CellPress

Review

Circuitous Genetic Regulation Governs a Straightforward Cell Migration

Afsoon Saadin[1] and Michelle Starz-Gaiano[1,*]

Drosophila **border cells undergo a straightforward and stereotypical collective migration during egg development. However, a complex genetic program underlies this process. A variety of approaches, including biochemical, genetic, and imaging strategies have identified many regulatory components, revealing layers of control. This complexity suggests that the active processes of evaluating the environment, remodeling the cytoskeleton, and coordinating movements among cells, demand rapid systems for modulating cell behaviors. Multiple signaling inputs, nodes of integration, and feedback loops act as molecular rheostats to fine-tune gene expression levels and physical responses. Since key genetic regulators of border cell migration have been shown to be required in other types of cell migration, this model system continues to provide an important avenue for genetic discovery.**

How Did That Get There? Moving towards Understanding

Moving cells captured the attention of biologists centuries ago when the first microscopes focused upon mysterious protists swimming through water. An appreciation of cell motility in multicellular animals developed much later, but the essential role for this process in development and disease is now clear. In the postgenomic era, many of the molecular mechanisms underlying cell movement have been established. Generally, we know that cells perceive chemical and physical cues and respond by reorganizing cytoskeletal and adhesive molecules, thus pushing and pulling themselves in the appropriate direction. With a basic understanding of individual cell motility, more attention has been paid to examining how groups of cells coordinate their movements, and how they navigate the complex, cell-rich environments of animal tissues. The genetic regulation of these collective migrations is now being uncovered.

Here, we examine the **genetic circuits** (see Glossary) that induce and maintain cell motility in a cohort of epithelial cells, called border cells, found in the developing *Drosophila melanogaster* egg chamber. *Drosophila* is advantageous for examining cell migrations given the extensive genetic tools available, molecular signaling that is well-conserved to humans, and small transparent tissues that allow direct observations of cells within their normal environments. A framework for the specification and guidance of border cells was determined over a decade ago, but newer experiments have uncovered additional inputs and layers of regulatory elements, fleshing out how this intricate system works. **Gene regulatory networks** have been described in a number of contexts, such as neural crest, embryonic patterning, and various cancers [1–4], and many of these reveal unidirectional cascades ending in cell fate specification. In the context of border cells, we first discuss transcriptional networks. These networks are essential during specification and remain active to instruct collective, directional migration. Thus, genetic circuitry for cell fate specification and motility are intimately intertwined. Next we review the multiple

Trends

With many research strategies available, *Drosophila* border cells provide a powerful system for elucidating genetic circuits that govern collective cell migrations.

Transcriptional regulatory networks required for cell identity and cell motility are interconnected but also have independent outputs.

Multiple, integrated input signals and feedback loops fine-tune gene expression, creating genetic circuits. These allow optimization of coordinated cell migration and timely responses to environmental cues.

Multiple types of cell-to-cell signaling impact collective cell movement. Proper border cell specification and migration depend on diffusible signals, cell adhesion contacts, and physical forces exerted by neighbors. Such signals influence pathway activity at the protein level, but can also impact gene expression.

[1]Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21250, USA

*Correspondence: starz@umbc.edu (M. Starz-Gaiano).

 http://dx.doi.org/10.1016/j.tig.2016.08.001
© 2016 Elsevier Ltd. All rights reserved.

CrossMark

inputs, different levels of regulation, and feedback loops that act to fine-tune responses to the environment and to coordinate cell behaviors.

Interestingly, collective cell migrations have been found and studied in a variety of animals, including fish lateral line development, vertebrate neural crest movements, and certain carcinoma metastases (reviewed in [5–7]). Researchers continue to identify critical mechanisms to help explain how different cells of the migratory system contribute to the process. Such interactions ensure timely and accurate morphogenesis. Many questions remain; however, it is clear that the cell migration factors characterized through genetic analyses in *Drosophila* ovary can provide broadly-applicable insights into the movements of cell collectives [8].

Wiring the Border Cell Circuitry

Within the *Drosophila* ovary, eggs develop over 14 different characteristic stages [9]. In each egg chamber, a single epithelial layer of somatic follicle cells envelops 16 germline cells, including the oocyte. Follicle cells adopt a number of different fates (Figure 1, Key Figure). Early in development, cells at the anterior and posterior pole become the polar cells, a fate later restricted to two cells at each end [10]. Later, four to six neighboring follicle cells change from stationary epithelial cells to motile border cells (Figure 1B), which will adhere tightly to the polar cells as they all journey together to the oocyte, traveling between germ line cells (Figure 1C). Development of a fertilizable egg depends on the successful completion of border cell migration.

At least five signaling pathways contribute to the specification and efficient movement of the border cells (Figure 1): Janus kinase/signal transducer and activator of transcription (**JAK/STAT**), **ecdysone** steroid hormone, Jun kinase (**JNK**), Hippo (**Hpo**)/Warts (Wts), and two receptor tyrosine kinases (**RTK**) activated by chemoattractants. Signaling through these pathways largely generate transcriptional outputs, often targeting adhesion, cytoskeletal, and polarity gene expression. In addition, multiple other transcription factors are required. Loss of function analysis demonstrates there are many different factors required for efficient border cell migration (Figure 2, Table 1). While the five major pathways initiate independently, they share some common targets. It is not yet known if transcription factors synergistically promote downstream target expression by binding to the same loci, but given that genome-wide analysis shows that many enhancers are highly occupied [11], it seems likely that signaling may converge on a few critical targets.

Post-transcriptionally, protein regulatory circuits transduce cell identity information and environmental cues into cluster movement (Figure 1C). Subcellular and cluster-level protein localization and activity are established. Strong adhesions confine polar cells into the center of the cluster. Apical–basal polarity proteins maintain cluster organization, partly through restricting localization of adhesion molecules. Front/back polarity is generated by activated RTK signaling in response to graded chemoattractants at the leading edge. These patterns of polarized protein localization rely on vesicle trafficking components, particularly those in the Rab family, to be established and maintained. Finally, changes in the cytoskeleton are mediated both transcriptionally and post-translationally through the Rho family of GTPases and a variety of regulatory proteins. These multi-tiered regulatory networks provide a robust and rapidly adjustable mechanism to move cells accurately, coordinately, and efficiently.

JAK/STAT Signaling

The polar cells play an important role in specifying border cells: they secrete cytokines, particularly Unpaired 1 and 3 (Upd1 and Upd3), which bind to a transmembrane receptor to trigger activation of JAK [12–17]. JAK phosphorylates STAT, which moves to the nucleus and activates transcription of a host of target genes [18,19]. This pathway has well-conserved roles in

Glossary

Cell protrusion: a general name for a small, elongated extension (at least 1 micron long) of the cell filled with cytoskeleton. Filopodia and lamellopodia are specialized types of protrusions, most easily characterized in cells moving across a two-dimensional surface. Protrusions in border cells are generated by F-actin polymerization and often arise due to Rac GTPase activity.

Ecdysone: the single steroid hormone in flies, responsible for many developmental transitions. Ecdysone activates a nuclear steroid hormone receptor, made up of Ecdysone Receptor (EcR) and Ultraspiracle, which acts as transcriptional regulator.

Gene regulatory network (GRN): parallel sets of regulatory cascades that function together to modulate gene expression resulting in an output such as cell fate.

Genetic circuit: a specialized type of genetic regulatory network that features feedback regulation, in which downstream targets regulate their upstream regulators. Feedback mechanisms can act positively or negatively.

Hippo (Hpo): a kinase and key regulator in a signaling pathway originally characterized for essential roles in organ size regulation. In flies, Salvador, Warts, and Mats are other core components of the pathway, and these signal to repress Yorkie, which regulates transcription. All signaling components have conserved members in mammals.

Janus kinase (JAK): a kinase activated when an associated receptor binds to its activator (Upd in the follicle cells). Activated JAK phosphorylates and activates STAT. *Drosophila* JAK is encoded by the single gene, *hopscotch*, while mammals have four family members.

Jun N-terminal kinase (JNK): kinase which canonically activates the Jun transcription factor (Jra in *Drosophila*). In border cells, this pathway promotes cluster cohesion.

Rac: part of the Rho family of small GTPases that direct changes in actin organization and dynamics.

Receptor Tyrosine Kinase (RTK): a cell surface molecule activated by chemoattractants secreted by the oocyte. In border cells, one RTK is an ortholog of mammalian epidermal growth factor receptor (EGFR), and

embryonic development, immunity, and stem cell fate, and contributes to cancer metastasis [19]. After activation by Upd, only four to eight cells with the highest STAT signaling levels complete the transition to become motile, while the rest shut down the pathway. A critical downstream target is *slow border cells* (*slbo*), which encodes a C/EBP transcription factor [20], and determines border cell fate. In addition, STAT promotes expression of another transcription factor, the pathway inhibitor *apontic* (*apt*) [21]. These studies provided a minimal genetic circuit sufficient to explain border cell specification. Recent studies paint a more complex picture of the required network and the importance of its tight regulation, and implicate other signal transduction cascades in this process.

