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VIEWPOINT

Basement membrane dynamics in living animals: Insights and pitfalls

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Recent studies with fluorophore-tagged basement membrane (BM) components have led to remarkable discoveries about BMs but also inconsistent interpretations. Here, we review types of BM dynamics, discuss how we conduct and interpret fluorophore-tagged BM studies, and highlight experimental conditions that are important to consider.

Introduction

BM is a thin, dense extracellular matrix (ECM) that underlies tissues. During development, BMs form and diversify to regulate numerous cell and tissue functions, such as tissue shaping, barrier formation, and cell differentiation (Jayadev, 2022). BMs are constructed from two planar laminin and type IV collagen networks that anchor to cell-associated receptors and interconnect via cross-bridging proteins, such as nidogen and perlecan. BMs also harbor non-structural matricellular proteins, growth factors, and proteases. Together, over 200 different proteins localize to vertebrate BMs, and variants in over 100 BM genes are associated with human phenotypes (Jayadev, 2022). Thus, understanding how BMs form and function has crucial basic and clinical significance.

A fundamental, yet underappreciated, element of BM construction and function is its dynamics—component assembly and disassembly during formation, growth, and homeostasis, along with the plasticity of the polymerized network. However, historically, BMs have been viewed as static, rigid structures that passively support tissues and anchor signaling molecules that influence cellular functions (Diaz-de-la-Loza and Stramer, 2024). Analysis of BM dynamics has been limited by indirect and challenging approaches, such as pulse-chase experiments with radioactive amino acids and silver granules that accumulate in BMs, and injection of BM component-specific antibodies in living animals (Diaz-de-la-Loza and Stramer, 2024; Price and Spiro, 1977). These early studies hinted that BMs might have dynamic properties, especially during development. Yet, the limitations and complexities of these experimental approaches have precluded our understanding of the breadth of BM dynamics and regulatory mechanisms controlling BM assembly, disassembly, and plasticity.

BM fluorescent tagging and dynamics during development

Led by studies in *Drosophila* and *Caenorhabditis elegans*, and more recent findings in zebrafish and mice, genetically encoded fluorophore-tagged BM components are revealing fascinating insights into BMs. Fluorescent recovery after photobleaching (FRAP) and photoconvertible fluorophore experiments have revealed that BM components have dynamic, diverse, and tissue-specific turnover (assembly/disassembly rates) within BM during development. Structural components, such laminin and type IV collagen have the longest documented stability, with half-lives ranging from ∼1 to 10 h (Keeley, 2020; Matsubayashi, 2020; Soans, 2022; Wuergezhen et al., 2023, *Preprint*). In contrast, non-structural components, such as fibulin and even some structural proteins such as nidogen, have more rapid turnover of ∼15–30 min (Keeley, 2020). Even within the same BM, the scaffolding components laminin and collagen can have distinct half-lives (Keeley, 2020). Live imaging of laminin and type IV collagen have also revealed that BMs are pliable and can dynamically slide, polymerize, and be molded into fibers to shape tissues (Diaz-de-la-Loza and Stramer, 2024). Furthermore, many BM components rapidly diffuse within the BM in response to physical forces, suggesting BMs may quickly adapt to changing mechanical environments (Keeley, 2020).

Considerations and pitfalls in conducting experiments and interpreting BM dynamics

It is important to be aware that FRAP and photoconversion of BM proteins, especially when performed over short timescales, primarily examine component diffusion and binding kinetics of the most dynamic subpopulation of protein and do not necessarily indicate degradation and synthesis. For example, loss of a photoconverted BM component in a specific BM region and then gain of the unconverted component has been interpreted as degradation and synthesis (Morgner, 2023). However, BM components in *Drosophila* and *C. elegans* are often made at distant cellular sources and recruited from the extracellular fluid (Fig. 1) (Diaz-de-

