed in cultured mammalian epithelial cells have indicated that distinct pathways exist for polarized trafficking of BM versus transmembrane proteins. Treatment with NH_4Cl or colchicine, which perturbs the acidification of intracellular compartments and microtubule dynamics, respectively, disrupts the polarized secretion of BM proteins without affecting basolateral transmembrane proteins. Under these conditions, BM proteins are secreted from both the apical and basal epithelial surfaces (Boll, Partin, Katz, Caplan, & Jamieson, 1991; Caplan, Stow, & Newman, 1987; De Almeida & Stow, 1991; Natori et al., 1992). Conversely, disruption of Cdc42 function disrupts polarized secretion of basolateral transmembrane proteins but has no effect on BM proteins (Cohen, Müsch, & Rodriguez-Boulan, 2001). In fact, even integrins appear to move through a different trafficking pathway than BM proteins (Boll et al., 1991). These studies revealed that a specific pathway for polarized BM secretion exists, but offered little insight into its molecular details.

Recent genetic studies in *Drosophila* have begun to identify the major molecular players that control polarized BM secretion in this system. The DENN domain-containing protein Crag (calmodulin-binding protein

related to a Rab3 GDP−GTP exchange protein) was discovered in a forward genetic screen for novel regulators of apicobasal polarity in the follicular epithelium (Denef et al., 2008). Similar to the early observations in mammalian cells, BM proteins accumulate on both the apical and basal surfaces of *Crag* mutant follicle cells, whereas apical and basolateral transmembrane proteins localize normally. Apical accumulation of perlecan was also observed in the epidermis of *Crag* mutant embryos. Importantly, this paper confirmed that the apical deposition of BM proteins is not due to transcytosis of protein from the existing BM, but rather due to the aberrant secretion of newly synthesized proteins. Crag was initially observed to localize to apical and lateral cell membranes and to Rab5- and Rab11-positive endosomes, although an important population near the basal surface has since been described (see below).

DENN domain-containing proteins commonly function as guanine nucleotide exchange factors (GEFs) for Rab-family GTPases (Marat, Dokainish, & McPherson, 2011), which are molecular switches that cycle between an active GTP-bound state and an inactive GDP-bound state. GEFs transition Rabs to the GTP-bound state, whereas GTPase activating proteins (GAPs) induce transition to the GDP-bound state. The presence of specific active Rab proteins confers identity to membrane-bound compartments within the cell, such as organelles and trafficking vesicles (Barr, 2013). Rabs are also master regulators of vesicle activity, controlling their formation, sorting, targeting, fission, and fusion (Hutagalung & Novick, 2011). It was therefore speculated that Crag might activate a Rab that plays one or more roles in the polarized secretion of BM proteins.

Crag was later found to be a GEF for a known exocytic Rab, Rab10, first in mammals and then in flies (Lerner et al., 2013; Xiong et al., 2012; Yoshimura, Gerondopoulos, Linford, Rigden, & Barr, 2010). Consistent with this result, Rab10 depletion also causes BM proteins to accumulate on both the apical and basal surfaces of the follicular epithelium (Lerner et al., 2013). One key function of a GEF is to recruit its cognate Rab to the correct intracellular membranes (Blümer et al., 2013). Interestingly, Rab10 and Crag colocalize on membrane-bound compartments that are tightly associated with the basal surfaces in the follicle cells, proximal to the ER compartment where BM proteins are synthesized. This basally localized population of Rab10 is lost in *Crag* mutant cells, which suggests that BM proteins likely pass through these compartments on their way to the basal surface.

Although Rab10's exact role(s) in polarized BM secretion remains to be determined, one appealing hypothesis is that this protein functions in an

endosomal recycling compartment (ERC) to help sort BM cargos into a basally directed trafficking pathway. Biosynthetic cargo sorting commonly occurs in the trans-Golgi network (Anitei & Hoflack, 2011; Santiago-Tirado & Bretscher, 2011). However, in polarized epithelial cells most exocytic traffic also passes through ERCs, where additional sorting occurs (Fölsch, Mattila, & Weisz, 2009; Gonzalez & Rodriguez-Boulan, 2009). Rab10 localizes to endocytic compartments in both *Caenorhabditis elegans* and mammalian cells (Babbey et al., 2006; Chen et al., 2006; Shi et al., 2010). Moreover, although Rab11 (another Rab that localizes to ERCs) is not normally required for BM traffic, when Rab10 or Crag are depleted, the BM proteins that travel to the apical surface do so through a Rab11-dependent mechanism (Lerner et al., 2013). This observation suggests that Crag and Rab10 promote the sorting of BM proteins away from a Rab11-dependent pathway.

Crag and Rab10 have been established as the core components of a BM-specific trafficking pathway, though how this pathway recognizes, sorts, and targets BM proteins to the basal surface remains to be discovered, as do additional components involved in this process. Two additional proteins have been identified that are required for polarized BM secretion, although the mechanisms by which they do so are less well understood. These are phosphatidylinositol synthase (Pis), an enzyme involved in the production of phosphoinositides (Devergne, Tsung, Barcelo, & Schupbach, 2014), and Scarface, a secreted serine protease-like protein that lacks catalytic activity (Sorrosal et al., 2010).

The phosphoinositides are a family of phospholipids that regulate a stunning array of cellular processes (Balla, 2013). Various phosphoinositide isoforms are created by kinase- and phosphatase-mediated interconversion between different phosphorylation states of a common phospholipid backbone, phosphatidylinositol. Pis synthesizes phosphatidylinositol and is therefore required for formation of all phosphoinositides; however, the authors focused their analyses on the role of phosphatidylinositol 4,5-bisphosphate (PIP2) in polarized BM secretion (Devergne et al., 2014). PIP2 functions as an apical determinant in the regulation of epithelial polarity (Martin-Belmonte et al., 2007). It also regulates multiple steps of polarized vesicle trafficking, including cargo sorting and membrane fusion (Balla, 2013). In the follicular epithelium, PIP2 is enriched on apical and lateral membranes. Loss of Pis or other PIP2 biosynthetic enzymes decreased PIP2 levels and caused a loss of Crag from apical and lateral membranes (Devergne et al., 2014). How the lateral and apical Crag populations would feed into basal

protein secretion is difficult to say. It is also unclear whether loss of these populations upon Pis depletion is specific, or is an indirect effect of a general reduction in Crag protein levels throughout the cell.

In *scarface* mutant embryos, laminin accumulates on the apical surface of the lateral epidermis and an adjacent extraembryonic epithelium called the amnioserosa; this phenotype is also seen in *Crag* mutant embryos (Sorrosal et al., 2010). Interestingly, expression of Scarface exclusively in the lateral epidermis is sufficient to rescue the BM secretion defect in the amnioserosa of *scarface* mutants, which suggests that this protein can act at a distance. Consistent with this finding, expression of a tagged version of Scarface in a specific region of the wing disc epithelium caused the protein to accumulate on the apical surface and within the endosomal system of nonexpressing cells. Three hypotheses have been proposed for Scarface's function in polarized BM secretion (Eastburn & Mostov, 2010; Sorrosal et al., 2010). Scarface could function within an endosomal compartment to help sort BM proteins into a basally directed trafficking pathway. This possibility is appealing as it aligns well with the likely function of Rab10. Alternatively, Scarface could function at the apical plasma membrane to prevent targeting of BM-containing vesicles to this domain, a function that has also been proposed for Crag (Denef et al., 2008; Devergne et al., 2014). Finally, Scarface could remove BM proteins from the apical surface either by stimulating a proteolytic cascade or via endocytosis. Study of Scarface in the better-understood follicular epithelium may help to build a coherent model for how Crag, Rab10, and Scarface function together to control polarized BM secretion.

Finally, while the Crag/Rab10 pathway has not yet been shown to control polarized BM trafficking in vertebrates, the utilization of distinct secretory pathways for BM and basolateral transmembrane proteins in both *Drosophila* and mammalian cells is intriguing. Such a condition could arise based on special accommodations that certain BM proteins require to move through the secretory pathway (i.e. an acidic environment, enlarged vesicles, etc.), or due to differences in recognizing transmembrane and soluble proteins by the sorting machinery. Where, precisely, these proteins leave the cell could also play a role. Basolateral transmembrane proteins appear to exit the cell though an apical region of the lateral plasma membrane, just basal to the adherens junctions (Grindstaff et al., 1998). In contrast, BM proteins are more likely to exit at or very near the basal surface; indeed, Rab10 has been found on secretory vesicles bound for a basal region of the lateral plasma membrane in mammalian cells (Cao et al., 2008).

Further characterization of the proteins already known to be involved in basal targeting of BM proteins and the identification of other key players will provide a rich area for future research.

