Seminars in Cell and Developmental Biology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Seminars in Cell and Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

The *C. elegans* anchor cell: A model to elucidate mechanisms underlying invasion through basement membrane

Isabel W. Kenny-Ganzert, David R. Sherwood *

Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA

ARTICLE INFO

Keywords:
Basement membrane
Cell invasion
Transcriptional networks
Ribosome biogenesis
Endomembrane
Invadopodia
Adaptive invasion
Glycolysis
Mitochondria
ATP

ABSTRACT

Cell invasion through basement membrane barriers is crucial during many developmental processes and in immune surveillance. Dysregulation of invasion also drives the pathology of numerous human diseases, such as metastasis and inflammatory disorders. Cell invasion involves dynamic interactions between the invading cell, basement membrane, and neighboring tissues. Owing to this complexity, cell invasion is challenging to study in vivo, which has hampered the understanding of mechanisms controlling invasion. *Caenorhabditis elegans* anchor cell invasion is a powerful in vivo model where subcellular imaging of cell-basement membrane interactions can be combined with genetic, genomic, and single-cell molecular perturbation studies. In this review, we outline insights gained by studying anchor cell invasion, which span transcriptional networks, translational regulation, secretory apparatus expansion, dynamic and adaptable protrusions that breach and clear basement membrane, and a complex, localized metabolic network that fuels invasion. Together, investigation of anchor cell invasion is building a comprehensive understanding of the mechanisms that underlie invasion, which we expect will ultimately facilitate better therapeutic strategies to control cell invasive activity in human disease.

1. Introduction

Basement membrane (BM) is a dense, sheet-like, extracellular matrix (ECM) that underlies or enwraps most animal tissues [1,2]. BMs are highly conserved and are composed of self-associating networks of laminin and covalently crosslinked networks of type IV collagen, which are bridged by the glycoprotein nidogen and the heparan sulfate proteoglycans perlecan and agrin [3,4]. In addition to these core structural components, BMs also harbor a variety of matricellular proteins, proteases, and growth factors [5]. The diverse composition of BMs facilitates its numerous functions, such as mechanically supporting tissues, shaping organs, and harboring signals that regulate cell polarity, growth, survival, and cell migration [6–8]. Electron micrograph and dextran diffusion studies have revealed that BMs are dense and have pore sizes ~10–100 nm in diameter, which is well below the ~2 μm pore size that limits cell migration in vitro [9–12]. Thus, BMs also act as formidable barriers that separate tissues and prevent the exchange of cells.

Despite its barrier properties, many cells in development acquire the unique ability to cross BMs to exit and enter tissues. For example, cells undergoing epithelial-mesenchymal transition (EMT), such as vertebrate neural crest cells and mesenchymal limb progenitor cells, breach the epithelial BM at the site of EMT transition to leave a tissue and then migrate to reach distant sites and differentiate [13,14]. Cells also breach BM to enter new tissues to reach locations of differentiation and function, such as sea urchin pigment cells that invade and embed in epithelia, mammalian cytotrophoblast cells that penetrate the uterine wall BM to implant, and vertebrate sensory neurons that extend pioneering axons through BM to synapse with the spinal cord [15–17]. Immune cells also breach BMs to reach sites of infection and injury [18–20]. Dysregulated cell invasion underlies diseases such as the pregnancy disorder preeclampsia, rheumatoid arthritis, and cancer metastasis [21–23]. Thus, there is great clinical and basic biological importance in elucidating the mechanisms that cells use to breach BM barriers.

Studying cell invasion through BM in vivo is challenging, as invasion events are often stochastic, rapid, and occur deep in tissues, and are thus hard to visualize and experimentally perturb [20,24]. As a result, most

Abbreviations: BM, Basement membrane; AC, Anchor cell; ER, Endoplasmic reticulum; ECM, Extracellular matrix; VPCs, Vulval precursor cells; MMPs, Matrix metalloproteinases; F-actin, Filamentous actin; EMT, Epithelial-mesenchymal transition; VU, Ventral uterine; PM, Plasma membrane; MT, Microtubules; ATP, Adenosine triphosphate; DIC, Differential Interference Contrast Microscopy.

* Corresponding author.
E-mail address: david.sherwood@duke.edu (D.R. Sherwood).

https://doi.org/10.1016/j.semcdb.2023.07.002
Received 11 June 2023; Received in revised form 30 June 2023; Accepted 1 July 2023

cell invasion investigations have relied on in vitro assays using reconstituted ECM, which do not recapitulate the composition or biophysical properties of native BM [18,25]. To circumvent these limitations, studies have examined invasion through decellularized native BMs [18,26]. However, these assays lack complex cell and tissue environments, including the endogenous cues that regulate invasion. Some progress has been made studying invasion in vivo using the chick chorioallantoic membrane assay and zebrafish invasion models [20,27,28]. Yet, because of the stochastic nature of invasion and difficulty in imaging BM, it has been challenging to visualize dynamic interactions between invasive cells and BM in these in vivo models. Despite limitations, in vitro and in vivo models have advanced knowledge of how invasive cells break through BM barriers—particularly in vitro studies examining the invasive protrusions, actin-myosin generated forces, and proteases that breakdown and displace BM barriers [18,29]. Many of these findings, however, require confirmation of in vivo relevance. Further, because of the lack of studies of cell invasion in native contexts, many key mechanisms regulating cell invasion are likely unknown.

C. elegans anchor cell (AC) invasion is an in vivo model that allows visualization and molecular dissection of the mechanisms driving BM invasion. The AC is a specialized uterine cell that plays many roles in organizing uterine and vulval development [30]. One AC function is to initiate uterine-vulval connection by invading through the juxtaposed uterine and ventral epidermal BMs that separate the uterine and vulval tissue during a specific 90-minute window in the L3 larval stage (Fig. 1A) [31]. Over the past twenty years of studying AC invasion [31], many approaches and molecular tools have been developed to analyze different aspects of AC invasion (Tables 1 and 2). Most notably, the highly stereotyped nature of AC invasion allows visualization of specific cell biological events that occur during different stages of invasion and powerful unbiased genetic and RNAi screening to identify genes regulating different steps in the invasion process [32,33]. The rapidity of *C. elegans* development, amenability to germline manipulation, and the development of efficient genome editing selection protocols [34], also allows for quick, cost-effective, and comprehensive endogenous tagging of proteins that regulate invasion with genetically encoded fluorophores. This has led to important insight into protein regulation and function. For example, over ~60 *C. elegans* BM proteins have now been endogenously tagged with various fluorophores, which is facilitating unprecedented insight into BM remodeling and dynamics during invasion [5,35–37]. Here, we review how examination of AC invasion is building a holistic understanding of the mechanisms that control invasion through BM barriers in native settings.

2. Preparing for invasion through BM

The well-defined cell lineage of *C. elegans* and highly stereotyped nature of AC invasion is facilitating a detailed understanding of how an invasive cell prepares to invade. A theme emerging from these studies is that the AC requires many specialized features to invade, including a pro-invasive transcriptional network, distinct gene expression, and expansion of the translation machinery and endomembrane system.

2.1. Specifying the AC—the absence of Notch signaling

Two founder cells in the L1 larva, Z1 and Z4, give rise to all somatic gonad cells, including the uterine AC, which is born at the L2 larval stage (Fig. 1B) [65]. Initially, either of the two Z1 and Z4 descendants, Z1.ppp and Z4.aaa, respectively, can become the AC through a Notch mediated signaling interaction. Both Z1.ppp and Z4.aaa cells initially express the receptor LIN-12 (vertebrate Notch), and the Notch ligand LAG-2 (DSL). Through feedback interactions, however, one cell ultimately expresses only LIN-12 and becomes a ventral uterine (VU) cell, and one cell expresses only LAG-2, and becomes the AC [66,67]. A key component of this feedback loop is the conserved basic helix-loop-helix transcription factor HLH-2 (E2A/TCF3,5,12). HLH-2 is expressed prior to AC invasion in both the Z1.pp and Z4.aa cells, and is required for either cell to have the potential to become the AC. LIN-12 (Notch) activity results in degradation of HLH-2 protein in the VU fated cell, and HLH-2 in the fated AC promotes expression of the Notch ligand *lag-2* [67,68]. Interestingly, differences in the initial levels of HLH-2 protein inheritance in the Z1.ppp and Z4.aaa biases the AC/VU decision, such that the cell receiving greater levels of HLH-2 expresses higher levels of the *lin-12* Notch receptor and becomes the VU cell [69]. Counterintuitively then, the cell that initially harbors more HLH-2 protein expresses more *lin-12*, and is fated to become VU, which then directs HLH-2 to be degraded. Two other transcription factors, the nuclear hormone receptor NHR-67 (TLX/NR2E1) and zinc finger transcription factor EGL-43 (EVI1/MECOM) are also expressed in the Z1.ppp and Z4.aaa and independently promote *lin-12* expression and NHR-67 also promotes *lag-2* expression to mediate the AC/VU decision [70,71]. How these transcriptional inputs are coordinated with HLH-2 during AC/VU specification remains unclear, but they likely play roles in the feedback mechanisms that mediate AC/VU specification.

Interestingly, although the cell fated to be the AC is born in the early L2 stage, ~15 h prior to AC invasion [31,52], no known external signals promote AC differentiation into an invasive fate. Consistent with this, laser ablation of all gonad cells prior to or at the time of Z1.ppp/Z4.aaa birth, do not affect AC differentiation and invasion [31,52]. This implies that a cell autonomous program(s) within the AC guides the differentiation of the AC after its birth to an invasive fate. Supporting this idea, HLH-2, NHR-67, and EGL-43, continue their expression in the AC after AC specification (Fig. 1B) and form an integrated gene regulatory network that directs AC invasive differentiation, which we outline below.

