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Group choreography: mechanisms orchestrating the collective movement of border cells

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Abstract

Cell movements are essential for animal development and homeostasis but also contribute to disease. Moving cells typically extend protrusions towards a chemoattractant, adhere to the substrate, contract and detach at the rear. It is less clear how cells that migrate in interconnected groups *in vivo* coordinate their behaviour and navigate through natural environments. The border cells of the *Drosophila melanogaster* ovary have emerged as an excellent model for the study of collective cell movement, aided by innovative genetic, live imaging, and photomanipulation techniques. Here we provide an overview of the molecular choreography of border cells and its more general implications.

Cell migration is a fascinating, complex and essential cellular behaviour. Without it embryos would not develop, wounds would not heal and the nervous and immune systems would neither form nor function[1,2]. Cell movements also contribute to morbidity and mortality from disease, most notably tumour metastasis, immune deficiencies, inflammatory diseases and various birth defects[3–5]. In addition, in order to achieve a major goal of regenerative medicine, which is the creation of artificial organs and tissues, it is necessary not only to specify all of the appropriate cell types, but also to control their organization, communication and movements. So, it is important to decipher the mechanisms that govern when, where, whether and how cells move *in vivo*.

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FURTHER INFORMATION
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Cell Migration Consortium Website: http://www.cellmigration.org/resource/discovery/montell/montell_super2.cgi

SUPPLEMENTARY INFORMATION
See online article: S1 (movie) | S2 (movie)
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Studies of single cells moving unobstructed on hard surfaces *in vitro* have provided us with our current understanding of this process (reviewed in Refs 6–12). To move, a migrating cell must polarize, protrude and adhere primarily at the front and contract and release at the back. Each of these processes is complex at the molecular level and must be integrated with the other events in space and time. Great progress has been made in identifying molecules that participate in each of these steps, although understanding cell motility in complex environments remains a challenge. It is difficult, if not impossible, to reproduce *in vitro* the intricate molecular, cellular and extracellular components found *in vivo*[13].

In addition, many cells move in diverse types of interconnected groups, rather than migrating individually. Some cells, such as trunk neural crest cells and mouse embryonic germ cells, maintain loose connections[14,15]. Other cells, including cranial neural crest cells, move with more stable cell–cell adhesions[16,17]. Keratinocytes stay tightly connected in a sheet as they fill in a wound, and during development of cellular tubes, such as blood vessels and *Drosophila melanogaster* trachea and salivary glands, distal cells lead while towing the rest of the cells behind them. Cells of the developing mammary gland do not exhibit forward directed protrusions[18]; instead their dynamic movements seem constrained and sculpted by a myoepithelial cell layer. Cells of the lateral line primordium in developing fish and of some carcinomas (for example, melanoma, rhabdomyosarcoma, colorectal and breast carcinomas) migrate as well-connected cohorts[19–22]. This fascinating diversity raises new and fundamental questions, including how migratory populations coordinate their behaviour with each other and their surroundings, and what molecules mediate this communication. The powerful combination of genetics and live imaging has made the border cells of the *D. melanogaster* ovary an important model for the cohort type of collective cell migration (Table 1). These approaches have uncovered signalling pathways that mediate communication between migrating border cells and their microenvironment, as well as proteins involved in the mechanics of movement.

In this Review article, we provide a comprehensive overview of the mechanisms that govern the collective movement of border cells, including specification of the migratory population, developmental timing signals, guidance cues, polarity and the cytoskeletal changes that are required for border cell motility. Moreover, we discuss similarities and differences between individual and collective cell migrations of border cells, and how the insights gained from this model can improve our general understanding of collective cell migration.

Overture

Whereas some moving cells perform solo, the border cell cluster moves as a group composed of two inter-dependent cell types: a pair of cells called polar cells, which nucleate the cluster but cannot move on their own; and four to eight outer, motile border cells, which carry the polar cells to their destination[23–26]. Both polar and border cells are subtypes of ovarian follicle cells.

The basics of border cell migration

The fruitfly ovary is composed of ovarioles, which are strings of egg chambers progressing through 14 developmental stages[27] (Fig. 1a,b). Egg chambers contain one oocyte and 15

support cells, called nurse cells, surrounded by a monolayer of about 650 somatic epithelial follicle cells. From stage 1, each egg chamber possesses polar cells at each end[28–30] (Fig. 1b), but it is not until stage 8 that anterior polar cells recruit four to eight nearby cells to form the migratory border cell cluster[24]. During stage 9 (Fig. 1c), in response to the cytokine Unpaired (UPD), which is secreted by polar cells, border cells round up, extend protrusions in between nurse cells (Fig. 1d), detach from their epithelial neighbours and the basal lamina that surrounds the egg chamber, and they actively navigate between nurse cells, ultimately arriving at the anterior dorsal border of the oocyte[31,32].

Live imaging reveals that the border cell cluster is dynamic[25,33,34]: outer cells extend and retract protrusions, which are preferentially stabilized in the forward direction, and the whole cluster can pirouette and rearrange, with cells taking turns leading and following[25] (see Supplementary information S1 (movie)). Although the non-motile polar cells always remain at the centre of the cluster (Fig. 1e,f) and are carried along, they can also roll and spin with the cluster (see Supplementary information S2 (movie)). The group moves at variable speeds, a bit faster at first and slower towards the end, with an average speed of about 0.5 micrometre per minute[25,33,35]. Live imaging analysis reveals that detachment from neighbouring cells and from the basal lamina that surrounds the egg chamber can be a slow struggle, or can occur swiftly and seemingly effortlessly. When they arrive at the oocyte, border cells secrete a crucial patterning signal[36] and sculpt the micropyle[24], which is an eggshell structure that permits sperm entry[24,36]. Thus, incomplete migration or loss of either the polar cells or border cells results in infertility.

Selecting the company

STAT activity specifies and maintains cell motility

Of the approximately 650 epithelial follicle cells, only four to eight migrate[24]. Genetic screens (Table 1) have revealed the molecular pathway that governs the selection of this migratory group.

The two polar cells, which are determined early in oogenesis, secrete UPD. This cytokine activates the JAK–STAT (Janus kinase–signal transducer and activator of transcription) pathway in nearby cells[37–42]. As in mammalian cytokine signalling, the ligand UPD binds to a transmembrane receptor, resulting in activation of the tyrosine kinase JAK, which phosphorylates both itself and the UPD receptor. STAT then binds to the activated receptor complex and becomes phosphorylated. Phosphorylated STAT dimerizes and translocates to the nucleus, where it activates transcription (reviewed in Refs 43, 44) (Fig. 2).

In the egg chamber, the cells with the highest levels of STAT activate the expression of many target genes, including *slow border cells* (*slbo*), which encodes a C/EBP (CCAAT/enhancer-binding protein)-type transcription factor[24,40,45–47]. Mutation of any known component of the JAK–STAT pathway results in failure of border cell specification and migration. More impressively, activation of STAT in anterior cells that normally remain within the epithelium is sufficient to induce their migration in between nurse cells[40]. By contrast, in *slbo* mutants, the usual number of cells cluster together, but they are incapable of generating protrusions or migrating[24,25]. Ectopic expression of SLBO is also insufficient for

motility. Interestingly, if STAT is inactivated midmigration, some clusters fail to reach the oocyte even if earlier STAT activity was sufficiently high to allow movement to begin[48]. Thus, STAT activity is required for both specification and migration (Box 1). The steril e 20-like kinase Misshapen (which is encoded by the *msn* locus) is also required independently of STAT and SLBO for specification of the appropriate number of border cells and for their migration[49]. Unlike STAT and SLBO, this kinase is pleiotropic and is required independently in polar cells, nurse cells and border cells to promote border cell migration; however the relevant substrates are not yet clear.