3. MECHANICAL CONTRIBUTIONS OF THE BM TO MORPHOGENESIS

Morphogenesis is the process by which cells and tissues change their shapes to create the complex form of adult tissues and organs. BM sheets are well designed to physically assist and regulate morphogenetic processes—by modifying the movement of cells via adhesive interactions, by resisting the contractile forces exerted by cells, or by restricting the expansion of growing tissues. Furthermore, a large number of studies, primarily in in vitro cell culture systems, have found that changing the physical properties of ECMs can modulate the dynamic activities of cells. For instance, changes in matrix stiffness or cell-matrix adhesion affect cellular contractile dynamics and downstream signals within the cell (Charras & Sahai, 2014). Matrix stiffness also appears to regulate cell migration, as many cells tend to migrate from softer to stiffer ECM substrates or toward ECM that is under deformation forces (Lo, Wang, Dembo, & Wang, 2000; Reinhart-King, Dembo, & Hammer, 2008; Roca-Cusachs, Sunyer, & Trepat, 2013). A softer matrix, meanwhile, can promote cellular invasion through the network (Gu et al., 2014). The organization of the matrix also influences migration dynamics (Kim, Provenzano, Smith, & Levchenko, 2012); directionally aligned matrices have been found to orient cellular migration in the direction of alignment and to increase migration speed (Diehl, Foley, Nealey, & Murphy, 2005; Provenzano et al., 2006; Provenzano, Inman, Eliceiri, Trier, & Keely, 2008; Tan & Saltzman, 2002). Confirmation of these observations in vivo remains an important task; *Drosophila* offers an enticing opportunity to explore these concepts within a developing animal.

In vivo, the BMs of developing tissues undergo heavy remodeling (Bernfield, Banerjee, Koda, & Rapraeger, 1984; Daley, Peters, & Larsen, 2008). Moreover, it is clear that different BMs, and even the same BMs over time, exhibit vastly different compositions and, therefore, physical properties. It is likely that the characteristics of these matrices are tuned to appropriately contribute to morphogenesis, although the changes in physical properties, their mechanisms, and their effects on morphogenesis are largely unclear.

3.1 Contributions of the BM to egg chamber elongation

The development of the egg chamber is perhaps the best understood example of how a coordinated progression of changes to BM structure and cellular activity interact to drive tissue morphogenesis (Horne-Badovinac, 2014). Egg chambers, which number in the hundreds within the adult ovary, are each responsible for the maturation of a single oocyte. They are composed of a central cluster of germ cells—one posteriorly localized oocyte and 15 supporting nurse cells—that are surrounded by the follicular epithelium that was discussed in section 2. This somatic tissue is made up of roughly 800–1000 cells, and the BM it produces ensheathes the entire organlike structure (Figure 4(A)). Initially small and spherical, egg chambers proceed through 14 distinct morphological stages, during which they grow to nearly 1000 times their initial volume (Cummings & King, 1969). Between stages 5 and 10, growth is channeled anisotropically to induce elongation along the anterior–posterior (A–P) axis, a process that creates the elliptical shape of the egg (Figure 4(C)).

BM structure has been examined across egg chamber development and found to shift dramatically with the onset of elongation. The BM surrounding early egg chambers does not show any obvious structure by light microscopy. However, concurrent with the onset of elongation at stage 5, dense, linear fibril-like aggregates of collagen IV, laminin, and perlecan begin to be incorporated into the existing planar matrix (Cetera et al., 2014; Gutzeit, Eberhardt, & Gratwohl, 1991; Haigo & Bilder, 2011; Schneider et al., 2006) (Figure 4(C)). These structures all align perpendicular to the A–P axis, effectively polarizing the BM. Arrays of linear actin bundles in the adjacent basal cortex of each follicle cell, which are physically coupled to the BM via integrin-based focal adhesions, align in the same direction as the BM fibrils (Bateman, Reddy, Saito, & Van Vactor, 2001; Delon & Brown, 2009; Gutzeit, 1990). Together, the BM fibrils and basal actin bundles are thought to act as a "molecular corset" that directionally constrains egg chamber growth, thereby providing the anisotropic force that drives elongation (Gutzeit et al., 1991) (Figure 4(B)). This hypothesis is supported by the observation that disruption of the tissue-level organization of these structures leads to the production of rounded eggs (Bateman et al., 2001; Cetera et al., 2014; Conder, Yu, Zahedi, & Harden, 2007; Frydman & Spradling, 2001; Gutzeit et al., 1991; Haigo & Bilder, 2011; Horne-Badovinac, Hill, Gerlach, Menegas, & Bilder, 2012; Lewellyn, Cetera, & Horne-Badovinac, 2013; Viktorinová, König, Schlichting, & Dahmann, 2009).

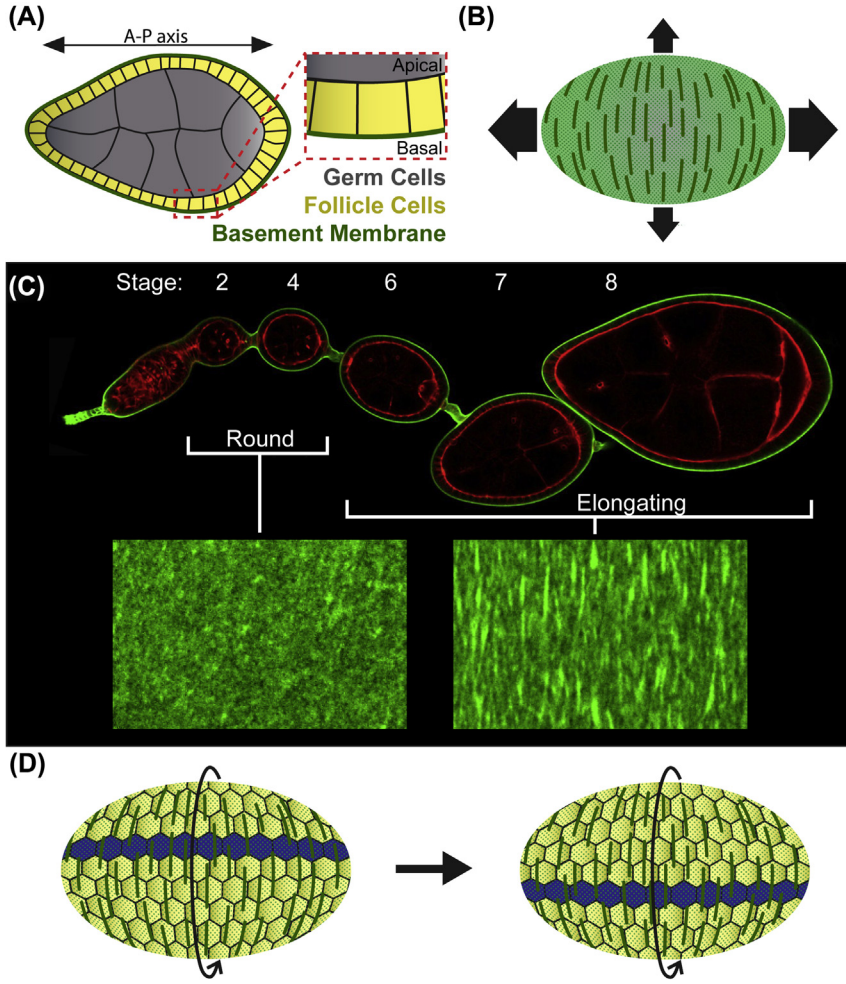


Figure 4 ***Basement membrane (BM) function in egg chamber elongation.*** (A) Illustration of a transverse section through an egg chamber. Central germ cells are surrounded by a somatic epithelium of follicle cells, which assemble a BM on their basal surfaces. (B) Model of the molecular corset. Polarized linear fibril-like structures in the BM are hypothesized to constrain egg chamber growth in the direction of polarization, biasing growth to occur along the A—P axis. Arrows indicate direction and magnitude of growth. (C) BM structural dynamics during elongation. Top: a developmental array of egg chambers showing cell outlines (actin) and the BM (Viking-GFP). Bottom: fluorescent micrographs of collagen IV (Viking-GFP) in the BM. Young, round egg chambers exhibit no obvious BM structure, while older, elongating egg chambers display polarized fibrils within the BM. (D) Overview of egg chamber rotation. In this illustration, the BM is partially transparent to reveal the cells underneath. The dark row of cells in each image represents the same cells at two different time points. The egg chamber rotates within a stationary BM, in the direction of BM fibrils. Curved arrows indicate direction of egg chamber rotation. (See color plate)

Formation of the molecular corset depends on a dramatic rotational motion of the egg chamber. Between stages 1 and 8, the basal surfaces of the follicle cells migrate along the BM, orthogonal to the A—P axis (Cetera et al., 2014; Haigo & Bilder, 2011). Because the apical surfaces of the follicle cells are physically attached to the germ cell cluster, this collective motion causes the entire egg chamber to rotate within the surrounding BM, which remains largely stationary throughout the process (Figure 4(D)). Failure of the follicle cells to migrate disrupts the polarity of the actin and BM networks and prevents egg chamber elongation (Cetera et al., 2014; Haigo & Bilder, 2011; Lerner et al., 2013; Viktorinová & Dahmann, 2013).

