Review TRENDS in Cell Biology Vol.16 No.5 May 2006

Cell invasion through basement membranes: an anchor of understanding

David R. Sherwood

DCMB Group, Department of Biology, Box 91000, Duke University, Durham, NC27708, USA

To metastasize, cancer cells must acquire the ability to breach several basement membrane barriers. Cell invasions through basement membranes also occur during normal development and immune system function, enabling organ formation and cell dispersal. The mechanisms that cells use to cross basement membranes *in vivo* remain elusive. In cancer and development, these invasions occur in complex and inaccessible environments, which are difficult to study *in vivo*. Anchor-cell invasion in *Caenorhabditis elegans* is a simple, visually and experimentally accessible model of basement membrane invasion that is beginning to reveal a network of cellular and molecular control mechanisms that regulate the fundamental cellular process of invasion through basement membranes.

Introduction

The acquisition of cell-invasive behavior, which includes the ability to adhere to and migrate through the extracellular matrix, is an essential step in cancer metastasis. There has been great interest in understanding the mechanisms that promote cell invasion in an effort to design therapies to control this aspect of cancer progression [1–3]. Cell invasions occur frequently in the course of normal animal development and during leukocyte trafficking but, in contrast to cancer, these invasions are tightly regulated [4–8]. Key genes regulating invasive cell traits in normal contexts, such as epithelial–mesenchymal transitions and migratory ability, often have oncogenic roles in metastasis [5,9–12], demonstrating that a crucial element in the acquisition of invasive behavior in cancer is the inappropriate regulation of genes whose normal function is to control cell invasion.

Though necessary for successful invasion, the mechanisms that guide the movement and penetration of cells through the extracellular matrix remain one of the least understood aspects of cell-invasive behavior. The extracellular matrix is a highly diverse and dynamic protein network that provides tissue level structural support and influences the differentiation and functioning of surrounding cells [13]. Extracellular matrices take on different structures, ranging from matrix packed bone and cartilage, to more porous connective tissue, to the dense, sheet-like basement membrane. Cell invasions occur through all of these extracellular matrices in normal development and during cancer metastasis [14–17]. Given the diversity of structures, invasive cells probably have a range of mechanisms to navigate through these matrices. Even within one type of matrix, the connective tissue, invasive cells can use several distinct mechanisms to mediate migration [18–20].

A crucial bottleneck in understanding the process of cell invasion through extracellular matrices has been the challenge of studying this process *in vivo* [15,21]. *In vitro* assays, such as the Boyden chamber assay through Matrigel, have important roles in revealing potential regulators of adhesion and invasion through extracellular matrix [22–24]. *In vitro* assays, however, must be interpreted with caution, as they are outside the context of normal cellular interactions and the cell-associated matrix. Furthermore, unlike endogenous matrix, extracted Matrigel is not crosslinked [25], and important differences in invasion through Matrigel and *in vivo* connective tissue matrix have been observed recently [18,20]. Anchor cell (AC) invasion in *Caenorhabditis elegans* is a new model for examining cell invasion through basement membranes, in which cellular, genetic and molecular dissection of invasive behavior can be directly addressed *in vivo* [26]. Here, I focus on the importance of cell invasion through basement membranes in cancer and normal physiology and on new insights being gained by studying AC invasion in *C. elegans*.

Invasion through basement membranes in cancer and normal development

The basement membrane is a cell-associated extracellular matrix that underlies epithelia and endothelia and surrounds peripheral nerve fibers, muscles and fat cells. Basement membranes regulate tissue structure, cell function and act as barriers between tissue compartments [27]. All vertebrate basement membranes contain laminin, type IV collagen, nidogen and proteoglycans, which together form dense sheet-like structures that are 50–100 nm thick [27,28]. These major basement membrane proteins have orthologs in *C. elegans* that participate in basement membrane assembly and function [29,30], suggesting that this structure is an ancient form of matrix.

Epithelial-derived tumors, also known as carcinomas, represent ~90% of all cancers in the USA and Europe [31]. The metastatic spread of these cancers is the most common cause of death in cancer patients [21,32]. When epithelial-derived tumors metastasize, the first barrier that invading cells breach is the basement membrane

Corresponding author: Sherwood, D.R. (david.sherwood@duke.edu).
Available online 3 April 2006

 0962-8924/$ - see front matter doi:10.1016/j.tcb.2006.03.004

underlying the epithelium. Freed cells traverse the stromal connective tissue, a gelatinous extracellular matrix rich in proteoglycans that surrounds glands and blood vessels, and then cross the basement membranes of blood or lymphatic vessels to disperse in these vessels, a process known as intravasation [15]. During extravasation, the invasive cells cross back through the vascular basement membrane into new tissues where they can form micrometastases [21].

Invasions through basement membranes also occur during normal development and immune system functioning, in which tightly regulated cells undergo basement membrane crossings to help construct tissues and disperse cells. Examples include primate trophoblast cells that breach basement membrane underlying the endometrium to establish a functional placenta [4], neural crest cells and myoblasts that cross basement membranes during their migrations [33,34], capillary sprouts that penetrate through the surrounding vessel basement membrane to form new vessels [6,35] and leukocytes crossing through perivascular basement membrane to sites of injury and infection [8]. Despite the significant barrier function of basement membranes, invasive cells have adopted successful strategies to target and breach this dense and highly cross-linked matrix repeatedly.