STAT levels control the number of motile cells

Although most or all cells of the follicular epithelium are competent to respond to UPD, only the four to eight cells closest to the polar cells differentiate into border cells[38–40,50]. Selecting the right number of cells is essential because the percentage of border cell clusters that reach the oocyte on time is proportional to the number of motile cells initially recruited to the cluster[48,51,52]. Interestingly, the ligand UPD is limiting, and apical localization of *upd* mRNA within polar cells, together with local secretion of the UPD protein, is necessary to achieve a high enough local concentration for successful border cell recruitment[51].

However, excess STAT activity can also perturb border cell migration. Increased levels of STAT can be achieved by overexpressing UPD, by expressing a constitutively active allele of JAK (which is encoded by *hopscotch* (*hop*), or by loss of function mutations in the transcription factor Apontic (which is encoded by *apt*) or its downstream target microRNA-279 (miR-279) (Refs 38–40,50,53,54). APT and miR-279 function as feedback inhibitors of STAT in a genetic circuit that converts the initially graded pattern of STAT activation into discrete on and off states: high STAT activity in a small number of cells that migrate away, and low or no activity in the cells that stay behind[53,54] (Fig. 2). So, the variability in the length of time it takes for border cells to detach completely from the follicle cells that stay behind might reflect heterogeneity in the efficiency of these feedback mechanisms.

The cells with the highest levels of active STAT promote expression of more than 200 direct and indirect target genes, including *slbo* and *shotgun* (*shg*; which encodes *D. melanogaster* epithelial cadherin (DE-cadherin)), and thereby acquire the ability to initiate migration[45,46]. Expression of another set of over 200 genes is reduced in migratory cells[45,46]; at least some of these genes are functionally significant, including the nucleotide diphosphate kinase encoded by the *abnormal wing discs* (*awd*) gene[55]. The number of STAT targets that are essential and what truly distinguishes motile from stationary follicle cells remain to be elucidated. Since the discovery that STAT has a key role in border cell migration, STAT proteins have been widely implicated in cell motility, both in other *D. melanogaster* cell types[56–58] and in ovarian and other tumour cells (reviewed in Refs 44,59–62).

Keeping time

Ecdysone coordinates the timing of cell migration

Similar to dancers in an ensemble, moving cells need timing cues to know when to start and stop and when to coordinate their movements with each other and the rest of the

'production'. UPD is expressed early in ovarian development[40,63], but border cells do not migrate until stage 9. So, in addition to the spatial selection of the migratory group, the cells require a developmental timing cue. The insect steroid hormone ecdysone serves this purpose. The ecdysone titre rises late in stage 8 and peaks in stage 10 (Ref. 64), probably through local production of the hormone within the egg chamber[65]. Ecdysone activates a hetero dimer consisting of ecdysone receptor (EcR) and Ultraspiracle (USP), which binds to DNA[66,67]. The conserved co-activato r Taiman (TAI) then binds to this heterodimer in a ligand-dependent manner, and all three proteins are necessary for border cell movement[68,69]. Ecdysone is required globally for egg chamber progression through stages 9 and 10 (Ref. 65). Surprisingly though, reporters for EcR transcriptional activity reveal that the response to the hormone is not spatially uniform, but instead is increased in anterior follicle cells, including the border cells[70,71] (Fig. 2).

Integration of ecdysone and STAT signalling

The mechanism governing the spatiotemporal localization of the ecdysone response has been elucidated at least in part. Anterior cells exhibit slightly enhanced expression of the EcRB1 isoform of EcR, and this creates an initial inequality in concentration. This leads to a commensurate increase in signal strength that is amplified in a feedback circuit that integrates JAK–STAT and ecdysone signalling pathways[71,72]. The widely expressed BTB and zinc-finger domain-containing protein Abrupt attenuates ecdysone signalling by direct binding to the basic helix–loop–helix (bHLH) domain of TAI. STAT signalling antagonizes Abrupt, thus indirectly promoting the ecdysone response. Abrupt localizes to the nucleus in most cells, but STAT activity progressively excludes Abrupt from border cell nuclei during migration[71]. Thus, Abrupt serves as a node of integration for the spatial (JAK–STAT) and temporal (ecdysone) cues that govern border cell migration (Fig. 2). This integration may occur simply and directly at the promoters of individual target genes that require both factors for optimal expression. Alternatively, and perhaps more likely, integration of these inputs may be complex, with some common and some divergent target genes and pathways. The two inputs may cooperate or act in opposition on target genes. For example, DE-cadherin levels are reduced in *Stat92E* and *slbo* mutants compared with wild type but increased in *tai* mutant cells. Global analysis of ecdysone pathway target genes would be useful in deciphering the mechanisms by which these two pathways are integrated.

Additional transcription factors downstream of STAT and EcR include Jing[73], SIX4 (Ref. 45), Yan (also known as pokkuri)[74], Similar (also known as HIF1α)[75], Hindsight (HNT)[76] and Jun-related antigen[77], emphasizing the importance of transcriptional regulation during border cell migration (Box 1). Although the complete set of targets for each factor is not known, many of them affect the expression level of DE-cadherin protein, which is required for border cell migration. Excess DE-cadherin, which is observed in *tai*, *yan* and *hnt* mutant cells, also impedes movement[69,74,76].

Stage directions

Polarity determination in individually moving cells

All performers need good direction, and migrating cells are no exception. In the simplest case, single cells polarize in response to a chemoattractant gradient such that protrusions are both more probable to form and more stable at the front, whereas retraction is more likely to occur at the back. In amoeboid cells such as neutrophils or *Dictyostelium discoideum*, enhanced phosphoinositide 3-kinase (PI3K) activity leads to an increased phosphat idylinositol-3,4,5-triphosphate (PtdIns(3,4,5) P_3) concentration at the front of the cell. This provides binding sites for pleckstrin homology (PH) domain-containing proteins, including activators of the small GTPase RAC, which leads to actin polymerization and protrusion[78,79]. PTEN (phosphatase and tensin homologue) segregates to the back of the cell, and feedback signalling then amplifies the differences between the front and the back[10,80,81]. Cells can develop polarity between their leading and lagging edges even in very shallow gradients, and many different stimuli can polarize migrating cells, including electrical and mechanical gradients. Moreover, cells can polarize spontaneously even in uniform chemoattractant, although each cell chooses a random direction to move in. The ‘biased excitable network’ model has also been proposed as a mechanism that generates polarity in response to a chemoattractant gradient[82]. This model proposes that positive and negative signalling feedback loops that generate ‘excitability’ analogous to action potentials in neurons are directionally biased by external chemoattractant gradients to generate polarized cellular morphology and directed cell motility even in very shallow gradients.