The presence of linear, fibril-like aggregates in the follicular BM is surprising, as a BM architecture of this type has not been described in other systems. Because flies do not produce fibril-forming collagens or fibronectin, it is intriguing to speculate as to whether these unusual BM structures might perform some of the same functions as the true fibrillar matrices found in other organisms. Future work will be required to understand the molecular organization of the BM fibrils, as well as the mechanism by which they form. Although egg chamber rotation is required for their formation (Haigo & Bilder, 2011), it is clearly not sufficient, as rotation begins at stage 1 and BM fibrils do not begin to form until stage 5 (Cetera et al., 2014). Fibril formation does, however, happen at a time when collagen IV levels are increasing in the matrix (Haigo & Bilder, 2011), which could indicate a role for new protein secretion in this process. Further, because fibrils appear to play an important functional role in this BM, it will be intriguing to explore to what extent BM superstructural elements are used in other systems to regulate tissue dynamics.

In addition to polarized fibril formation, other structural changes in the BM likely also contribute to its proposed corset function. As mentioned in the previous paragraph, collagen IV levels drastically increase in the BM between stages 5 and 8 (Haigo & Bilder, 2011). Precise control of collagen IV cross-linking also appears to influence elongation, as increasing peroxidasin-dependent collagen IV cross-linking enhances elongation while decreasing peroxidasin activity inhibits elongation without grossly affecting BM superstructure (McCall et al., 2014). In the future, it will be important to determine whether the levels or properties of other BM components are also dynamically regulated as part of the elongation program. It will also be interesting to explore whether the complex interplay that occurs between different BM components in other tissues similarly affects the physical

properties of the follicular BM (Pastor-Pareja & Xu, 2011). Finally, methods will need to be developed to directly measure how each of these changes in matrix architecture alter BM strength and stiffness.

The BM also may influence the tissue-level alignment and activity of the basal actin bundles during elongation. Global actin bundle alignment depends on the rotational motion of the egg chamber at early developmental stages, but this organization becomes rotation-independent concurrent with the establishment of matrix polarity (Cetera et al., 2014). Although rotation ceases at stage 8, maintenance of basal actin bundle alignment is likely to be important for the final phase of elongation, which begins at stage 9. At this stage, periodic myosin-based contraction of the basal actin bundles contributes to elongation (He, Wang, Tang, & Montell, 2010). Proper basal actin alignment is likely required for directionality of this force. Interaction with the BM also directly influences contractile activity of the cells. Decreasing expression of the focal adhesion protein talin shortens the period of myosin contraction, while overexpressing paxillin, another focal adhesion protein, prolongs the period (He et al., 2010). Surprisingly, treatment of egg chambers with collagenase, which might be expected to mimic a decrease in cell-BM adhesion, has also been observed to prolong the period of myosin contraction (Koride et al., 2014). Myosin-based contractions, therefore, are likely regulated by complex inputs from BM structure and cell-BM adhesion.

Once growth has stopped and the elongation program is complete, the BM now plays an important role in maintaining the egg chamber's elliptical shape. When stage 13 egg chambers are treated with collagenase to disrupt the BM, they rapidly become rounder, shortening along the A—P axis, and expanding along the orthogonal axis (Haigo & Bilder, 2011). Thus the BM is required, in varying capacities, to support morphogenesis of the egg chamber for the entirety of its development.

As a final note, studies of egg chamber elongation have also revealed interesting potential effects of BM architecture and cell-BM adhesion on cell migration speed. Although the collective migration of the follicle cells that causes the egg chamber to rotate begins at stage 1, the speed of this migration increases sharply at stage 6, just after the BM fibrils begin to form and collagen IV levels begin to rise (Cetera et al., 2014). However, further investigation will be required to determine whether changes in BM architecture play a causal role in this acceleration. Altering integrin levels in the follicle cells also modifies their migration speed, such that decreasing integrins increases speed and increasing integrins decreases speed (Lewellyn et al., 2013).

3.2 Contributions of the BM to the morphogenesis of other tissues

Loss of function studies have revealed that, in addition to the egg chamber, BMs also appear to play critical roles in the morphogenesis of many other fly tissues and organs. To date, however, few of these initial observations have been followed up with mechanistic studies. Two tissues that have been examined more intensely in this regard are the wing imaginal disc epithelium and migrating glial cells in the imaginal eye disc. These studies are detailed below.

In the wing disc epithelium, local matrix degradation is required for two developmental processes. During larval development, adjacent tracheal tissue must invade through the wing disc BM and contact the underlying epithelium to form the air sac primordium. This process requires local BM degradation by matrix metalloprotease 2 (Mmp2) (Guha, Lin, & Kornberg, 2009). In the pupa, the internal wing disc protrudes through the body wall to form the external wing in a process known as disc eversion. This morphogenesis also requires Mmp2-mediated BM degradation (Srivastava, Pastor-Pareja, Igaki, Pagliarini, & Xu, 2007). A unique pattern of collagen IV cleavage is seen during disc eversion, suggesting precise, context-specific modifications of BM structure during morphogenesis (Fessler, Condic, Nelson, Fessler, & Fristrom, 1993).

Maintenance of proper cell shape in the larval wing disc epithelium also depends on interaction with an appropriately structured BM. Integrin-based adhesion to the BM is crucial for maintaining proper cell shape (Domínguez-Giménez, Brown, & Martín-Bermudo, 2007). Maintenance of cell shape also depends on balanced and opposing forces contributed by collagen IV and perlecan. Loss of collagen IV causes flattening of wing disc epithelial cells and the entire tissue, while loss of perlecan causes disc compaction and elongation of cells along their apicobasal axes (Pastor-Pareja & Xu, 2011). A similar antagonistic role for collagen IV and perlecan has been observed in the *C. elegans* neuromuscular junction, where the two molecules differentially regulate growth of presynaptic boutons (Qin, Liang, & Ding, 2014).

Finally, regulation of BM structure, specifically its stiffness, impacts the migration of glial cells within the eye disc. A stiffer matrix is known to promote cell migration in vitro (Lo et al., 2000; Roca-Cusachs et al., 2013). It was recently demonstrated that increasing the activity of the collagen cross-linking enzyme lysyl oxidase (Lox) or integrins increases BM stiffness in vivo

in this system (Kim et al., 2014). This study further found that migrating glial cells upregulate Lox and integrin expression to promote their own migration. This is superficially similar to the observation, discussed above, that altering integrin levels affects cell migration in the egg chamber. However, during glial migration upregulation of integrins promotes migration, while in the egg chamber integrin upregulation slows migration or, when severe enough, inhibits it completely (Lewellyn et al., 2013). Integrin levels therefore appear to play a complex and context-specific role in migration dynamics.

4. CONTRIBUTIONS OF THE BM TO CELL–CELL SIGNALING DURING DEVELOPMENT

Cell-to-cell signaling, primarily through several major secreted molecules and their receptors, is crucial to regulate and coordinate tissue development. Although the predominant view of the BM tends to be structure-centric, it also serves as a major extracellular signaling platform. By interacting directly with secreted signaling proteins, the BM can act to limit their diffusion or modify their interactions with cell surface receptors. Genetic and biochemical evidence from flies and vertebrates indicates that the BM regulates most, if not all, major developmental signaling pathways, including TGF-β/BMP (Paralkar, Vukicevic, & Reddi, 1991; Wang, Harris, Bayston, & Ashe, 2008), FGF (Folkman et al., 1988; Klagsbrun, 1990; Lin, Buff, Perrimon, & Michelson, 1999; Park et al., 2003), Wingless/Wnt (Binari et al., 1997; Perrimon & Bernfield, 2000), and Hedgehog (Datta et al., 2006; Park et al., 2003; Rubin, Choi, & Segal, 2002; The, Bellaiche, & Perrimon, 1999). An advantage of the developmental focus of *Drosophila* research is that the early studies performed in this system offered not only evidence for these interactions, but also immediate indications of their developmental relevance. Since the connection was made between the BM and signaling, a diverse set of examples have emerged in *Drosophila* of the roles these interactions play in guiding specific developmental processes. Three such processes will be discussed here: axonal pathfinding, Malpighian tubule morphogenesis, and regulation of stem cell activity.