AC invasion in *C. elegans*: a stereotyped cell invasion event

The simplicity, complete transparency and amenability to genetic and genomic approaches have made *C. elegans* an important model system for addressing fundamental biological processes [36]. During larval development in *C. elegans*, the uterine and vulval tissues attach together to form a uterine–vulval connection that is an essential structure in the adult for mating and moving embryos from the uterus to the outside environment. The uterine and vulval cells in *C. elegans* initially develop separately, isolated from one another by distinct gonadal and ventral epidermal basement membranes [37]. The AC, a single specialized gonadal cell, crosses these basement membranes during the mid L3 larval stage with a basolateral process that then moves between the central vulval cells (the 1° vulval lineage cells) to initiate attachment and properly align the future uterine–vulval connection (Figure 1) [26,38]. AC invasion is invariant from animal to animal: the AC breaks through both basement membranes in ~30 min and always invades at the mid L3 larval stage between the central 1° vulval lineage cells [26].

The cellular mechanisms that control the targeting and timing of AC invasion

During the late L2 to early L3 larval stage, the AC induces three epidermal precursor cells along the ventral midline to generate the vulval cells of the hermaphrodite through the epidermal growth factor (EGF) signaling pathway [39]. The epidermal precursor cell P6.p, which is closest to the AC, is induced to a 1° fate and gives rise to the eight specialized vulval cells positioned at the center of the vulva by the end of the L3 stage. The 1° vulval cells have a crucial role in regulating the timing and targeting of AC invasion by means of a diffusible cue(s) [26]. When 1° vulval cells are removed, the AC fails to break down and invade through the basement membranes in most animals. Experimental manipulations moving the 1° vulval cells from their normal central position results in ACs that invade toward these inappropriately placed cells in response to their chemotactic invasion cue(s) (Figure 2a–c).

The ability of the AC to respond to the 1° vulval cue also controls the timing of its invasion. Taking advantage of mutations in *C. elegans* that alter developmental timing events in specific tissues (heterochronic mutants), it has been possible to examine AC invasion in animals in which the 1° vulval cells form precociously during the late L2 larval stage [40,41]. Rather than invading precociously, the AC invades at or near the normal time of invasion at the mid L3 larval stage [26]. Thus, although the signal to invade is present, apparently the AC is not ready to invade at the L2 stage.

A third mechanism regulates the timing of AC invasion. Whereas most ACs fail to invade in vulvaless animals, ~20% of ACs nevertheless break through the basement membrane with small protrusions, which enter the underlying hypodermis during the mid L3 stage, the normal time of invasion [26]. This AC behavior indicates that an unidentified extrinsic signal from nonvulval cells or an AC cell-autonomous program helps drive AC invasion weakly during the mid L3 stage. Together, the 1° vulval cue, the vulval-independent mechanism and the capacity of the AC to invade ensure that AC invasion is executed with spatial and temporal precision.

Similar mechanisms regulate invasion in other cells. In a mouse model for intravasation, cells from metastatic MnTLn3 rat tumors polarize, migrate towards and cross through the basement membranes of blood vessels that generate a chemotactic signal, possibly EGF from tumor-associated macrophages [42–44]. Many metastatic cancers express the chemokine receptor CXCR4, which could help target invasion during extravasation to particular tissues expressing the CXCR4 ligand CXCL12 [45–47]. The chemokine receptor CCR1 is also expressed on trophoblast cells as they differentiate to an invasive phenotype [48]. Furthermore, similar to the AC, the time that trophoblast cells can invade is tightly regulated [49].

The AC is polarized

During invasion, only the basolateral portion of the AC extends an invasive process through the basement membrane and between the 1° vulval cells. The apical end of the AC always remains attached to neighboring uterine cells. The AC is thus polarized; the basolateral region of the AC has migratory mesenchymal characteristics and the apical portion has cell–cell adhesion epithelial qualities. Molecular markers further confirm this polarity. The Fat-like cadherin CDH-3 localizes specifically to the invasive basolateral membrane of the AC during invasion [50], whereas AJM-1, an apical adherens protein expressed in epithelial cells, forms junctions in the apical region of the AC (Sherwood, D.R., unpublished). Several cells in development and cancer maintain cell–cell junctions yet have active cellular