Switching on the Activator

A number of factors influence Upd production and release from polar cells. First, multiple signaling pathways converge to specify the polar cells, including Hedgehog and Notch, which may induce *upd* expression [10,22–24]. Interestingly, recent studies suggest that restricting polar cell fate solely to two cells depends on STAT activity [25], and that Hpo signaling contributes to polar cell identity [26,27]. Reduction of Hpo and/or Wts function increases the number of polar cells and reduces STAT activity in border cells. Reduction of the negative regulator of the pathway, Yorkie (Yki), increases Upd expression levels and border cell numbers [26]. Further, Tousled-like kinase (Tlk) is an important signal in polar cells [28]. *upd* mRNA levels and the number of border cells are reduced upon *tlk* knockdown [28]. After transcription, motor protein complexes transport *upd* mRNA apically along microtubules, concentrating protein production and subsequent release to one side of the cell [16]. Upd extracellular diffusion towards neighboring anterior cells is likely influenced by the heparin sulfate proteoglycans, as in posterior follicle cells [29]. In addition, Upd distribution is likely altered by gaps between follicle cells and germ cells [30] (Box 1). Ultimately, approximately 6 cells receive high enough levels of STAT signaling to become motile border cells.

Rheostat Circuits Keep STAT Signaling Just Right

Border cells are very sensitive to STAT activity levels even after specification–too much or too little disrupts migration [31]. Thus, additional components act on the minimal genetic circuit, adding layers of regulation to optimize signaling. Interestingly, multiple genes turned on by STAT activity inhibit the pathway. These include *mir-279* [32] and *Socs36E* [31,33], both activated by Apontic. *mir-279* binds *Stat92E* mRNA, reducing its abundance and protein production, whereas Socs36E promotes Cullin-2-mediated ubiquitination and proteosomal destruction of a pathway component [34]. The STAT target Ken inhibits *mir-279* [32]. Protein tyrosine phosphatase at 61F (Ptp61F), often downstream of STAT activity, also functions in border cells to keep STAT signaling in check [35,36]. Brahma, a SWI/SNF-type chromatin remodeling protein, is required for efficient border cell recruitment via STAT signaling [36]. This recent result hints at the idea that chromatin remodeling may also regulate the acquisition of cell motility, adding an additional mechanism for signaling control.

In addition to its role in specification, STAT signaling is continuously required during migration, as evidenced using temperature-sensitive alleles of *Stat92E* [31]. The non-motile polar cells act as an Upd signaling center such that tightly-associated cells in the cluster have a migratory advantage. Negative feedback through Apontic continues; however, additional regulators are necessary after specification to maintain a 'steady' level of STAT activation and an optimal amount of the key STAT target, *slbo*. For example, the border cells require the PDZ-containing protein Big bang to promote activated, nuclear STAT [37]. During movement, STAT also signals to the Hedgehog (Hh) pathway, and canonical Hh signaling components including the transcription factor Cubitus interruptus (Ci) have essential roles within the cluster [38]. While Hh signaling is parallel to that of *slbo*, it affects cytoskeleton, adhesion, and polarity, and is necessary to maintain migration efficiency.

another is similar to both platelet-derived growth factor (PDGF) receptor and vascular endothelial growth factor (VEGF) receptor called PVR. Binding of ligand causes the receptors to become phosphorylated, and triggers a number of downstream events including activation of effectors in the Rho family and transcriptional activation via MAP kinase signaling.

Signal transducer and activator of transcription (STAT): a transcription factor that dimerizes and translocates to the nucleus after phosphorylation by JAK. Regulates expression of key border cell identity genes. There is one STAT protein in *Drosophila*, while there are seven in mammals.

Key Figure

Regulatory Circuits in Cell Specification and Cohort Migration

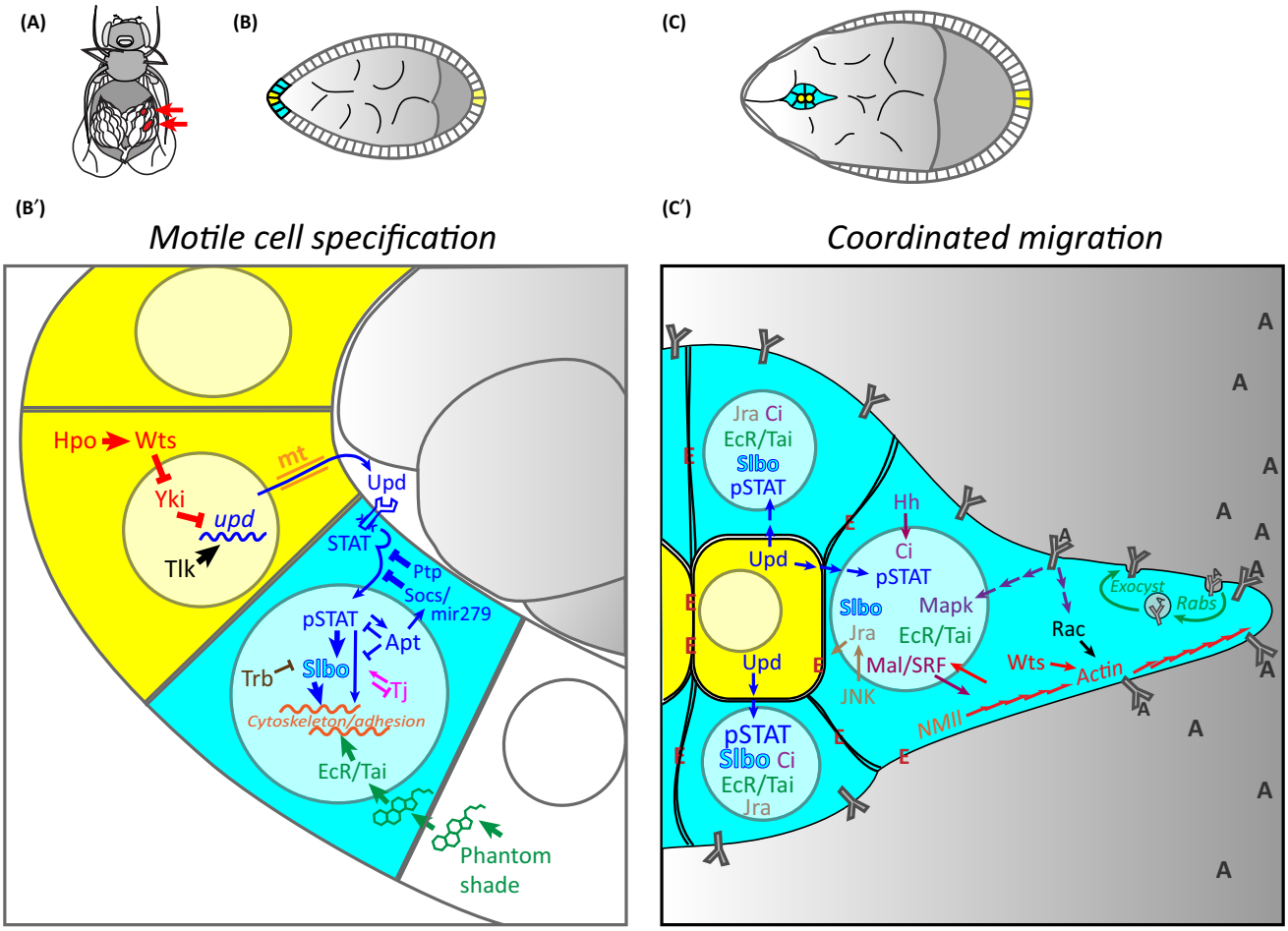

Figure 1. (A) The ovary fills the female *Drosophila* abdomen. Key oogenesis stages are red. (B) In a stage 8 egg chamber, the follicle cell epithelium surrounds large germline cells (gray), including the oocyte (dark gray). Anteriorly, border cells (cyan) neighbor the polar cells (yellow). (B′) Separate transcriptional programs govern polar cell and border cell function. In polar cells, Upd production depends upon Hpo signaling and Tlk, then it becomes localized apically along microtubules (mt). Secreted Upd is received by nearby cells, triggering activation of the STAT pathway and expression of Slbo, a border cell determinant. Transcriptional regulators converge on activated STAT (pSTAT) and Slbo in the nucleus; high expression of cytoskeletal and adhesion molecules depends on these and steroid hormone signaling through EcR and Tai. (C) Later, border cells detach from the epithelium and invade between adjacent germline cells. Other follicle cells rearrange. (C′) Regulation from stage 8 continues at stage 9, acting on STAT, Slbo, and EcR, and new layers of regulation are added as the cells interpret environmental cues. In the nuclei, only predominating transcriptional regulators are depicted. JNK signaling promotes cluster cohesion; E-cadherin 'E' maintains cell adhesions. Hh signals throughout the cluster. Border cells move up a concentration gradient of chemoattractant, denoted as 'A' and shading. Binding of chemoattractant to a receptor activates local changes in actin via Rac and MAPK signaling. Rab and exocyst components recycle plasma membrane, including guidance receptors. Wts, Mal-d, and NMII contribute to actin regulation; NMII maintains cluster shape. Apical–basal polarity would be oriented into the plane of the page. (See text for details.)