Similarly, single mesenchymal cells polarize spontaneously and migrate in random directions in uniform chemoattractant but orient predictably in gradients. During animal development, many cell types undergo an epithelial to mesenchymal transition (EMT), which switches cells from a stationary, adherent monolayer to dynamic individual migratory cells[83]. Examples include gastrulating mesodermal cells, neural crest cells and limb muscle precursors[20,21,84–86]. These groups of cells move in the same direction, but associate loosely or not at all with one another. They lose expression of cell–cell adhesion mole cules such as E-cadherin, dismantle apical–basal polarity and adopt a polarity between their leading and lagging edges that is characteristic of individually migrating cells. This has led to the notion that loss of apical–basal polarity and cell–cell adhesion are both necessary and sufficient for motility[87,88]. However, improvements in imaging techniques facilitated by a palette of fluorescent proteins have now allowed the direct observation of many different morphogenetic movements in living embryos and organ cultures. Such studies reveal that cell movements are extraordinarily diverse and that many cells move in interconnected groups that retain cell–cell adhesion and apical–basal polarity while moving. Collectively migrating cells are thus polarized along more than one axis. Border cells, for example, exhibit three distinct polarities: front–back, apical– basal and also an inside–outside polarity with respect to the cell cluster (Fig. 3).

Border cells adopt a leading–lagging edge polarity

The border cell cluster overall has a leading edge, generated in response to chemotactic cues. One or two leading cells extend long and relatively stable actin-rich pro-trusions[89]. By

contrast, cells at the side and back of the cluster extend fewer protrusions, which tend to be shorter and less stable[25,90]. Front–back polarity is generated upon binding of multiple chemoattractants to two receptor tyrosine kinases on all follicle cells, including border cells. These chemoattractants, which are secreted by germline cells and are normally produced at highest concentration in the oocyte relative to nurse cells[91–94], include PVF1 (platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF)-related factor 1), Spitz and Keren. Ectopic expression of these ligands can reroute border cells[91,92]. A fourth ligand, Gurken, is present only at the dorsal–anterior corner of the oocyte and guides border cells dorsally as they get close to the oocyte[91–93].

All follicle cells, including border cells, express two receptor tyrosine kinases: PVR (PDGF- and VEGF-receptor related), which is equally related to PDGF and VEGF receptors and binds PVF1; and epidermal growth factor receptor (EGFR), which is related to EGFR and binds Spitz, Keren and Gurken. But only border cells move up the concentration gradient. EGFR, PVR and their ligands are partially redundant in function, but loss of both receptors results in greatly reduced movement[91–94]. A third receptor, termed Tie, may make a minor contribution; however, its ligand remains to be identified[46]. Although these guidance receptors were thought to be required for extending motile projections, live imaging shows that when PVR and EGFR signalling is blocked, border cells maintain protrusive activity but lack the usual strong forward bias[25,90].

It is likely that receptor activation is slightly higher at the front of the cell cluster than at the back, although so far this has only been demonstrated for over expressed protein[25,90,95]. Some downstream amplification of the signalling output difference between the cell front and back is necessary for the dramatic morphological polarization that occurs in response to a much smaller difference in receptor activation. However, the PI3K– PTEN signalling loop that provides this function in *D. discoideum* is not required in border cells[89], so other mechanisms must operate, and even in *D. discoideum* multiple redundant pathways contribute[96]. Endocytosis and vesicle recycling also have a key role in this signalling amplification process, and their effects in border cells are mediated in part by Dynamin, Rab5, Missing in metastasis (MIM), Rab11 and other associated proteins[97–99]. It has been suggested that active receptors might be recycled directionally to the front of the cell to increase their concentration there, and this amplifies the difference between signalling levels between the front and the back[95,97]. However, this conclusion has been questioned[99]. Although phosphotyrosine (pTyr) accumulates noticeably at the leading edge, particularly at the beginning of migration, labelling with an antibody against the active form of PVR is not detectably enriched at the front of the cluster unless PVR is overexpressed. So other kinases and/or phosphorylated proteins may be recycled to the leading edge that enrich pTyr staining, and multiple mechanisms probably contribute to amplifying the front–back asymmetry.

Migrating border cells require apical–basal polarity

A key distinction between cells that migrate collectively and those that migrate individually is that cells in groups can maintain epithelial character even as they gain front–back polarity. Migrating border cells retain asymmetric localization of apical markers along an axis that is

typically orthogonal to the direction of movement[23,100] (Fig. 3). Before invasion, polar cells and outer border cells, similarly to the follicular epithelium, localize PAR3 (partitioning defective 3; also known as Bazooka), PAR6, atypical protein kinase C (aPKC), Crumbs, Stardust and PALS1-associated tightjunction protein (PATJ) at the apical surface[100,101], which is in contact with nurse cells (Fig. 3a). Lethal giant larva (LGL), Fasciclin 2 (FAS2), Neuroglian, Scribble and Discs large localize basolaterally[102–104]. Basolateral contacts are required to maintain the single-layer epithelium and must be remodelled to allow migration[103,105]. As border cells become motile, protrusions arise from the basolateral surface and, as the cells move in between nurse cells, the cluster turns such that the less dynamic apical surface is oriented approximately orthogonal to the direction of migration (Fig. 3b,c). Apical proteins help to keep the cells well connected to one another, which facilitates cohesive movement, and knockdown of PAR3 or PAR6 by RNA interference (RNAi) causes the cluster to splay apart, reducing the efficiency of border cell migration[100]. Another polarity protein, the Ser/Thr kinase PAR1, localizes basolaterally within the epithelium and restricts PAR3 to the apical surface[106]. PAR1 is also required in border cells for their detachment from the epithelium. Unlike PAR3 though, PAR1 also has a direct role in protrusion dynamics[106]. PAR1 phosphorylates and inactivates an inhibitor of the motor protein myosin II at the rear of the cell, resulting in myosin II activation and lagging edge retraction[107]. This suggests that polarity proteins may have multiple roles in collective movement.

Motile cell groups exhibit internal–external polarit y

Cells at the edge of a collectively migrating cluster have an additional axis of polarization because they are in contact with cells of the group on one or more sides and lack that contact on the free edge. This confers an inside–outside polarity. Within the border cell cluster, the polar cells are always located in the centre and each outer, migratory cell typically contacts a polar cell on one side and other border cells on two sides, while keeping a more dynamic contact with nurse cells (Fig. 1c, e, f).

The phenomenon of contact inhibition of locomotion, or more precisely in this context, contact inhibition of protrusion, may explain why protrusions are never observed at border cell–polar cell or border cell–borde r cell contacts. The concept of contact inhibition of locomotion was introduced over 50 years ago by Abercrombie and Heaysman[108], who noticed that encounters between individually migrating cells cause them to stop, change direction and to migrate away from each other. More recently, in the context of collectively migrating cranial neural crest cells, neural cadherin (N-cadherin)-mediated adhesion between cells restricts protrusions to free edges, and this combined with an external chemoattractant gradient produces collective chemotaxis[16]. Although border cells do not express DN-cadherin detectably, DE-cadherin is present at polar cell–border cell and border cell–borde r cell junctions[23] (Figs 1f,3c); however, this is not the only mechanism that maintains close contact between the migrating cells and regulates contact inhibition of protrusion.

Although individually migrating cells adopt one major axis of polarity (that is, the leading and lagging edges), collectively migrating cells can actively maintain three axis of polarity:

front–back, apical–basal and inside–outside. The three axes of polarity adopted by collectively migrating cells are all important for efficient movement and arise by distinct means. Front–back polarity requires guidance factor signalling, which is needed for chemotaxis, whereas the apical–basal axis is retained from the epithelial origin of the cohort and is required for cluster cohesion and coordination (Fig. 3). Inside–outside polarization results from the relative position of cells within the cluster and seems to restrict protrusion to the outside surface. This may be a consequence of the lower level of DE-cadherin (Fig. 3c) and possibly other adhesion molecules at the border cell–nurse cell junctions, the presence of the activated guidance receptors specifically at that interface or other unidentified mechanisms.