4.1 Modulation of Slit/Robo and Semaphorin/Plexin signaling during axonal pathfinding

As the nervous system develops, growth of axons away from neuronal cell bodies is required to appropriately innervate the body and promote

connections with other neurons or tissues. As discussed above, the BM is an important permissive substrate for migration of many cell types, including neurons (Takagi et al., 1996), but it also acts to regulate the response of extending axons to multiple environmental signals that provide attractive or repulsive cues to guide their growth. Slit and Robo are a highly conserved signaling duo that were first discovered in forward genetic screens for developmental defects in *Drosophila* embryos (Nüsslein-Volhard, Wieschaus, & Kluding, 1984; Seeger, Tear, Ferres-Marco, & Goodman, 1993). Subsequent work showed that Slit is an extracellular protein that primarily functions as a repellent cue for axons that express the Robo receptor (Brose et al., 1999; Dickson & Gilestro, 2006). There is evidence that Slit may bind laminin in vertebrates (Brose et al., 1999), and laminin misexpression causes axon guidance defects in *Drosophila* (García-Alonso, Fetter, & Goodman, 1996; Kraut, Menon, & Zinn, 2001). Further, decreasing laminin or integrin expression enhances the axon pathfinding defects in a *Slit* hypomorphic allele, suggesting that the BM modulates axonal responsiveness to Slit signals (Stevens & Jacobs, 2002). Slit binding to heparin also enhances the Slit—Robo interaction (Hussain et al., 2006), and the transmembrane HSPG syndecan, which can function as a BM receptor (Carey, 1997), acts with Robo as a Slit coreceptor (Johnson et al., 2004; Smart et al., 2011; Steigemann, Molitor, Fellert, Jäckle, & Vorbrüggen, 2004). Whether this interaction occurs cooperatively with or independently of the BM is unclear, although perlecan, the BM HSPG, does not appear to exhibit similar activity (Steigemann et al., 2004).

Semaphorin-based axon guidance also relies on interactions with the BM. Semaphorin-1A is a transmembrane protein expressed at axon guidance decision points that signals to the axonal receptor Plexin A, which mediates repulsion at sites of Semaphorin contact (He, Wang, Koprivica, Ming, & Song, 2002). Similar to Slit, vertebrate Semaphorin activity is enhanced by heparin (De Wit, De Winter, Klooster, & Verhaagen, 2005). In this case, however, the HSPG utilized appears to be perlecan, which is heavily deposited at axon branch points and is required to augment a *Semaphorin-1A* gain-of-function mutation in *Drosophila* (Cho, Chak, Andreone, Wooley, & Kolodkin, 2012). *Syndecan* showed no genetic interaction with *Semaphorin-1A* in this study. It is intriguing that two signaling pathways, Slit/Robo and Semaphorin-1A/Plexin A, which regulate similar axonal guidance events by different molecular means, exhibit nonoverlapping reliance on two HSPG proteins. The coincidence of HSPG utilization among these pathways is mysterious, although utilization of different HSPG

cofactors may enhance signaling diversity while maintaining signal distinction and resolution in the crowded neuronal milieu.

4.2 Modulation of BMP signaling during Malpighian tubule morphogenesis

Epithelial tubes are organized with their apical surfaces surrounding an internal lumen and their basal surfaces covered by a BM. Tubular outgrowth and branching are important in the development of several organs, including lungs, kidney, and salivary and mammary glands (Andrew & Ewald, 2010). Work in several vertebrate systems has identified mechanical roles for the BM and other ECMs in branching morphogenesis (Varner & Nelson, 2014). In *Drosophila*, the BM has been implicated in signaling during tubule morphogenesis as well. The *Drosophila* kidney ortholog, the Malpighian tubules, exhibits stereotyped outgrowth guided by a leading group of "kink cells" at the anterior of the tissue (Denholm, 2013). Local expression of the BMP homolog decapentaplegic (Dpp) in tissues along the tubule outgrowth route is necessary and sufficient to guide this morphogenesis (Bunt et al., 2010). Further, local deposition of collagen IV by hemocytes on the outgrowing tubules is required to transduce the Dpp signal in kink cells (Figure 5(A)). Supporting this observation, Dpp directly binds the C-terminus of collagen IV, which enhances interaction of Dpp with its receptors (Wang et al., 2008). This interaction appears to be conserved in mammals (Paralkar et al., 1991).

In the case of the Malpighian tubules, collagen IV helps to localize and concentrate the Dpp signal for its reception by target cells. Two other examples where Dpp and collagen IV interact in a similar manner will be discussed in the next section. Interestingly, Dpp is also known to regulate several developmental processes in *Drosophila* by formation of precise morphogen gradients. Because collagen IV appears to restrict diffusion of Dpp, it has been proposed that the BM may play an important role in these instances of Dpp signaling as well (Sawala, Sutcliffe, & Ashe, 2012; Umulis, Shimmi, O'Connor, & Othmer, 2010; Wang et al., 2008). It will therefore be intriguing to examine the role of the BM during Dpp signaling in other contexts.

4.3 Regulation of stem cell maintenance, differentiation, and division

Stem cells are multipotent progenitors that provide a key source of new cells during development. They also play important roles in tissue maintenance

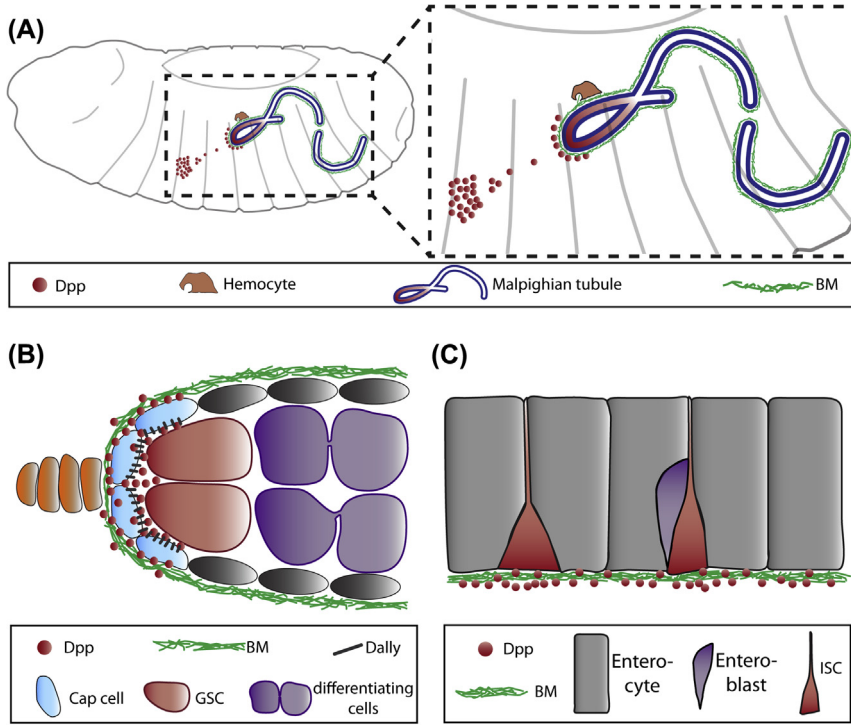


Figure 5 ***Basement membrane (BM) regulation of Dpp signaling in three developmental contexts.*** (A) Embryonic Malpighian tubule morphogenesis. Diffusible Dpp protein attracts the growing Malpighian tubule. Hemocyte-deposited collagen IV around the Malpighian tubule promotes reception of the Dpp signal by tubule cells. (B) Ovarian germ line stem cell (GSC) maintenance. GSCs are maintained within the stem cell niche via interaction with cap cells. Collagen IV in the BM and the HSPG Dally concentrate cap cell-derived Dpp to promote signal reception by GSCs but not differentiating daughter cells. (C) Intestinal stem cell (ISC) maintenance. ISCs exhibit basally positioned cell bodies that adhere to the BM. Dpp is concentrated by collagen IV within the BM to promote a high level of signal reception by ISCs but not the more apically localized, differentiating enteroblasts. (See color plate)

and repair in adults. The defining feature of stem cells is that they divide asymmetrically to produce two distinct cells: one stem cell to replace the mother and one cell that will differentiate. In *Drosophila*, the capacity to identify and observe stem cells in vivo under different genetic conditions has revealed distinct roles for the BM in several tissues, three of which are discussed here.

In *Drosophila*, the female germ line stem cells (GSCs) sit at the anterior end of the ovary in a specialized signaling environment deemed the niche (Losick, Morris, Fox, & Spradling, 2011; Xie & Spradling, 1998). The

primary cells that make up the niche, the cap cells, bind GSCs through cadherin-based adhesions (Song, Zhu, Doan, & Xie, 2002). When GSCs divide, one daughter remains attached to the cap cells, while the other is expelled from this environment and, lacking the niche signals, differentiates. One key niche signal, secreted by the cap cells, is Dpp (López-Onieva, Fernández-Miñán, & González-Reyes, 2008; Wang et al., 2008). Collagen IV in the BM surrounding the niche binds Dpp and restricts its diffusion (Figure 5(B)); in the absence of collagen IV, the Dpp signaling field expands to reach GSC daughter cells outside of the niche, which prevents them from differentiating and leads to an overabundance of stem cells (Wang et al., 2008). The HSPG Dally is also enriched around the niche and appears to concentrate Dpp and promote its reception by GSCs (Guo & Wang, 2009; Hayashi, Kobayashi, & Nakato, 2009).