Fine-tuning Slbo

Multiple modes of regulation also act on the border cell determinant Slbo. Slbo promotes its own transcription in a positive feedback loop [21,39,40]. The transcription factor Traffic jam (Tj) normally activates *slbo* expression; however, at high levels it induces Socs36E-mediated repression of STAT activity and reduces *slbo* mRNA, as well as reducing the amount of

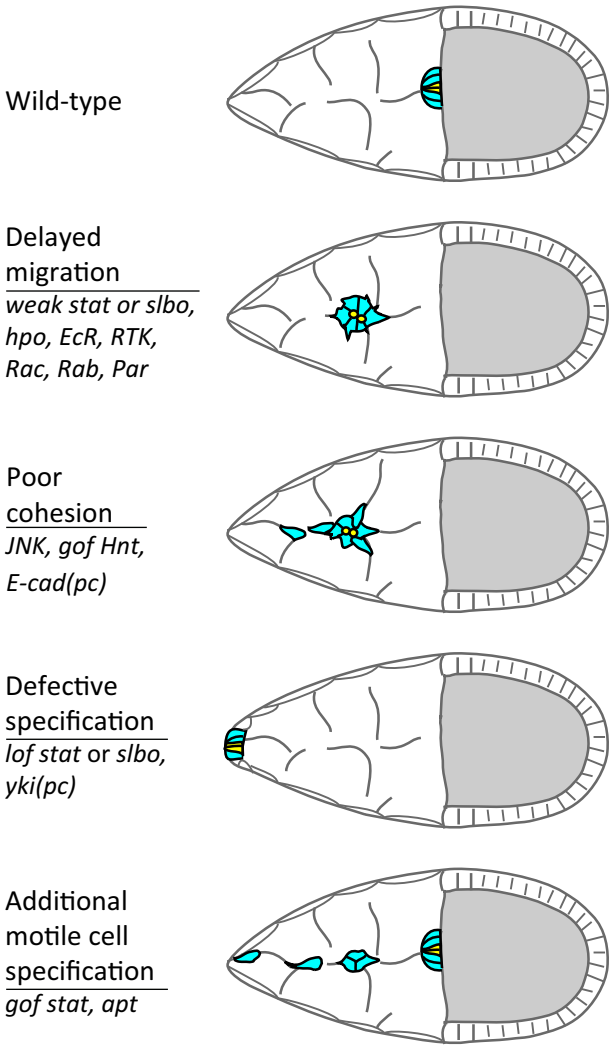

Figure 2. Classes of Border Cell Migration Mutant Phenotypes. Schematics of stage 10 egg chambers, a time at which border cell normally have reached the oocyte (gray), as in the wild type cartoon. Mutations in different transcription factors can result in each class of phenotype, and the 5 major classes of signaling pathways have overlapping phenotypic effects. Representative examples of genes that cause each phenotype upon disruption are listed; note that positive effector genes in the same pathway can result in similar mutant phenotypes. 'Delayed migration' is a commonly observed phenotype; this can also be caused by failures to detach from the anterior (not shown). Hypomorphic (weak) mutations in STAT pathway components or *slbo* can produce this effect; disruption of steroid hormone receptor (EcR), receptor tyrosine kinase (RTK) signaling, actin regulators, Rabs, and polarity regulators often yield this phenotype. Reduction of Jun kinase (JNK) signaling, overexpression of Hnt, or reduction of E-cad in polar cells results in splayed-out cluster morphology, as in the 'poor cohesion' example. Null mutations (loss of function, lof) in *Stat92E* or *slbo* result in few to no motile cells ('defective specification'), although with some alleles or mutations in pathway components, a small number of cells become motile (not shown). Increased activation of those pathways (gain of function, gof), or mutations in negative regulators like *apt* result in 'additional motile cell specification', with some cells trailing behind the main cluster. When additional motile cells arise, the main cluster may or may not be able to complete migration.

E-cadherin [41]. Thus, Tj must be downregulated in border cells at the start of their migration. Similarly, Hindsight (Hnt) can downregulate Slbo and STAT activity [42]. The Tribbles (Trbl) kinase dampens Slbo function at the protein level, probably by phosphorylating it to promote its turnover [40,43]. However, Slbo reduces Trbl expression, so this regulator (like Tj) normally drops as border cells migrate [43]. Slbo marked for turnover can be restored by a ubiquitin hydrolase [40]. Eventually, sufficient Slbo must be present for border cell movement. When Slbo 'wins' the expression battles downstream of STAT, it induces expression of a number of motility effectors including E-cadherin (*shotgun*) [44], Jing [45], and focal adhesion kinase [39,46].

Wiring a Clock: Ecdysone and Timing

Specification is not sufficient for border cell migration away from the epithelium; instead, cells require a 'go' signal provided by the steroid hormone ecdysone. Ecdysone receptor (a heterodimer of EcR and Ultraspiracle) and the coactivator Taiman mediate the transcriptional response to this timing cue [47–50]. Interestingly, all follicle cells can synthesize the active ecdysone, but only the border cell cluster and a few other anterior cells respond to it. Large mutant clones of key synthesis enzymes (encoded by *phantom* and *shade*), encompassing the majority of follicle and

Table 1. Transcription Factors Required in Border Cell Specification and/or Movement

Symbol	Full name	Upstream signal	Human orthologs	Refs
Ab (*repressed*)	Abrupt	(repressed by STAT and EcR)	ZBTB37, ZBTB22	[48]
Aop	Anterior open	STAT, N, Map kinases downstream of RTKs	ETV6, ETV7	[46,100]
Apt	Apontic	STAT, Eya, (repressed by Slbo), feedback inhibition on STAT signaling	Fibrinogen Silencer Binding Protein	[21]
Brk	Brinker	Dpp signaling (indirect evidence)	DMRT-like	[101]
Ci	Cubitus interruptus	Hh signaling	Gli 1-3	[38]
Ct (*repressed*)	Cut	Notch, (Slbo represses)	CUX1, CUX2	[102]
EcR/USP (heterodimer)	Ecdysone receptor/ Ultraspiracle	Ecdysone steroid hormone	nuclear steroid hormone receptor/ retinoid x receptor	[47–50]
pErk (present, not shown to be required)	Rolled	Map kinases downstream of RTKs	Erk1, 2	[65]
Eya	Eyes absent	Suppressed by *ci*, N	Eya 1-4	[103]
Hnt (Peb)	Hindsight (aka Pebbled)	Notch	RREB1	[10,42]
Jing	Jing	Slbo	AEPB2	[45]
Jra	Jun related antigen	JNK signaling	Jun (AP-1)	[42,52]
Ken	Ken and Barbie	STAT, feedback inhibits	BCL-6	[32]
N/ Su(H) (follicle cells)	Notch/ Suppressor of Hairless	Delta	Notch1-3/ RBPJ1	[100,104,105]
Six4	Six4	Slbo (indirect evidence)	Six4, Six5	[46]
Slbo	Slow border cells	STAT signaling, Slbo, (repressed by Hnt, Apt, Ct)	C/EBP	[12,14,20,40,102]
SRF/Mal-d (MRTF)	Serum response factor/ Myocardin-related transcription factor	Actin	SRF/Myocardin related (MRTF)	[93–95]
STAT	Signal transducer and activator of transcription	Extracellular cytokines (Upd family), transmembrane receptor, Janus Kinase	Stat 3, Stat 5A,5B	[12–17]
Tai (EcR coactivator)	Taiman	Binding to EcR complex + ecdysone	SRC3 (aka AIB3, NCOA3)	[50]
Tj	Traffic Jam	Slbo inhibits	MAF	[41]
Ttk	Tramtrack	Slbo	ZBTB45, 39, 37, 24, 17	[39]
Yki (polar cells)	Yorkie	Hpo signaling	YAP1, WWTR1	[26,69]

nurse cells, still permitted border cell migration, even when border cells or germline cells were mutant, demonstrating a nonautonomous function of this signal [51]. Interestingly, there appears to be a threshold level needed, as larger clones resulted in less efficient cluster migration. To promote cluster movement, Ecdysone signaling mediates the subcellular localization of E-cadherin towards the interior of the cluster, maintaining lower levels at the periphery. EcR transcriptional target(s) mediating this are not known. It is also not clear how ecdysone signaling is integrated with other signaling pathways, although Abrupt provides one point of overlap [48].