Staying in touch on the road

A major difference between collective cell migration and single cell motility is the importance of continued communication between cells. In particular, the regulation of cell–cell adhesion differs substantially. Whereas cells migrating alone eliminate cell–cell adhesion to facilitate their movement, collectively migrating cells by definition maintain cell–cell attachments. An open question is what mechanisms maintain relatively stable adhesion between the cells of the cluster so that they stay together but allow transient adhesion on the outside surfaces of the same cells, so that they gain traction without getting stuck.

Maintaining adhesion within the border cell cluster

In the border cell cluster, DE-cadherin[23] and its binding partners Armadillo (ARM; also known as β-catenin)[109] and α-catenin[110] are concentrated between polar cells and outer border cells as well as in aggregates that may be adherens junctions between neighbouring border cells, reflecting their persistent epithelial character. DE-cadherin is also present, albeit at lower levels, at border cell–nurse cell interfaces where it is clearly required, as border cell migration fails when nurse cells lack DE-cadherin[23]. Overexpression of DE-cadherin to very high levels in border cells also prevents them from moving[74]. Thus, border cells require precise regulation of DE-cadherin levels to balance cohesion and traction.

Many mutations affect the expression level and/ or localization of DE-cadherin in border cells, including mutations in *Stat*, *slbo*[45,46], *tai*[69], *yan*[74], *hnt*[76] and *msn*[49], suggesting that this regulation is both important and complex. Post-transcriptional mechanisms are also involved in destabilizing DE-cadherin to allow border cell detachment[111]. In mammalian cells, phosphorylation of β-catenin by tyrosine kinases leads to dissociation of the β-catenin-α-catenin-E-cadherin complex, which results in phosphorylation of E-cadherin and downregulation of E-cadherin-mediated adhesion[112]. However fusion of α-catenin to DE-cadherin obviates the need for β-catenin in border cells[113], indicating that regulation of this linkage is not essential.

DE-cadherin-independent cluster cohesion

Although loss of DE-cadherin from border cells prevents efficient recruitment of border cells to the cluster and their movement, it does not result in cluster dissociation once a

cluster forms[23]. This suggests that additional molecules contribute to cluster cohesion, and several factors are known to be involved. Downregulation of Jun amino-terminal kinase (JNK) signalling causes clusters to dissociate and extend long protrusions[77], although the phenotype only appears late in stage 9 and the effect on migration is mild. Expression of a dominant-negative form of the RHO-family GTPase CDC42 has a similar effect[77], as does inhibition of apical polarity determinants such as PAR3 and PAR6 (Ref. 100), and results in reduced cluster co hesion. Splayed border cells with reduced JNK activity exhibit reduced expression of PAR3, DE-cadherin and Paxillin and relocalization of β-integrin and myosin VI (also known as Jaguar) to the tips of their protrusions. However, the functional significance of this localization is unclear, as the known integrin ligands are not concentrated in between nurse cells.

One essential negative regulator of JNK is the transcription factor HNT, which seems to reduce cluster cohesion[76]. *hnt* mutant cells accumulate excess adhesion molecules and appear very compact, whereas overexpression of HNT in border cells reduces JNK pathway activity and causes cluster dissociation. However, cells overexpressing HNT exhibit a more severe delay in migration than JNK-pathway mutants, probably due to reduced levels of STAT and SLBO. Downregulation of the human homologue of HNT, RREB1 (RAS responsive element binding protein 1), increases cell-cell adhesion and reduces the ability of mammalian epithelial cells to migrate in wound-healin g assays[76], suggesting that the role of HNT in motility is well conserved.

Detachment of border cells from the extracellular matrix

Cells adhere to the extracellular matrix (ECM) via proteins of the integrin family, and this adhesion is essential for movement on or through the ECM. Although cultured cells can be plated on dishes coated with uniform sheets of ECM components such as laminin, fibronectin and collagen, migrating cells encounter more varied terrains *in vivo*. Here, cells may move along collagen fibres, basement membranes, blood vessels or other cells. Before migration, border cells contact a basal lamina at their basal surface, as do all the epithelial follicle cells[114,115]. During stages 6–8, most follicle cells move in concert along the ECM (akin to hamsters running on a wheel), and this causes the whole egg chamber to rotate[116]. By contrast, border cells disassociate from the basal lamina to invade between nurse cells. This detachment from the ECM and neighbouring cells requires Notch activity[25,117] and PAR1 (Ref 106). The precise mechanism by which Notch signalling promotes detachment from the basal lamina is not yet known. One clue is that Notch signalling causes MCF10A mammary epithelial cells to detach from ECM substrates due to downregulation of p63, a p53 family member that promotes the expression of multiple genes encoding ECM components[118]. If such a mechanism is conserved, it could explain how Notch signalling promotes border cell detachment.

During migration, border cells move in direct contact with, and surrounded on all sides by, nurse cells. For this reason, the role of integrin is different in this setting compared with its role during cell migration on, or through, the ECM. Integrins are expressed in border cells, and integrin depletion enhances migration defects associated with other mutations but causes little or no loss-of-function phenotype alone[77,119–121]. Integrins seem to contribute more to

cluster cohesion than to cell-ECM interactions[77,119,120], which is consistent with the observed loss of cell-ECM interactions as border cells detach from the basal lamina. In addition, microarray studies reveal reduced expression of the integrin ligands collagen IV (which is encoded by *viking* and *Cg25)* and laminin A in migrating border cells[46], consistent with the idea that they do not need to continue to secrete, or adhere to, ECM proteins as they move.

Classic models of EMTs suggest that E-cadherin must be downregulated for epithelial cells to move out of a tissue, and that integrins then mediate adhesion to the ECM during movement. However, in the case of border cells, migration occurs directly on the surface of nurse cells and is mediated by DE-cadherin, which thereby minimizes the requirement for integrins. Thus, the regulation of adhesion during cell migration can vary depending on the cell type and the particular migratory microenvironment.

Power and control

Just as dancers depend on well-developed and precisely controlled skeletal muscles, cells rely on the actomyosin cytoskeleton to produce the forces necessary for movement (reviewed in Ref 122). Much of the prevailing view of how actin polymer kinetics are regulated and how this contributes to cell migration comes from work on cultured cells moving on a hard, flat surface (Box 2). In the next section we compare concepts that have arisen from such studies of cell migration in culture to what occurs during an *in vivo* collective cell migration.

RHO-family GTPases regulate actin dynamics

In vivo just as *in vitro*, filamentous actin (F-actin) dynamics take centre stage during cellular movements. Members of the RHO-family of small GTPases have emerged as crucial nodes in the network that controls actin organization and dynamics, myosin activity and cell–matrix adhesions downstream of signalling from the cell surface[8,122–125]. In cultured cells, activation of the GTPases CDC42, RAC and RHO promotes different cytoskeletal organizations, resulting in the formation of filopodia, lamellipodia and actomyosin stress fibre formation, respectively (Box 2). Despite the clear importance of these proteins in virtually all cells, the details vary from one cell type to another. In addition, *in vivo*, migratory cells exhibit extremely varied morphologies, which implies differences in the organization of the actin filaments that underlie cell shape[126]. Therefore, it is valuable to elucidate and compare the functions of the RHO GTPases in different cell types.