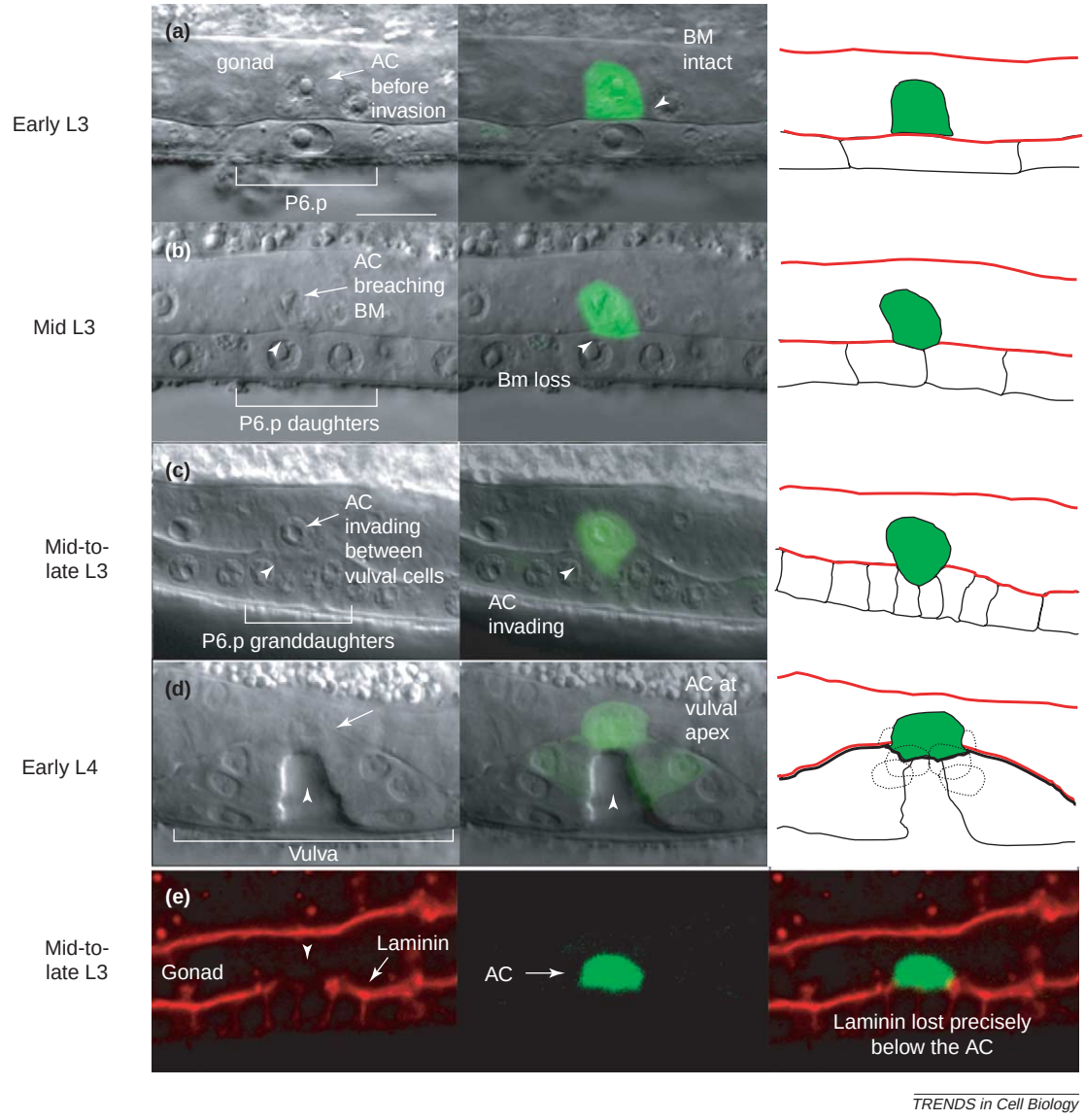

Figure 1. AC invasion through basement membrane initiates uterine–vulval attachment. Panels **(a–d)** viewed with Nomarski optics (left), overlaid with the GFP fluorescence signal from a *cdh-3*::GFP transcriptional reporter that drives GFP expression in the AC (center), and in a schematic showing the basement membrane (BM) in red (right). Anterior is to the left, ventral is down and the scale bar is 10 μm. (a) At the early L3 stage, the ectodermal precursor cell P6.p (bracket) has been specified to a 1° vulval cell fate. The gonadal AC (arrow) is directly over the P6.p cell and does not cross the gonadal and ventral epidermal basement membranes (arrowhead) that separate the gonad and vulval cells. With Nomarski optics increased contrast is observed where there are differences in thickness and refractive index of the specimen. This is the case when the basement membrane is intact between the uterine and vulval cells: a dark high contrast line represents the intact basement membrane. (b) During the mid L3 stage, the 1° fated P6.p cell has divided (bracket) and the basolateral portion of the AC (arrowhead) has broken through and initiated movement across the basement membranes and begun to directly contact the 1° vulval cells. (c) At the mid-to-late L3 stage, the basolateral region of the AC has completed crossing through the basement membranes, is firmly attached to the 1° fated vulval cells, which have now formed four cells (bracket), and is invading between the central 1° vulval cells (arrowhead). (d) By the early L4 stage, the AC has completed invasion and moved between the 1° vulval cells to the apex of the invaginating vulva (arrowhead). The 1° vulval cells have divided again (eight cells total) and shifted out of the plane of focus. Their position on one side of the AC is indicated by the dotted lines in the schematic image. **(e)** Immunolocalization of the basement membrane component laminin (red) reveals the basement membrane around the gonad. The gonadal and ventral epidermal basement membranes are tightly juxtaposed, thus they are difficult to distinguish. The hole in the basement membranes (arrow), corresponds with the invasive basolateral region of the AC (green, center; overlaid, right). Reproduced, with permission, from [26].

protrusions. For example, many carcinomas, including epithelial prostate cancer, lobular breast cancer and melanoma, invade *en masse* rather than as single cells: the invading cells are attached by cell–cell contacts at one end, while extending cellular protrusions from the other [51]. The mechanisms that generate and maintain asymmetry of dynamic cytoskeletal activity at the protrusive membrane while promoting adhesions at the apical membrane are only beginning to be understood [5,51,52]. Thus, it will be important to understand the mechanisms that establish AC polarity and how this polarization impacts AC invasion.