Ready, Set, Go! Protein Activity Circuits Induce and Reinforce Directional Movement

Soon after motility is established by transcription factors, protein regulatory networks convey information about cell cluster morphology and the direction to the oocyte. Transduction cascades downstream of these signals refine and update transcriptional responses, for example upregulating *actin* expression, which feeds forward to allow rapid and robust responses to all inputs.

Box 1. Impact of Tissue Structure on Signaling

In addition to genetic tools, another important advantage of studying *Drosophila* is the ability to observe cell behaviors within their normal environment. Many studies have examined movements of cells cultured in substrates such as Matrigel. The small transparent tissue of the ovary and ability to culture egg chambers allows direct observation of the interactions between cells that could not otherwise be appreciated [8]. Several recent studies illustrate this idea.

Tissue architecture plays an unexpected role in border cell specification. Since Upd is released apically from two anterior cells amidst a field of follicle cells, one would expect all neighboring cells to have activated STAT. Surprisingly, gaps often arise in the STAT activation patterns (Figure IA, activation in green) around the polar cells (yellow), even though all follicle cells are competent to respond to the pathway [30]. Imaging, genetic, and mathematical modeling data support the idea that the contours of large, adjacent germline cells impact diffusion of the activator (Upd) and thus receipt of the signal. It will be interesting to explore this nonautonomous effect of tissue topology in more detail.

Other work is starting to examine physical forces and how these influence signaling. Aranjuez *et al.* uncovered a role for nonmuscle myosin II in the border cells as well as the substrate through which they migrate, the germline cells [92]. This study reveals that myosin is needed to maintain the streamlined shape of the moving cluster to resist forces from surrounding cells, and that greater force from germline cells increased myosin levels, presumably as an oppositional force. Similar types of haptotaxis, the movement along graded substrate stiffness, are observed in mammalian morphogenesis events and cancer (reviewed in [99]).

The dimensions of the border cell cluster and how it fits between cells along the migration path are also important for efficient movement (Figure IB). Border cells squeeze between germline cells to get to the oocyte, but they can be misguided, indicating that they are chemotaxing, not just following the path of least resistance [60,61]. Manipulations of cell number or organization and mathematical modeling support the idea that there is an optimal range for cluster size [106,107]. Small clusters likely have less traction, but larger ones also delay migration, probably due to resistance from the germline. Overall, tissue architecture plays a key role in determining how cells move in space and time, and multidisciplinary research will improve our understanding of this phenomenon.

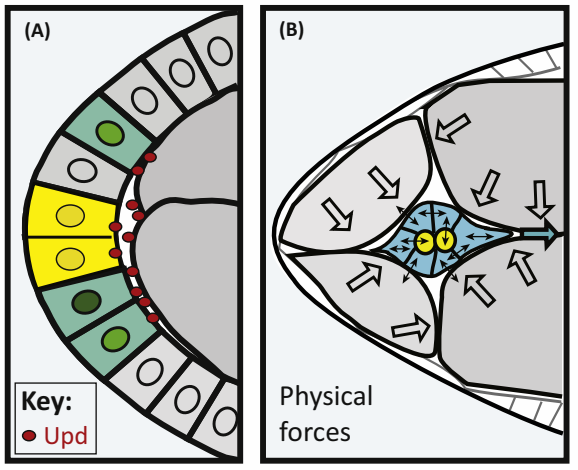

Figure I. Interaction of Tissue Structure, Signaling, and Collective Cell Migration. (A) At the anterior of the egg chamber (in cross section), polar cells (yellow) release Upd (red circles) apically towards the large germline cells. In the follicle cell epithelium, the pattern of STAT activity (green) has a gap (single gray cell above polar cells), suggesting that the Upd activator may be be unevenly distributed spatially due to underlying tissue contours. Cells with highest STAT signaling will become the motile border cells. (B) As the border cells (cyan) and polar cells (yellow) invade between nurse cells (gray) to migrate, many physical forces influence their movements. Forces within the cluster (small arrows) mediated by actin and myosin pull the cells together but also resist opposing forces from the germline (large arrows). In addition, the border cells actively extend protrusions forward (cyan arrow) to push their way towards the oocyte.

Polarity Prepares the Cluster from the Inside Out

The Jun N-terminal kinase (JNK) signaling cascade is essential for maintenance of cluster cohesion [42,52], which is necessary for efficient migration. JNK regulates apical–basal polarity

Box 2. Connecting with the Neighbors

There is much interest in understanding how cells of a motile collective communicate with each other to allow coordinated movement. Adhesion molecules clearly help by transducing mechanical forces from one cell to the next, and may have more active signaling roles as well. In border cells, the homophilic adhesion molecule E-cadherin plays an active role in conveying physical forces from one cell to another. E-cadherin at high levels between polar cells and border cells holds the cluster together, while at lower levels at the border cell periphery and in germline cells, it allows the border cells to 'grab on' to their substrate and pull forward [44]. An E-cadherin fluorescent tension-sensor expressed in border cells indicated that force promotes cell–cell communication, and that E-cad 'feels' attachments along nurse cells for the cluster to proceed forward. This signal is continuously modulated as actin-based protrusive activity changes the amount of tension to promote forward movement and cluster cohesion around the polar cells [57].

The vesicle trafficking regulator Rab11 is also required for cell-cell communication. Usually, if Rac activity is blocked in one leading cell of the cluster, other cells will extend protrusions frontward. Intriguingly, this does not occur in clusters bearing a dominant-negative form of Rab11; instead cells extend protrusions in all directions, suggesting they cannot follow the leader [108]. However, the molecular machinery underlying this communication is not yet known.

Recently, Anllo and Schüpbach showed that the G-protein coupled receptor Rickets (Rk) and its ligand, Bursicon, function in border cells to regulate cluster detachment and migration [109]. Rk is involved in localization of apical proteins and enrichment of E-cadherin to border cell–border cell contacts. Mosaic clonal analysis indicates that differences in Rk signaling between cells of the cluster disrupted detachment and migration, but migration was normal if all cells of the cluster were mutant. This suggests that *rk* mediates cell–cell communication and collective behavior of the cells in the group. Together, these findings provide a basis for further study of this interesting aspect of collective cell migration.

and a rounded cluster morphology [52], potentially acting downstream of actin regulator Cdc42. This cascade ends in activation of the transcription factor Jun, Jra in flies. Hnt opposes JNK signaling. Hnt overexpression experiments suggest that low JNK can be rescued by high expression of the adhesion molecule E-cadherin [42]. Homophilic E-cadherin adhesions seem to aid in cell–cell communication, along with several other factors (Box 2). JNK may also function through integrin regulation; low integrin leads to cell dissociation [53]. The inability of non-clustered cells to migrate effectively reflects the importance of cohesiveness, cell communication, and cluster organization.

Apical–basal polarity is required for efficient border cell migration, presumably because it allows organization of the cluster so that high adhesion is towards the center, and signaling receptors are concentrated towards the outside. JNK, Par1, Pak3 and canonical apical–basal polarity proteins such as Scribble, Crumbs, Par6, aPKC, Patj1, and Discs large, are all needed for polarization [52,54–56]. The maintenance of apical–basal complexes reflects the sustained epithelial character of the border cell cluster, and their acquisition of motility is sometimes called a partial epithelial-to-mesenchymal transition. The polar cells are most obviously polarized, and their high expression of E-cadherin is required to organize the structure of the collective, which improves migratory efficiency [57]. Interestingly, dominant negative Rac, which alters actin filaments, also disrupts polarity markers and the cluster becomes disorganized, as in *Pak3* and *Jra* mutants [56]. This result links the cytoskeleton to apical–basal polarity maintenance.