2.2. A gene regulatory network that directs AC differentiation to an invasive fate

After AC specification, HLH-2, NHR-67, and EGL-43 form an interdependent transcriptional network with the addition of a fourth transcription factor, the basic leucine zipper transcription factor FOS-1 (Fos) to direct invasion (Fig. 1C). How this network is rewired to become interdependent is unknown but might involve changes in chromatin accessibility as chromatin regulators such as the SWI/SNF complex alter transcription factor expression and AC differentiation [57]. Timed RNAi-mediated knockdown, measurement of endogenous transcription factor protein levels, chromatin immune-precipitation and sequencing (ChiP-seq), transcription factor binding site removal, and assessment of AC invasion, has led to the identification of a transcriptional network that controls invasive differentiation [41,49,71,72]. A key component of this network is NHR-67, which maintains the AC in a post-mitotic G0 arrested cell cycle state, in part through promoting cyclin-dependent kinase inhibitor CKI-1 (p21CIP1/p27KIP1) expression [41,57]. Loss of NHR-67 results in non-invasive, mitotic ACs that fail to generate most invasive features—the AC does not express genes that promote BM breaching and does not form invadopodia, specialized invasive protrusions that breach the BM [41]. Cell cycle arrest is now recognized as a common feature of invasive cells, which has implications for many cancer therapies that target proliferation and might select for more aggressive tumors [73]. Another key transcription factor is FOS-1, which depends on NHR-67 mediated cell cycle arrest to control the expression of genes that promote BM breaching, such as the matrix metalloproteinases *zmp-1, zmp-3* and *zmp-6*, and the protocadherin *cdh-3* [62,74]. Two other transcription factors, EGL-43 and HLH-2, promote the expression of NHR-67 (cell cycle arrest) and each also controls the expression of some pro-invasive genes [49,71,72,75]. There is complex positive feedback regulation in the expression of all these transcription factors (Fig. 1C). Positive feedback might help stably maintain the invasive fate. Feedback might also be critical in the co-regulation of the precise levels of downstream targets. For example, an incoherent feed-forward circuit sets the precise expression level of the gene encoding

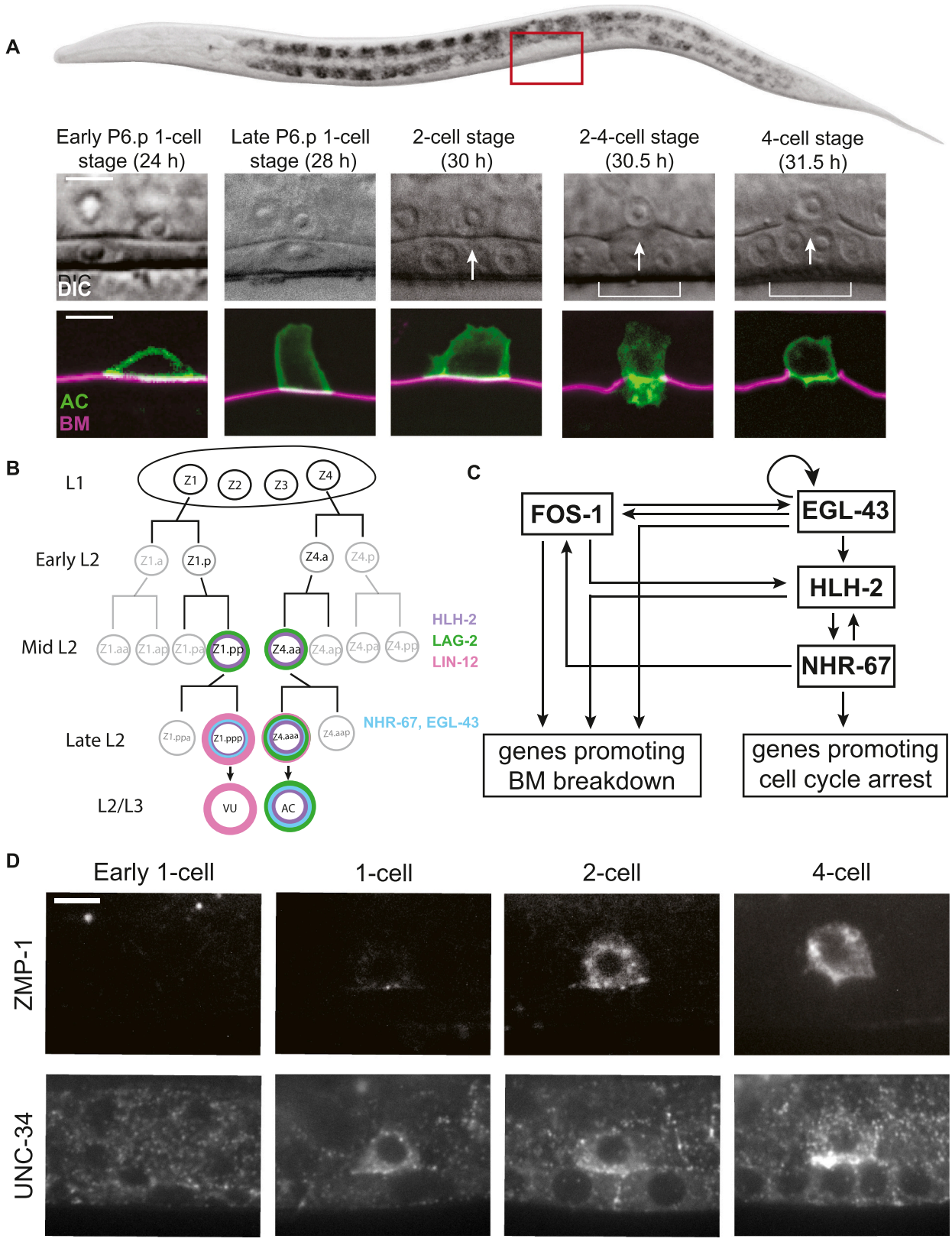


Fig. 1. The pro-invasive transcriptional network and protein translation prior to anchor cell (AC) invasion. A) An L3 larva (top image) and Differential Interference Microscopy (DIC) images (top panel) and fluorescence images (bottom panel) show a developmental time series of AC (visualized with *lin-29p*::2xmKate2::PLCδ^{PH}, green) invasion through basement membrane (BM) (marked with laminin::mNG, magenta) from the 1° vulval precursor cells (VPC) P6.p early 1-cell stage to the P6.p 4-cell stage. The AC invades (arrows) between the central 1° VPCs (brackets). B) Cell lineage of the AC and ventral uterine cell (VU) and proteins involved in AC/VU specification. C) The pro-invasive gene regulatory network that mediates AC differentiation. D) ZMP-1::mNG (MMP) and UNC-34::mNG (Ena/VASP) proteins, which promote BM breaching, ramp up dramatically prior to invasion and peak at the time of BM breaching. Scale bars: 5 μm.
(c) Adapted from [71]. (d) Adapted from [32].

Table 1
Methods used to study AC invasion.

Method	Ref
Quantitative live-cell multi-dimensional image of the AC-BM interface	[35]
DIC microscopy and Protruded vulval genetic/RNAi screens for invasion defects	[31,38]
Stimulated Raman scattering microscopy	[39]
Transmission electron microscopy	[37]
Uterine-specific RNAi	[37]
AC transcriptome	[32]
NanoDam transcription factor binding profile	[40]
AC smFISH for mRNA transcript localization	[41]
Photoconversion for protein dynamics	[42,43]
Fluorescence Recovery After Photobleaching for protein dynamics	[44]
Fluorescence Loss In Photobleaching for protein dynamics	[43]
Auxin induced TIR-1 mediated targeted protein degradation	[45–47]
ZIF-1 mediated targeted protein degradation	[32,48]
FRT-FLP Recombination for site-directed removal of DNA	[49]
Microfluidic Immobilization for long-term timelapse imaging	[50,51]
Cell Ablation	[31,52]
2-DG - Competitive Glucose Inhibitor	[39]
unc-6 ectopic expression	[53,54]
CK666 - Small molecule inhibitor of the Arp2/3 complex	[55]

MIG-10 (Lamellipodin), which stabilizes INA-1/PAT-3 integrin mediated AC-BM adhesion [76]. FOS-1 positively controls expression of the gene encoding MIG-10 (Lamellipodin) and the transcription factor EGL-43, while the EGL-43 protein negatively regulates *mig-10* gene expression. Such networks likely tightly control the expression of effector targets that mediate BM invasion [76]. Recent findings have also revealed that the conserved DNA pre-replication complex, which functions to limit DNA replication to a single round per cell cycle, has a non-canonical role in the AC to promote pro-invasive gene expression [58]. How the DNA pre-replication complex regulates pro-invasive gene transcription, however, is unclear.

Several interesting findings have emerged from this work. One is that the key pro-invasive transcription factors EGL-43, NHR-67, and HLH-2 are expressed prior to AC specification, regulate AC specification, and then are maintained in the AC and mediate AC differentiation. Throughout this time they appear to control distinct transcriptional targets at different stages [75]. The reiterative use of a transcription factor may be a common mechanism to link specification with the differentiated state of a cell. Another important observation is that the transcription factors that promote AC invasion are strongly implicated in EMT and cell invasion in normal development and multiple tumor types (Table 3) [77–81]. Thus, the transcriptional networks that program invasiveness might be conserved. Finally, additional transcriptional mechanisms controlling AC invasion likely exist, as many genes that regulate BM breaching, such as those encoding PAT-3 (β Integrin), MIG-2 (Rac GTPase), and CDC-37 (Hsp 90 co-chaperone), are upregulated in the AC, but are not controlled by any known transcriptional regulators [38].

2.3. Ribosome biogenesis and endomembrane expansion prior to invasion

Cell invasion through BM requires many proteins, such as cytoskeletal proteins, matrix degrading enzymes, adhesion receptors, and metabolic enzymes. Several of the genes encoding these pro-invasive proteins have been characterized during AC invasion, and the expression of many are controlled by the AC's invasive transcriptional network, such as the MMPs *zmp-1*, *zmp-3,* and *zmp-6* [62,74]. Endogenous levels of these pro-invasive proteins ramp up dramatically prior to invasion and peak at the time of BM breaching (Fig. 1D). A recently generated AC transcriptome revealed that genes encoding ribosomal proteins are enriched during invasion [32]. Endogenous ribosome labeling with split-GFP and analysis of ribosome biogenesis indicated that a burst of ribosome biogenesis occurs shortly after AC specification [32]. Early ribosome biogenesis appears to be required to expand the translation capacity of the AC to produce the many pro-invasive proteins that mediate BM breaching (Fig. 1D). Consistent with this notion, a modest reduction in ribosome levels blocks AC invasion and reduces the translation of pro-invasive proteins [32].

In addition to expansion of the ribosome pool, the endomembrane system—endoplasmic reticulum (ER), Golgi apparatus, and secretory vesicles—expands during AC differentiation. AC ribosomes also concentrate in the region of the ER Sec61 translocon, where ribosomes

Table 2
Molecular sensors, probes, and markers used to study AC invasion.