The mechanics of basement membrane crossing

The *in vivo* interactions between invading cells and the basement membrane have been difficult to visualize and understand. For example, in studying the metastatic spread of epithelial-derived tumors, it has been hard to

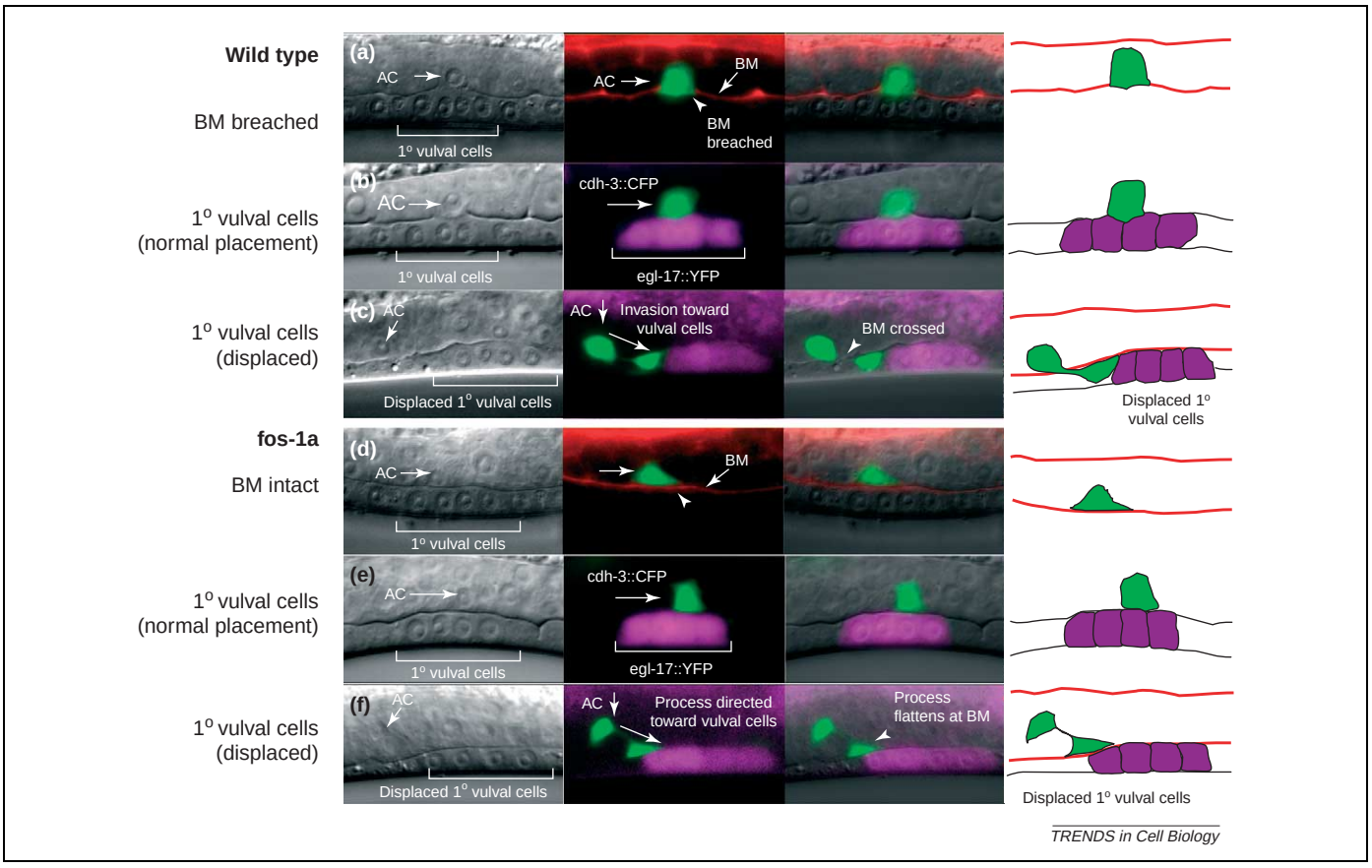

Figure 2. AC invasion in wild-type animals and *fos-1a* mutants. All animals viewed at the mid-to-late L3 stage and shown with Nomarski (left), fluorescence (left center), overlaid (right center), and schematic (right) images. **(a)** Basement membrane staining (red, small arrow) and *cdh-3*::CFP expression in the AC (green, arrow) show an AC in a wild-type animal that has breached and crossed through a gap in the underlying basement membranes (arrowhead), and is now attached to the central 1° vulval cells (bracket). **(b)** A wild-type animal in which the AC (expressing *cdh-3*::CFP in green) has invaded and contacted the underlying 1° vulval cells that express the marker *egl-17*::YFP (purple, bracket). **(c)** A wild-type animal in which the 1° vulval cells (expressing *egl-17*::YFP in purple) have been displaced from their normal site directly under the AC. In response to a chemotactic cue from the 1° vulval cells, the AC directs an invasive process through the basement membrane (arrowhead) and toward the vulval cells (small arrow). **(d)** and **(e)** *Fos-1* is expressed in the AC. In a *fos-1a* loss-of-function mutant, the AC fails to remove the underlying basement membrane and contact the 1° vulval cells, although they express *egl-17*::YFP normally (bracket, e). **(f)** Moving the 1° vulval cells in a *fos-1a* mutant reveals that the 1° vulval cells generate a chemotactic cue and the AC can extend a basolateral process (small arrow) towards this signal; however, this process flattens at the site of the basement membrane, indicating an inability to breach this barrier. Reproduced, with permission, from [50].