Finding a Destination and Moving: a Chemical GPS

In spite of the complex gene expression circuitry, the border cell cluster follows a direct and linear path to the oocyte. Border cells move towards several chemoattractants that are predominantly made in the oocyte and expressed in a graded fashion across the egg chamber (reviewed in [5,6,58,59]). Misdirection experiments established three chemoattractants that are sufficient for guidance [60,61]. The chemoattractants bind and activate the PVR and EGFR receptor tyrosine kinases (RTKs), establishing the leading edge of the cluster and forward direction for movement. RTKs, activated by increasing concentrations of their ligands along the migration path, turn on the small GTPase **Rac** near the cluster front, via the guanyl-nucleotide exchange factors Myoblast city (Mbc) [62,63] and Vav [64]. This increases formation of Rac-mediated actin-based **cell protrusions**. The receptor tyrosine kinases additionally activate signaling by MAPK

[65], and intersect the JNK signaling pathway [66]. In response, cellular extensions occur around the cluster periphery but are reinforced pointing forward, towards the oocyte, stimulating forward translocation. Early on, one cell of the cluster tends to lead the collective movement, but later, the cluster can 'tumble' and leading cells will cycle in and out of the front [65,67]. While tumbling may be the most efficient mechanism to move the cluster forward, it is not clear that it is required. Intriguingly, the migratory polarity of the cluster overall appears to depend on apical–basal polarity, as disruptions of the latter lead to smaller and shorter-lasting actin-based forward protrusions [56,68]. This result suggests there is feedback regulation between apical–basal polarity complexes and leading-lagging polarity regulators, which promotes actin outgrowth at the leading edge.

Refining Cytoskeletal Outputs via Hippo Signaling

New research shows that the Hpo/Wts pathway also plays a key role in cytoskeletal regulation in the border cells (in addition to its role in the polar cells) [26,69]. Hpo activators, including Expanded, Merlin, and Kibra, localize to the border cell–border cell junctions near the apical polarity components aPKC and Crumbs [69]. In canonical signaling, Hpo phosphorylates Wts, whose activity suppresses Yki [27], as in polar cells [26]. While *hpo* and *wts* are needed to maintain F-actin and activated nonmuscle myosin II (NMII) localization towards the outside of the migrating border cell cluster [26,69,70], *yki* is not required in outer border cells, and its over-expression does not slow migration [69]. Lucas *et al*. suggest a noncanonical role for Wts in directly regulating actin polymerization by phosphorylating Enabled (Ena). Higher Hpo signaling led to mislocalization/accumulation of F-actin [69]. Interestingly, in some contexts, Hpo signaling responds to cell tension and cytoskeletal structure [71]. It is possible that α- and β-spectrin may work in this way to promote *hpo* signaling in border cells and maintain F-actin organization, cluster morphology, and efficient migration [72].

Recycling Circuit: Endocytosis/Exocytosis Regulation

Leading-edge activation to promote forward movement in response to chemoattractants utilizes a number of endocytic regulators, presumably to localize/enrich receptors and responses to one area of the cell. Prior work established requirements for dynamin (Shibire), Rab5, and the Rab GEF Sprint in endocytosis of the activated receptors [31,73–75]. In contrast, missing in metastasis (DMim) inhibits endocytic turnover of membrane proteins, independently of clathrin, by blocking the proendocytic complex cortactin/cindr (CD2AP)[76]. This helps to balance positive and negative regulators.

Recent studies using dominant-negative mutants and RNA interference for vesicle-trafficking proteins showed requirements for recycling endosomes in border cells. Disruption of Rab 11 [74], the Rab11 negative regulator Evi5 [77], or exocyst components including Sec3 [78] and Sec 15 [74] tends to cause widespread localization of activated guidance receptors (instead of mainly at the front) and reduces the ability of clusters to complete migration, probably due to poorly-localizated actin regulation [74] (Box 2). A sensible model is that RTKs are continuously removed, reset, and recycled to the front. Wan *et al.* argue that the enrichment of exocyst components and Rab11-containing recycling vesicles at the leading edges of the cluster primes the front cell to respond more quickly to guidance receptor activation, thereby amplifying the response [78]. Rac signaling and F-actin promoted this Rab11 + vesicle enrichment and E-cadherin-containing vesicles required *sec3* function to be deposited at interior cluster cell surfaces. Given the rotation of the cluster, though, it is still unclear how activation signaling can rapidly change as cells switch frontal positions, or how cell-cell communication occurs, and more studies are needed.

Vesicle trafficking is also implicated in cluster polarization. The Myopic (Mop) enzyme interacts with Rab4 to localize beta-PS-integrin at cell cortices [79] and maintain cluster integrity/coordination (independent of its possible phosphatase activity). This signaling may connect to integrin regulation

Box 3. Common Genetic Regulators between Border Cell Migration and Tumor Invasion

Physical similarities are evident between the movements of border cells and certain carcinomas that migrate as a collective; both undergo a partial type of epithelial to mesenchymal transition that preserves epithelial polarity and some adhesions (reviewed in [7,99]). Recent studies demonstrate similarities at the level of the gene regulatory networks, and a few are highlighted here.

Both the Notch and STAT pathways contribute to cancer progression, and they may synergize. Patient sample studies correlated metastatic prostate cancer [110] and poor breast cancer prognosis [111] with high expression of the Notch ligand Jagged1. Upregulation of Jagged1 in human breast cancer cell lines led to frequent metastases to bone [112,113]. Studies in mice indicate that Jagged1 from the cancer cells activates Notch in the bone microenvironment, which supports metastasis by two mechanisms: Notch induces expression of STAT activator IL-6 in the osteoblast cells, which feeds back to the breast cancer cells and promotes proliferation, and it supports osteoclast cell identity. This resembles the regulatory relationship between Notch and UPD/STAT in the polar and border cells. It would be interesting to know if the human homologs of other border cell regulators affect the paracrine signaling in bone metastasis of breast cancer.

Several studies reinforce the concept that STAT activation promotes metastasis by cytoskeletal regulation [98]. In human prostate cancer cells, JAK2/STAT3 contributes to metastatic ability by altering the actin and microtubule dynamics [114] and STAT5 activation is linked to metastasis to bone and distant organs [115]. Activated STAT5 increased microtubule polymerization, reduced E-cadherin, and enhanced cancer cell adhesion to endothelial cells, which may explain its pro-metastatic effects.

In mice, loss of a copy of Neurofibromatosis type 2 (Nf2), the mammalian ortholog of *Drosophila* Hippo pathway component Merlin, leads to highly metastatic osteosarcoma [116]. Since loss of Nf2 in human only leads to benign tumors [117], it is tempting to speculate about a connection between Nf2 and F-actin dynamics, similar to the function suggested for Merlin in flies [69].

Functional conservation between *Drosophila* and human genes used during cell migration is not limited to transcriptional regulators, but also includes effector molecules. For example, Lamellipodin, like its fly ortholog Pico, regulates actin dynamics to promote formation of cell protrusions in various cells, including a breast cancer cell line [88].

Another example is that human HDPTP affects the migratory characteristic of breast cancer cells in a similar manner to that of its *Drosophila* homolog Mop. HDPTP contributes to distribution of actin cytoskeleton regulators and recycling of integrin in a Rab4-dependent manner, the same mechanism by which Mop regulates border cell migration [79].

by JNK signaling, as it does in other contexts. This is one of multiple cases of genetic-based regulation found to be shared between cancer invasiveness and border cell migration (Box 3). Vesicle transport studies in flies suggest that endocytic and exocytic regulation could be key nodes in cell migration dynamics.

Dynamic Hardwiring: Actin Regulation

Actin dynamics governs most types of cell movements, and a host of proteins act to change actin organization from monomers to polymers, filaments, and branched structures [80]. In border cells, laser-mediated activation experiments demonstrated that Rac activation is sufficient for guided forward movement, and this signal likely acts immediately downstream of guidance receptor signaling [81]. Coordinated by Rac function, a number of actin regulators influence border cell migration, including Cofilin [82], Profilin [83], Enabled (Ena/VASP), and Capping proteins [84], and newer players Diap1 [85] and Psidin [86] (reviewed in [87]). Newer studies show that Rac can function through the Lamellopodin homolog Pico, which interacts with Scar/WAVE–ARP2/3 complexes to promote branched actin filaments, create forward-directed cellular extensions, and move cells forward [88]. Additionally, Glial Maturation Factor (GMF) acts to modulate directional protrusion dynamics and migration speed by destabilization of branched actin filaments, allowing new protrusions to form [89]. GMF strongly interacts genetically with *flare*, which encodes the cofilin cofactor Aip1, and, like *cofilin* mutants, loss of *flare* resulted in poor border cell migration and accumulation of F-actin [89]. Thus, regulated loss and gain of branched actin filaments can optimize cell dynamics.

Disruptions in signaling between the chemoattractant receptors and cytoskeletal elements can yield abnormal rotational behavior of the migrating cluster, well appreciated in live imaging studies. Such is the case when Pico is overexpressed and actin filaments are enriched throughout the cluster perimeter [88]. Similarly, myosin II (NMII), which is required downstream of Par-1 for efficient detachment from the epithelium [90], also functions downstream of guidance receptors to promote rotation. Myosins generate contractile forces along actin networks. Knockdown of NMII or upregulation of a negative regulator decreased both overall speed and rotational speed, while expression of a constitutive activator decreased speed overall but increased early rotational speed, with less persistent linear movement [91]. Genetic epistasis experiments suggest that in the early phase when the border cells do not rotate, the predominant RTK inhibits NMII; however, during the late rotational migration phase, a different RTK activates NMII. Additional work shows that NMII is needed both in the border cell cluster to maintain a streamlined morphology, and in germline cells to balance physical, migratory forces [92] (Box 1).