Cell Biological Process		Sensor, probe, marker	Use	Ref
Cell Cycle		CDK Biosensor	G1 or G0 cell cycle state biosensor	[56, 57]
		GFP::MCM-7	Marker for proliferating cells	[58]
		RNR-1::GFP	S-phase marker	[58]
Plasma Membrane (PM)		PLCδPH	Sensor for ($PI(4,5)P_2$) in inner leaflet of plasma membrane	[59]
		CAAX::GFP	Inner leaflet of PM marker	[53]
		GFP::GPI	Outer leaflet PM marker	[53]
		FM1–43 Dye	Stains outer plasma membrane leaflet	[60, 61]
Cytoskeleton	Actin	MoesinABD:: mCherry Lifeact::GFP Dendra2::ACT-1	Probes for F-actin/actin	[43, 55, 59]
		ARX-2::GFP	Marker for site of F-actin nucleation	[32]
	MT	EBP-2::GFP	Marker of growing microtubules (MTs)	[32]
		emtb::GFP	Probe for microtubules	[32]
Metabolism		Green Glifon 4000	Glucose biosensor	[39]
		aTEAM	FRET-based ATP biosensor	[62]
		PercevalHR	Ratiometric ADP:ATP biosensor	[39, 63]
		Biotracker ATP-Red 1	Mitochondrial ATP dye	[39]
		MitoTracker CMXRos	Mitochondrial membrane-potential-sensitive dye	[39]
		PFK-1.1::mNG, PYK-1a::mNG	Markers for glycolysis	[39]
Organelles		GFP::CUP-5, LMP-1::GFP	Lysosomes markers	[53]
		RPL-4::GFP11, RPL-31::GFP11	Localization of ribosomes	[32]
		mCherry::RAB-5	Early endosome marker	[43, 53]
		mChmerry::RAB-7	Late endosome marker	[43, 53]
		mCherry::RAB-11	Recycling endosome marker	[43, 53]
		mNG::RAB-11.1	Recycling endosome marker	[32]
		CYTB-5.1::GFP	Endoplasmic reticulum (ER) Marker	[53]
		SEC-61B::GFP11	ER Translocon marker	[32]
		XBP-1::eGFP	ER Stress/unfolded protein response	[32]
		AMAN-2::GFP AMAN-2:: mScarlet	Golgi apparatus markers	[32, 43]
		DiOC6(3) iodide	Mitochondrial dye	[62]
Basement membrane		Laminin::Dendra Laminin::GFP Laminin::mNG Laminin::mKate2 Collagen::mNG Collagen::GFP Collagen::mEos2 Collagen:: mCherry	Basement membrane markers: Laminin and type IV collagen are crucial scaffolding components	[36, 59,62, 64]

Table 3
Key *C. elegans* AC invasion regulators and vertebrate counterparts.

Cell Biological Process	*C. elegans* protein	Vertebrate protein, protein family or complex	Association with vertebrate invasion, EMT or metastasis
Transcriptional regulation	HLH-2	E2A/TCF3/TCF4/TCF12	[80]
	NHR-67	TLX/NR2E1	[111]
	EGL-43	EVI1/MECOM	[78]
	FOS-1	Fos	[77,79]
	SWSN-4	SWI/SNF complex	[112,113]
	SWSN-8		
	PBRM-1		
	MCM-7	Pre-replication complex	[114]
	HDA-1	HDAC	[115]
Translation regulation	RPL-31	60S ribosomal subunit	[116]
	RPL-4		
	NIFK-1	NIFK	[83]
Endoplasmic reticulum/ Secretory apparatus	SEC-61	Sec61 translocon complex	[117]
	IRE-1	IRE1	[82]
	XBP-1	XBP1	[82]
Chaperone proteins	CDC-37	CDC37	[118]
	TCT-1	TCTP	[119]
	CCT-7	CCT/TRiC complex	[120]
Cell cycle	CKI-1	p21CIP1/p27KIP1	[121]
BM adhesion	INA-1	α3, α6, and α7 Integrin	[122]
	MIG-10	Lamellipodin	[123]
	PAT-4	ILK	[124]
	TLN-1	Talin	[125]
Invadopodia	CDC-42	Cdc42	[126]
	CED-10	Rac	[126]
	MIG-2	RhoG	[127]
	UNC-34	Ena/VASP	[128]
	UNC-60	ADF/cofilin	[29,129]
	ZMP-1	Matrix	[29,130]
	ZMP-3	Metalloproteinase	
	ZMP-6		
	GDI-1	GDI	[131]
	WSP-1	N-WASP	[29,132]
	WVE-1	WAVE	[132]
	ARX-2	Arp2/3	[29,92]
Invasive Protrusion	UNC-6	Netrin	[97]
	UNC-40	DCC	[97]
	DGN-1	Dystroglycan	[133]
	PPK-3	PIKfyve	[134]
	SNAP-29	SNAP29	[135]
	EXOC-8	Exocyst complex	[136]
Metabolism	ANT-1.1	Adenine nucleotide translocator	[137]
	FDGT-1	GLUT	[138]
	FDGT-2		
	TRAK-1	TRAK1	[139]
	OGT-1	OGT	[140]
	PYK-1	Pyruvate kinase	[141]

bind to direct cotranslational protein import of secreted and transmembrane proteins into the ER. Many of the proteins required to breach BM must enter the endomembrane system for proper trafficking, suggesting that there is there is a concomitant expansion of the endomembrane system with ribosomes to accommodate increased translation of transmembrane and secreted proteins. Supporting this idea, the AC is under ER stress prior to invasion, as there is a buildup of the IRE-1 (IRE1) stress sensor transcription factor target XBP-1 (XBP1) in the AC nucleus prior to invasion [32]. XBP1 upregulates chaperones that promote ER protein folding and secretion and increases lipid synthesis for ER expansion [82].

Ribosome biogenesis and sensitivity to ER stress has been linked to EMT events in vertebrate neural crest cells and several cancers [82,83]. Thus, expanding translation capacity and increasing endomembrane trafficking might be a common feature of invasive cells that allows production and delivery of proteins to the cell surface that mediate BM breaching.

2.4. AC-vulval cell alignment prior to invasion: EGF signaling and BM-BM connection

Developmental cell invasion events occur in complex tissue environments where invasive cells must find the appropriate locations to breach BMs and enter tissues. In wild type animals, the AC always invades between the central 1° fated P6.p vulval precursor cells (VPCs) (Fig. 1A) [31]. However, prior to invasion, the uterine and vulval tissues are separated by the juxtaposed gonadal and ventral epidermal BMs and the tissues shift independently of each other as the animal moves, creating dynamic misalignment of the tissues prior to invasion [31,64,84,85].

Cell ablation experiments and mutant backgrounds that augment mispositioning of the AC and 1° fated VPCs cells, have shown that the 1° fated VPC and its descendants move towards and directly under the AC prior to invasion [31,85]. LIN-3 (EGF) growth factor secretion by the AC, induces the closest VPC, P6.p, to adopt a 1° VPC fate at the early L3 larval stage [86]. In addition to its role in fate specification, LIN-3 also serves as an attractive signal that guides the 1° VPC and its descendants under the AC (Fig. 2) [85,87]. Besides the LIN-3 (EGF) alignment mechanism, the AC also secretes the large ~5000 amino acid matrix protein HIM-4 (hemicentin) ~2 h prior to invasion. HIM-4 (hemicentin) localizes between the juxtaposed gonadal and ventral epidermal BMs and connects the BMs together (Fig. 2) [37], thus locking the two tissues with the AC appropriately aligned for invasion. The AC adheres to the region of the HIM-4 mediated BM-BM linkage via the integrin heterodimer αINA-1/βPAT-3, which links to the AC cytoskeleton through the cytolinker VAB-10 (plakin) (Fig. 2). This further stabilizes the AC at the appropriate location for invasion and facilitates the simultaneous breaching and removal of both BMs. The *him-4* gene is a transcriptional target of FOS-1, indicating that BM linkage is a component of the AC invasion program [74].

3. Breaching and clearing the BM barrier

A key limitation in understanding of how cells overcome BM barriers is the challenge of visualizing invasive cells interacting with BM. The development multi-dimensional time-lapse microscopy and quantitative image analysis to visualize the dynamic protrusions the AC uses to breach BM are helping to fill this experimental gap and are revealing how BM is removed during invasion [35]. Key discoveries emerging from these studies are robust, adaptive mechanisms of BM clearance during invasion and the findings of a dynamic, complex, and localized metabolic system that fuels BM breaching.

3.1. Invadopodia initiate BM breaching

The development of quantitative live imaging approaches to visualize the interface of the AC and BM revealed that dynamic, force-producing, ~1.0 μm diameter F-actin (filamentous actin) rich membrane protrusions initiate BM breaching (Fig. 3A,B) [42,55,62]. These small protrusions are similar in composition and function to invadosomes, which were originally observed in transformed fibroblasts and human cancer cell lines, and that have now been seen in a wide variety of invasive cell types in vitro and in some tumor cell implantation models [29,88,89]. Often referred to as invadopodia in cancer cells and podosomes in normal cells, these invasive protrusions are likely a continuum of related membrane-associated structures regulated by intrinsic and extrinsic factors [29,90,91]. AC invadosomes have been referred to as invadopodia because of their similar protrusive nature to cancer cell invadopodia [42]. Study of invadosomes in vivo has been particularly challenging, because of their small size, transient nature, and as outlined above, the difficulty of visualizing cells in the act of BM invasion.

