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Previews

Tissue Sculpting by Fibrils

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In this issue of *Developmental Cell*, Isabella and Horne-Badovinac (2016) show that Rab10 directs site-specific secretion of basement membrane components, which assemble into fibrils that spool out to elongate the *Drosophila* egg chamber. These findings establish the basement membrane's active role in tissue sculpting.

Basement membranes are thin, dense, sheet-like extracellular matrices that surround most tissues (Yurchenco, 2011). Basement membranes are conventionally viewed as static scaffolds that provide tissue support, provide barrier functions, and harbor growth factors. Recent in vivo studies have shown that this matrix is far more active than originially envisioned. For example, global translocation of basement membrane accompanies vertebrate salivary-gland branching morphogenesis, and basement membrane deposition polarizes entire organs in *C. elegans* and *Drosophila* (Morrissey and Sherwood, 2015). These observations suggest that basement membranes are actively constructed and altered to shape tissues. In this issue of *Developmental Cell*, Isabella and Horne-Badovinac (2016) reveal a fascinating mechanism in which the mode of basement membrane deposition is specifically modified during development to elongate an entire structure, thus providing the clearest example to date that dynamic basement membrane assembly directs tissue morphogenesis.

The *Drosophila* egg chamber is composed of a germ cell cluster surrounded by an epithelial layer of follicle cells (Figure 1A). The follicular epithelium secretes a sheet-like, planar basement membrane, which localizes to its basal surface and envelops the egg chamber. Egg chambers progress through 14 developmental stages before forming the mature egg. Starting around stage 5, the spherical egg chamber begins elongating to form an ellipsoidal shape. Strikingly, during the time of rapid elongation (stages 5–9), the follicle cells collectively migrate within the encasing basement membrane, causing the entire egg chamber to rotate within the surrounding basement membrane. As the chamber rotates, the follicular epithelium deposits basement membrane fibrils that orient perpendicular to the A-P axis. These oriented fibrils are thought to act as a molecular corset that promotes egg chamber elongation along the A-P axis by restricting circumferential expansion along the D-V axis (Figure 1A). However, prior to the work of Isabella and Horne-Badovinac (2016), this hypothesis had not been formally tested, and the mechanisms that govern the formation of these fibrils were unclear.

To determine how these fibrils form, the authors combined elegant live-cell imaging of GFP-tagged type IV collagen (a major basement membrane protein) with the powerful genetic analysis tools available in the fly. The authors observed that at the time of fibril formation, newly synthesized collagen is specifically secreted between follicle cells along their lateral membranes, where it forms into nascent fibrils. Upon contacting the basement membrane, these nascent fibrils adhere and then spool out into the basement membrane, drawn out by the force of the collectively migrating follicle cells (Figure 1A). Laminin and perlecan (two other major basement membrane components) also appear to be secreted laterally and form nascent fibrils with collagen, providing the first evidence that a complete basement membrane matrix can form into fibrils instead of a planar sheet.

The specific timing of pericellular basement membrane secretion suggested that a unique trafficking pathway may direct this polarized secretion. In previous studies, the authors observed that the Rab10 GTPase, a regulator of vesicle trafficking, localizes basally in the follicle epithelial cells (Lerner et al., 2013). Further examination of Rab10 revealed that it also accumulates on the lateral surfaces of follicle cells and that its expression is upregulated at the time of lateral secretion, suggesting that it might be the switch that redirects basement membrane secretion. Consistent with Rab10 directing lateral secretion, overexpression of Rab10 and its effector Ehbp1 increased the quantity of collagen in the pericellular space and the amount and length of fibrils in the basement membrane. Notably, the overall amount of collagen IV in the basement membrane did not change; rather, the fibril fraction increased with a concomitant reduction in the amount of planar basement membrane. Thus, the Rab10-driven secretion pathway appears to compete with another unknown pathway that mediates basal secretion of basement membrane components into the planar matrix (Figure 1A).

One of the most exciting aspects of this work is the authors' use of Rab10 and Ehbp1 overexpression as a means to increase basement fibril formation. This allowed Isabella and Horne-Badovinac (2016) to manipulate fibril levels and determine their role in regulating tissue geometry. A modest increase in the fibril fraction further lengthened the egg chamber, consistent with these fibrils playing a key role in egg chamber elongation. Paradoxically, however, a strong increase in the fibril fraction decreased the length of the egg chamber. This unexpected result was likely due to a global weakening of the planar basement membrane (Figure 1B). Together, this work has revealed a finely tuned molecular switch that alters the mode of basement membrane deposition to modulate the shape of an entire structure.

