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Mobilizing the Matrix for Organ Morphogenesis

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How a basement membrane continuously surrounds an organ that is growing and changing shape is not yet understood. In this issue of *Developmental Cell*, Matsubayashi et al. and Keeley et al. address this question by showing that individual basement membrane proteins are more dynamic than previously thought.

Basement membranes (BMs) are planar extracellular matrices that surround most organs in our bodies. Built on a foundation of laminin and type IV collagen (collagen IV), these complex protein networks provide attachment points for cells, mechanically shape tissues, and facilitate cell-cell and cell-matrix signaling, among other functions (Jayadev and Sherwood, 2017; Ramos-Lewis and Page-McCaw, 2019). Some studies have suggested that BMs are remarkably stable, with individual BM proteins having half-lives on the order of weeks (Decaris et al., 2014; Trier et al., 1990). This finding raises a conundrum. How can developing organs grow and change shape if they are surrounded by such a stable matrix? In this issue of *Developmental Cell*, complementary studies in *Drosophila* (Matsubayashi et al., 2020) and *C. elegans* (Keeley et al., 2020) reveal that BM proteins are, in fact, highly dynamic and that this dynamism is required for proper organ development. Keeley et al. (2020) also make the surprising discovery that a subset of BM components can actually flow within the plane of the matrix.

Both papers show that collagen IV has a higher turnover rate than previously thought. Matsubayashi et al. (2020) used a *Drosophila* strain in which collagen IV is endogenously tagged with GFP to monitor how this protein is incorporated into BMs over embryonic development. They found that collagen IV incorporation fits a logistic growth curve and combined this observation with mathematical modeling to predict a half-life of 7–10 h for the protein. They then tested this prediction in two ways. First, they expressed a fluorescently tagged collagen IV for a defined period and watched the fluorescence decay across the embryo over time. Second, they expressed a photo-convertible version of collagen IV, converted the fluorophore from green to red on the surface of the ventral nerve cord (VNC), and again watched the fluorescence decay. Both experiments showed a half-life for collagen IV close to the predicted value. Keeley et al. (2020) also used photo-conversion to probe the rate of collagen IV removal from the BM surrounding the gonad in adult *C. elegans* hermaphrodites. Their data similarly suggested a half-life for collagen IV on the order of hours. With this high collagen IV turnover being observed in two organisms, and in both embryos and adults, it may be a general feature of BM biology.

Both papers also explore how and why collagen IV undergoes rapid turnover in developing tissues. Matsubayashi et al. (2020) approached these questions by examining matrix metalloproteases (MMPs), which are known to cleave and remove collagen IV from BMs. Using their joint imaging/modeling approach, they estimated that collagen IV turnover rates are reduced by 20% in *MMP1* mutant embryos. They then asked if this condition disrupts organ morphogenesis. The VNC shortens by half during late embryogenesis through a process that requires an intact BM (Olofsson and Page, 2005; Urbano et al., 2009). In *MMP1* mutants, VNC shortening is slowed, suggesting a link between collagen IV turnover and morphogenesis (Figure 1A). Counterintuitively, collagen IV levels in the VNC BM are also reduced when MMP1 is mutated. This observation led the authors to speculate that BM turnover may be required to incorporate new collagen IV into the existing matrix, similar to how osteoclast-based matrix removal is required to build new, healthy bone.

Keeley et al. (2020) similarly found a connection between collagen IV turnover and organ development, but their starting point was very different. This paper introduces a collection of *C. elegans* strains in which most known BM proteins and their receptors are endogenously tagged with mNeonGreen. Using this resource, they found that a lesser-studied protein called papilin is present at higher levels in the gonadal BM than all other BM proteins combined during the first larval stage. Moreover, depleting papilin reduces the dramatic growth of this organ by half over the course of larval development (Figure 1B). Closer inspection of the gonadal BM in papilin-depleted animals revealed that collagen IV turnover is reduced, its levels are increased, and its distribution is uneven and fibrous. Although papilin lacks a catalytic domain, it has homology to ADAMTS metalloproteinases and is known to regulate ADAMTS activity (Kawano et al., 2009; Kramerova et al., 2000). Keeley et al. (2020) found that levels of the ADAMTS, GON-1, are increased in the gonadal BM when papilin is depleted and that co-depletion of GON-1 with papilin partially restores smooth collagen IV patterning. Altogether, these data suggest a model in which papilin promotes collagen IV removal from the BM by preventing the accumulation of GON-1 and that these interactions produce a sufficiently pliable matrix to allow organ growth.