The identification and study of invadopodia during AC invasion has

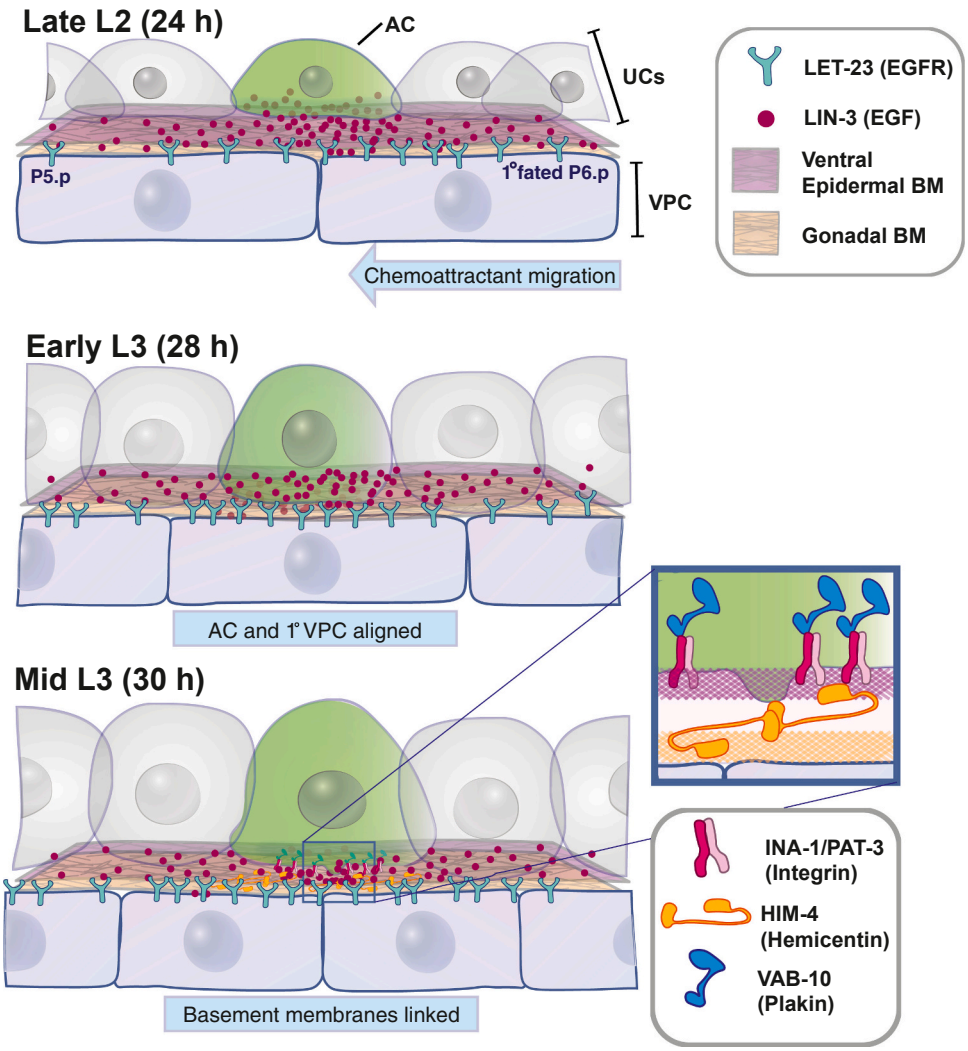
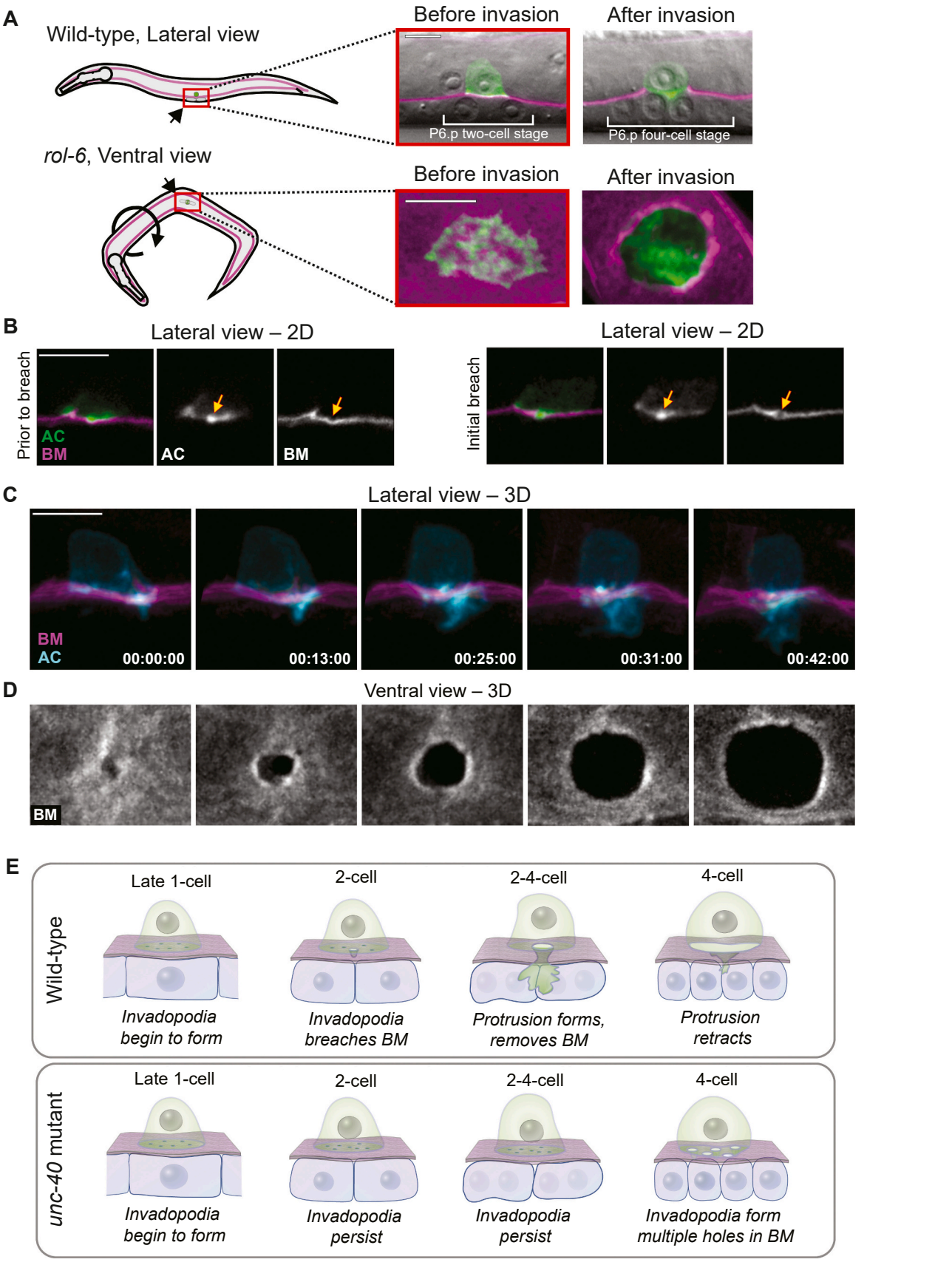


Fig. 2. The AC and 1° VPCs align prior to invasion. A schematic diagram showing LIN-3 (EGF) mediated chemoattraction and HIM-4 (hemicentin) directed BM-BM linkage that aligns the AC with the 1° VPCs prior to invasion.

been important in confirming the existence and function of invadopodia in native tissue settings and in revealing new insights into their regulation. AC invadopodia formation is induced ~3 h prior to invasion by an unidentified diffusible cue from the 1° VPCs [33,42]. The vulval signal promotes invadopodia formation, in part, through activation of the Rho GTPase CDC-42 (Cdc42) [33]. CDC-42 in turn, nucleates invadopodia through its effectors WSP-1 (N-WASP) and WVE-1 (WAVE), which activate the Arp2/3 complex, an actin polymerization nucleator that facilitates generation of branched F-actin that produces forces against the plasma membrane [33,55,92]. The Arp2/3 complex is crucial for invadopodia activity, as inhibiting Arp2/3 in the AC strongly reduces the ability of the AC to generate force on the BM and inhibits the capacity of invadopodia to breach the BM [55]. The actin filament severing protein UNC-60 (ADF/cofilin) promotes invadopodia turnover—UNC-60 localizes to invadopodia and *unc-60* loss blocks invasion and leads to large static aggregates of F-actin along the invasive membrane [43]. Matrix metalloproteinases (MMPs) are also required for invadopodia to breach the BM [62,74]. Three MMPs are expressed in the AC (*zmp-1 zmp-3* and *zmp-6)* and endogenous tagging of *zmp-1* with mNeonGreen revealed that ZMP-1 localizes to invadopodia [62]. MMPs are similarly enriched in invadopodia in cancer cells, where they mediate ECM degradation [89].

AC-invadopodia also require the dynamic trafficking of a specialized invadopodial membrane containing the lipid phosphatidylinositol 4,5 bisphosphate (PI(4,5)P2) and the membrane anchored Rac GTPases, CED-10 and MIG-2 [43,91]. The invadopodial membrane is actively recycled through the endolysosome during invadopodia formation and disassembly, and its delivery to the invasive membrane requires UNC-60 (ADF/cofilin) and the Rab GDP dissociation inhibitor GDI-1 (GDI1) [43]. How F-actin-mediated formation of invadopodia at the invasive cell membrane is coordinated with delivery of the invadopodial membrane is unclear, but may rely on cofilin at invadopodia, which might break the cortical actin barrier to allow vesicle delivery [33]. Recycling of the invadopodial membrane through the endolysosome likely provides a flexible source of membrane, allowing the AC to generate dynamic protrusions that penetrate the BM. It might also facilitate the delivery of proteases such as ZMP-1, which is a membrane associated glycosylphosphatidylinositol (GPI)-anchored MMP. Cancer cells also traffic membrane tethered MT1-MMP through the endolysosome to invadopodia, which supports the idea that dynamic endolysosome trafficking is a shared feature of invadopodia [91,93].

Many of the genes that regulate AC invadopodia have been implicated cancer cell invadopodia and tumor invasion [28,94,95], strongly suggesting that invadopodia are a conserved subcellular structure within invasive cells. A sensitized whole genome RNAi screen has identified numerous additional genes that may have roles in AC invadopodia formation, turnover, or function [33]. Determining how these genes regulate invadopodia will significantly expand the understanding of



(caption on next page)

Fig. 3. Mechanisms of BM breaching and clearance during AC invasion. A) A schematic diagram shows two viewpoints used for time-lapse imaging of AC invasion. In the top image, the wild-type animal is laying on its side, which allows lateral imaging of AC invasion. On the bottom, the *rol-6* mutant animal is twisted, allowing ventral imaging along the entire AC-BM interface. The BM is visualized using laminin::GFP and the AC with the AC-specific F-actin probe mCherry::moeABD. B) Prior to BM breaching (left panels), AC invadopodia (visualized with mCherry::moesinABD, green) press (yellow arrows) on the intact BM (magenta, laminin::GFP). At the time of breach (right panels), an invadopodium creates a small hole in the BM (yellow arrows). C) A lateral time series of the AC's large invasive protrusion (*cdh-3p::* mCherry::PLCδPH, cyan) that forms and expands at the BM breach site (laminin::GFP, magenta). D) A ventral perspective time series shows the widening hole in the BM that occurs during invasive protrusion growth. E) A schematic diagram of invadopodia and protrusion formation during wild-type AC invasion. In wild-type animals (top), the UNC-40 (DCC) receptor traffics to the invadopodium mediated breach site and directs the formation of a large protrusion that clears a wide opening in the BM. UNC-40 acts as a molecular sink that depletes F-actin regulators from invadopodia, thus shutting down invadopodia formation. In *unc-40* mutant animals (bottom), a large protrusion fails to form, invadopodia persist, and multiple breaches in the BM occur.
Adapted from [42].

invadopodia in native settings and might help inform therapies targeting invadopodia in cancer [96].

3.2. An UNC-40/DCC directed large invasive protrusion clears a path through the BM

Invadopodia have been well studied in vitro on glass slides covered with ECM [89]. What occurs after invadopodia penetrate matrix, however, is less clear. *C. elegans* AC invasion has been particularly useful in determining how invadopodia BM breaching leads to large scale BM removal to facilitate wide openings in BM barriers.