RAC regulates border cell protrusions and dynamics

As they initiate movement, border cells typically extend F-actin protrusions that are 10–20 micrometres long and about 2–4 micrometres in diameter[89,127,128] (Fig. 1d). These protrusions are thicker than typical filopodia but narrower than lamellipodia. The dynamics of this protrusive activity is best appreciated in live-imaging studies[25,129] (see Supplementary information S1 (movie)). Microarray analyses of border cells reveal that cytoskeletal regulators and vesicle trafficking proteins are enriched in these migratory cells relative to stationary follicle cells[45,46]. Despite the marked differences in the sizes and

shapes of border cell protrusions compared with the leading lamellipodium of a migrating fibroblast, RAC is required in both cell types for protrusion and migration.

Studies in *D. melanogaster* were the first to demonstrate the *in vivo* functions of RAC in axon outgrowth[130], F-actin accumulation[131] and cell protrusion and migration[128]. Subsequently, RAC has been implicated in nearly every type of cell migration, whether collective or individual, in normal or tumour cells[123,125]. GTP exchange factors, which are required to activate RAC, are also conserved, and in flies include Vav[132,133] and a heterodimer of Myoblast city and ELMO (Engulfment and cell motility).

Injection of an active form of RAC into a serumstarved cell produces striking membrane ruffling all over the cell[134], leading to the idea that localized RAC activation might cause local actin polymerization and protrusion[124]. This hypothesis has now been tested directly by using a photo-activatable analogue of RAC (PA-RAC)[135]. Strikingly, focal stimulation of PA-RAC leads to local membrane ruffling and directed movement of border cell collectives[129] and single neutrophils[136] *in vivo*. In fact, activation of RAC in just a single cell of the border cell collective is sufficient to guide the cluster forward, backward or sideways[129]. Furthermore, RAC activation or inactivation in just one cell inhibits protrusive activity of the other cells of the cluster. This non-autonomous effect depends on cluster cohesion and requires JNK signalling[129]. The mechanism mediating this cell–cell communication is not yet clear. But a study of isolated cells in culture suggests that membrane tension inhibits protrusion from the rear of a migrating neutrophil[137]. So, it will be interesting to test whether membrane or cytoskeletal tension also mediates communication between collectively migrating cells.

The roles of CDC42 and RHO are less dramatic in migrating border cells. Expression of a dominant-negative form of CDC42 causes a phenotype that is similar to JNK knockdown or loss of apical polarity proteins, suggesting a role for CDC42 in cluster cohesion more so than actin polymerization[77]. Similarly, expression of dominantnegative RHO causes clusters to splay apart[138]. Although there is a rich literature describing potential crossregulation between RHO, RAC and CDC42 in cultured cells, these relationships have not been thoroughly explored in border cells.

Actin regulatory proteins with conserved roles

Many proteins modulate actin polymer dynamics, and this is simplest to understand in the context of a cell moving across a flat surface (Box 2). Some actin regulatory proteins serve the same function in border cells and fibroblasts, despite differences in cell morphologies and expression profiles. For example, Profilin (also known as Chickadee in flies) promotes protrusion in border cells as in other cell types[139,140]. Diaphanous (DIA) is a member of the formin protein family, which promotes linear rather than branched actin polymerization. In border cells, as in mouse fibroblast cells, activated DIA causes nuclear translocation of Myocardin-related transcription factor (MRTF; also known as MAL-D), possibly in response to membrane tension, which then regulates transcription together in a complex with serum response factor (SRF)[141–143]. Both *mal-d* and *srf* are required for border cell migration[143].

Cofilin modulates actin dynamics at the leading edge

Cofilin (also known as ADF), which is encoded by *twinstar (tsr)* in *D. melanogaster*, promotes cell migration by severing filaments, which increases the concentration of uncapped barbed ends and thus can stimulate actin polymerization. But Cofilin also stimulates de polymerization of actin filaments, replenishing the supply of actin monomers available for polymerization[144]. The phosphatase Slingshot activates Cofilin because Cofilin is inactivated by phosphorylation. Cofilin is required for border cell migration[145]. At the front of the border cell cluster, there is a lower ratio of phosphorylated Cofilin to the unphosphorylated form of Cofilin and thus greater Cofilin activity compared with the rest of the cluster, and this asymmetry depends on guidance receptor signalling[146]. A puzzling result is that RAC activity inactivates Cofilin, but RAC and Cofilin activities are both higher at the front of the border cell cluster. One possible explanation for these seemingly contradictory results is that Slingshot activity may also be high at the front of the cluster and thus override inactivation of Cofilin by RAC; however this hypothesis remains to be tested. The protein tyrosine kinase Twinfilin is also expressed in border cells and inhibits long F-actin filament production[129]. Twinfilin is an actin monomer-binding protein that negatively regulates F-actin formation by sequestering globular actin (G-actin), and it is also necessary for border cell movement[129,147].

The unexpected roles of other actin regulators

Both positive and negative regulators of actin dynamics contribute to individual and collective cell migration (Box 2; Table 2), sometimes in unanticipated ways. For example, although Enabled (ENA) and related proteins promote actin polymerization, surprisingly they also inhibit motility in individually migrating fibroblasts[3,148]. An explanation for this counter-intuitive result is that polymerization proceeds rapidly in the presence of ENA, but leads to relatively unstable protrusions that do not support motility as effectively as those generated by the slow and steady polymerization that occurs when ENA is sequestered away (reviewed in Ref. 149). By contrast, in border cells, sequestration of ENA away from the leading edge impedes migration[150], suggesting that the effect of ENA depletion is cell type-specific. Increased ENA protein levels in border cells also impede migration and produce many excess fine filopodia[150], strikingly similar to the effect observed in fibroblasts when capping protein, which usually prevents polymerization at the barbed end of F-actin, is knocked down. This finding supports the idea that ENA directly antagonizes capping protein at the barbed ends of growing filaments[151]. Together, these findings suggest that the precise contribution of each actin regulatory protein to motility varies from one cell type to another, probably due to the different combinations of various actin regulatory proteins present in each cell type, some of which have overlapping activities. Cortactin, for example, promotes F-actin formation in border cells, but null mutations cause a very mild defect in migration[98,152], suggesting that other actin regulatory proteins may act redundantly.

Actin filament stabilizing factors

Several classes of proteins crosslink and stabilize actin polymers[122], including myosin, tropomyosin, fascin (known as Singed in *D. melanogaster*)[153] and filamin (also known as Cheerio)[139]. Singed and Cheerio are enriched in border cells, and although Cheerio is

required for efficient border cell movement, Singed is not[139,153]. Myosin II is a pointed end directed motor protein that crosslinks actin filaments and provides contractile force. In *D. melanogaster*, it is composed of a heavy chain encoded by the *zipper* (*zip*) locus and a regulatory light chain encoded by *spaghetti squash* (*sqh*). ZIP and SQH are required for border cell migration, and cells mutant for either generate unusually long protrusions that are unable to retract efficiently and have defects in retraction of the cell rear[89,107,154,155,156]. By contrast, myosin VI is a barbed end directed motor that is required during border cell migration to stabilize DE-cadherin-based adhesion[157]. Given that myosin VI can physically associate with DE-cadherin and ARM, its motor function may promote extensions by pushing existing actin filaments outward.