Actin Acting on Actin

Another instance of feedback from physical signaling to molecular signaling is found in an actin-sensing mechanism in which cytoskeletal effectors signal back to gene expression in the nucleus. Serum response factor (SRF) DNA binding protein and its transcription cofactor Mal (MRTF/Mal-d) are required during border cell migration to sense tension and promote movement [93]. Biochemical analysis and studies in cell culture [94] demonstrated that Mal function is modulated by free actin monomers. Studies of the border cells and other actin-rich structures suggest the key downstream target for SRF/Mal-d is the *Actin 5C* gene: overexpression of Actin 5C is sufficient to rescue loss of *MRTF* and restore migration [95]. This mechanism provides a simple homeostatic feedback circuit to maintain an optimal amount of free actin even as it is lost to polymer growth. How physical inputs influence molecular signaling will continue to be of great interest as more relationships like these are found.

Additional Contributions of Cytoskeletal Components

While actin regulation is critical for border cell migration, microtubules (MT) play an important but less dynamic role. Proper MT organization is required in the polar cells for the polarized release of the JAK/STAT activator [16]. Treatment of egg chambers with drugs that disrupt microtubules reduce border cell cluster movement speed, suggesting an active requirement. Loss of function mutations in the MT depolymerizing factor Stathmin result in slower migration and defects in detachment [96]. Knockdown of Dynein and motor-associated proteins also slowed migration speed, reduced the size and frequency of initial cell protrusions, and altered adhesion proteins. This suggests the overall organization of the cluster depends in part on MT organization, which promotes efficient movements.

A recent screen for genes impacting border cell migration identified a variant of tropomyosin, Tm1-I/C, that polymerizes into intermediate filament-like structures [97]. It had been proposed that fly cytoskeleton did not include intermediate filament proteins, but this work suggests there is a functional ortholog with low sequence conservation. Filaments of Tm1-I/C are necessary for epithelial architecture, germ line development, and border cell migration. Further *Drosophila* genetics is poised to continue to uncover functions for novel genes.

Concluding Remarks

The field of cell migration has itself moved rapidly over the past few decades, revealing much of the molecular machinery that governs this process. Progress is particularly notable in understanding how groups of cells, like the fly ovarian border cells, undergo coordinated movements *in vivo*. Remarkably, many of the genetic regulators controlling border cell migration have homologs involved in tumor invasion [7,98,99] (Box 3). *In vitro* systems have provided invaluable

Outstanding Questions

How does tissue environment contribute to cell signaling and movement? Border cell specification and migration provides several examples in which gaps or tension in tissue structure alters cell signaling. Interdisciplinary approaches and the simple structure of the egg chamber can be leveraged to explore these physical influences.

What are the key, conserved nodes within migration genetic networks? Multiple genetic pathways are required for border cell migration, but it is unclear what kinds of 'cross-talk' enable the signals to synergize. What are the nodes of integration? Do pathways converge on a few critical effectors? Which components are functionally conserved in other collective cell migrations?

How does chromatin regulation affect cell migration? With tight regulation on transcriptional networks during cell migration, chromatin remodeling factors may impinge on signaling. It will be interesting to explore how chromatin organization contributes to cell motility and its genetic circuitry.

Do additional adhesion molecules contribute to cluster cohesion and movement? E-cadherin plays a central role in border cell organization, but other adhesion molecules are expressed as well. These may function redundantly. With newer genetic tools, adhesion proteins can be disrupted in sets to determine overlapping roles.

How is steroid hormone signaling regulated, and what are its targets? How is the action of widely-produced steroid hormone restricted to a subset of cells? What key transcriptional targets mediate the effect of ecdysone signaling on cell adhesion? How do nuclear hormone receptor targets compare across different organisms?

How is polarity maintained within a motile cell cluster? As it moves, the border cell cluster requires cell polarity along three axes: apical–basal, internal–peripheral, and leading–lagging. How these patterns are maintained and coordinated at the subcellular and supracellular level is an active area of further study.

information about the physical mechanics of cell migration, especially with respect to the cytoskeleton and its regulators, implying that transcription is not really needed. In border cells, we find that multiple signal transduction cascades continuously provide information to motile cells to change their transcriptional output, and these events are highly regulated. We speculate that continued transcriptional regulation during movement allow cells to adjust to a changing context as they migrate, which might not be necessary in the stable environment of a tissue culture dish. Decoding the genetic circuits of cells like these *in vivo* may ultimately allow researchers to 're-wire' cell types and thereby control cell behaviors, with major implications in tissue engineering and medicine.

As always, increased knowledge has led to new questions in how cohorts of cells move (see Outstanding Questions). The amenability of *Drosophila* to genetics, live imaging, modeling, and biochemical experiments positions this organism well to continue to help us advance our understanding of cell migration.

Acknowledgments

We thank Cale Whitworth and Cynthia Wagner for helpful comments on the manuscript, and Denise Montell for sharing data prior to publication. This work was supported in part by grant NIH-NIGMS 2-R25-GM55036 to A.S., and an NSF-CAREER award IOS-1054422 to M.S.G.

References

1. Simões-Costa, M. and Bronner, M.E. (2015) Establishing neural crest identity: a gene regulatory recipe. *Development* 142, 242–257
2. Levine, M. and Davidson, E.H. (2005) Gene regulatory networks for development. *Proc. Natl. Acad. Sci. U.S.A.* 102, 4936–4942
3. Madhamshettiwar, P.B. *et al.* (2012) Gene regulatory network inference: evaluation and application to ovarian cancer allows the prioritization of drug targets. *Genome Med.* 4, 41
4. Emmert-Streib, F. *et al.* (2014) The gene regulatory network for breast cancer: integrated regulatory landscape of cancer hallmarks. *Front. Genet.* 5, 15
5. Aman, A. and Piotrowski, T. (2010) Cell migration during morphogenesis. *Dev. Biol.* 341, 20–33
6. Mayor, R. and Etienne-Manneville, S. (2016) The front and rear of collective cell migration. *Nat. Rev. Mol. Cell Biol.* 17, 97–109
7. Friedl, P. and Gilmour, D. (2009) Collective cell migration in morphogenesis, regeneration and cancer. *Nat. Rev. Mol. Cell Biol.* 10, 445–457
8. Hudson, A.M. and Cooley, L. (2014) Methods for studying oogenesis. *Methods* 68, 207–217
9. Spradling, A.C. (1993) Developmental genetics of oogenesis. In *The Development of Drosophila melanogaster* (Bate, M. and Martinez Arias, A., eds), pp. 1–70, Cold Spring Harbor Laboratory Press
10. Klusza, S. and Deng, W.M. (2011) At the crossroads of differentiation and proliferation: precise control of cell-cycle changes by multiple signaling pathways in *Drosophila* follicle cells. *Bioessays* 33, 124–134
11. Roy, S. *et al.* (2010) Identification of functional elements and regulatory circuits by *Drosophila* modENCODE. *Science* 330, 1787–1797
12. Silver, D.L. and Montell, D.J. (2001) Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in *Drosophila*. *Cell* 107, 831–841
13. Ghiglione, C. *et al.* (2002) The *Drosophila* cytokine receptor Domeless controls border cell migration and epithelial polarization during oogenesis. *Development* 129, 5437–5447
14. Beccari, S. *et al.* (2002) The JAK/STAT pathway is required for border cell migration during *Drosophila* oogenesis. *Mech. Dev.* 111, 115–123
15. Xi, R. *et al.* (2003) A gradient of JAK pathway activity patterns the anterior–posterior axis of the follicular epithelium. *Dev. Cell* 4, 167–177
16. Van de Bor, V. *et al.* (2011) Asymmetric localisation of cytokine mRNA is essential for JAK/STAT activation during cell invasiveness. *Development* 138, 1383–1393
17. Wang, L. *et al.* (2014) Pleiotropy of the *Drosophila* JAK pathway cytokine Unpaired 3 in development and aging. *Dev. Biol.* 395, 218–231
18. Arbouzova, N.I. and Zeidler, M.P. (2006) JAK/STAT signalling in *Drosophila*: insights into conserved regulatory and cellular functions. *Development* 133, 2605–2616
19. Amoyel, M. *et al.* (2014) JAK/STAT pathway dysregulation in tumors: a *Drosophila* perspective. *Semin. Cell Dev. Biol.* 28, 96–103
20. Montell, D.J. *et al.* (1992) slow border cells, a locus required for a developmentally regulated cell migration during oogenesis, encodes Drosophila C/EBP. *Cell* 71, 51–62
21. Starz-Gaiano, M. *et al.* (2008) Feedback inhibition of Jak/STAT signaling by apontic is required to limit an invasive cell population. *Dev. Cell* 14, 726–738
22. Dobens, L.L. and Raftery, L.A. (2000) Integration of epithelial patterning and morphogenesis in *Drosophila* ovarian follicle cells. *Dev. Dyn.* 218, 80–93
23. Assa-Kunik, E. *et al.* (2007) *Drosophila* follicle cells are patterned by multiple levels of Notch signaling and antagonism between the Notch and JAK/STAT pathways. *Development* 134, 1161–1169
24. Horne-Badovinac, S. and Bilder, D. (2005) Mass transit: epithelial morphogenesis in the *Drosophila* egg chamber. *Dev. Dyn.* 232, 559–574
25. Borensztejn, A. *et al.* (2013) JAK/STAT autocontrol of ligand-producing cell number through apoptosis. *Development* 140, 195–204
26. Lin, T.H. *et al.* (2014) The Hippo pathway controls border cell migration through distinct mechanisms in outer border cells and polar cells of the Drosophila ovary. *Genetics* 198, 1087–1099
27. Irvine, K.D. and Harvey, K.F. (2015) Control of organ growth by patterning and hippo signaling in *Drosophila*. *Cold Spring Harb. Perspect. Biol.* 7, a019224
28. Xiang, W. *et al.* (2016) Tousled-like kinase regulates cytokine-mediated communication between cooperating cell types during collective border cell migration. *Mol. Biol. Cell* 27, 12–19
29. Hayashi, Y. *et al.* (2012) Glypicans regulate JAK/STAT signaling and distribution of the Unpaired morphogen. *Development* 139, 4162–4171