Live imaging revealed that usually only one or two AC invadopodia breach the BM and then a large protrusion forms from only one of these sites and clears a wide opening in the BM (Fig. 3C-E) [42]. The UNC-40 (DCC) receptor localizes to the invadopodia-mediated BM breach site and in response to UNC-6 (netrin) secreted from the 1° VPCs recruits F-actin regulators, such as UNC-34 (Ena/VASP), CED-10 (Rac), and MIG-2 (RhoG), and stimulates the exocytosis of lysosomes through the exocyst and the t-SNARE SNAP-29 to form the large invasive protrusion [42,53]. UNC-40 activation at the BM breach site acts as a molecular sink that depletes F-actin regulators from invadopodia, thus shutting down invadopodia formation—in the absence of *unc-40,* a large protrusion fails to form, invadopodia persist, and multiple breaches in the BM occur (Fig. 3E) [42]. Netrin might be a common mechanism to stimulate invasive protrusion formation, as vertebrate netrin-1 is overexpressed in many metastatic cancers and promotes invasion in pancreatic, breast, and colorectal cancer cells [97].

The invasive protrusion has a distinct composition from other regions of the plasma membrane of the AC and concentrates lipid anchored Rho and Rac GTPases, transmembrane proteins, and harbors the GPI-membrane anchored MMP ZMP-1 [53]. Live imaging, genetic analysis and photobleaching experiments revealed that the BM receptor DGN-1 (dystroglycan) localizes to the neck of the invasive protrusion in contact with the BM and forms a membrane diffusion barrier that restricts the flow of lipid anchored proteins in the invasive protrusion from the rest of the AC [53]. DGN-1 is not only required to maintain the diffusion barrier, but is also necessary for protrusion growth, possibly by allowing focused vesicle trafficking and addition of lysosomal membrane to the protrusion. Interestingly, as the edge of the BM gap extends beyond the protrusion, the diffusion barrier breaks down and the protrusion retracts, suggesting that loss of the diffusion barrier might also be a mechanism to end invasion [53].

Optical highlighting of laminin and type IV collagen tagged with the photoconvertible Dendra fluorophore also revealed that the invasive protrusion uses a combination of proteolytic degradation and physical displacement to remove the BM: ~ 30% of the BM under the AC is displaced during invasion, while ~70% appears to be removed by proteolytic degradation [42]. These observations were surprising, as BM transmigration had been previously thought to be solely mediated by proteolytic removal. Notably, recent studies have confirmed the existence of non-proteolytic BM invasion. Macrophages and cancer associated fibroblasts use actomyosin-generated forces to physically create openings in BMs to facilitate BM transmigration [18,20,26]. Understanding how invasive cell breach BM through force without using proteolytic degradation is important, as therapies targeting MMPs, the major matrix degrading proteases in metastatic cancer, have thus far failed in clinical trials [98].

3.3. The AC adapts its invasion program in the absence of MMPs

MMPs are a family of zinc-dependent endopeptidases that cleave ECM proteins, including the major BM structural components of laminin and type IV collagen [99]. MMP overexpression is strongly associated with metastasis and invasion in cancer in animal models and human patients [100]. As stated above, MMPs have been a target of unsuccessful clinical trials. The reasons for the failure of these trials are unclear. A key challenge is clearly defining the role of MMPs in cell invasion, as vertebrates encode over 20 MMPs, which has made genetic analysis daunting [101].

C. elegans AC invasion offers an attractive model to determine the role of MMPs in cell invasion through BM, as the *C. elegans* genome harbors only six MMP genes, which are named zinc metalloproteinases (*zmp-1–6)* [102]. Expression profiling revealed that *zmp-1, zmp-3,* and *zmp-6* are expressed in the AC and that *zmp-4* and *zmp-5* are expressed in neighboring cells [62]. Strikingly, genetical removal of all the MMPs expressed at the time of AC invasion (quintuple mutant) delayed but did not block AC invasion. Examination of the AC-BM interface with live imaging revealed that invadopodia still form in the absence of MMPs, but fail to breach the BM. Instead of breaching the BM, invadopodia transition into a large protrusion that repeatedly stretches the BM and then ultimately breaks through the BM [62]. Optical highlighting revealed that in the absence of MMPs, the BM is predominantly removed through physical displacement instead of proteolysis. Analysis of actin-based mechanisms that generate force and a large-scale synthetic RNAi screen indicated that in the absence of MMPs the AC produces more force on the BM through an increase in the levels of the F-actin nucleator Arp2/3 at the invasive plasma membrane. Further, there is also a further enrichment of mitochondria at the invasive front to generate ATP to fuel increased Arp2/3 mediated F-actin generation and possibly other components of the invasion program that require energy [62].

It is likely that AC adaptation to the absence of MMPs is a built-in component of the invasion program, which balances proteolytic degradation and physical displacement in response to the physical environment and cellular state. Further supporting this idea, in the absence of *unc-40* and the invasive protrusion, the AC still clears the BM with only invadopodia, albeit in a delayed manner [42]. Invadopodia-mediated invasion relies almost exclusively on proteolytic degradation to remove the BM (~90% of the BM is removed by degradation) [42]. These observations illustrate the robustness of the invasion program, which might explain the failure of clinical trials solely targeting MMPs in cancer. An important consideration is that the AC is only one example of invasion. Many intrinsic factors within a cells programing, as well as extrinsic factors, such as environmental cues and makeup and cross-linking of the BM, likely influence how a cell can invade through BM. For example, using de-cellularized mouse mesentery BM, recent studies using the pan-specific MMP inhibitor Batimastat (BB-94) revealed that inhibition of MMPs prevents breast cancer and fibrosarcoma cell

invasion through BM, but doesn't halt macrophage invasion [18]. This suggests that it will likely be necessary to tailor therapeutic treatments to inhibit invasion for different cancers or cell types.

3.4. A localized metabolic network at the invasive front fuels BM invasion

Like the AC, mitochondria localize to the leading edge of many cancer cells, including pancreatic, prostate, ovarian and glioblastoma (Fig. 4A) [103–105]. ATP is not stored in cells and ATP diffusion is restricted by the dense cytoplasm and organelles within cells [106,107]. It is thought that localized ATP production fuels energy consuming molecular processes required for invasion and motility, such as F-actin turnover, proteinase trafficking, cell matrix adhesion, and membrane trafficking [108,109]. How ATP production is coordinated with invasion and how cells acquire a carbon source to fuel high levels of ATP, however, has been unclear.

AC-specific expression of the genetically encoded ATP biosensor PercevalHR and the genetically encoded glucose biosensor Green Glifon 4000 revealed that a burst of glucose import and ATP production at the AC's invasive front accompanies BM breaching (Fig. 4B) [39]. Through extensive RNAi screening, two facilitated glucose transporters, FDGT-1 and FDGT-2, were identified that localize to the AC's invasive cell membrane. Interestingly, each has distinct roles in glucose import. FDGT-1 is predominantly localized to the AC cell membrane and gradually increases in enrichment along the invasive cell membrane leading up to the time of BM breaching. In contrast, FDGT-2 largely localizes to intracellular vesicles prior to invasion, but then sharply increases localization to the invasive cell membrane during BM breaching [39]. FDGT-1 appears to be the dominant glucose importer, as its loss leads to delays in AC invasion, reduced glucose import, reduced F-actin generation, and a smaller invasive protrusion at the time of BM breaching (Fig. 4C) [39]. FDGT-2 seems to have a supportive and likely adaptive role in providing a further boost to glucose import under increased energy demands for invasion. Consistent with this idea, more FDGT-2 enriches at the invasive membrane in *fdgt*-1 null mutants [39].

Glucose is processed into pyruvate, which is imported into mitochondria as a fuel source for ATP production [110]. Glucose processing into pyruvate is referred to as glycolysis and is mediated by 10 glycolytic enzymes that are primarily found in the cytosol of cells [110]. Strikingly, fluorophore tagging of several genes encoding glycolytic enzymes at the entry and exit points of glycolysis, including PFK-1.1 (phosphofructokinase) and PYK-1 (pyruvate kinase), revealed that they specifically form aggregates on mitochondria at the AC's invasive front (Fig. 4D). This suggests that glucose processing into pyruvate is likely channeled rapidly at the surface of the invasive mitochondria by the clustering of glycolytic enzymes. Staining mitochondria with the membrane-potential-sensitive dye Mitotracker CMXRos and mitochondrial ATP reporter dye Biotracker ATP-Red 1 also revealed that mitochondria at the invasive front have a high membrane potential, which is harnessed to make ATP, and contain elevated levels of ATP. Thus, the mitochondria at the site of BM breaching are primed to rapidly process glucose and generate ATP to fuel the invasive machinery [39].

Together, the studies on AC invasion have revealed a localized and adaptable glucose uptake, processing, and mitochondrial ATP generation and delivery system that fuels BM invasion. It will be important to determine if similar metabolic systems are deployed by other invasive