Discovering new players in actin dynamics

Genetic screens for mutants that affect border cell migration have identified several motility genes that unexpectedly have roles in actin dynamics. One of these genes encodes *D. melanogaster* Inhibitor of apoptosis 1 (DIAP1; also known as Thread), which had previously been well known for its inhibitory effect on caspases in cell death control[158]. In border cells, DIAP1 is not required to prevent death, but instead is required for their movement. Overexpression of DIAP1 rescues migration defects caused by dominant-negative RAC, as does inhibition of the caspase Dronc (also known as NEDD2-like caspase (Nc)). DIAP1 forms a biochemica l complex with RAC and Profilin and, through these interactions, modulates actin dynamics[158]. A screen for mutations that enhance border cell migration defects due to dominant-negative PVR also turned up mutations in DIAP1 (Ref. 159), confirming the migratory role of this protein.

Another mutagenesis screen identified two alleles of the *phagocyte signalling impaired* (*psidin*) locus, which exhibit defects in border cell migration and actin-dependent processes in germline cells[160]. Similarly, knockdown of the human homologue in breast epithelial cells decreases their motility. Overexpression of *psidin* results in longer, more persistent border cell protrusions as well as increased projections and membrane ruffling in cultured *D. melanogaster* cells. Purified Psidin protein physically interacts with F-actin and, although Psidin alone does not alter the dynamics of polymer formation, it blocks Tropomyosin–actin interactions. In mammalian cells, tropomyosin binding to F-actin inhibits binding of the actin-related protein 2/3 (ARP2/3) complex, cofilin and potentially other regulators[160].

Despite the drastic differences in cell morphologies between individually migrating cells and those moving as part of a cluster, some aspects of the mechanics of movement are shared. Both cell types generate protrusions at the front and retract the rear, and many conserved actin regulatory proteins mediate these changes. Studies of border cells have led to several surprises, including the finding that some proteins, such as ENA, have unforeseen or more complex roles in migration and the discovery of new players in basic actin dynamics, such as Psidin and DIAP1 (Box 2; Table 2). More studies are needed to elucidate precisely how cytoskeletal changes are governed in different migratory cell types.

Finale

Migrating cells are a tremendously diverse set of soloists and ensembles that move through an ever-changing scenery, and we are really only just beginning to elucidate how they are selected and directed, how they keep time and they coordinate their steps. Because of this diversity it is important to study motility mechanisms in many different cell types in their natural settings. The combination of two decades of genetic screening and candidate gene testing with more recent developments in live imaging and photomanipulation have significantly advanced our understanding of the mechanisms that govern collective movement of border cells (Tables 1,2). Signalling pathways with intricate feedback loops carry out the crucial function of specifying the migratory population and ensuring proper developmental timing. Guidance cues, adhesion molecules and cytoskeletal regulators control spatially segregated protrusion, adhesion and retraction events that propel the cells in the correct direction.

Many interesting questions remain unanswered and present opportunities for the future. Key open questions include how the lead cell communicates with the following cells (via biochemical signals, mechanical forces or some combination of the two) to achieve coordinated directional behaviour. Elucidating the complete biochemical pathways from guidance receptors to small GTPases, F-actin regulators and adhesion molecules will also be important and has yet to be fully defined even in cultured cells. Unravelling the crosstalk between RHO, RAC and CDC42 in this *in vivo* context may contribute significant new insights into the functions of these crucial regulators of protrusion, adhesion and contractility. New tools such as photo-activatable and photoinhibitable GTPases, and fluorescence resonance energy transfer (FRET) activity probes will have increasingly strong roles in all of these efforts. Although it is clear that border cells can change positions during migration so that new leaders emerge, it is less clear what the causes and functional importance of this behaviour are. Does the lead cell eventually undergo adaptation and become desensitized to the guidance signals, allowing a more sensitive trailing cell to take over the lead? Can individual cells sense direction better or worse than the group? Is collective migration ultimately more or less efficient than individual migration?

A broader open question is whether each collective cell movement is a unique performance with a choreography of its own, or whether there might be a repertoire of subroutines that can be combined in different ways to generate diversity. Repeated use of proteins such as RAC in different motile cell types, both collective and individual, suggests that there is a repertoire of functional modules, so the challenge will be to determine what they are and how they are combined in different ways to produce just the right performance for each cell type and biological setting. Now that molecules with either major or supporting roles in collective cell migration have been identified, current challenges are to decipher how mechanical forces and biochemical signals are integrated and feed back to one another to coordinate protrusion, contractility, cell–cell and cell–ECM adhesion in space and time at subcellular, cellular and multicellular scales. It will be important to use the insights gained from such studies to obtain better control of cell motility and ultimately be able to enhance wound healing and tissue regeneration and to prevent tumour invasion and metastasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Border cells	Six to eight somatic ovarian cells that originate in the follicle epithelium, then coalesce around the polar cells, detach and migrate collectively between germline cells to the border of the oocyte, where they are required for patterning and egg fertilization.
Polar cells	Two somatic follicle cells that are specified at each end of the developing egg chamber. Anterior polar cells secrete signals that specify neighbouring cells to become border cells.
Nurse cells	Auxiliary germline cells that supply the oocyte with synthesized mRNAs, proteins and organelles during insect oogenesis.
Basal lamina	A thin sheet of laminin, collagen IV and proteoglycans that underlies the basal surface of an epithelium.
Ecdysone	The single *Drosophila melanogaster* steroid hormone, which activates a nuclear hormone receptor complex and initiates transcriptional regulation.
RAC	A 21 kDa GTPase of the RHO-family that is activated by chemoattractants and in turn stimulates polymerization of branched actin networks, resulting in lamellipodial protrusion.
Epithelial to mesenchymal transition	(EMT). A morphological change that is characteristic of some developing tissues and certain forms of cancer. During EMT, cells lose intercellular junctions and apical–basal polarity, become migratory and, in the case of cancer, become invasive.
Filopodia	Thin, dynamic, cellular extensions that contain actin filaments. They are aligned in parallel with their barbed ends pointing towards the tip and are often found in growth cones and at the leading edge of migrating cells.
Lamellipodia	Broad, flat protrusions at the leading edge of a moving cell that are enriched with a branched network of elongating actin filaments, which generate the force to push the cell membrane forward.

Stress fibres	Consist of contractile actin filament bundles that are typically anchored at one or both ends to the extracellular matrix via focal adhesions.