30. Manning, L.A. *et al.* (2015) Tissue landscape alters adjacent cell fates during *Drosophila* egg development. *Nat. Commun.* 6, 7356
31. Silver, D.L. *et al.* (2005) Requirement for JAK/STAT signaling throughout border cell migration in *Drosophila*. *Development* 132, 3483–3492
32. Yoon, W.H. *et al.* (2011) miRNA-mediated feedback inhibition of JAK/STAT morphogen signalling establishes a cell fate threshold. *Nat. Cell Biol.* 13, 1062–1069
33. Monahan, A.J. and Starz-Gaiano, M. (2013) Socs36E attenuates STAT signaling to optimize motile cell specification in the *Drosophila* ovary. *Dev. Biol.* 379, 152–166
34. Monahan, A.J. and Starz-Gaiano, M. (2015) Socs36E limits STAT signaling via Cullin2 and a SOCS-box independent mechanism in the *Drosophila* egg chamber. *Mech. Dev.* 138 (Pt 3), 313–327
35. Baeg, G.H. *et al.* (2005) Genome-wide RNAi analysis of JAK/STAT signaling components in *Drosophila*. *Genes Dev.* 19, 1861–1870
36. Saadin, A. and Starz-Gaiano, M. (2016) Identification of novel regulators of the JAK/STAT signaling pathway that control border cell migration in the Drosophila ovary. *G3 (Bethesda)* 6, 1991–2002
37. Aranjuez, G. *et al.* (2012) On the role of PDZ domain-encoding genes in Drosophila border cell migration. *G3 (Bethesda)* 2, 1379–1391
38. Geisbrecht, E.R. *et al.* (2013) Genetic interaction screens identify a role for hedgehog signaling in *Drosophila* border cell migration. *Dev. Dyn.* 242, 414–431
39. Wang, X. *et al.* (2006) Analysis of cell migration using whole-genome expression profiling of migratory cells in the *Drosophila* ovary. *Dev. Cell* 10, 483–495
40. Rørth, P. *et al.* (2000) The level of C/EBP protein is critical for cell migration during *Drosophila* oogenesis and is tightly controlled by regulated degradation. *Mol. Cell* 6, 23–30
41. Gunawan, F. *et al.* (2013) The Maf factor Traffic jam both enables and inhibits collective cell migration in *Drosophila* oogenesis. *Development* 140, 2808–2817
42. Melani, M. *et al.* (2008) Regulation of cell adhesion and collective cell migration by hindsight and its human homolog RREB1. *Curr. Biol.* 18, 532–537
43. Masoner, V. *et al.* (2013) The kinase domain of *Drosophila* Tribbles is required for turnover of fly C/EBP during cell migration. *Dev. Biol.* 375, 33–44
44. Niewiadomska, P. *et al.* (1999) DE-cadherin is required for intercellular motility during *Drosophila* oogenesis. *J. Cell Biol.* 144, 533–547
45. Liu, Y. and Montell, D.J. (2001) Jing: a downstream target of slbo required for developmental control of border cell migration. *Development* 128, 321–330
46. Borghese, L. *et al.* (2006) Systematic analysis of the transcriptional switch inducing migration of border cells. *Dev. Cell* 10, 497–508
47. Cherbas, L. *et al.* (2003) EcR isoforms in *Drosophila*: testing tissue-specific requirements by targeted blockade and rescue. *Development* 130, 271–284
48. Jang, A.C. *et al.* (2009) Border-cell migration requires integration of spatial and temporal signals by the BTB protein Abrupt. *Nat. Cell Biol.* 11, 569–579
49. Hackney, J.F. *et al.* (2007) Ras signaling modulates activity of the ecdysone receptor EcR during cell migration in the *Drosophila* ovary. *Dev. Dyn.* 236, 1213–1226
50. Bai, J. *et al.* (2000) Regulation of invasive cell behavior by taiman, a *Drosophila* protein related to AIB1, a steroid receptor coactivator amplified in breast cancer. *Cell* 103, 1047–1058
51. Domanitskaya, E. *et al.* (2014) Phantom, a cytochrome P450 enzyme essential for ecdysone biosynthesis, plays a critical role in the control of border cell migration in *Drosophila*. *Dev. Biol.* 386, 408–418
52. Llense, F. and Martín-Blanco, E. (2008) JNK signaling controls border cell cluster integrity and collective cell migration. *Curr. Biol.* 18, 538–544
53. Dinkins, M.B. *et al.* (2008) Integrin alpha chains exhibit distinct temporal and spatial localization patterns in epithelial cells of the *Drosophila* ovary. *Dev. Dyn.* 237, 3927–3939
54. Pinheiro, E.M. and Montell, D.J. (2004) Requirement for Par-6 and Bazooka in *Drosophila* border cell migration. *Development* 131, 5243–5251
55. Goode, S. *et al.* (2005) Novel spatiotemporal patterns of epithelial tumor invasion in *Drosophila* discs large egg chambers. *Dev. Dyn.* 232, 855–864
56. Felix, M. *et al.* (2015) Pak3 regulates apical–basal polarity in migrating border cells during *Drosophila* oogenesis. *Development* 142, 3692–3703
57. Cai, D. *et al.* (2014) Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. *Cell* 157, 1146–1159
58. Rørth, P. (2002) Initiating and guiding migration: lessons from border cells. *Trends Cell Biol.* 12, 325–331
59. Montell, D.J. (2003) Border-cell migration: the race is on. *Nat. Rev. Mol. Cell Biol.* 4, 13–24
60. McDonald, J.A. *et al.* (2003) PVF1, a PDGF/VEGF homolog, is sufficient to guide border cells and interacts genetically with Taiman. *Development* 130, 3469–3478
61. McDonald, J.A. *et al.* (2006) Multiple EGFR ligands participate in guiding migrating border cells. *Dev. Biol.* 296, 94–103
62. Duchek, P. *et al.* (2001) Guidance of cell migration by the *Drosophila* PDGF/VEGF receptor. *Cell* 107, 17–26
63. Murphy, A.M. and Montell, D.J. (1996) Cell type-specific roles for Cdc42, Rac, and RhoL in Drosophila oogenesis. *J. Cell Biol.* 133, 617–630
64. Fernández-Espartero, C.H. *et al.* (2013) GTP exchange factor Vav regulates guided cell migration by coupling guidance receptor signalling to local Rac activation. *J. Cell Sci.* 126 (Pt 10), 2285–2293
65. Bianco, A. *et al.* (2007) Two distinct modes of guidance signalling during collective migration of border cells. *Nature* 448, 362–365
66. Mathieu, J. *et al.* (2007) A sensitized PiggyBac-based screen for regulators of border cell migration in *Drosophila*. *Genetics* 176, 1579–1590
67. Prasad, M. and Montell, D.J. (2007) Cellular and molecular mechanisms of border cell migration analyzed using time-lapse live-cell imaging. *Dev. Cell* 12, 997–1005
68. McDonald, J.A. *et al.* (2008) PAR-1 kinase regulates epithelial detachment and directional protrusion of migrating border cells. *Curr. Biol.* 18, 1659–1667
69. Lucas, E.P. *et al.* (2013) The Hippo pathway polarizes the actin cytoskeleton during collective migration of *Drosophila* border cells. *J. Cell Biol.* 201, 875–885
70. Zhao, M. *et al.* (2008) Basolateral junctions utilize warts signaling to control epithelial-mesenchymal transition and proliferation crucial for migration and invasion of *Drosophila* ovarian epithelial cells. *Genetics* 178, 1947–1971
71. Rauskolb, C. *et al.* (2014) Cytoskeletal tension inhibits Hippo signaling through an Ajuba-Warts complex. *Cell* 158, 143–156
72. Wong, K.K. *et al.* (2015) β-Spectrin regulates the hippo signaling pathway and modulates the basal actin network. *J. Biol. Chem.* 290, 6397–6407
73. Jékely, G. *et al.* (2005) Regulators of endocytosis maintain localized receptor tyrosine kinase signaling in guided migration. *Dev. Cell* 9, 197–207
74. Assaker, G. *et al.* (2010) Spatial restriction of receptor tyrosine kinase activity through a polarized endocytic cycle controls border cell migration. *Proc. Natl. Acad. Sci. U.S.A.* 107, 22558–22563
75. Medioni, C. and Noselli, S. (2005) Dynamics of the basement membrane in invasive epithelial clusters in *Drosophila*. *Development* 132, 3069–3077
76. Quinones, G.A. *et al.* (2010) I-BAR protein antagonism of endocytosis mediates directional sensing during guided cell migration. *J. Cell Biol.* 189, 353–367
77. Laflamme, C. *et al.* (2012) Evi5 promotes collective cell migration through its Rab-GAP activity. *J. Cell Biol.* 198, 57–67