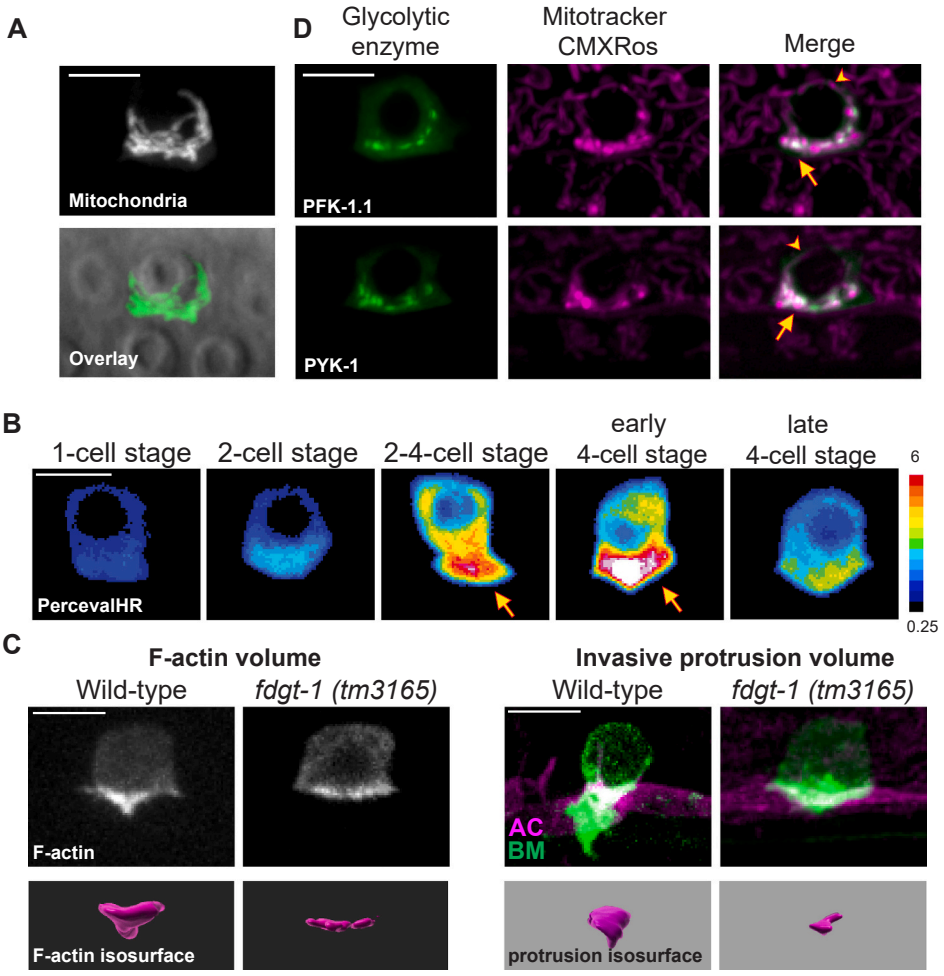


Fig. 4. Fueling AC invasion. A) Anchor cell mitochondria (visualized with *zmp-1p::*MLS::GFP) are enriched at the invasive front (top, fluorescence; bottom, overlay on DIC). B) A spectral fluorescence intensity map of the ATP:ADP ratio in the AC visualized by the ATP:ADP ratiometric sensor PercevalHR (*lin-29p::*PercevalHR) prior to (P6.p 1-cell and 2-cell stage), during (2–4-cell and early 4-cell stage), and after BM breaching (P6.p late 4-cell stage). The ATP:ADP ratios peak at the site of invasion (arrows) during BM breaching and clearance. C) (left) Maximum intensity projections of the AC's F-actin network (*cdh-3p::*mCherry::moeABD) at the time of BM breaching and isosurface renderings of F-actin volume (magenta) in wild-type and *fdgt-1* mutant animals. (Right) Maximum intensity projections (of the largest volume of the invasive protrusion (magenta, BM in green) during a 60-min time series. Isosurface rendering (magenta) of the volume of invasive protrusion beneath the BM. D) (Left) Glycolytic enzymes PFK-1.1 and PYK-1A (*lin-29p::*pfk-1.1::mNG and *lin-*29p::pyk-1a::mNG, green) colocalize with mitochondria stained with Mitotracker CMXRos (magenta) at the AC's invasive front. Arrows indicate glycolytic enzyme puncta that colocalize with basal mitochondria; arrowheads show apical mitochondria with no glycolytic enzyme colocalization in merged images.
Adapted from [39].

cells and whether these metabolic networks can be therapeutically targeted to halt invasion in disease contexts such as cancer and immune disorders.

4. Concluding remarks

Over the past twenty years, AC invasion has powerfully expanded the understanding of mechanisms cells use to breach BM (Table 3). Key findings include: (1) The requirement for cell cycle arrest during cell invasion, an aspect of invasion that appears shared with most cancers; (2) the extensive transcriptional control of invasion and specialization of the translational and secretory apparatus prior to invasion; (3) the mechanisms that ensure the precise targeting of invasion; (4) the validation of the key role of invadopodia in BM breaching in vivo and new insights into invadopodia regulation, such as a distinct invadopodial membrane; (5) the mechanisms that shut down invadopodia formation after BM breaching and the transition to a large invasive protrusion that removes BM; (6) the identification of a robust, adaptive invasion mechanism that relies on a flexible combination of proteolytic degradation and physical displacement; and finally (7) a localized, adaptable metabolic network that provides a burst of ATP to fuel BM breaching.

It will be important for future work to strengthen emerging connections between the different aspects of invasion. For example, it will be crucial to determine how the ATP burst generated by the mitochondria is coordinated with the needs of the invasive machinery that removes the BM. Also, it will also be important to understand how the AC senses an intact, non-degraded BM after loss of MMPs and how that detection is linked to the response of enriching mitochondria and F-actin production at the invasive front. To extend our understanding of AC invasion, it will also be vital to continue to develop new methods and technologies to study invasion. For example, a recently constructed NanoDam toolkit now allows AC-specific profiling of transcriptional targets of the transcription factors that promote AC differentiation [40]. NanoDam will allow the identification of many additional genes regulating invasion and provide further insight into how the invasive transcriptional network is integrated and controlled. Finally, many important aspects of AC invasion remain unknown. For example, it will be crucial to determine how lipid production is regulated in the AC to facilitate the generation of numerous lipid anchored proteins and the production of the large invasive protrusion. Further, since the AC secretes the conserved matrix component MIG-10 (papilin) into the BM prior to invasion and MIG-10 loss perturbs AC invasion [33,75], it will be vital to determine how MIG-10 (papilin) facilitates invasion, and investigate whether other BM modifications occur to foster BM breaching and removal. Addressing these questions and continuing to leverage careful observation during live cell imaging of AC invasion with unbiased screening, promises to significantly extend our understanding of the fascinating and clinically important mechanisms by which invasive cells overcome BM barriers.

Declaration of Competing Interest

The authors have no conflicts of interests to declare.

Acknowledgements

The authors thank Laura Kelley, Siddharthan Balachandar Thendral and Ranjay Jayadev for helpful comments on the manuscript and figures. IWK and DRS were supported by National Institutes of Health, USA grants R35GM118049, R21OD028766, and R21OD032430.

References

[1] R. Jayadev, D.R. Sherwood, Basement membranes, Curr. Biol. 27 (6) (2017) R207–R211.
[2] A. Pozzi, P.D. Yurchenco, R.V. Iozzo, The nature and biology of basement membranes, Matrix Biol. 57–58 (2017) 1–11.
[3] E. Hohenester, P.D. Yurchenco, Laminins in basement membrane assembly, Cell Adh Migr. 7 (1) (2013) 56–63.
[4] N. Khalilgharibi, Y. Mao, To form and function: on the role of basement membrane mechanics in tissue development, homeostasis and disease, Open Biol. 11 (2) (2021), 200360.
[5] R. Jayadev, et al., A basement membrane discovery pipeline uncovers network complexity, regulators, and human disease associations, Sci. Adv. 8 (20) (2022) eabn2265.
[6.] P.D. Yurchenco, Basement membranes: cell scaffoldings and signaling platforms, Cold Spring Harb. Perspect. Biol. 3 (2011) 2.
[7.] M.A. Morrissey, D.R. Sherwood, An active role for basement membrane assembly and modification in tissue sculpting, J. Cell Sci. 128 (9) (2015) 1661–1668.
[8] D.R. Sherwood, Basement membrane remodeling guides cell migration and cell morphogenesis during development, Curr. Opin. Cell Biol. 72 (2021) 19–27.
[9] G.A. Abrams, et al., Nanoscale topography of the basement membrane underlying the corneal epithelium of the rhesus macaque, Cell Tissue Res 299 (1) (2000) 39–46.
[10] P.D. Yurchenco, G.C. Ruben, Basement membrane structure in situ: evidence for lateral associations in the type IV collagen network, J. Cell Biol. 105 (6 Pt 1) (1987) 2559–2568.
[11] A. Gaiko-Shcherbak, et al., The acinar cage: basement membranes determine molecule exchange and mechanical stability of human breast cell Acini, PLoS One 10 (12) (2015), e0145174.
[12] K. Wolf, et al., Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force, J. Cell Biol. 201 (7) (2013) 1069–1084.
[13] C.E. Leonard, L.A. Taneyhill, The road best traveled: Neural crest migration upon the extracellular matrix, Semin Cell Dev. Biol. 100 (2020) 177–185.
[14] J. Gros, C.J. Tabin, Vertebrate limb bud formation is initiated by localized epithelial-to-mesenchymal transition, Science 343 (6176) (2014) 1253–1256.
[15.] G. Moser, et al., Human trophoblast invasion: new and unexpected routes and functions, Histochem Cell Biol. 150 (4) (2018) 361–370.
[16] A.W. Gibson, R.D. Burke, Migratory and invasive behavior of pigment cells in normal and animalized sea urchin embryos, Exp. Cell Res 173 (2) (1987) 546–557.
[17] E.L. Nichols, C.J. Smith, Pioneer axons employ Cajal's battering ram to enter the spinal cord, Nat. Commun. 10 (1) (2019) 562.
[18] J.C. Bahr, et al., Divergent regulation of basement membrane trafficking by human macrophages and cancer cells, Nat. Commun. 13 (1) (2022) 6409.
[19] J. Wang, P. Kubes, A reservoir of mature cavity macrophages that can rapidly invade visceral organs to affect tissue repair, Cell 165 (3) (2016) 668–678.
[20] M.C.W. van den Berg, et al., Proteolytic and opportunistic breaching of the basement membrane zone by immune cells during tumor initiation, Cell Rep. 27 (10) (2019) 2837–2846, e4.
[21] S.J. Fisher, Why is placentation abnormal in preeclampsia? Am. J. Obstet. Gynecol. 213 (4 Suppl) (2015) S115–S122.
[22] R. Xing, et al., Interleukin-21 induces migration and invasion of fibroblast-like synoviocytes from patients with rheumatoid arthritis, Clin. Exp. Immunol. 184 (2) (2016) 147–158.
[23] R. Reuten, et al., Basement membrane stiffness determines metastases formation, Nat. Mater. 20 (6) (2021) 892–903.
[24] E. Beerling, et al., Intravital microscopy: new insights into metastasis of tumors, J. Cell Sci. 124 (Pt 3) (2011) 299–310.
[25] Y. Dong, et al., Angiogenesis invasion assay to study endothelial cell invasion and sprouting behavior, Methods Mol. Biol. 2608 (2023) 345–364.
[26] A. Glentis, et al., Cancer-associated fibroblasts induce metalloprotease-independent cancer cell invasion of the basement membrane, Nat. Commun. 8 (1) (2017) 924.
[27] T.L. Liu, et al., Observing the cell in its native state: Imaging subcellular dynamics in multicellular organisms, Science 360 (2018) 6386.
[28] K.C. Williams, et al., Invadopodia are chemosensing protrusions that guide cancer cell extravasation to promote brain tropism in metastasis, Oncogene 38 (19) (2019) 3598–3615.
[29] E.K. Paterson, S.A. Courtneidge, Invadosomes are coming: new insights into function and disease relevance, FEBS J. 285 (1) (2018) 8–27.
[30] A.J. Schindler, D.R. Sherwood, Morphogenesis of the caenorhabditis elegans vulva, Wiley Inter. Rev. Dev. Biol. 2 (1) (2013) 75–95.
[31] D.R. Sherwood, P.W. Sternberg, Anchor cell invasion into the vulval epithelium in C. elegans, Dev. Cell 5 (1) (2003) 21–31.
[32] D.S. Costa, et al., The Caenorhabditis elegans anchor cell transcriptome: ribosome biogenesis drives cell invasion through basement membrane, Development 150 (2023) 9.
[33] L.L. Lohmer, et al., A sensitized screen for genes promoting invadopodia function in vivo: CDC-42 and Rab GDI-1 direct distinct aspects of invadopodia formation, PLoS Genet 12 (1) (2016), e1005786.
[34] D.J. Dickinson, et al., Engineering the Caenorhabditis elegans genome using Cas9-triggered homologous recombination, Nat. Methods 10 (10) (2013) 1028–1034.
[35] L.C. Kelley, et al., Live-cell confocal microscopy and quantitative 4D image analysis of anchor-cell invasion through the basement membrane in Caenorhabditis elegans, Nat. Protoc. 12 (10) (2017) 2081–2096.
[36] D.P. Keeley, et al., Comprehensive endogenous tagging of basement membrane components reveals dynamic movement within the matrix scaffolding, Dev. Cell 54 (1) (2020) 60–74, e7.
[37] M.A. Morrissey, et al., B-LINK: a hemicentin, plakin, and integrin-dependent adhesion system that links tissues by connecting adjacent basement membranes, Dev. Cell 31 (3) (2014) 319–331.