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Box 1|The role of transcription in cell motility

Cell migration research resides at the intersection between the fields of cell biology and developmental biology, which are converging as methods for imaging and manipulating cells within developing tissues *in vivo* achieve spatial and temporal resolution closer to that achieved *in vitro*. Occasionally the two fields collide. An example is a disagreement concerning the significance of transcriptional regulation in cell motility. Some cell biologists dismiss transcriptional regulation in cell migration as uninteresting because its influence is indirect. By contrast, many developmental biologists find that a complete understanding of cell migration has to include elucidating the contributions of transcriptional regulation[161]. Cell biologists point to experiments in which an enucleated cell fragment can move around for hours independently of new gene transcription as evidence that transcriptional regulation is unimportant. However, this seemingly compelling experiment only works with terminally differentiated cell types that are constitutively motile, such as neutrophils[162, 163]. By contrast, developmental biologists have repeatedly identified transcription factors as key regulators of cell motility *in vivo*. The regulation of border cell migration by STAT (signal transducer and activator of transcription) is a good example. Without STAT activation, border cells remain immobile within the epithelium[38, 39, 50, 54, 115]. Even if they activate STAT and start moving, their behaviour is labile, and inactivating STAT during migration impedes their progress[48]. Moreover, activating STAT in other epithelial follicle cells is sufficient to cause them to migrate like border cells. This is not the case, for example, for guidance receptors; all follicle cells express PVR (platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF)-related) and EGFR (epidermal growth factor receptor) and can presumably sense the ligands. But only border cells migrate towards the chemoattractants, and this ability to chemotax is conferred by STAT. So does STAT regulate border cell fate, differentiation or migration? The answer is that STAT regulates all three, and all three are intimately related. Neural crest cells and gastrulating mesodermal cells are similar in that it is their fate to migrate[85, 86, 161].

Box 2|Actomyosin dynamics during single-cell movement

Cell movement is largely mediated through changes in the actin cytoskeleton. Studies in cell culture and *in vitro* have yielded models of how actin polymer kinetics are regulated (see the figure; reviewed in Refs 6,7). Globular actin (G-actin) monomers in the cytosol are added to the barbed ends of filamentous actin (F-actin) 'seeds', which rapidly increases the length of these filaments. Many regulators influence the dynamic equilibrium between filament lengthening and shortening. Formins such as diaphanous (DIA) nucleate seeds, an otherwise rate-limiting event. ENA/VASP (enabled/vasodilator-stimulated phosphoprotein) proteins promote filament elongation by antagonizing capping proteins, which, as their name suggests, terminate elongation. Elongating F-actin can push out the plasma membrane, forming narrow protrusions called filopodia. Myosins, tropomyosin and other F-actin-binding proteins can stabilize actin filaments and protect them from severing and depolymerizing factors such as cofilin. Along existing filaments, the actin-related protein 2/3 (ARP2/3) complex nucleates formation of an F-actin branch. These branched configurations drive formation of the broad and dynamic protrusions called lamellipodia, which promote cell migration across a flat surface. Stable actin filaments are also found in stress fibres, which associate with adhesion molecules and are rich in myosin. The movement of myosin motors along stress fibres contracts the rear of the cell during cell translocation. Focal adhesions are complex macromolecular assemblies that include adaptor proteins such as paxillin, which link the F-actin cytoskeleton to the ECM via integrin receptors. Focal adhesions serve both mechanical and biochemical signalling functions, and focal adhesion dynamics are important for cell movement on or through ECM substrates. For more information see the Cell Migration Consortium website.

Signals at the cell membrane promote cell movement by changing the actin cytoskeleton, largely via the local activities of the RHO-family of small GTPases (reviewed in Refs 123,125,134,164). In fibroblasts, active CDC42 promotes filopodia formation, active RAC stimulates membrane ruffling and protrusion of lamellipodia, and RHO causes focal adhesions (see the figure, bottom inset) and the associated tension-bearing actomyosin stress fibres to form. However, there is significant crosstalk between GTPases, cell type specificity to the responses and additional activities of these key regulators. Thus, it is important to examine signalling in different contexts. Many actin regulatory proteins are required in border cell migration *in vivo* (see Table 2). However, their roles *in situ* are sometimes unanticipated, and in particular their functions in collective motility are still under investigation.