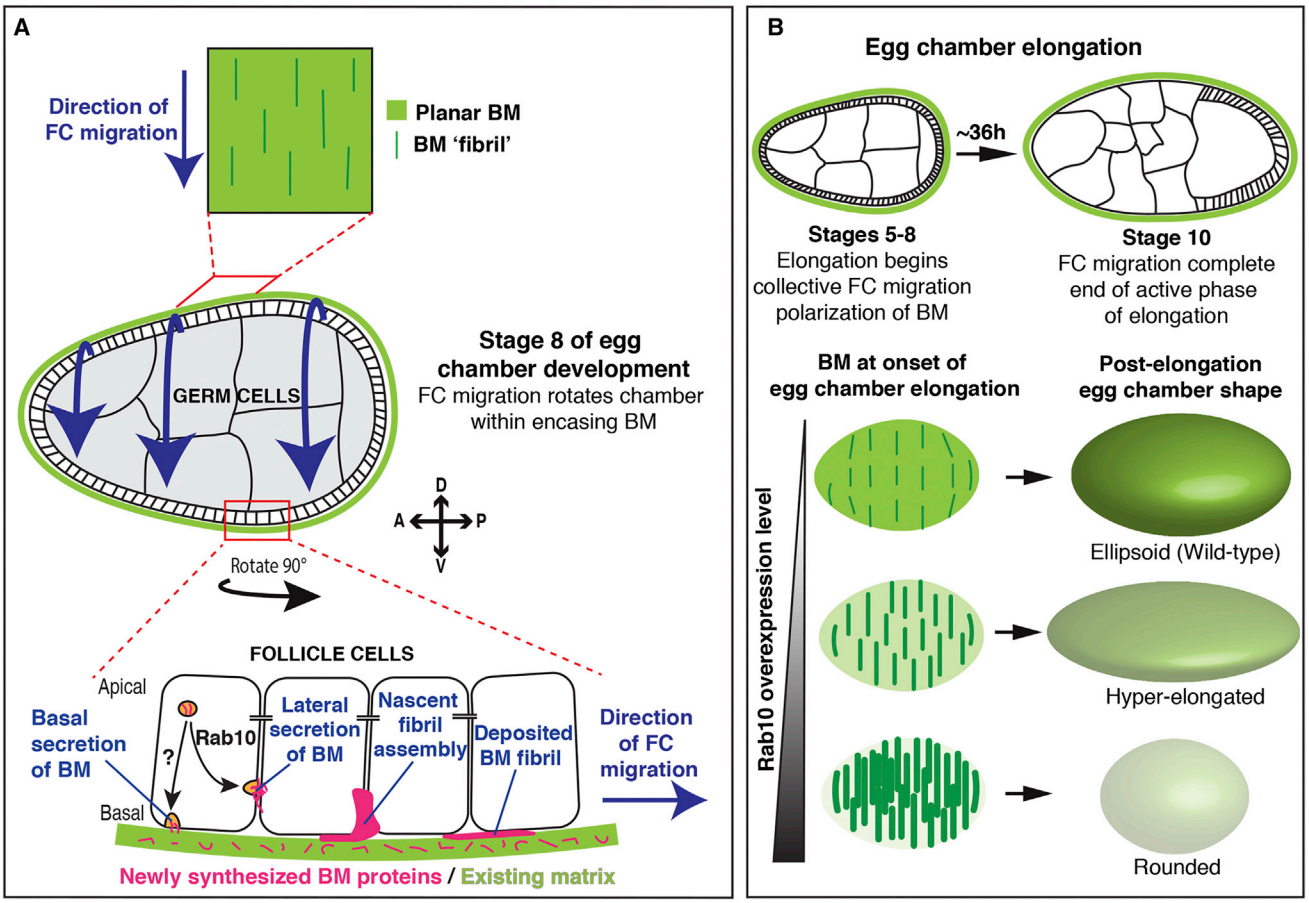

Figure 1. Basement Membrane Fibril Formation and Function in the *Drosophila* Egg Chamber

(A) The elongating *Drosophila* egg chamber (middle) is encased in a basement membrane (BM, green) composed of a planar matrix and perpendicularly oriented fibrils (top). The migrating follicle cells (FCs) synthesize and secrete basement membrane proteins laterally through a Rab10-dependent pathway and basally through an unknown pathway. Laterally deposited basement membrane assembles into nascent fibrils that are then drawn into the planar matrix by the movement of FCs.

(B) The egg chamber elongates significantly between stages 5 and 8 when basement membrane fibrils are deposited in the planar matrix. Increasing Rab10 enhances fibril formation at the expense of planar matrix. A modest fibril increase further elongates the egg chamber, while a more significant fibril increase results in a round egg chamber, likely as a result of the reduction in the stabilizing planar matrix.

