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Tissue Remodeling: Making Way for Cellular Invaders

Cellular invasion through protein matrices is a critical process during epithelial–mesenchymal transitions. A recent study of *Caenorhabditis elegans* vulval development reports a novel invasive mechanism in which cells coordinate spatially restricted degradation and sliding of a basement membrane during cellular ingression and tissue formation.

Mark Schramp and Jeff Hardin

The invasion of cells through the basement membrane is a critical process during animal development, when mesenchymal cells detach from their resident epithelia to migrate within the embryo [1]. Additionally, the re-acquisition of invasiveness is one of the earliest steps in metastasis [2]. The 'standard' model of cellular invasion through an underlying basement membrane involves two premises: decreased or altered expression of genes whose protein products are essential to the structural integrity of the extracellular matrix (ECM), leading to a more porous or labile matrix; and/or the localized and regulated secretion of proteases to degrade ECM components to form a hole in the matrix through which cells can move. Examples of each of these processes have been well documented, and include the loss of certain basement-membrane-associated proteins and increased secretion of the glycoprotein fibronectin during tumor cell invasion [3] and the enhanced secretion and activity of matrix metalloproteases during neurulation [4]. Studies of other invasive events, however, such as leukocyte invasion into endothelial-based tissues, suggest that collaborative processes between multiple cell types are critical to form basement membrane gaps [5]. Recent work by Ihara *et al.* [6] indicates that another, novel mechanism exists to promote cell invasion. In this case, migrating cells expand a previously formed gap in the basement membrane by sliding the perforated ECM apart, allowing them to move through it.

During development of the vulva in *Caenorhabditis elegans* hermaphrodites, epithelial cells known as vulval precursor cells (VPCs) are born on the ventral surface of the animal. The VPCs then invaginate, giving rise to a stack of seven toroids (vulA, ventral-most, through vulF, dorsal-most; Figure 1) that form an epithelial lumen through which mating and the passage of fertilized eggs or embryos occurs [7]. Vulval invagination is preceded by the localized secretion of proteases from the anchor cell (AC) and its subsequent movement adjacent to the 1°-fated VPCs, which form direct attachments with uterine epithelial cells [8]. This invasive event creates a gap in both the gonadal and ventral basement membranes, through which the invaginating cells will ultimately pass (Figure 1). Thus, *C. elegans* vulval development provides a unique *in vivo* system to further define the molecular mechanisms of cell invasion, and its consequences for other concurrent morphogenetic events.

Ihara *et al.* [6] began their analysis by using a tried-and-true approach in *C. elegans* — laser ablation — to identify which cells are involved in widening the perforation that normally forms at the site of AC invasion and found that both VPCs and ventral uterine cells are required. Ihara *et al.* [6] went on to use several important technical approaches to identify how regulated formation of basement membrane perforations occurs during AC invasion, and which cells are involved. One is the use of Dendra — a stable, photoconvertible, fluorescent protein [9] — fused to components of the basement membrane (such as laminin) to track ECM movement. Using this technology, Ihara *et al.* [6] showed that the basement membrane adjacent to the ECM gap induced by AC invasion remains intact while the diameter of the gap increases. Furthermore, photobleached basement membranes proximal and distal to the expanding gap had similar rates of fluorescence recovery, suggesting that decreased membrane deposition does not