determine whether tumor associated macrophages, the activities of the tumor cells themselves, or both actively create breaches in basement membrane to enable tumor cell dispersal [53]. Similarly, the mechanisms used by leukocytes to cross through perivascular basement membranes are unknown [8]. Visual observations combined with forward genetic and molecular approaches are beginning to reveal how the AC interacts with basement membrane to break through this barrier during its invasion.

The *fos-1* gene is the *C. elegans* ortholog of the vertebrate *fos* gene family. Fos proteins form heterodimers with Jun or other bZIP proteins to create the AP-1 transcription factor complex [54]. An isoform of the *fos-1* gene, *fos-1a*, is a crucial regulator of basement membrane removal during AC invasion. In *fos-1a* mutants, ACs never invade at the mid L3 stage (Figure 2d,e) and only occasionally invade in a delayed and partial manner [50]. In *fos-1a* mutants, the AC continues to respond to the chemotactic cue generated by the 1° vulval cells and extends cellular processes toward 1° vulval cells. These processes, however, cannot cross through the basement membrane and instead flatten at this site (Figure 2f). FOS-1A is expressed at high levels in the AC during invasion and restoration of FOS-1A expression in the AC in *fos-1a* mutants rescues invasion [50]. The other isoform of *fos-1*, *fos-1b*, does not rescue invasion in *fos-1a* mutants, indicating that these two isoforms of *fos-1* have distinct activities [50]. In vertebrates there are four *fos* genes (*c-fos*, *fosB*, *fra-1* and *fra-2*) that, similar to *fos-1a* and *fos-1b*, have different functional properties. *c-fos* and *fra-1* are overexpressed in several metastatic cancers [55–57], including breast, prostate, pancreatic and thyroid, and their ectopic expression in cell lines stimulates cell-invasive behavior through reconstituted extracellular matrix [58,59]. These observations suggest that *fos* is a conserved component of a regulatory pathway underlying cell-invasive behavior in development and cancer and that *c-fos* and *fra-1* are required in metastatic cancer cells to promote their movement across basement membranes.

Three AC-specific transcriptional targets of *fos-1a* have been identified that are diverse in structure and function: ZMP-1, a GPI-linked matrix metalloproteinase (MMP) with matrix degrading activity [60,61], CDH-3, a Fat-like

protocadherin [62] that functions in vertebrates to regulate the dynamics of cellular protrusions [63] and hemicentin, a recently identified conserved extracellular member of the immunoglobulin superfamily [64] that promotes cell associations with basement membranes. These proteins localize at the site of invasion. CDH-3 is found on the invasive basolateral membrane and ZMP-1 localizes to apparent lipid raft microdomains concentrated near the invasive membrane. Hemicentin is deposited in the basement membrane under the AC presaging the future site of invasion where it might mediate AC adherence to the basement membrane to target it for removal [50]. Surprisingly, animals lacking *zmp-1*, *cdh-3* and hemicentin function have only a slight delay in AC invasion, indicating that there are additional and perhaps many *fos-1a* targets that contribute to basement membrane removal. Whereas *in vitro* studies have largely focused on the role of MMPs and other proteases in breaching the basement membrane [22], these *in vivo* observations indicate that basement membrane removal is probably a complex and highly regulated process involving MMPs, as well as several other proteins with diverse functions.

How do the 1° vulval cue and FOS-1A activity coordinate invasion?

The 1° vulval cue and the activity of *fos-1a* are required to execute AC invasion in the mid L3 larval stage. An attractive model for how AC invasive activity could be coordinated would be for the 1° vulval cue to stimulate *fos-1a* expression or activity. This, however, is not the case. In vulvaless animals FOS-1A is expressed and localized to the AC nucleus normally. Furthermore, the expression of the *fos-1a* targets, *zmp-1* and *cdh-3* are not altered and hemicentin is deposited normally in the basement membrane under the AC [50]. Thus, in vulvaless animals, *fos-1a* is active, and it functions to initiate invasion, as evidenced by the deposition of hemicentin in the basement membrane. However, the 1° vulval signal is required to complete invasion, as invasion fails to occur in most vulvaless animals [26]. The 1° vulval signal might provide the AC with directional information to target invasive processes more effectively or could contribute a key component necessary for the AC to break through the basement membrane.