A mechanism similar to that seen with the GSCs also regulates adult intestinal stem cells (ISCs). While the ISC niche is not clearly understood, these cells are scattered throughout the intestinal epithelium and adhere directly to the BM (Micchelli & Perrimon, 2006; Ohlstein & Spradling, 2006). The BM appears to play a role in defining the niche, as it was recently discovered that Dpp maintains stem cell identity and that this protein is confined to the basal surface by collagen IV. This localization allows higher signal reception by the ISCs than by the differentiating ISC daughter cells, the enteroblasts, whose cell bodies are located more apically within the epithelium (Tian & Jiang, 2014) (Figure 5(C)). Dpp has also been proposed to regulate ISC proliferation, although the role of the BM in this case has not been elucidated (Guo, Driver, & Ohlstein, 2013; Li, Zhang, Han, Shi, & Lin, 2013). The BM may therefore play a common role in regulating signals within stem cell niches.

Asymmetric division of *Drosophila* neuroblasts, the stem cells that give rise to the nervous system, occurs via an intrinsic mechanism that can, at least in part, occur independently of the cells' external environment (Broadus & Doe, 1997). This is achieved by uneven segregation of fate-determining factors into one of the two daughter cells (Knoblich, 2008). While the importance of the environment in neuroblast fate is not well understood, the BM does play a role in determining when and to what extent neuroblasts divide. *Drosophila* perlecan was first identified as a factor that promoted activation of quiescent neuroblasts in larvae by counteracting the antiproliferative activity of the secreted glycoprotein Anachronism—hence its name, *terribly reduced optic lobes* (Datta, 1995; Voigt, Pflanz, Schäfer, & Jäckle, 2002). Perlecan was also found to promote neuroblast proliferation by binding to and

enhancing the signaling of Hedgehog and the FGF homolog Branchless (Park et al., 2003). The BM, therefore, appears to modulate several signaling pathways to ensure proper stem cell function in many contexts.

5. CONCLUSION

Developing tissues require precise control of their size, shape, activity, and signaling environment to robustly create the adult organism. The BM has been found to integrally regulate all of these processes, thereby contributing an important external input to guide coordinated cellular activity. While studies of the BM to this point have been informative, they have also revealed how much we still have to learn. Regarding the polarized deposition and assembly of BM proteins, it will be crucial to continue to identify new factors that regulate this process. Proteins that are already known to function with Rab10 in *C. elegans* and mammalian cells are excellent candidates in this regard, and forward genetic screening strategies in *Drosophila* are likely to identify even more. In terms of understanding the functional properties of BMs once they are built, it will be important to better map their structural diversity. Although evidence from *Drosophila* and vertebrates suggests that BM architecture can vary from one tissue to the next, the precise structures of individual matrices are still largely unexplored. It will therefore be interesting to examine the composition (both of core and accessory proteins) and architecture of a diverse set of BMs, establish the levels of heterogeneity between them, and connect the physical characteristics of individual BMs to their functional properties. Expanding the library of fluorescently tagged BM proteins using modern genome editing techniques will greatly assist this endeavor. Additionally, recent evidence suggests that a BM may be more than the sum of its parts—that individual elements modify and collaborate with other proteins in the BM, on the cell surface, and within the local environment to create a complex interactive network. Understanding the nature of such interactions, in synergy with an enhanced understanding of BM structure, will further reveal the dynamic inputs of these matrices to cellular activity.

ACKNOWLEDGMENTS

We are grateful to Joel Collier and to members of the Horne-Badovinac Lab for helpful discussions and critical comments on the manuscript. This work was supported by NIH T32 HD055164 and a National Science Foundation Graduate Research Fellowship to A.J.I., and grants from the National Institutes of Health (R01-GM094276 and the American Cancer Society to S.H-B.

REFERENCES

Andrew, D. J., & Ewald, A. J. (2010). Morphogenesis of epithelial tubes: insights into tube formation, elongation, and elaboration. *Developmental Biology, 341*(1), 34–55.

Anitei, M., & Hoflack, B. (2011). Exit from the trans-Golgi network: from molecules to mechanisms. *Current Opinion in Cell Biology, 23*(4), 443–451.

Babbey, C., Ahktar, N., Wang, E., Chen, C., Grant, B., & Dunn, K. (2006). Rab10 regulates membrane transport through early endosomes of polarized Madin-Darby canine. *Molecular Biology of the Cell, 17*, 3156–3175.

Balla, T. (2013). Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiological Reviews, 93*(3), 1019–1137.

Barr, F. A. (2013). Rab GTPases and membrane identity: causal or inconsequential? *The Journal of Cell Biology, 202*(2), 191–199.

Bateman, J., Reddy, R. S., Saito, H., & Van Vactor, D. (2001). The receptor tyrosine phosphatase Dlar and integrins organize actin filaments in the *Drosophila* follicular epithelium. *Current Biology, 11*(17), 1317–1327.

Bernfield, M., Banerjee, S. D., Koda, J. E., & Rapraeger, A. C. (1984). Remodelling of the basement membrane: morphogenesis and maturation. In *Basement membranes and cell movement* (pp. 179–196).

Binari, R., Staveley, B., Johnson, W., Godavarti, R., Sasisekharan, R., & Manoukian, A. (1997). Genetic evidence that heparin-like glycosaminoglycans are involved in wingless signaling. *Development, 124*, 2623–2632.

Blümer, J., Rey, J., Dehmelt, L., Mazel, T., Wu, Y.-W., Bastiaens, P., et al. (2013). RabGEFs are a major determinant for specific Rab membrane targeting. *The Journal of Cell Biology, 200*(3), 287–300.

Boll, W., Partin, J. S., Katz, A. I., Caplan, M. J., & Jamieson, J. D. (1991). Distinct pathways for basolateral targeting of membrane and secretory proteins in polarized epithelial cells. *Proceedings of the National Academy of Sciences of the United States of America, 88*(19), 8592–8596.

Bramham, C. R., & Wells, D. G. (2007). Dendritic mRNA: transport, translation and function. *Nature Reviews Neuroscience, 8*(10), 776–789.

Broadus, J., & Doe, C. Q. (1997). Extrinsic cues, intrinsic cues and microfilaments regulate asymmetric protein localization in *Drosophila* neuroblasts. *Current Biology, 7*(11), 827–835.

Brose, K., Bland, K., Wang, K., Arnott, D., Henzel, W., Goodman, C., et al. (1999). Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. *Cell, 96*, 795–806.

Bunt, S., Hooley, C., Hu, N., Scahill, C., Weavers, H., & Skaer, H. (2010). Hemocyte-secreted type IV collagen enhances BMP signaling to guide renal tubule morphogenesis in *Drosophila*. *Developmental Cell, 19*(2), 296–306.

Buszczak, M., Paterno, S., Lighthouse, D., Bachman, J., Planck, J., Owen, S., et al. (2007). The Carnegie protein trap library: a versatile tool for *Drosophila* developmental studies. *Genetics, 175*, 1505–1531.

Cao, Z., Li, C., Higginbotham, J. N., Franklin, J. L., Tabb, D. L., Graves-Deal, R., et al. (2008). Use of fluorescence-activated vesicle sorting for isolation of Naked2-associated, basolaterally targeted exocytic vesicles for proteomics analysis. *Molecular & Cellular Proteomics, 7*(9), 1651–1667.

Caplan, M., Stow, J., & Newman, A. (1987). Dependence on pH of polarized sorting of secreted proteins. *Nature, 329*, 632–635.

Carey, D. (1997). Syndecans: multifunctional cell-surface co-receptors. *Biochemical Journal, 327*, 1–16.