[38] D.Q. Matus, et al., In vivo identification of regulators of cell invasion across basement membranes, Sci. Signal 3 (120) (2010) ra35.
[39] A. Garde, et al., Localized glucose import, glycolytic processing, and mitochondria generate a focused ATP burst to power basement-membrane invasion, Dev. Cell 57 (6) (2022) 732–749, e7.
[40] C. Yee, et al., A NanoDam toolkit for tissue-specific transcription factor profiling in C. elegans, bioRxiv (2023), p. 2023.05.31.543105.
[41] D.Q. Matus, et al., Invasive cell fate requires G1 cell-cycle arrest and histone deacetylase-mediated changes in gene expression, Dev. Cell 35 (2) (2015) 162–174.
[42] E.J. Hagedorn, et al., The netrin receptor DCC focuses invadopodia-driven basement membrane transmigration in vivo, J. Cell Biol. 201 (6) (2013) 903–913.
[43] E.J. Hagedorn, et al., ADF/cofilin promotes invadopodial membrane recycling during cell invasion in vivo, J. Cell Biol. 204 (7) (2014) 1209–1218.
[44] Kenny-Ganzert, I., Q. Chi, and D. Sherwood, Differential production rates of cytosolic and transmembrane GFP reporters in C. elegans L3 larval uterine cells. 2023, microPublication Biology.
[45] K. Daniel, et al., Conditional control of fluorescent protein degradation by an auxin-dependent nanobody, Nat. Commun. 9 (1) (2018) 3297.
[46] M.A.Q. Martinez, et al., Rapid degradation of caenorhabditis elegans proteins at single-cell resolution with a synthetic auxin, G3 10 (1) (2020) 267–280.
[47] M.A.Q. Martinez, D.Q. Matus, Auxin-mediated protein degradation in Caenorhabditis elegans, Bio-Protoc. 10 (2020) 8.
[48] S.T. Armenti, et al., Repurposing an endogenous degradation system for rapid and targeted depletion of C. elegans proteins, Development 141 (23) (2014) 4640–4647.
[49] T. Deng, et al., The Caenorhabditis elegans homolog of the Evi1 proto-oncogene, egl-43, coordinates G1 cell cycle arrest with pro-invasive gene expression during anchor cell invasion, PLoS Genet 16 (3) (2020), e1008470.
[50] S. Berger, et al., Microfluidic-based imaging of complete Caenorhabditis elegans larval development, Development 148 (18) (2021) 1.
[51] S. Spiri, et al., Reciprocal EGFR signaling in the anchor cell ensures precise inter-organ connection during Caenorhabditis elegans vulval morphogenesis, Development 149 (2022) 1.
[52] J. Kimble, Alterations in cell lineage following laser ablation of cells in the somatic gonad of Caenorhabditis elegans, Dev. Biol. 87 (2) (1981) 286–300.
[53] K.M. Naegeli, et al., Cell Invasion In Vivo via Rapid Exocytosis of a Transient Lysosome-Derived Membrane Domain, Dev. Cell 43 (4) (2017) 403–417, e10.
[54] Z. Wang, et al., UNC-6 (netrin) stabilizes oscillatory clustering of the UNC-40 (DCC) receptor to orient polarity, J. Cell Biol. 206 (5) (2014) 619–633.
[55] R. Caceres, et al., Forces drive basement membrane invasion in Caenorhabditis elegans, Proc. Natl. Acad. Sci. USA 115 (45) (2018) 11537–11542.
[56] R.C. Adikes, et al., Visualizing the metazoan proliferation-quiescence decision in vivo, Elife (2020) 9.
[57] J.J. Smith, et al., The SWI/SNF chromatin remodeling assemblies BAF and PBAF differentially regulate cell cycle exit and cellular invasion in vivo, PLoS Genet 18 (1) (2022), e1009981.
[58] E. Lattmann, et al., A DNA replication-independent function of pre-replication complex genes during cell invasion in C. elegans, PLoS Biol. 20 (2) (2022), e3001317.
[59] J.W. Ziel, et al., UNC-6 (netrin) orients the invasive membrane of the anchor cell in C. elegans, Nat. Cell Biol. 11 (2) (2009) 183–189.
[60] E.J. Hagedorn, et al., Integrin acts upstream of netrin signaling to regulate formation of the anchor cell's invasive membrane in C. elegans, Dev. Cell 17 (2) (2009) 187–198.
[61.] E. Amaral, S. Guatimosim, C. Guatimosim, Using the fluorescent Styryl Dye FM1-43 to visualize synaptic vesicles exocytosis and endocytosis in motor nerve terminals, in: H. Chiarini-Garcia, R.C.N. Melo (Eds.), Light Microscopy: Methods and Protocols, Humana Press, Totowa, NJ, 2011, pp. 137–148.
[62] L.C. Kelley, et al., Adaptive F-actin polymerization and localized ATP production drive basement membrane invasion in the absence of MMPs, Dev. Cell 48 (3) (2019) 313–328, e8.
[63] A. Garde, D.R. Sherwood, Visualizing cytoplasmic ATP in C. elegans larvae using PercevalHR, STAR Protoc. 3 (2) (2022), 101429.
[64] S. Ihara, et al., Basement membrane sliding and targeted adhesion remodels tissue boundaries during uterine-vulval attachment in Caenorhabditis elegans, Nat. Cell Biol. 13 (6) (2011) 641–651.
[65] J. Kimble, D. Hirsh, The postembryonic cell lineages of the hermaphrodite and male gonads in Caenorhabditis elegans, Dev. Biol. 70 (2) (1979) 396–417.
[66] H.A. Wilkinson, K. Fitzgerald, I. Greenwald, Reciprocal changes in expression of the receptor lin-12 and its ligand lag-2 prior to commitment in a C. elegans cell fate decision, Cell 79 (7) (1994) 1187–1198.
[67] X. Karp, I. Greenwald, Post-transcriptional regulation of the E/Daughterless ortholog HLH-2, negative feedback, and birth order bias during the AC/VU decision in C. elegans, Genes Dev. 17 (24) (2003) 3100–3111.
[68] J.M. Benavidez, J.H. Kim, I. Greenwald, Influences of HLH-2 stability on anchor cell fate specification during Caenorhabditis elegans gonadogenesis, G3 (Bethesda) 12 (4) (2022).
[69] M.A. Attner, et al., HLH-2/E2A expression links stochastic and deterministic elements of a cell fate decision during C. elegans gonadogenesis, Curr. Biol. 29 (18) (2019) 3094–3100, e4.
[70] E. Verghese, et al., The tailless ortholog nhr-67 functions in the development of the C. elegans ventral uterus, Dev. Biol. 356 (2) (2011) 516–528.
[71] T.N. Medwig-Kinney, et al., A developmental gene regulatory network for C. elegans anchor cell invasion, Development 147 (2020) 1.
[72] I. Rimann, A. Hajnal, Regulation of anchor cell invasion and uterine cell fates by the egl-43 Evi-1 proto-oncogene in Caenorhabditis elegans, Dev. Biol. 308 (1) (2007) 187–195.
[73] A.Q. Kohrman, D.Q. Matus, Divide or Conquer: Cell Cycle Regulation of Invasive Behavior, Trends Cell Biol. 27 (1) (2017) 12–25.
[74] D.R. Sherwood, et al., FOS-1 promotes basement-membrane removal during anchor-cell invasion in C. elegans, Cell 121 (6) (2005) 951–962.
[75] A.J. Schindler, D.R. Sherwood, The transcription factor HLH-2/E/Daughterless regulates anchor cell invasion across basement membrane in C. elegans, Dev. Biol. 357 (2) (2011) 380–391.
[76] L. Wang, et al., MIG-10 (Lamellipodin) stabilizes invading cell adhesion to basement membrane and is a negative transcriptional target of EGL-43 in C. elegans, Biochem Biophys. Res Commun. 452 (3) (2014) 328–333.
[77.] L. Casalino, et al., FRA-1 as a regulator of EMT and metastasis in breast cancer, Int J. Mol. Sci. 24 (2023) 9.
[78] A.M. Grandits, et al., EVI1 promotes the proliferation and invasive properties of human head and neck squamous cell carcinoma cells, Int J. Mol. Sci. 23 (2022) 3.
[79] B.W. Ozanne, et al., Transcription factors control invasion: AP-1 the first among equals, Oncogene 26 (1) (2007) 1–10.
[80] W. Lin, et al., Kindlin-2-miR-1258-TCF4 feedback loop promotes hepatocellular carcinoma invasion and metastasis, J. Gastroenterol. 57 (5) (2022) 372–386.
[81] S.J. Renaud, et al., The FOS transcription factor family differentially controls trophoblast migration and invasion, J. Biol. Chem. 289 (8) (2014) 5025–5039.
[82] C.M. Limia, et al., Emerging roles of the endoplasmic reticulum associated unfolded protein response in cancer cell migration and invasion, Cancers (Basel) (2019) 11.
[83] V. Prakash, et al., Ribosome biogenesis during cell cycle arrest fuels EMT in development and disease, Nat. Commun. 10 (1) (2019) 2110.
[84] J.E. Sulston, J.G. White, Regulation and cell autonomy during postembryonic development of Caenorhabditis elegans, Dev. Biol. 78 (2) (1980) 577–597.
[85] S. Grimbert, et al., Anchor cell signaling and vulval precursor cell positioning establish a reproducible spatial context during C. elegans vulval induction, Dev. Biol. 416 (1) (2016) 123–135.
[86] R.J. Hill, P.W. Sternberg, The gene lin-3 encodes an inductive signal for vulval development in C. elegans, Nature 358 (6386) (1992) 470–476.
[87] L. Mereu, et al., Polarized epidermal growth factor secretion ensures robust vulval cell fate specification in Caenorhabditis elegans, Development 147 (2020) 11.
[88] W.T. Chen, Proteolytic activity of specialized surface protrusions formed at rosette contact sites of transformed cells, J. Exp. Zool. 251 (2) (1989) 167–185.
[89] A. Cambi, P. Chavrier, Tissue remodeling by invadosomes, Fac. Rev. 10 (2021) 39.
[90] J. Di Martino, et al., The microenvironment controls invadosome plasticity, J. Cell Sci. 129 (9) (2016) 1759–1768.
[91] E.L. Hastie, D.R. Sherwood, A new front in cell invasion: the invadopodial membrane, Eur. J. Cell Biol. 95 (11) (2016) 441–448.
[92] V. Papalazarou, L.M. Machesky, The cell pushes back: The Arp2/3 complex is a key orchestrator of cellular responses to environmental forces, Curr. Opin. Cell Biol. 68 (2021) 37–44.
[93] A. Castro-Castro, et al., Cellular and molecular mechanisms of MT1-MMP-dependent cancer cell invasion, Annu Rev. Cell Dev. Biol. 32 (2016) 555–576.
[94] B. Gligorijevic, et al., N-WASP-mediated invadopodium formation is involved in intravasation and lung metastasis of mammary tumors, J. Cell Sci. 125 (Pt 3) (2012) 724–734.
[95] J. Di Martino, et al., Cdc42 and Tks5: a minimal and universal molecular signature for functional invadosomes, Cell Adh Migr. 8 (3) (2014) 280–292.
[96] L. Perrin, B. Gligorijevic, Proteolytic and mechanical remodeling of the extracellular matrix by invadopodia in cancer, Phys. Biol. 20 (2022) 1.
[97] I. Ylivinkka, J. Keski-Oja, M. Hyytiainen, Netrin-1: a regulator of cancer cell motility? Eur. J. Cell Biol. 95 (11) (2016) 513–520.
[98] G.B. Fields, The rebirth of matrix metalloproteinase inhibitors: moving beyond the Dogma, Cells 8 (2019) 9.
[99] S. Ricard-Blum, S.D. Vallet, Fragments generated upon extracellular matrix remodeling: biological regulators and potential drugs, Matrix Biol. 75–76 (2019) 170–189.
[100] S. Quintero-Fabian, et al., Role of matrix metalloproteinases in angiogenesis and cancer, Front Oncol. 9 (2019) 1370.
[101] A. Page-McCaw, A.J. Ewald, Z. Werb, Matrix metalloproteinases and the regulation of tissue remodelling, Nat. Rev. Mol. Cell Biol. 8 (3) (2007) 221–233.
[102] B. Altincicek, et al., Role of matrix metalloproteinase ZMP-2 in pathogen resistance and development in Caenorhabditis elegans, Dev. Comp. Immunol. 34 (11) (2010) 1160–1169.
[103] A. Garde, D.R. Sherwood, Fueling cell invasion through extracellular matrix, Trends Cell Biol. 31 (6) (2021) 445–456.
[104] B. Cunniff, et al., AMPK activity regulates trafficking of mitochondria to the leading edge during cell migration and matrix invasion, Mol. Biol. Cell 27 (17) (2016) 2662–2674.
[105] V. Papalazarou, et al., The creatine-phosphagen system is mechanoresponsive in pancreatic adenocarcinoma and fuels invasion and metastasis, Nat. Metab. 2 (1) (2020) 62–80.
[106] D. Zala, et al., The advantage of channeling nucleotides for very processive functions, F1000Res 6 (2017) 724.
[107] M.R. Zanotelli, et al., Regulation of ATP utilization during metastatic cell migration by collagen architecture, Mol. Biol. Cell 29 (1) (2018) 1–9.
[108] Y. Li, et al., On the energy efficiency of cell migration in diverse physical environments, Proc. Natl. Acad. Sci. USA 116 (48) (2019) 23894–23900.