Future prospects

Analysis of the mechanisms that control AC invasion through the basement membrane has only recently been initiated (Figure 3). Forward genetic and genomic approaches [65–67] combined with the visual cell biological assays described here should identify the molecular pathways that stimulate and target invasion, the mechanisms that establish cell polarity, and the genes that promote basement membrane removal. It is likely that these studies will continue to identify genes with known oncogenic functions (e.g. *fos-1*), as well as those not previously implicated in regulating invasion or metastasis (e.g. hemicentin). Reverse genetic approaches will also be useful. AC and vulval-specific *cis*-regulatory elements [68] can knock down or target overexpression of genes in *C. elegans* that are implicated in regulating invasion in metastatic cancers. The specific functions and interactions of genes and genetic networks in the invasion process can then be determined to gain a systems level understanding of how distinct steps in AC invasion are controlled and coordinated. The knowledge gained from these studies might ultimately contribute to the development of novel cancer therapeutics. An understanding of cell invasion through basement membranes is a daunting challenge, but the AC is beginning to break down barriers that have impeded our understanding of this fundamental biological process.

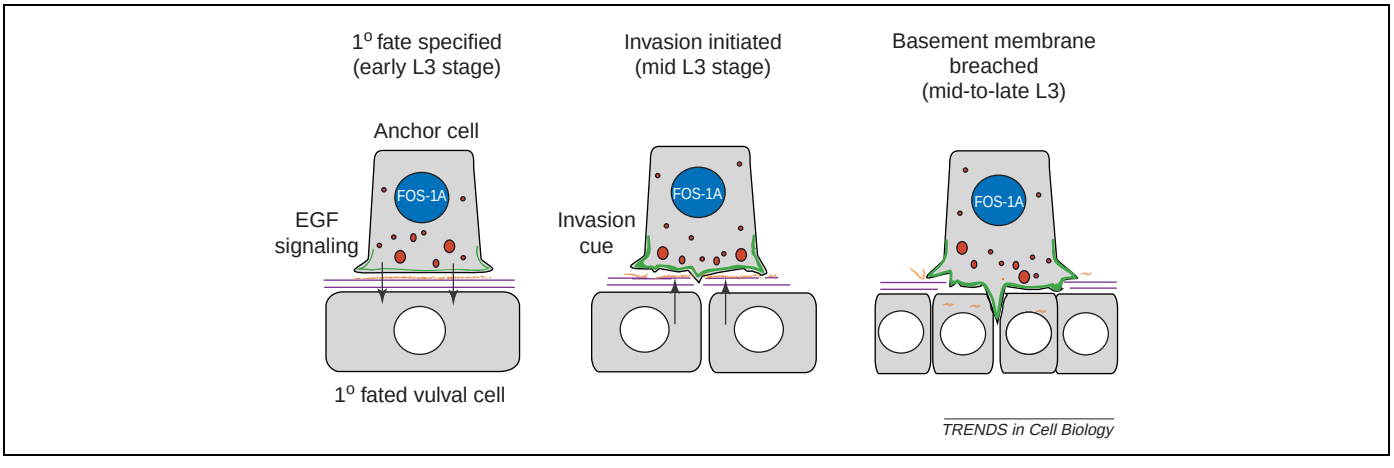

Figure 3. The cellular and molecular networks regulating AC invasion through basement membrane. During the late L2 to early L3 larval stage, the AC induces the 1° fate in the underlying P6.p ectodermal precursor cells through the EGF signaling pathway. FOS-1A is also expressed at high levels and active in the nucleus of the AC at this time (blue circle), where it controls the expression of (1) ZMP-1 (red spots), a GPI-linked MMP that localizes to apparent lipid raft microdomains located near the invasive basolateral membrane; (2) CDH-3 (green line), a Fat-like protocadherin that is found on the invasive basolateral membrane of the AC; and (3) hemicentin (orange, rough line), an extracellular immunoglobulin superfamily member that is secreted directly under the AC before invasion. During the mid L3 stage, the 1° fated vulval cell divides and generates a chemotactic cue that initiates invasion. ZMP-1, CDH-3 and hemicentin appear to be members of a large number of transcriptional targets of FOS-1A that promote removal of the gonadal and ventral epidermal basement membranes (purple lines) during this process. By the mid-to-late L3 stage, invasion is completed, hemicentin is removed under the AC and found in large aggregates surrounding the site of invasion, and the basolateral region of the AC begins inserting itself between the central 1° vulval cells. The apical region remains attached to neighboring cells within the gonad and retains an epithelial character.

Acknowledgements

I am extremely grateful for the support of Paul Sternberg during my postdoctoral studies and conversations about cell invasion with Denise Montell, Pernille Rorth and Stephen Weiss. I thank Ginger Miley, Sarah Durbin, Carrie Johnson, Jonah Cool and Nina Sherwood for critical reading of the article. D.R.S was supported by a Leukemia and Lymphoma Special Fellow Award and a Howard Temin Award (K01 CA098316–01).