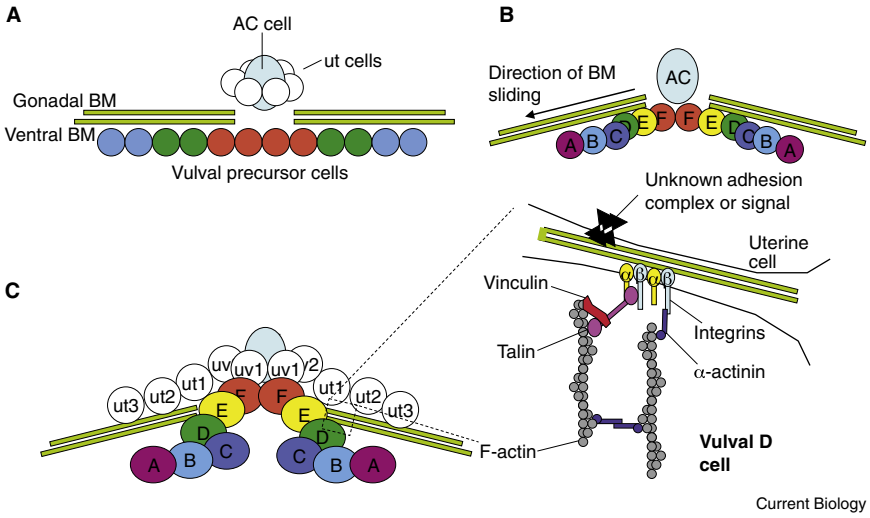

Figure 1. Diagram of vulval morphogenesis.

(A) During the late L3 stage, the anchor cell (AC) is responsible for gap formation in both the gonadal and ventral basement membranes (BM). (B) As the vulval precursor cells invaginate, the gonadal and ventral basement membranes appear to slide until (C) the edges of the gap formed by the actions of the AC come to rest over the vulD cells. To prevent excessive basement membrane sliding, integrin activity and VAB-19/Kank are required in the vulD cells, in addition to an as yet unknown function in the ventral uterine cells.

mediate expansion. Instead, Ihara *et al.* [6] proposed that the basement membranes slide along the invaginating vulval cells until they come into proximity with the vulD cells, which prevent further sliding.

To identify which cell surface receptors regulate the interaction of the basement membrane with uterine and vulval cells, Ihara *et al.* [6] used a plethora of cell-specific RNA interference (RNAi) constructs to deplete endogenous proteins or express dominant-negative proteins in specific VPCs or surrounding cells. Dominant-negative PAT-3/β-integrin expression, loss of either subunit of the specific integrin heterodimer involved in this process (INA-1/α-integrin and PAT-3) or loss of an integrin-associated protein (VAB-19/Kank) in vulval cells resulted in an excessively wide breach in the ECM. Even though the laser ablation data suggested that uterine cells are also required for gap widening, tissue-specific knockdown of integrins in these cells surprisingly does not result in over-expansion of the gap. This suggests that ventral uterine cells are able to compensate for the loss of integrins using other adhesion systems.

This work by Ihara *et al.* [6] challenges previous notions of cell invasion and provides an exciting system for investigating regulated degradation of the ECM during organogenesis. It shows that one cell (or cells) can, at least in some cases, initiate basement membrane perforations, while others modulate the size of the perforation once it is formed. In addition to such cellular teamwork, this work identifies a new role for integrin-based adhesion: vulD cells mediate a 'stop' mechanism for the expansion of a hole in the basement membrane once it is formed. While the precise lineages of cells in the uterine/vulval complex allow for analysis of these processes in the vulva, it will be interesting to see whether basement membranes in other organisms undergo similar tightly choreographed perforation and subsequent remodeling.

Although the study by Ihara *et al.* [6] has identified an exciting new mechanism of precise basement membrane remodeling, several questions remain.

First, why do vulval cells and ventral uterine cells have differing requirements for integrin-based attachment to the ECM? One possibility is that ventral uterine cells use hemidesmosome-like structures, evolutionarily conserved attachment structures found in some epithelial cells in *C. elegans* that link intermediate filaments to the underlying ECM [10]. Another possible attachment system used by uterine cells could be the dystrophin–glycoprotein complex, which binds various ECM components (notably laminin) in other systems and is conserved in worms [11]. Given the tightly choreographed signals that specify cell fates within this system, an alternative explanation is that ventral uterine cells signal to the vulD cells, which respond by increasing their adhesion to the basement membrane.

Second, what mechanism(s) might drive basement membrane sliding across multiple cell diameters to widen gaps? The invagination of vulval cells at the late-L3/early-L4 stage coincides with extensive growth and elongation of the worm. How cellular growth and division impact the movement of the basement membrane in relation to the anchor cell requires further study. If sliding does not involve growth, but simple translocation, one might expect to see increased fluorescence intensity somewhere along the basement membrane as the ECM is 'reeled in' by cells lateral to the edges of the gap. It is possible, however, that such accumulation of fluorescence might go undetected if changes in the turnover of basement-membrane-associated proteins occur simultaneously with gap widening.