[109] N. Shah, et al., Three alphaSNAP and 10 ATP molecules are used in SNARE complex disassembly by N-ethylmaleimide-sensitive factor (NSF), J. Biol. Chem. 290 (4) (2015) 2175–2188.
[110] M. Bonora, et al., ATP synthesis and storage, Purinergic Signal 8 (3) (2012) 343–357.
[111] A.T. Nelczyk, et al., The nuclear receptor TLX (NR2E1) inhibits growth and progression of triple- negative breast cancer, Biochim Biophys. Acta Mol. Basis Dis. 1868 (11) (2022), 166515.
[112] M. Xu, et al., MiR-199a-5p-regulated SMARCA4 promotes oral squamous cell carcinoma tumorigenesis, Int J. Mol. Sci. 24 (2023) 5.
[113] X. Zhang, et al., ARID1A mutations in cancer development: mechanism and therapy, Carcinogenesis 44 (3) (2023) 197–208.
[114] W. Kang, et al., MCM7 serves as a prognostic marker in diffuse-type gastric adenocarcinoma and siRNA-mediated knockdown suppresses its oncogenic function, Oncol. Rep. 31 (5) (2014) 2071–2078.
[115] T. Singh, et al., Differential molecular mechanistic behavior of HDACs in cancer progression, Med Oncol. 39 (11) (2022) 171.
[116] M.J. Ko, et al., RPL17 promotes colorectal cancer proliferation and stemness through ERK and NEK2/beta-catenin signaling pathways, J. Cancer 13 (8) (2022) 2570–2583.
[117] Q. Zhang, Z. Guo, SEC61G participates in endoplasmic reticulum stress by interacting with CREB3 to promote the malignant progression of lung adenocarcinoma, Oncol. Lett. 24 (1) (2022) 233.
[118] A. El Hamidieh, N. Grammatikakis, E. Patsavoudi, Cell surface Cdc37 participates in extracellular HSP90 mediated cancer cell invasion, PLoS One 7 (8) (2012), e42722.
[119] J. Gao, et al., Translationally controlled tumor protein: the mediator promoting cancer invasion and migration and its potential clinical prospects, J. Zhejiang Univ. Sci. B 23 (8) (2022) 642–654.
[120] A. Cox, et al., Chaperonin containing TCP-1 (CCT/TRiC) is a novel therapeutic and diagnostic target for neuroblastoma, Front Oncol. 12 (2022), 975088.
[121] B. Bayarmagnai, et al., Invadopodia-mediated ECM degradation is enhanced in the G1 phase of the cell cycle, J. Cell Sci. 132 (2019) 20.
[122] R.L. Stewart, K.L. O'Connor, Clinical significance of the integrin alpha6beta4 in human malignancies, Lab Invest 95 (9) (2015) 976–986.
[123] G. Carmona, et al., Lamellipodin promotes invasive 3D cancer cell migration via regulated interactions with Ena/VASP and SCAR/WAVE, Oncogene 35 (39) (2016) 5155–5169.
[124] A. Gorska, A.J. Mazur, Integrin-linked kinase (ILK): the known vs. the unknown and perspectives, Cell Mol. Life Sci. 79 (2) (2022) 100.
[125] Z. Sun, M. Costell, R. Fassler, Integrin activation by talin, kindlin and mechanical forces, Nat. Cell Biol. 21 (1) (2019) 25–31.
[126] B. Humphries, Z. Wang, C. Yang, Rho GTPases: big players in breast cancer initiation, metastasis and therapeutic responses, Cells 9 (2020) 10.
[127] H. Al-Koussa, et al., The role of Rho GTPases in motility and invasion of glioblastoma cells, Anal. Cell Pathol. 2020 (2020) 9274016.
[128] D.D. Wang, et al., The significance of ENAH in carcinogenesis and prognosis in gastric cancer, Oncotarget 8 (42) (2017) 72466–72479.
[129] S. Shishkin, et al., Cofilin-1 and Other ADF/Cofilin Superfamily Members in Human Malignant Cells, Int J. Mol. Sci. 18 (2016) 1.
[130] N.M. Abdel-Hamid, S.A. Abass, Matrix metalloproteinase contribution in management of cancer proliferation, metastasis and drug targeting, Mol. Biol. Rep. 48 (9) (2021) 6525–6538.
[131] X. Xie, et al., Overexpression of GDP dissociation inhibitor 1 gene associates with the invasiveness and poor outcomes of colorectal cancer, Bioengineered 12 (1) (2021) 5595–5606.
[132] K. Takahashi, K. Suzuki, WAVE2, N-WASP, and Mena facilitate cell invasion via phosphatidylinositol 3-kinase-dependent local accumulation of actin filaments, J. Cell Biochem 112 (11) (2011) 3421–3429.
[133] P.A. Brennan, et al., Dystroglycan complex in cancer, Eur. J. Surg. Oncol. 30 (6) (2004) 589–592.
[134] A. Oppelt, et al., PIKfyve, MTMR3 and their product PtdIns5P regulate cancer cell migration and invasion through activation of Rac1, Biochem J. 461 (3) (2014) 383–390.
[135] Y. Wang, et al., Silencing DAPK3 blocks the autophagosome-lysosome fusion by mediating SNAP29 in trophoblast cells under high glucose treatment, Mol. Cell Endocrinol. 502 (2020), 110674.
[136] B. Wu, W. Guo, The exocyst at a glance, J. Cell Sci. 128 (16) (2015) 2957–2964.
[137] M. Raudenska, et al., Engine shutdown: migrastatic strategies and prevention of metastases, Trends Cancer 9 (4) (2023) 293–308.
[138] R.J. Gillies, Cancer heterogeneity and metastasis: life at the edge, Clin. Exp. Metastas-.-. 39 (1) (2022) 15–19.
[139] Y. Onodera, et al., Arf6-driven cell invasion is intrinsically linked to TRAK1-mediated mitochondrial anterograde trafficking to avoid oxidative catastrophe, Nat. Commun. 9 (1) (2018) 2682.
[140] X.F. He, et al., O-GlcNAcylation in cancer development and immunotherapy, Cancer Lett. 566 (2023), 216258.
[141] S. Zhu, et al., Pyruvate kinase M2 (PKM2) in cancer and cancer therapeutics, Cancer Lett. 503 (2021) 240–248.