References

1 Fidler, I.J. (2003) The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat. Rev. Cancer* 3, 453–458
2 Friedl, P. and Wolf, K. (2003) Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat. Rev. Cancer* 3, 362–374
3 Wang, W. *et al*. (2005) Tumor cells caught in the act of invading: their strategy for enhanced cell motility. *Trends Cell Biol.* 15, 138–145
4 Duc-Goiran, P. *et al*. (1999) Embryo-maternal interactions at the implantation site: a delicate equilibrium. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 83, 85–100
5 Starz-Gaiano, M. and Montell, D.J. (2004) Genes that drive invasion and migration in Drosophila. *Curr. Opin. Genet. Dev.* 14, 86–91
6 Pepper, M.S. (1997) Manipulating angiogenesis. From basic science to the bedside. *Arterioscler. Thromb. Vasc. Biol.* 17, 605–619
7 Affolter, M. *et al*. (2003) Tube or not tube: remodeling epithelial tissues by branching morphogenesis. *Dev. Cell* 4, 11–18
8 Yadav, R. *et al*. (2003) Migration of leukocytes through the vessel wall and beyond. *Thromb. Haemost.* 90, 598–606
9 Yang, J. *et al*. (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 117, 927–939
10 Kunwar, P.S. and Lehmann, R. (2003) Developmental biology: Germ-cell attraction. *Nature* 421, 226–227
11 Balkwill, F. (2004) Cancer and the chemokine network. *Nat. Rev. Cancer* 4, 540–550
12 Wang, W. *et al*. (2004) Identification and testing of a gene expression signature of invasive carcinoma cells within primary mammary tumors. *Cancer Res.* 64, 8585–8594
13 Schwarzbauer, J. (1999) Basement membranes: Putting up the barriers. *Curr. Biol.* 9, R242–R244
14 Sohara, Y. *et al*. (2005) Mechanisms of bone invasion and metastasis in human neuroblastoma. *Cancer Lett.* 228, 203–209
15 Condeelis, J. and Segall, J.E. (2003) Intravital imaging of cell movement in tumours. *Nat. Rev. Cancer* 3, 921–930
16 Egeblad, M. and Werb, Z. (2002) New functions for the matrix metalloproteinases in cancer progression. *Nat. Rev. Cancer* 2, 161–174
17 Nix, P.A. and Salvage, D. (2004) Neoplastic invasion of laryngeal cartilage: the significance of cartilage sclerosis on computed tomography images. *Clin Otolaryngol Allied Sci* 29, 372–375
18 Wolf, K. *et al*. (2003) Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *J. Cell Biol.* 160, 267–277
19 Chun, T.H. *et al*. (2004) MT1-MMP-dependent neovessel formation within the confines of the three-dimensional extracellular matrix. *J. Cell Biol.* 167, 757–767
20 Sabeh, F. *et al*. (2004) Tumor cell traffic through the extracellular matrix is controlled by the membrane-anchored collagenase MT1-MMP. *J. Cell Biol.* 167, 769–781
21 Chambers, A.F. *et al*. (2002) Dissemination and growth of cancer cells in metastatic sites. *Nat. Rev. Cancer* 2, 563–572
22 Albini, A. *et al*. (2004) The "chemoinvasion assay": a tool to study tumor and endothelial cell invasion of basement membranes. *Int. J. Dev. Biol.* 48, 563–571
23 Shaw, L.M. (2005) Tumor cell invasion assays. *Methods Mol. Biol.* 294, 97–105
24 Wang, G.K. and Zhang, W. (2005) The signaling network of tumor invasion. *Histol. Histopathol.* 20, 593–602
25 Kleinman, H.K. *et al*. (1982) Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma. *Biochemistry* 21, 6188–6193
26 Sherwood, D.R. and Sternberg, P.W. (2003) Anchor cell invasion into the vulval epithelium in *C. elegans*. *Dev. Cell* 5, 21–31
27 Kalluri, R. (2003) Basement membranes: structure, assembly and role in tumour angiogenesis. *Nat. Rev. Cancer* 3, 422–433
28 Erickson, A.C. and Couchman, J.R. (2000) Still more complexity in mammalian basement membranes. *J. Histochem. Cytochem.* 48, 1291–1306
29 Kramer, J.M. (2005) Basement membranes. *WormBook, ed*
30 Hutter, H. *et al*. (2000) Conservation and novelty in the evolution of cell adhesion and extracellular matrix genes. *Science* 287, 989–994
31 Pantel, K. and Brakenhoff, R.H. (2004) Dissecting the metastatic cascade. *Nat. Rev. Cancer* 4, 448–456
32 Hanahan, D. and Weinberg, R.A. (2000) The hallmarks of cancer. *Cell* 100, 57–70
33 Hughes, S.M. and Blau, H.M. (1990) Migration of myoblasts across basal lamina during skeletal muscle development. *Nature* 345, 350–353
34 Poelmann, R.E. *et al*. (1990) The extracellular matrix during neural crest formation and migration in rat embryos. *Anat. Embryol. (Berl.)* 182, 29–39
35 Paku, S. and Paweletz, N. (1991) First steps of tumor-related angiogenesis. *Lab. Invest.* 65, 334–346
36 Barr, M.M. (2003) Super models. *Physiol. Genomics* 13, 15–24
37 White, J.G. *et al*. (1976) The structure of the ventral nerve cord of *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 275, 327–348
38 Newman, A.P. and Sternberg, P.W. (1996) Coordinated morphogenesis of epithelia during development of the *Caenorhabditis elegans* uterine-vulval connection. *Proc. Natl. Acad. Sci. U. S. A.* 93, 9329–9333
39 Moghal, N. and Sternberg, P.W. (2003) The epidermal growth factor system in *Caenorhabditis elegans*. *Exp. Cell Res.* 284, 150–159
40 Ambros, V. and Horvitz, H.R. (1984) Heterochronic mutants of the nematode *Caenorhabditis elegans*. *Science* 226, 409–416
41 Euling, S. and Ambros, V. (1996) Heterochronic genes control cell cycle progress and developmental competence of *C. elegans* vulva precursor cells. *Cell* 84, 667–676
42 Wang, W. *et al*. (2002) Single cell behavior in metastatic primary mammary tumors correlated with gene expression patterns revealed by molecular profiling. *Cancer Res.* 62, 6278–6288
43 Wyckoff, J.B. *et al*. (2000) A critical step in metastasis: *in vivo* analysis of intravasation at the primary tumor. *Cancer Res.* 60, 2504–2511
44 Wyckoff, J. *et al*. (2004) A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res.* 64, 7022–7029
45 Muller, A. *et al*. (2001) Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410, 50–56
46 Scotton, C.J. *et al*. (2002) Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res.* 62, 5930–5938
47 Koshiba, T. *et al*. (2000) Expression of stromal cell-derived factor 1 and CXCR4 ligand receptor system in pancreatic cancer: a possible role for tumor progression. *Clin. Cancer Res.* 6, 3530–3535
48 Sato, Y. *et al*. (2003) Trophoblasts acquire a chemokine receptor, CCR1, as they differentiate towards invasive phenotype. *Development* 130, 5519–5532
49 Genbacev, O.D. *et al*. (2003) Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface. *Science* 299, 405–408
50 Sherwood, D.R. *et al*. (2005) FOS-1 promotes basement-membrane removal during anchor-cell invasion in *C. elegans*. *Cell* 121, 951–962
51 Friedl, P. *et al*. (2004) Collective cell migration in morphogenesis and cancer. *Int. J. Dev. Biol.* 48, 441–449
52 Rorth, P. (2002) Initiating and guiding migration: lessons from border cells. *Trends Cell Biol.* 12, 325–331
53 Pollard, J.W. (2004) Tumour-educated macrophages promote tumour progression and metastasis. *Nat. Rev. Cancer* 4, 71–78
54 Chinenov, Y. and Kerppola, T.K. (2001) Close encounters of many kinds: Fos-Jun interactions that mediate transcription regulatory specificity. *Oncogene* 20, 2438–2452
55 Milde-Langosch, K. *et al*. (2004) The role of the AP-1 transcription factors c-Fos, FosB, Fra-1 and Fra-2 in the invasion process of mammary carcinomas. *Breast Cancer Res. Treat.* 86, 139–152
56 Tsuchiya, H. *et al*. (1993) Human T-cell leukemia virus type 1 Tax activates transcription of the human fra-1 gene through multiple cis elements responsive to transmembrane signals. *J. Virol.* 67, 7001–7007