Finally, which cells provide the driving forces for basement membrane sliding? While the vulD cells are required for stopping gap widening, it is not clear, based on their position, that they initiate the sliding process. Cells that might be involved in initiation are the vulE cells; actomyosin-based contractile forces generated by these cells, coupled with coordinated disassembly of ECM adhesions could initiate the movement of the basement membrane. While many questions remain, the system Ihara *et al.* [6] have developed should allow many of them to be addressed in the near future. Their results provide the foundation for novel insights regarding how cells invade through basement membranes and then precisely regulate ECM remodeling. Such insights in turn have the potential to reshape our ideas of how cellular invasion occurs.

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Microbial Genomics: *E. coli* Relatives Out of Doors and Out of Body

Genome comparisons have shown that several clades of *Escherichia* isolated primarily from non-host habitats are adapted to life outside of hosts, and that these very close relatives of *E. coli* have historically not shared environments with gut-associated *E. coli*.

Frederick M. Cohan and Sarah M. Kopac

For decades, *Escherichia coli* has been an object of intense interest for its role as a model system in microbiology, for its role in causing disease [1], and for its use as a marker of fecal pollution in the environment [2]. Given the attention that has been paid to *E. coli* in the laboratory, as well as in surveys of its diversity [3], it comes as surprising news that there are several clades closely related to *E. coli* that are radically divergent from typical *E. coli* in their ecology. Three recent papers [4–6] demonstrate that three clades closely related to *E. coli* are primarily adapted to non-host environments such as freshwater beaches and surface water, and that historically these clades have spent little time with the classical *E. coli* living in the gastrointestinal tracts of mammals and birds.

This trio of studies begins with the discovery of three clades (C3–C5) associated with outside (non-host) environments and an additional clade (C1) found in the typical *E. coli* habitats of mammals and birds [6]. These clades were found to be closely related to the classical, gut-associated clades of *E. coli* as well as to *E. fergusonii* (Figure 1). The researchers set out to confirm and characterize the ecological distinctness of the environmental clades from typical *E. coli* through physiological [4] and genomic [5] approaches.

In their physiological study, Ingle *et al.* [4] showed the environmental clades to be genetically adapted to outside environments: they more readily produce biofilms, they can out-compete typical *E. coli* at low temperatures, and perhaps most significantly, they are non-pathogenic in a mouse septicemia model system. Luo *et al.* [5] then investigated the ecological capabilities of the environmental clades through an analysis of genome content, as seen in a growing number of studies of close relatives with different environmental preferences [7]. The environmental clades consistently differed from the gastrointestinal clades by producing lysozyme, presumably for killing other cells in the environment, and by having the entire pathway for utilizing diol as an energy substrate. Moreover, the gastrointestinal clades were distinguished from the environmental clades in having several genomic features adapting them to the gut environment, including the ability to utilize various molecules known to be in abundance in the gut: N-acetylglucosamine, gluconate, and five- and six-carbon sugars.

It will be interesting to apply further genomic methods to characterize the physiological differences between the environment- and gut-associated clades, including a search for shared genes showing evidence of positive selection [8] and comparisons of genome-wide gene expression of shared genes [9]. Expression studies could also confirm that genes unique to a clade are actually used under the expected natural conditions [10].

Luo *et al.* [5] also used the genomes to infer that the environmental clades have spent little of their history in the gut environment with typical *E. coli*. In one approach, they used the inventory of gene functions derived from the human microbiome project [11] to show that genes that are common in other gut bacteria are also contained in the gut-associated *E. coli*, but not in environmental clades. Also, the authors identified significant differences between the viruses contained in the genomes of environmental clades versus gut-associated *E. coli*, indicating historical barriers to sharing of phage.

Genome-based analysis of recombination rates also indicated a historical habitat difference. The authors found evidence of frequent recombination among the environmental clades and among the gut-associated *E. coli* clades, but not between environment- and gut-associated clades, constituting evidence for an ecological barrier to recombination. We note another possible interpretation: that the groups share few vectors of recombination, as suggested by the divergence in phage viromes. One factor not likely to totally impede recombination between environmental and gut clades, however, is the modest (<7%) sequence divergence between them, which would not eliminate recombination [12].