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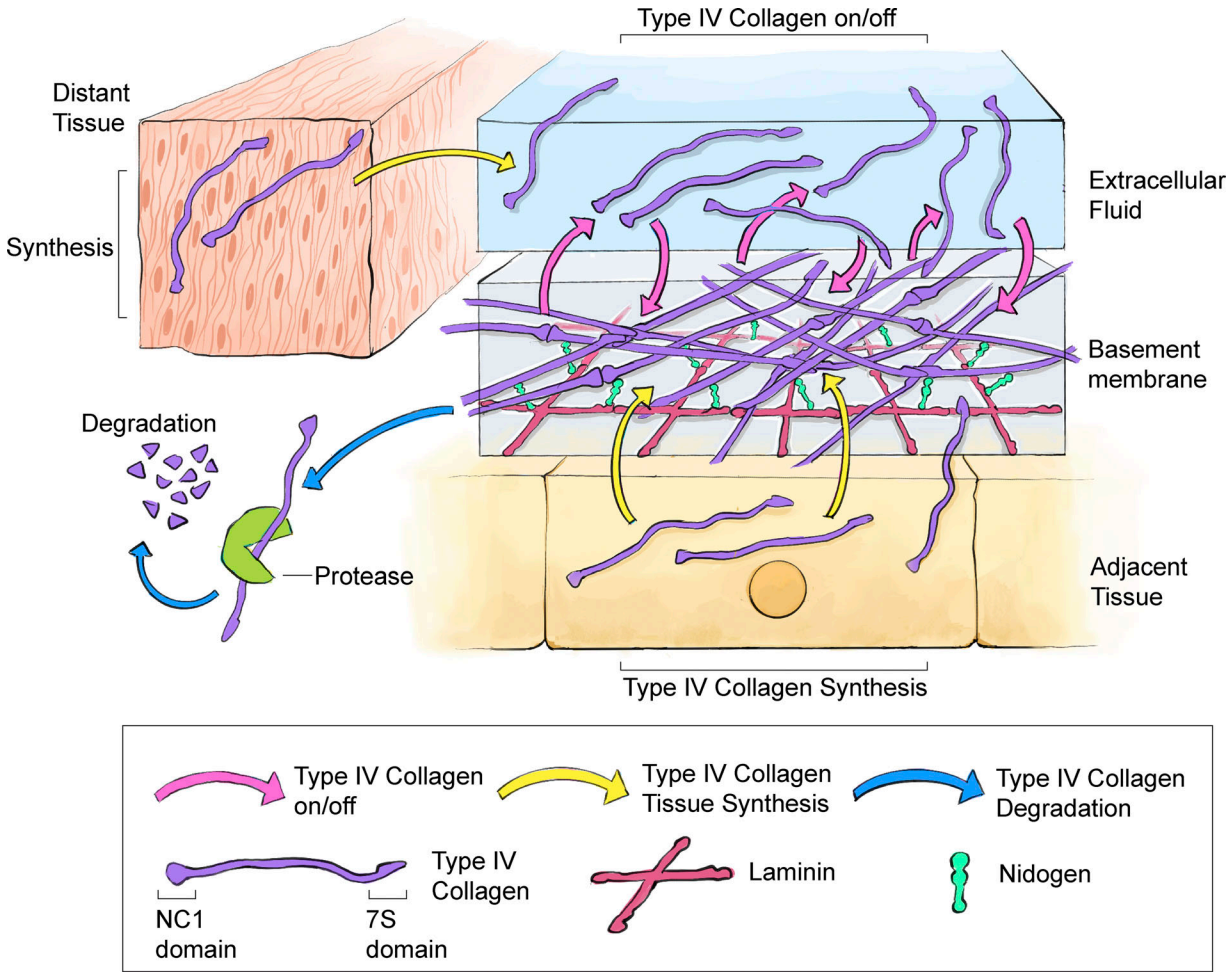

Figure 1. **BM component assembly and disassembly dynamics.** BM components can be made, secreted, and incorporated into BMs from cells immediately adjacent to the BM or from distant cellular sources (yellow arrows). BM proteins also disassemble and then reassemble on BMs (pink arrow) or can be degraded (blue arrow). BM proteins are enlarged for clarity and are not to scale. Image by Siddharthan Balachandar Thendral.

la-Loza and Stramer, 2024), and they are likely undergoing active exchange with a large pool of unconverted protein. The cellular sources of BM components have been studied less in vertebrates. However, expression analysis has revealed that BM components are produced by several cell types, aside from epithelial cells (Simon-Assmann, 1988; Tsutsui, 2021; Chew, 2024). Additionally, data suggest that mesenchymal fibroblasts may be a principal source of type IV collagen during development, and therefore collagen IV would also need to diffuse through the extracellular fluid in vertebrate tissues to assemble within BMs (Simon-Assmann, 1988; Tsutsui, 2021). To conclusively determine degradation and synthesis rates, pulse-chase experiments are required. A good example is where a Scarlet-tagged type IV collagen was transiently expressed in *Drosophila* embryos and the decay in fluorescence signal followed, which revealed a half-life of ∼14 h (Matsubayashi, 2020). Notably, this was much longer than the ∼4-h half-life association rate of photoconverted collagen (Matsubayashi, 2020). Thus, decay of the photoconverted collagen is primarily capturing the rapid dynamics of collagen that is repetitively coming on and off the BM and at most capturing only a small amount of degradation (Fig. 1).