These studies have a number of immediate implications. Although tissue rotation might seem like an unusual mode of morphogenesis, it is notable that mammary acini have been observed to rotate while assembling basement membrane in 3D culture (Tanner et al., 2012), suggesting that analogous basement membrane assembly mechanisms may shape other organs. Further, these studies indicate that basement membrane, which has previously been viewed as a flat, sheet-like structure, can form into fibrils outside of this planar matrix and is thus more plastic than originally thought.

More generally, these studies add further weight to the idea that basement membrane, a matrix that arose at the time of animal multicellularity over 580 million years ago, plays dynamic and instructive roles in all aspects of animal cell, tissue, and organismal biology. For example, a laminin proteolytic fragment directs early stem cell differentiation (Horejs et al., 2014), localized deposition of perlecan instructs muscle cell attachment and dendritic branching (Liang et al., 2015), and directed secretion of the basement membrane component hemicentin links neighboring tissues (Morrissey et al., 2014). Further, basement membrane accumulation and misregulation is associated with aging and numerous human diseases, while increased expression of matrix-remodeling factors enhance organismal longevity (Ewald et al., 2015; Halfter et al., 2015). We are only at the beginning of understanding the many functions of basement membrane in animals. We expect that continued study and advances in in vivo imaging will establish that basement membrane fibril formation in the *Drosophila* egg chamber is not a morphogenetic oddity, but rather one of many examples by which dynamic basement membrane assembly and modification regulate cell and tissue function in animals.

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A Pluripotency Platform for Prdm14

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The tripartite network of *Prdm14, Blimp1*, and *AP2γ* is essential for the important process of germ cell specification, but their precise molecular mechanisms of action remain lacking. Tu and colleagues (2016) report in *Nature* that the transcriptional co-repressor CBFA2T2 is an essential interactor protein regulating PRDM14 function, shedding light into the mechanisms directing germline formation and pluripotency.

The question of how a few cells are set aside in the developing embryo in order to generate the germline is of central importance in biology. The founder cells that give rise to the germ cells are called primordial germ cells (PGCs). In mice, PGCs first appear between embryonic day (E) 6.25 and E7.25 as a small subpopulation of around 30–40 cells in the posterior epiblast located proximally to the extraembryonic ectoderm. These cells are unique in their developmental properties. They are set apart from the embryonic program to transmit the genetic and potentially epigenetic information to the new generation. They are highly specialized and unipotent and resist the differentiation program that occurs in the rest of the embryo. They also re-induce the pluripotency gene network and ultimately have the potential to generate a totipotent zygote by undergoing extensive epigenetic changes, including global loss of the repressive DNA methylation and acquisition of active histone marks (reviewed in Surani et al., 2007). Three transcriptional regulators form a tripartite network that is critical for establishment of the PGCs: *Blimp1*, *Ap2γ*/*Tfap2c*, and *Prdm14* (Magnúsdóttir et al., 2013; Ohinata et al., 2005; Yamaji et al., 2008). In a recent issue of *Nature*, Tu and colleagues (2016) sought to investigate further the fundamental question of PGC specification, focusing on PRMD14, which has the earliest specific expression in the germ cell lineage (Yamaji et al., 2008).

Prdm14 is exclusively expressed during three crucial developmental windows characterized by reprogramming events: the 2- to 4-cell stage mouse preimplantation embryo, the inner cell mass of the early blastocyst, and during PGC development (Figure 1A) (Burton et al., 2013; Yamaji et al., 2008). In order to learn more about the function of PRDM14 in PGC development, Tu and colleagues (2016) identified interacting partners of PRDM14 using a proteomics screen in the human germ cell tumor line NCCIT. The top interactor was CBFA2T2, a co-repressor that they subsequently found to be highly correlated with PRDM14 in terms of chromatin binding, gene regulation, and function. They show that the two proteins co-localize broadly across the genome in both NCCIT and mouse embryonic stem cells (mESCs) and, as is the case for PRDM14, many targets of CBFA2T2 are genes involved in pluripotency (*Pou5f1*, *Klf4*, *Dax1*), lineage allocation (*Elf3*, *Cdx1*, *and Pitz2*) and chromatin modification (*Ehmt1*, *Dnmt3a*, *Dnmt3b*, *Dnmt3l*, *Tet2*, *Jarid 2*). However, very limited correlation with the Polycomb repressive complex was identified in contrast to previous reports in mESCs (for an extensive review on Prdm14, see Nakaki and Saitou, 2014). These results suggest that CBFA2T2 might act as a co-factor to PRDM14 in the regulation of PGC development.

In support of this, the authors determine that the ability of PRDM14 and CBFA2T2 to bind to chromatin each depends on the presence of the other factor and that the two factors exist in a large 600 kDa complex, suggesting the possibility for further interactors, which are likely to be functionally important for germ cell biology. Seven conserved amino acids predicted, based on studies of the CBFA2T2 homolog RUNX1T1 (Liu et al., 2006), to be required for