Cetera, M., Ramirez-San Juan, G. R., Oakes, P. W., Lewellyn, L., Fairchild, M. J., Tanentzapf, G., et al. (2014). Epithelial rotation promotes the global alignment of

contractile actin bundles during *Drosophila* egg chamber elongation. *Nature Communications, 5*, 5511.
Charras, G., & Sahai, E. (2014). Physical influences of the extracellular environment on cell migration. *Nature Reviews Molecular Cell Biology, 15*(12), 813–824.
Chen, C., Schweinsberg, P., Vashist, S., Mareiniss, D., Lambie, E., & Grant, B. (2006). RAB-10 is required for endocytic recycling in the *Caenorhabditis elegans* intestine. *Molecular Biology of the Cell, 17*, 1286–1297.
Cho, J. Y., Chak, K., Andreone, B. J., Wooley, J. R., & Kolodkin, A. L. (2012). The extracellular matrix proteoglycan perlecan facilitates transmembrane semaphorin-mediated repulsive guidance. *Genes & Development, 26*(19), 2222–2235.
Cohen, D., Müsch, A., & Rodriguez-Boulan, E. (2001). Selective control of basolateral membrane protein polarity by cdc42. *Traffic, 2*(8), 556–564.
Conder, R., Yu, H., Zahedi, B., & Harden, N. (2007). The serine/threonine kinase dPak is required for polarized assembly of F-actin bundles and apical-basal polarity in the *Drosophila* follicular epithelium. *Developmental Biology, 305*(2), 470–482.
Cummings, M., & King, R. (1969). The cytology of the vitellogenic stages of oogenesis in *Drosophila melanogaster* I. General staging characteristics. *Journal of Morphology, 128*, 427–442.
Daley, W. P., Peters, S. B., & Larsen, M. (2008). Extracellular matrix dynamics in development and regenerative medicine. *Journal of Cell Science, 121*(Pt 3), 255–264.
Datta, M. W., Hernandez, A. M., Schlicht, M. J., Kahler, A. J., DeGueme, A. M., Dhir, R., et al. (2006). Perlecan, a candidate gene for the CAPB locus, regulates prostate cancer cell growth via the Sonic Hedgehog pathway. *Molecular Cancer, 5*(9).
Datta, S. (1995). Control of proliferation activation in quiescent neuroblasts of the *Drosophila* central nervous system. *Development, 121*, 1173–1182.
De Almeida, J. B., & Stow, J. L. (1991). Disruption of microtubules alters polarity of basement membrane proteoglycan secretion in epithelial cells. *The American Journal of Physiology, 261*, C691–C700.
De Wit, J., De Winter, F., Klooster, J., & Verhaagen, J. (2005). Semaphorin 3A displays a punctate distribution on the surface of neuronal cells and interacts with proteoglycans in the extracellular matrix. *Molecular and Cellular Neurosciences, 29*(1), 40–55.
Delon, I., & Brown, N. H. (2009). The integrin adhesion complex changes its composition and function during morphogenesis of an epithelium. *Journal of Cell Science, 122*, 4363–4374.
Denef, N., Chen, Y., Weeks, S. D., Barcelo, G., & Schüpbach, T. (2008). Crag regulates epithelial architecture and polarized deposition of basement membrane proteins in *Drosophila*. *Developmental Cell, 14*(3), 354–364.
Denholm, B. (2013). Shaping up for action: the path to physiological maturation in the renal tubules of *Drosophila*. *Organogenesis, 9*(1), 40–54.
Devergne, O., Tsung, K., Barcelo, G., & Schupbach, T. (2014). Polarized deposition of basement membrane proteins depends on Phosphatidylinositol synthase and the levels of Phosphatidylinositol 4,5-bisphosphate. *Proceedings of the National Academy of Sciences of the United States of America, 111*(21), 7689–7694.
Dickson, B. J., & Gilestro, G. F. (2006). Regulation of commissural axon pathfinding by slit and its Robo receptors. *Annual Review of Cell and Developmental Biology, 22*, 651–675.
Diehl, K. A., Foley, J. D., Nealey, P. F., & Murphy, C. J. (2005). Nanoscale topography modulates corneal epithelial cell migration. *Journal of Biomedical Materials Research. Part A, 75*(3), 603–611.
Domínguez-Giménez, P., Brown, N. H., & Martín-Bermudo, M. D. (2007). Integrin-ECM interactions regulate the changes in cell shape driving the morphogenesis of the *Drosophila* wing epithelium. *Journal of Cell Science, 120*, 1061–1071.

Eastburn, D. J., & Mostov, K. E. (2010). Laying the foundation for epithelia: insights into polarized basement membrane deposition. *EMBO Reports, 11*(5), 329–330.
Fessler, L., Nelson, R., & Fessler, J. (1994). *Drosophila* extracellular matrix. *Methods in Enzymology, 245*, 271–294.
Fessler, L. I., Condic, M. L., Nelson, R. E., Fessler, J. H., & Fristrom, J. W. (1993). Site-specific cleavage of basement membrane collagen IV during *Drosophila* metamorphosis. *Development, 117*(3), 1061–1069.
Folkman, J., Klagsbrun, M., Sasse, J., Wadzinski, M., Ingber, D., & Vlodavsky, I. (1988). A heparin-binding angiogenic protein – basic fibroblast growth factor – is stored within basement membrane. *The American Journal of Pathology, 130*(2), 393–400.
Fölsch, H., Mattila, P. E., & Weisz, O. A. (2009). Taking the scenic route: biosynthetic traffic to the plasma membrane in polarized epithelial cells. *Traffic, 10*(8), 972–981.
Frydman, H. M., & Spradling, A. C. (2001). The receptor-like tyrosine phosphatase lar is required for epithelial planar polarity and for axis determination within *Drosophila* ovarian follicles. *Development, 128*(16), 3209–3220.
García-Alonso, L., Fetter, R., & Goodman, C. (1996). Genetic analysis of Laminin A in *Drosophila*: extracellular matrix containing laminin A is required for ocellar axon pathfinding. *Development, 122*, 2611–2621.
Gonzalez, A., & Rodriguez-Boulan, E. (2009). Clathrin and AP1B: key roles in basolateral trafficking through trans-endosomal routes. *FEBS Letters, 583*(23), 3784–3795.
Grindstaff, K. K., Yeaman, C., Anandasabapathy, N., Hsu, S.-C., Rodriguez-Boulan, E., Scheller, R. H., et al. (1998). Sec6/8 complex is recruited to cell–cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell, 93*(5), 731–740.
Gu, Z., Liu, F., Tonkova, E. A., Lee, S. Y., Tschumperlin, D. J., & Brenner, M. B. (2014). Soft matrix is a natural stimulator for cellular invasiveness. *Molecular Biology of the Cell, 25*(4), 457–469.
Guha, A., Lin, L., & Kornberg, T. B. (2009). Regulation of *Drosophila* matrix metalloprotease Mmp2 is essential for wing imaginal disc:trachea association and air sac tubulogenesis. *Developmental Biology, 335*(2), 317–326.
Gunn, P. A., Gliddon, B. L., Londrigan, S. L., Lew, A. M., van Driel, I. R., & Gleeson, P. A. (2011). The Golgi apparatus in the endomembrane-rich gastric parietal cells exist as functional stable mini-stacks dispersed throughout the cytoplasm. *Biology of the Cell, 103*(12), 559–572.
Guo, Z., Driver, I., & Ohlstein, B. (2013). Injury-induced BMP signaling negatively regulates *Drosophila* midgut homeostasis. *The Journal of Cell Biology, 201*(6), 945–961.
Guo, Z., & Wang, Z. (2009). The glypican Dally is required in the niche for the maintenance of germline stem cells and short-range BMP signaling in the *Drosophila* ovary. *Development, 136*(21), 3627–3635.
Gutzeit, H. (1990). The microfilament pattern in the somatic follicle cells of mid-vitellogenic ovarian follicles of *Drosophila*. *European Journal of Cell Biology, 53*(2), 349–356.
Gutzeit, H., Eberhardt, W., & Gratwohl, E. (1991). Laminin and basement membrane-associated microfilaments in wild-type and mutant *Drosophila* ovarian follicles. *Journal of Cell Science, 100*, 781–788.
Haigo, S. L., & Bilder, D. (2011). Global tissue revolutions in a morphogenetic movement controlling elongation. *Science, 331*(6020), 1071–1074.
Hanus, C., & Ehlers, M. D. (2008). Secretory outposts for the local processing of membrane cargo in neuronal dendrites. *Traffic, 9*, 1437–1445.
Hayashi, Y., Kobayashi, S., & Nakato, H. (2009). *Drosophila* glypicans regulate the germline stem cell niche. *The Journal of Cell Biology, 187*(4), 473–480.
He, L., Wang, X., Tang, H. L., & Montell, D. J. (2010). Tissue elongation requires oscillating contractions of a basal actomyosin network. *Nature Cell Biology, 12*(12), 1133–1142.

He, Z., Wang, K. C., Koprivica, V., Ming, G., & Song, H.-J. (2002). Knowing how to navigate: mechanisms of semaphorin signaling in the nervous system. *Science STKE*, re1.

Horne-Badovinac, S. (2014). The *Drosophila* egg chamber-a new spin on how tissues elongate. *Integrative and Comparative Biology, 54*(4), 667–676.

Horne-Badovinac, S., Hill, J., Gerlach, G., Menegas, W., & Bilder, D. (2012). A screen for round egg mutants in *Drosophila* identifies tricornered, furry, and misshapen as regulators of egg chamber elongation. *G3, 2*(3), 371–378.

Horton, A. C., Rácz, B., Monson, E. E., Lin, A. L., Weinberg, R. J., & Ehlers, M. D. (2005). Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis. *Neuron, 48*(5), 757–771.

Hussain, S.-A., Piper, M., Fukuhara, N., Strochlic, L., Cho, G., Howitt, J. A., et al. (2006). A molecular mechanism for the heparan sulfate dependence of slit-robo signaling. *The Journal of Biological Chemistry, 281*(51), 39693–39698.