78. Wan, P. *et al.* (2013) Guidance receptor promotes the asymmetric distribution of exocyst and recycling endosome during collective cell migration. *Development* 140, 4797–4806
79. Chen, D.Y. *et al.* (2012) The Bro1-domain-containing protein Myopic/HDPTP coordinates with Rab4 to regulate cell adhesion and migration. *J. Cell Sci.* 125 (Pt 20), 4841–4852
80. Pollard, T.D. and Borisy, G.G. (2003) Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 112, 453–465
81. Wang, X. *et al.* (2010) Light-mediated activation reveals a key role for Rac in collective guidance of cell movement *in vivo*. *Nat. Cell Biol.* 12, 591–597
82. Chen, J. *et al.* (2001) Cofilin/ADF is required for cell motility during *Drosophila* ovary development and oogenesis. *Nat. Cell Biol.* 3, 204–209
83. Verheyen, E.M. and Cooley, L. (1994) Profilin mutations disrupt multiple actin-dependent processes during *Drosophila* development. *Development* 120, 717–728
84. Gates, J. *et al.* (2009) Enabled and Capping protein play important roles in shaping cell behavior during *Drosophila* oogenesis. *Dev. Biol.* 333, 90–107
85. Geisbrecht, E.R. and Montell, D.J. (2004) A role for *Drosophila* IAP1-mediated caspase inhibition in Rac-dependent cell migration. *Cell* 118, 111–125
86. Kim, J.H. *et al.* (2011) Psidin, a conserved protein that regulates protrusion dynamics and cell migration. *Genes Dev.* 25, 730–741
87. Montell, D.J. *et al.* (2012) Group choreography: mechanisms orchestrating the collective movement of border cells. *Nat. Rev. Mol. Cell Biol.* 13, 631–645
88. Law, A.L. *et al.* (2013) Lamellipodin and the Scar/WAVE complex cooperate to promote cell migration *in vivo*. *J. Cell Biol.* 203, 673–689
89. Poukkula, M. *et al.* (2014) GMF promotes leading-edge dynamics and collective cell migration *in vivo*. *Curr. Biol.* 24, 2533–2540
90. Majumder, P. *et al.* (2012) Par-1 controls myosin-II activity through myosin phosphatase to regulate border cell migration. *Curr. Biol.* 22, 363–372
91. Combedazou, A. *et al.* (2016) Myosin II governs collective cell migration behaviour downstream of guidance receptor signalling. *J. Cell Sci.* Published online April 13, 2016. http://dx.doi.org/10.1242/jcs.179952
92. Aranjuez, G. *et al.* (2016) Dynamic myosin activation promotes collective morphology and migration by locally balancing oppositional forces from surrounding tissue. *Mol. Biol. Cell* 27, 1898–1910
93. Somogyi, K. and Rørth, P. (2004) Evidence for tension-based regulation of *Drosophila* MAL and SRF during invasive cell migration. *Dev. Cell* 7, 85–93
94. Miralles, F. *et al.* (2003) Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* 113, 329–342
95. Salvany, L. *et al.* (2014) The core and conserved role of MAL is homeostatic regulation of actin levels. *Genes Dev.* 28, 1048–1053
96. Yang, N. *et al.* (2012) Microtubules and Lis-1/NudE/dynein regulate invasive cell-on-cell migration in *Drosophila*. *PLoS ONE* 7, e40632
97. Cho, A. *et al.* (2016) An atypical tropomyosin in *Drosophila* with intermediate filament-like properties. *Cell Rep.* 16, 928–938
98. Naora, H. and Montell, D.J. (2005) Ovarian cancer metastasis: integrating insights from disparate model organisms. *Nat. Rev. Cancer* 5, 355–366
99. Haeger, A. *et al.* (2015) Collective cell migration: guidance principles and hierarchies. *Trends Cell Biol.* 25, 556–566
100. Schober, M. *et al.* (2005) Function of the ETS transcription factor Yan in border cell migration. *Development* 132, 3493–3504
101. Luo, J. *et al.* (2015) *In vivo* RNAi screen identifies candidate signaling genes required for collective cell migration in *Drosophila* ovary. *Sci. China Life Sci.* 58, 379–389
102. Levine, B. *et al.* (2010) Opposing interactions between *Drosophila* Cut and the C/EBP encoded by Slow Border Cells direct apical constriction and epithelial invagination. *Dev. Biol.* 344, 196–209
103. Bai, J. and Montell, D. (2002) Eyes absent, a key repressor of polar cell fate during *Drosophila* oogenesis. *Development* 129, 5377–5388
104. Wang, X. *et al.* (2007) Spatially localized Kuzbanian required for specific activation of Notch during border cell migration. *Dev. Biol.* 301, 532–540
105. Levine, B. *et al.* (2007) Notch signaling links interactions between the C/EBP homolog slow border cells and the GILZ homolog bunched during cell migration. *Dev. Biol.* 305, 217–231
106. Stonko, D.P. *et al.* (2015) A mathematical model of collective cell migration in a three-dimensional, heterogeneous environment. *PLoS ONE* 10, e0122799
107. Cai, D. *et al.* (2016) Modeling and analysis of collective cell migration in an *in vivo* three-dimensional environment. *Proc. Natl. Acad. Sci. U.S.A.* 113, E2134–E2141
108. Ramel, D. *et al.* (2013) Rab11 regulates cell–cell communication during collective cell movements. *Nat. Cell Biol.* 15, 317–324
109. Anllo, L. and Schüpbach, T. (2016) Signaling through the G-protein-coupled receptor Rickets is important for polarity, detachment, and migration of the border cells in *Drosophila*. *Dev. Biol.* 414, 193–206
110. Santagata, S. *et al.* (2004) JAGGED1 expression is associated with prostate cancer metastasis and recurrence. *Cancer Res.* 64, 6854–6857
111. Reedijk, M. *et al.* (2005) High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. *Cancer Res.* 65, 8530–8537
112. Sethi, N. and Kang, Y. (2011) Notch signalling in cancer progression and bone metastasis. *Br. J. Cancer* 105, 1805–1810
113. Sethi, N. *et al.* (2011) Tumor-derived JAGGED1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. *Cancer Cell* 19, 192–205
114. Abdulghani, J. *et al.* (2008) Stat3 promotes metastatic progression of prostate cancer. *Am. J. Pathol.* 172, 1717–1728
115. Gu, L. *et al.* (2010) Stat5 promotes metastatic behavior of human prostate cancer cells *in vitro* and *in vivo*. *Endocr. Relat. Cancer* 17, 481–493
116. McClatchey, A.I. *et al.* (1998) Mice heterozygous for a mutation at the Nf2 tumor suppressor locus develop a range of highly metastatic tumors. *Genes Dev.* 12, 1121–1133
117. Huson, S.M. and Hughes, R.A.C. (1994) *The Neurofibromatoses: A Pathogenetic and Clinical Overview,* Chapman & Hall