57 Serewko, M.M. *et al*. (2002) Alterations in gene expression and activity during squamous cell carcinoma development. *Cancer Res.* 62, 3759–3765
58 Reichmann, E. *et al*. (1992) Activation of an inducible c-FosER fusion protein causes loss of epithelial polarity and triggers epithelial-fibroblastoid cell conversion. *Cell* 71, 1103–1116
59 Kustikova, O. *et al*. (1998) Fra-1 induces morphological transformation and increases *in vitro* invasiveness and motility of epithelioid adenocarcinoma cells. *Mol. Cell. Biol.* 18, 7095–7105
60 Wada, K. *et al*. (1998) Cloning of three *Caenorhabditis elegans* genes potentially encoding novel matrix metalloproteinases. *Gene* 211, 57–62
61 Itoh, Y. *et al*. (1999) Membrane type 4 matrix metalloproteinase (MT4-MMP, MMP-17) is a glycosylphosphatidylinositol-anchored proteinase. *J. Biol. Chem.* 274, 34260–34266
62 Hill, E. *et al*. (2001) Cadherin superfamily proteins in *Caenorhabditis elegans* and *Drosophila melanogaster*. *J. Mol. Biol.* 305, 1011–1024
63 Moeller, M.J. *et al*. (2004) Protocadherin FAT1 binds Ena/VASP proteins and is necessary for actin dynamics and cell polarization. *EMBO J.* 23, 3769–3779
64 Vogel, B.E. and Hedgecock, E.M. (2001) Hemicentin, a conserved extracellular member of the immunoglobulin superfamily, organizes epithelial and other cell attachments into oriented line-shaped junctions. *Development* 128, 883–894
65 Fox, R.M. *et al*. (2005) A gene expression fingerprint of *C. elegans* embryonic motor neurons. *BMC Genomics* 6, 42
66 Dupuy, D. *et al*. (2004) A first version of the *Caenorhabditis elegans* Promoterome. *Genome Res.* 14(10B), 2169–2175
67 Poulin, G. *et al*. (2004) Genome-wide RNAi screens in *Caenorhabditis elegans*: impact on cancer research. *Oncogene* 23, 8340–8345
68 Kirouac, M. and Sternberg, P.W. (2003) *cis*-Regulatory control of three cell fate-specific genes in vulval organogenesis of *Caenorhabditis elegans* and *C. briggsae*. *Dev. Biol.* 257, 85–103