Hutagalung, A. H., & Novick, P. J. (2011). Role of Rab GTPases in membrane traffic and cell physiology. *Physiological Reviews, 91*, 119–149.

Hynes, R. O., & Naba, A. (2012). Overview of the matrisome–an inventory of extracellular matrix constituents and functions. *Cold Spring Harbor Perspectives in Biology, 4*, a004903.

Johnson, K., Ghose, A., Epstein, E., Lincecum, J., O'Connor, M., & Van Vactor, D. (2004). Axonal heparan sulfate proteoglycans regulate the distribution and efficiency of the repellent slit during midline axon guidance. *Current Biology, 14*, 499–504.

Khoshnoodi, J., Pedchenko, V., & Hudson, B. G. (2008). Mammalian collagen IV. *Microscopy Research and Technique, 71*(5), 357–370.

Kim, D.-H., Provenzano, P. P., Smith, C. L., & Levchenko, A. (2012). Matrix nanotopography as a regulator of cell function. *The Journal of Cell Biology, 197*(3), 351–360.

Kim, S. N., Jeibmann, A., Halama, K., Witte, H. T., Wälte, M., Matzat, T., et al. (2014). ECM stiffness regulates glial migration in *Drosophila* and mammalian glioma models. *Development, 141*(16), 3233–3242.

Klagsbrun, M. (1990). The affinity of fibroblast growth factors (FGFs) for heparin; FGF-heparan sulfate interactions in cells and extracellular matrix. *Current Opinion in Cell Biology, 2*, 857–863.

Knoblich, J. A. (2008). Mechanisms of asymmetric stem cell division. *Cell, 132*(4), 583–597.

Kondylis, V., Pizette, S., & Rabouille, C. (2009). The early secretory pathway in development: a tale of proteins and mRNAs. *Seminars in Cell & Developmental Biology, 20*(7), 817–827.

Kondylis, V., & Rabouille, C. (2009). The Golgi apparatus: lessons from *Drosophila*. *FEBS Letters, 583*(23), 3827–3838.

Koride, S., He, L., Xiong, L., Lan, G., Montell, D., & Sun, S. (2014). Mechanochemical regulation of oscillatory follicle cell dynamics in the developing *Drosophila* egg chamber. *Molecular Biology of the Cell, 25*(22), 3709–3716.

Kraut, R., Menon, K., & Zinn, K. (2001). A gain-of-function screen for genes controlling motor axon guidance and synaptogenesis in *Drosophila*. *Current Biology, 11*(6), 417–430.

Kusche-Gullberg, M., Garrison, K., MacKrell, A., Fessler, L., & Fessler, J. (1992). Laminin A chain: expression during *Drosophila* development and genomic sequence. *The EMBO Journal, 11*(12), 4519–4527.

Le Parco, Y., Knibiehler, B., Cecchini, J., & Mirre, C. (1986). Stage and tissue-specific expression of a collagen gene during *Drosophila melanogaster* development. *Experimental Cell Research, 163*, 405–412.

Lerner, D. W., McCoy, D., Isabella, A. J., Mahowald, A. P., Gerlach, G. F., Chaudhry, T. A., et al. (2013). A Rab10-dependent mechanism for polarized basement membrane secretion during organ morphogenesis. *Developmental Cell, 24*(2), 159–168.

Lewellyn, L., Cetera, M., & Horne-Badovinac, S. (2013). Misshapen decreases integrin levels to promote epithelial motility and planar polarity in *Drosophila*. *The Journal of Cell Biology, 200*(6), 721–729.

Li, Z., Zhang, Y., Han, L., Shi, L., & Lin, X. (2013). Trachea-derived dpp controls adult midgut homeostasis in *Drosophila*. *Developmental Cell, 24*(2), 133–143.

Lin, X., Buff, E., Perrimon, N., & Michelson, A. (1999). Heparan sulfate proteoglycans are essential for FGF receptor signaling during *Drosophila* embryonic development. *Development, 126*, 3715–3723.

Lo, C.-M., Wang, H.-B., Dembo, M., & Wang, Y.-L. (2000). Cell movement is guided by the rigidity of the substrate. *Biophysical Journal, 79*(1), 144–152.

López-Onieva, L., Fernández-Miñán, A., & González-Reyes, A. (2008). Jak/Stat signalling in niche support cells regulates dpp transcription to control germline stem cell maintenance in the *Drosophila* ovary. *Development, 135*(3), 533–540.

Losick, V. P., Morris, L. X., Fox, D. T., & Spradling, A. (2011). *Drosophila* stem cell niches: a decade of discovery suggests a unified view of stem cell regulation. *Developmental Cell, 21*(1), 159–171.

Lowenstein, P., Morrison, E., Bain, D., Shering, A., Banding, G., Douglas, P., et al. (1994). Polarized distribution of the trans-Golgi network marker TGN38 during the in vitro development of neocortical neurons: effects of nocodazole and brefeldin A. *European Journal of Neuroscience, 6*, 1453–1465.

Marat, A. L., Dokainish, H., & McPherson, P. S. (2011). DENN domain proteins: regulators of Rab GTPases. *The Journal of Biological Chemistry, 286*(16), 13791–13800.

Martin, D., Zusman, S., Li, X., Williams, E., Khare, N., DaRocha, S., et al. (1999). Wing blister, a new *Drosophila* laminin alpha chain required for cell adhesion and migration during embryonic and imaginal development. *The Journal of Cell Biology, 145*(1), 191–201.

Martin-Belmonte, F., Gassama, A., Datta, A., Yu, W., Rescher, U., Gerke, V., et al. (2007). PTEN-mediated apical segregation of phosphoinositides controls epithelial morphogenesis through Cdc42. *Cell, 128*(2), 383–397.

McCall, A. S., Cummings, C. F., Bhave, G., Vanacore, R., Page-McCaw, A., & Hudson, B. G. (2014). Bromine is an essential trace element for assembly of collagen IV scaffolds in tissue development and architecture. *Cell, 157*(6), 1380–1392.

Micchelli, C. A., & Perrimon, N. (2006). Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. *Nature, 439*, 475–479.

Mirre, C., Cecchini, J., Le Parco, Y., & Knibiehler, B. (1988). De novo expression of a type IV collagen gene in *Drosophila* embryos is restricted to mesodermal derivatives and occurs at germ band shortening. *Development, 102*, 369–376.

Morin, X., Daneman, R., Zavortink, M., & Chia, W. (2001). A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America, 98*(26), 15050–15055.

Myllyharju, J., & Kivirikko, K. I. (2004). Collagens, modifying enzymes and their mutations in humans, flies and worms. *Trends in Genetics, 20*(1), 33–43.

Natori, Y., O'Meara, Y., Manning, E., Minto, A., Levine, J., Weise, W., et al. (1992). Production and polarized secretion of basement membrane components by glomerular epithelial cells. *The American Journal of Physiology, 262*, 131–137.

Nüsslein-Volhard, C., Wieschaus, E., & Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* I. Zygotic loci on the second chromosome. *Roux's Archives of Developmental Biology, 193*, 267–282.

Ohlstein, B., & Spradling, A. (2006). The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. *Nature, 439*, 470–474.

Ozbek, S., Balasubramanian, P. G., Chiquet-Ehrismann, R., Tucker, R. P., & Adams, J. C. (2010). The evolution of extracellular matrix. *Molecular Biology of the Cell, 21*(24), 4300–4305.

Paralkar, V. M., Vukicevic, S., & Reddi, A. H. (1991). Transforming growth factor β type 1 binds to collagen IV of basement membrane matrix: implications for development. *Developmental Biology, 143*(2), 303–308.

Park, Y., Rangel, C., Reynolds, M. M., Caldwell, M. C., Johns, M., Nayak, M., et al. (2003). *Drosophila* perlecan modulates FGF and hedgehog signals to activate neural stem cell division. *Developmental Biology, 253*(2), 247–257.

Pastor-Pareja, J. C., & Xu, T. (2011). Shaping cells and organs in *Drosophila* by opposing roles of fat body-secreted collagen IV and perlecan. *Developmental Cell, 21*, 245–256.

Perrimon, N., & Bernfield, M. (2000). Specificities of heparan sulphate proteoglycans in developmental processes. *Nature, 404*, 725–728.

Pierce, J. P., Mayer, T., & McCarthy, J. B. (2001). Evidence for a satellite secretory pathway in neuronal dendritic spines. *Current Biology, 11*(5), 351–355.

Provenzano, P. P., Eliceiri, K. W., Campbell, J. M., Inman, D. R., White, J. G., & Keely, P. J. (2006). Collagen reorganization at the tumor-stromal interface facilitates local invasion. *BMC Medicine, 4*(1), 38.