Although collagen IV has a half-life of hours in the BMs of both flies and worms, Keeley et al. (2020) show that it is still one of the most stable components of the matrix. Armed with the mNeonGreen-tagged BM strain collection described above, these researchers used fluorescence recovery after photo-bleaching (FRAP) to probe the relative stabilities of the BM proteins surrounding the posterior pharynx. They found that the fluorescence

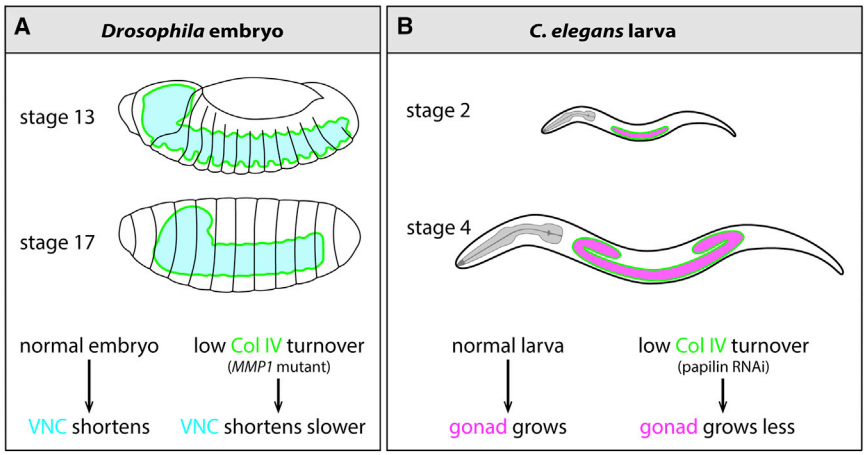

Figure 1. Reduced Collagen IV Turnover Disrupts Organ Development
(A) Reducing collagen IV turnover in the *Drosophila* embryo by mutating MMP1 slows the shortening of the ventral nerve cord (VNC).
(B) Reducing collagen IV turnover in the *C. elegans* larva by depleting papilin inhibits the growth of the gonad.

associated with collagen IV, laminin, collagen XVIII, and papilin was comparatively slow to recover and that recovery occurred uniformly across the bleached region, indicating that the new fluorescent protein was primarily incorporated from the extracellular fluid. By contrast, the fluorescence associated with fibulin, nidogen, agrin, spondin, and peroxidasin-1 recovered faster, and the recovery was biased toward the edge of the bleached region, indicating that the new fluorescent protein was primarily moving into the bleached BM region from adjacent unbleached regions. These observations suggest a new model for BM architecture in which some BM proteins dynamically flow within a more stable scaffold of laminins, collagens, and papilin.

Together, these studies show that individual BM proteins are highly dynamic, which may explain how the network as a whole can grow and change shape in concert with a developing organ. However, these discoveries also raise new questions about how these dynamics are regulated. First, both studies implicate proteases in collagen IV turnover, but the changes in collagen IV levels within the BM when the proteases are manipulated are counterintuitive in both cases. What are the substrates of these proteases, and how do they promote the removal of existing collagen IV and/or incorporation of new collagen IV? Second, Matsubayashi et al. (2020) report that nidogen slows collagen IV turnover, yet nidogen is one of the proteins that flows within the BM network. How does a protein with such fast dynamics stabilize collagen IV? Third, collagen IV is the only BM protein that undergoes covalent crosslinking. Does crosslinking slow collagen IV turnover, and does this change correlate with tissue maturation? No matter what the answers to these questions turn out to be, the idea that the BM is a stable and unchanging structure is being turned over almost as quickly as the matrix itself.

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