It is also important to consider BM complexity and regulation. For example, a study tagging a laminin chain in mice referred to laminin dynamics as a proxy for turnover of BMs (Morgner, 2023). Yet, evidence suggests that during development, each BM component has a unique turnover rate (Keeley, 2020), and thus one component's dynamics might not reflect the entire BM network. In addition, analysis of tissues ex vivo (Guerra Santillán et al., 2022; Wuergezhen et al., 2023; Jones, 2024, *Preprint*) should be interpreted with caution given that BM components are often recruited from the extracellular fluid, and supplied media may not have the endogenous concentrations.

Consideration of the fluorophore-tagged component is also important. Endogenously tagged BM components should be used where possible, as overexpressed transgenes compete with endogenous untagged counterparts. The fluorophore tag should also not cause a loss of organism viability and biochemical analysis should confirm that the fluorophore tag is not proteolytically removed (Keeley, 2020). Fluorophore self-quenching, maturation rate, and pH sensitivity should also be addressed depending upon the experiment.

Finally, interpreting BM dynamics requires both appropriate time scales of

analysis and calibration in the context of the tissue's morphodynamics. A recent study in developing murine skin with a tagged type IV collagen examined recovery only ∼10 min after photobleaching (Jones, 2024). The authors concluded that the ∼10% recovery revealed that collagen was extremely stable. However, we propose that this result is difficult to interpret. The ∼10% recovery of type IV collagen within ∼10 min could represent a dynamic subpopulation of type IV collagen coming on and off the BM rapidly with the remaining pool being more stable. Alternatively, an ∼10% recovery of type IV collagen within ∼10 min, if compounded over the 20 days of mouse gestation, could instead mean that the entire collagen network is being replaced many times over during embryogenesis, and thus, type IV collagen might be highly dynamic. Type IV collagen appears to be one of the most "stable" BM components during development; yet, in the growing *C. elegans* gonad and condensing *Drosophila* nerve cord BMs, collagen has half-lives of 2 and 4 h, respectively (measured by photoconversion decay). This is significantly more dynamic than the 48 h of gonad morphogenesis and 12 h of nerve cord condensation (Keeley, 2020; Matsubayashi, 2020). Notably, a different study examining mouse hair follicle morphogenesis using a tagged type IV collagen molecule and FRAP analysis over several hours revealed half-life replacement rates of ∼3 h, which led the authors to conclude that collagen turnover is rapid (Wuergezhen et al., 2023, *Preprint*). Therefore, interpretation of BM component turnover dynamics requires consideration of both analytical and developmental timescales—emerging evidence in both vertebrate and invertebrate models suggests that the embryonic and larval BM is dynamic with relatively high rates of component turnover.

Concluding thoughts

BMs regulate many developmental processes, and it will be crucial to extend studies on BM dynamics to more components, additional animal models, and distinct tissue contexts. In addition, it will also be important to examine adult BMs during homeostasis, disease, aging, and tissue repair, where few studies using fluorophore-tagged BM components have been conducted. Interestingly, the one study on BM dynamics in adult tissue—the mouse mammary gland—revealed that the laminin beta 1 chain has a half-life of ∼4 h (photoconversion decay) (Morgner, 2023), which is similar to dynamics found in developmental BMs (Keeley, 2020; Matsubayashi, 2020). Notably, the mammary gland undergoes constant growth and regression and might be more rapidly turned over compared with other adult BMs.

Understanding BM dynamics is clearly of emerging interest. Experimental approaches to quantify these dynamics are complex, and our viewpoint is not intended to flag any study as flawed. We ourselves have struggled (and made errors) with experimental interpretations of BM dynamics, and our intention is to help clarify analysis for the field. The outlined experimental conditions should also not necessarily preclude studies where these cannot be met, but instead, the caveats should be articulated so that accurate interpretations are possible.

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