Provenzano, P. P., Inman, D. R., Eliceiri, K. W., Trier, S. M., & Keely, P. J. (2008). Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization. *Biophysical Journal, 95*(11), 5374–5384.

Qin, J., Liang, J., & Ding, M. (2014). Perlecan antagonizes collagen IV and ADAMTS9/GON-1 in restricting the growth of presynaptic boutons. *The Journal of Neuroscience, 34*(31), 10311–10324.

Ramírez, O. A., & Couve, A. (2011). The endoplasmic reticulum and protein trafficking in dendrites and axons. *Trends in Cell Biology, 21*(4), 219–227.

Reinhart-King, C. A., Dembo, M., & Hammer, D. A. (2008). Cell-cell mechanical communication through compliant substrates. *Biophysical Journal, 95*(12), 6044–6051.

Roca-Cusachs, P., Sunyer, R., & Trepat, X. (2013). Mechanical guidance of cell migration: lessons from chemotaxis. *Current Opinion in Cell Biology, 25*(5), 543–549.

Rodriguez-Boulan, E., Kreitzer, G., & Müsch, A. (2005). Organization of vesicular trafficking in epithelia. *Nature Reviews Molecular Cell Biology, 6*, 233–247.

Rubin, J., Choi, Y., & Segal, R. (2002). Cerebellar proteoglycans regulate sonic hedgehog responses during development. *Development, 129*, 2223–2232.

Saito, K., Chen, M., Bard, F., Chen, S., Zhou, H., Woodley, D., et al. (2009). TANGO1 facilitates cargo loading at endoplasmic reticulum exit sites. *Cell, 136*(5), 891–902.

Santiago-Tirado, F. H., & Bretscher, A. (2011). Membrane-trafficking sorting hubs: cooperation between PI4P and small GTPases at the trans-Golgi network. *Trends in Cell Biology, 21*(9), 515–525.

Sawala, A., Sutcliffe, C., & Ashe, H. L. (2012). Multistep molecular mechanism for bone morphogenetic protein extracellular transport in the *Drosophila* embryo. *Proceedings of the National Academy of Sciences of the United States of America, 109*(28), 11222–11227.

Schneider, M., Khalil, A. A., Poulton, J., Castillejo-Lopez, C., Egger-Adam, D., Wodarz, A., et al. (2006). Perlecan and Dystroglycan act at the basal side of the *Drosophila* follicular epithelium to maintain epithelial organization. *Development, 133*, 3805–3815.

Seeger, M., Tear, G., Ferres-Marco, D., & Goodman, C. (1993). Mutations affecting growth cone guidance in *Drosophila*: genes necessary for guidance toward or away from the midline. *Neuron, 10*, 409–426.

Shahab, J., Baratta, C., Scuric, B., Godt, D., Venken, K. J. T., & Ringuette, M. R. (2015). Loss of SPARC dysregulates basal lamina assembly to disrupt larval fat body homeostasis in Drosophila melanogaster. *Developmental Dynamics, 244*(4), 540–552.

Shi, A., Chen, C. C., Banerjee, R., Glodowski, D., Audhya, A., Rongo, C., et al. (2010). EHBP-1 functions with RAB-10 during endocytic recycling in *Caenorhabditis elegans*. *Molecular Biology of the Cell, 21*, 2930–2943.

Smart, A. D., Course, M. M., Rawson, J., Selleck, S., Van Vactor, D., & Johnson, K. G. (2011). Heparan sulfate proteoglycan specificity during axon pathway formation in the *Drosophila* embryo. *Developmental Neurobiology, 71*(7), 608–618.

Song, X., Zhu, C., Doan, C., & Xie, T. (2002). Germline stem cells anchored by adherens junctions in the *Drosophila* ovary niches. *Science, 296*, 1855–1857.

Sorrosal, G., Pérez, L., Herranz, H., & Milán, M. (2010). Scarface, a secreted serine protease-like protein, regulates polarized localization of laminin A at the basement membrane of the *Drosophila* embryo. *EMBO Reports, 11*(5), 3–9.

Srivastava, A., Pastor-Pareja, J. C., Igaki, T., Pagliarini, R., & Xu, T. (2007). Basement membrane remodeling is essential for *Drosophila* disc eversion and tumor invasion. *Proceedings of the National Academy of Sciences of the United States of America, 104*(8), 2721–2726.

Steigemann, P., Molitor, A., Fellert, S., Jäckle, H., & Vorbrüggen, G. (2004). Heparan sulfate proteoglycan syndecan promotes axonal and myotube guidance by slit/robo signaling. *Current Biology, 14*(3), 225–230.

Stevens, A., & Jacobs, J. (2002). Integrins regulate responsiveness to slit repellent signals. *The Journal of Neuroscience, 22*(11), 4448–4455.

Stoops, E. H., & Caplan, M. J. (2014). Trafficking to the apical and basolateral membranes in polarized epithelial cells. *Journal of the American Society of Nephrology, 25*(7), 1375–1386.

Takagi, Y., Nomizu, M., Gullberg, D., MacKrell, A. J., Keene, D. R., Yamada, Y., et al. (1996). Conserved neuron promoting activity in *Drosophila* and vertebrate laminin alpha1. *Journal of Biological Chemistry, 271*(30), 18074–18081.

Tan, J., & Saltzman, W. M. (2002). Topographical control of human neutrophil motility on micropatterned materials with various surface chemistry. *Biomaterials, 23*(15), 3215–3225.

The, I., Bellaiche, Y., & Perrimon, N. (1999). Hedgehog movement is regulated through tout velu – dependent synthesis of a heparan sulfate proteoglycan. *Molecular Cell, 4*, 633–639.

Tian, A., & Jiang, J. (2014). Intestinal epithelium-derived BMP controls stem cell self-renewal in *Drosophila* adult midgut. *eLife, 3*, e01857.

Umulis, D. M., Shimmi, O., O'Connor, M. B., & Othmer, H. G. (2010). Organism-scale modeling of early *Drosophila* patterning via bone morphogenetic proteins. *Developmental Cell, 18*(2), 260–274.

Varner, V. D., & Nelson, C. M. (2014). Cellular and physical mechanisms of branching morphogenesis. *Development, 141*, 2750–2759.

Venditti, R., Scanu, T., Santoro, M., Di Tullio, G., Spaar, A., Gaibisso, R., et al. (2012). Sedlin controls the ER export of procollagen by regulating the Sar1 cycle. *Science, 337*, 1668–1672.

Vertel, B., Velasco, A., LaFrance, S., Walters, L., & Kaczman-Daniel, K. (1989). Precursors of chondroitin sulfate proteoglycan are segregated within a subcompartment of the chondrocyte endoplasmic reticulum. *The Journal of Cell Biology, 109*, 1827–1836.

Viktorinová, I., & Dahmann, C. (2013). Microtubule polarity predicts direction of egg chamber rotation in *Drosophila*. *Current Biology, 23*(15), 1472–1477.

Viktorinová, I., König, T., Schlichting, K., & Dahmann, C. (2009). The cadherin Fat2 is required for planar cell polarity in the *Drosophila* ovary. *Development, 136*(24), 4123–4132.

Voigt, A., Pflanz, R., Schäfer, U., & Jäckle, H. (2002). Perlecan participates in proliferation activation of quiescent *Drosophila* neuroblasts. *Developmental Dynamics, 224*, 403–412.

Wang, X., Harris, R. E., Bayston, L. J., & Ashe, H. L. (2008). Type IV collagens regulate BMP signalling in *Drosophila*. *Nature, 455*(7209), 72–77.

Wilson, D. G., Phamluong, K., Li, L., Sun, M., Cao, T. C., Liu, P. S., et al. (2011). Global defects in collagen secretion in a Mia3/TANGO1 knockout mouse. *The Journal of Cell Biology, 193*(5), 935–951.

Xie, T., & Spradling, A. C. (1998). decapentaplegic is essential for the maintenance and division of germline stem cells in the *Drosophila* ovary. *Cell, 94*(2), 251–260.

Xiong, B., Bayat, V., Jaiswal, M., Zhang, K., Sandoval, H., Charng, W.-L., et al. (2012). Crag is a GEF for Rab11 required for rhodopsin trafficking and maintenance of adult photoreceptor cells. *PLoS Biology, 10*(12), e1001438.

Yasothornsrikul, S., Davis, W. J., Cramer, G., Kimbrell, D. A., & Dearolf, C. R. (1997). viking: identification and characterization of a second type IV collagen in *Drosophila*. *Gene, 198*, 17–25.

Yoshimura, S., Gerondopoulos, A., Linford, A., Rigden, D. J., & Barr, F. A. (2010). Family-wide characterization of the DENN domain Rab GDP-GTP exchange factors. *The Journal of Cell Biology, 191*(2), 367–381.

Yurchenco, P. D. (2011). Basement membranes: cell scaffoldings and signaling platforms. *Cold Spring Harbor Perspectives in Biology, 3*(2), a004911.