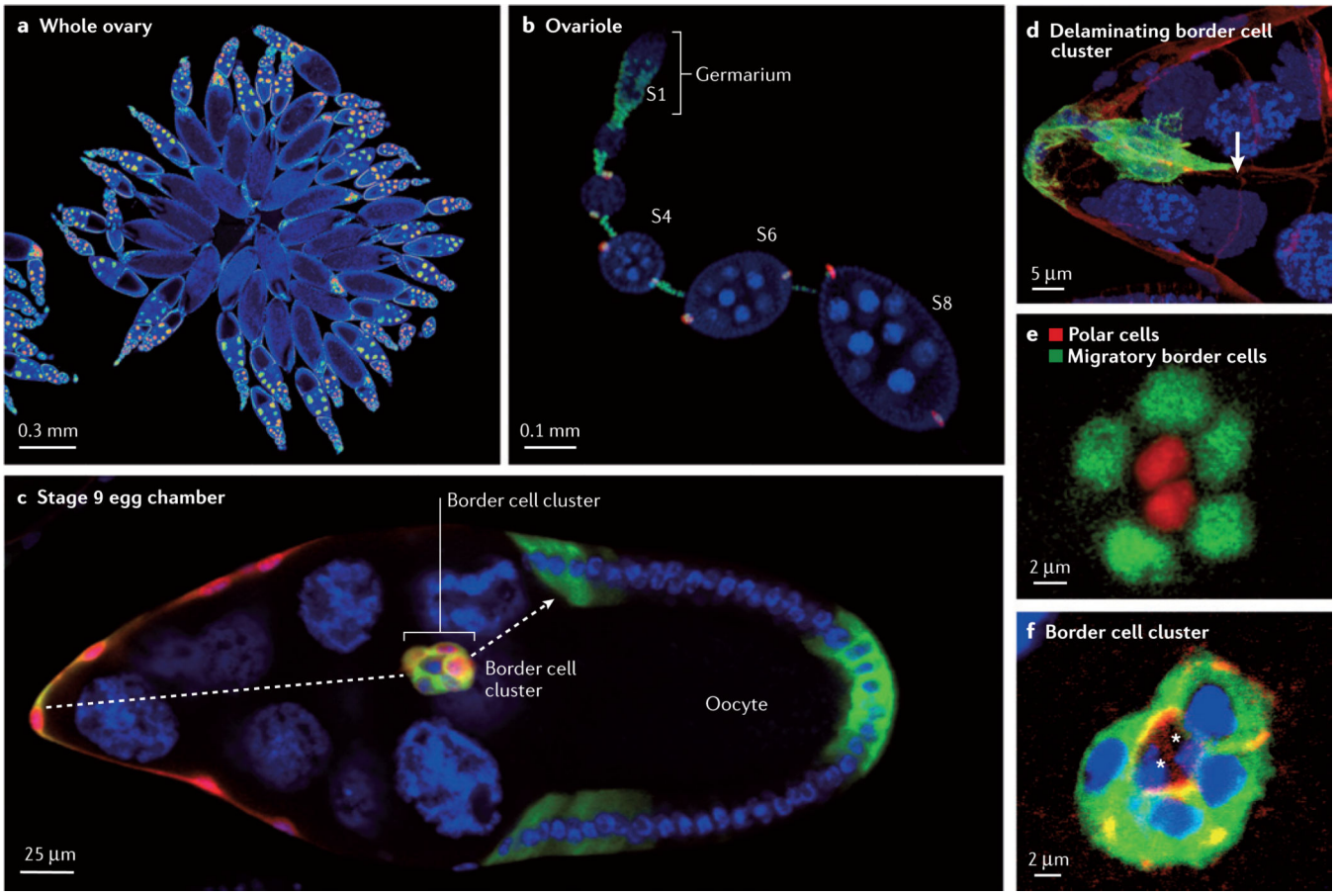

Figure 1. ***Drosophila melanogaster*** **oogenesis**

a | A whole ovary is shown, mounted flat to expose individual ovarioles. Hoechst dye labels DNA in the nuclei (blue) and the image is pseudo-coloured according to fluorescence intensity. Earliest developmental stages are oriented towards the periphery, and mature eggs are in the centre. **b** | Image of a single ovariole, stained with an antibody against Fasciclin 3 (FAS3) (shown in red), a protein that is expressed specifically in polar cells, and stalk cells are shown in green and nuclei are labelled by DAPI in blue. The germarium is shown at the top left, progressively older egg chambers are down and to the right, with a stage 8 (S8) egg chamber at the bottom right. FAS3-labelled polar cells are present from very young stages. **c** | Border cells migrating in a stage 9 egg chamber. The anterior is to the left, and the oocyte takes up most of the unstained space to the right. Border cells, posterior follicle cells (right) and centripetal cells (top and bottom centre) are marked by expression of a mouse CD8–GFP fusion protein driven by the yeast transcription factor GAL4 under the control of a *slbo* enhancer. Staining for β-galactosidase expressed from an ecdysone reporter is shown in red and shows highest activity in border cells and anterior follicle cells. DAPI marks nuclei in blue. The dashed lines indicate the migration path. **d** | Border cells delaminating from the epithelium and outer extracellular matrix display leading–lagging edge polarity. These cells are marked with GFP in green, filamentous actin (F-actin) is stained by phalloidin in red, and the nuclei are stained by DAPI in blue. The border cell cluster forms many protrusions and then retracts and de-adheres from other cells in the epithelium. The arrow indicates the

leading protrusion. **e** | A cluster of border cells mid-migration. Nuclei of the outer, migratory border cells are stained with antibody against Eyes absent (EYA) in green. Polar cell nuclei are labelled with antibodies recognizing β-galactosidase, which is expressed from the *Fas3* gene (shown in red). **f** | Within the moving cluster, cells adhere tightly together. Nuclei are marked with DAPI in blue. Migratory border cells express GFP (shown in green), whereas polar cells (*) do not. *D. melanogaster* epithelial cadherin (DE-cadherin) staining (shown in red or in yellow where it overlaps with GFP) is found at highest levels at polar cell–polar cell and polar cell–border cell junctions. Punctate junctions between migratory border cells also exhibit high levels of DE-cadherin. Lower levels of DE-cadherin staining are observed at border cell–nurse cell interfaces. Image in part **c** is modified, with permission, from Ref. 71© (2009) Macmillan Publishers Ltd. All rights reserved. The micrograph in part **e** is reproduced, with permission, from Ref. 165 © (2002) The Company of Biologists.

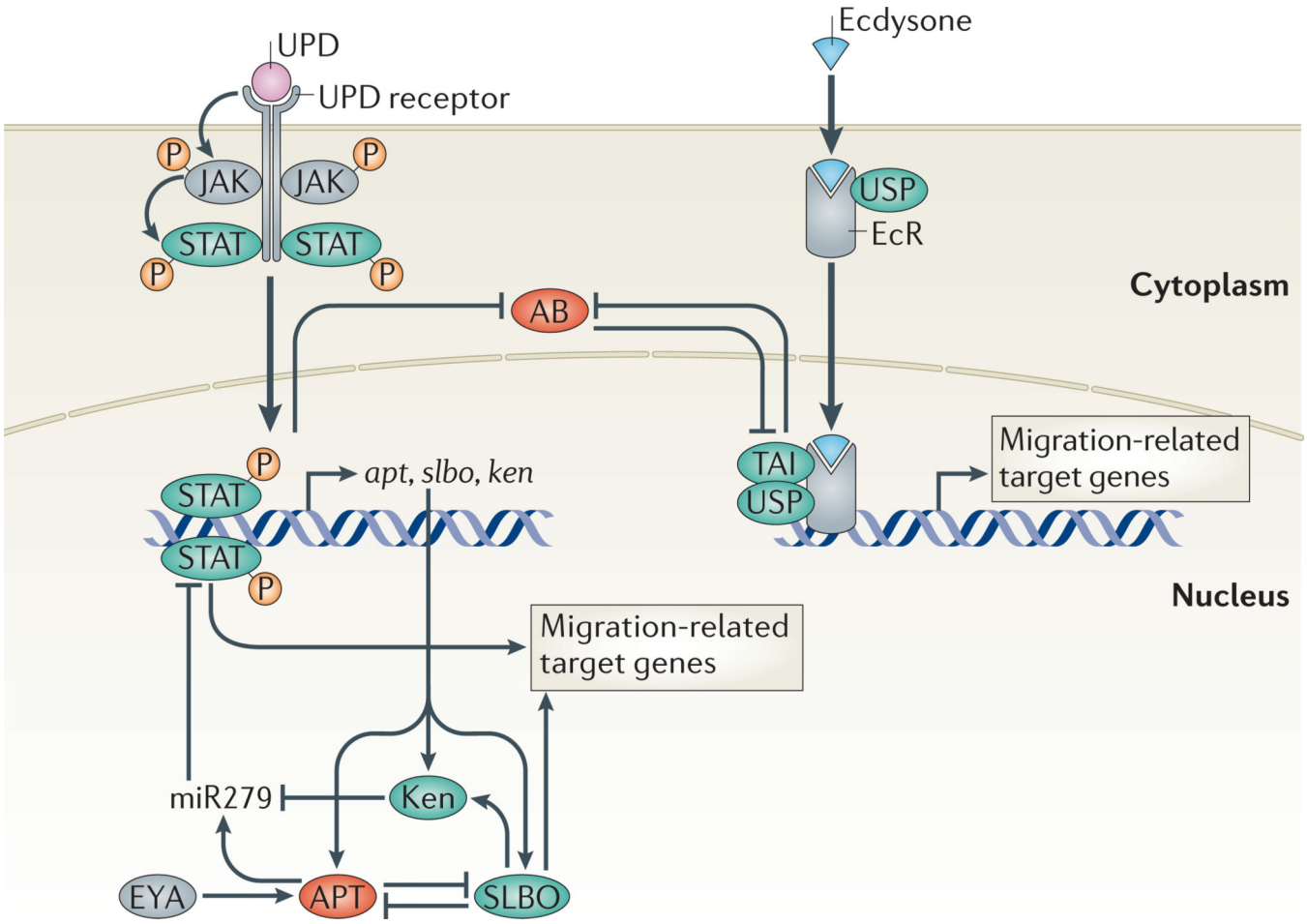

Figure 2. Converging signalling pathways regulate border cell migration

Local production of Unpaired (UPD) from polar cells activates the UPD receptor and JAK (Janus kinase), leading to phosphorylation and activation of STAT (signal transducer and activator of transcription). STAT signalling activates a regulatory circuit that is minimally comprised of Apontic (APT), Slow border cells (SLBO), microRNA-279 (miR-279) and Ken. APT is also activated by Eyes absent (EYA). Cells that surpass a signal threshold reinforce SLBO expression and activate downstream target genes that are needed for border cell movement. Mathematical modelling and simulation demonstrate that this set of interactions can convert a gradient of UPD into on and off states of STAT activity. The concentration of the steroid hormone ecdysone rises during stage 9, activating a nuclear hormone receptor complex comprised of Ecdysone receptor (EcR), Ultraspiracle (USP) and Taiman (TAI). Receptor activation leads to expression of downstream targets that promote border cell motility. Both the UPD and ecdysone pathways inhibit Abrupt (AB), which also feeds back to inhibit TAI.

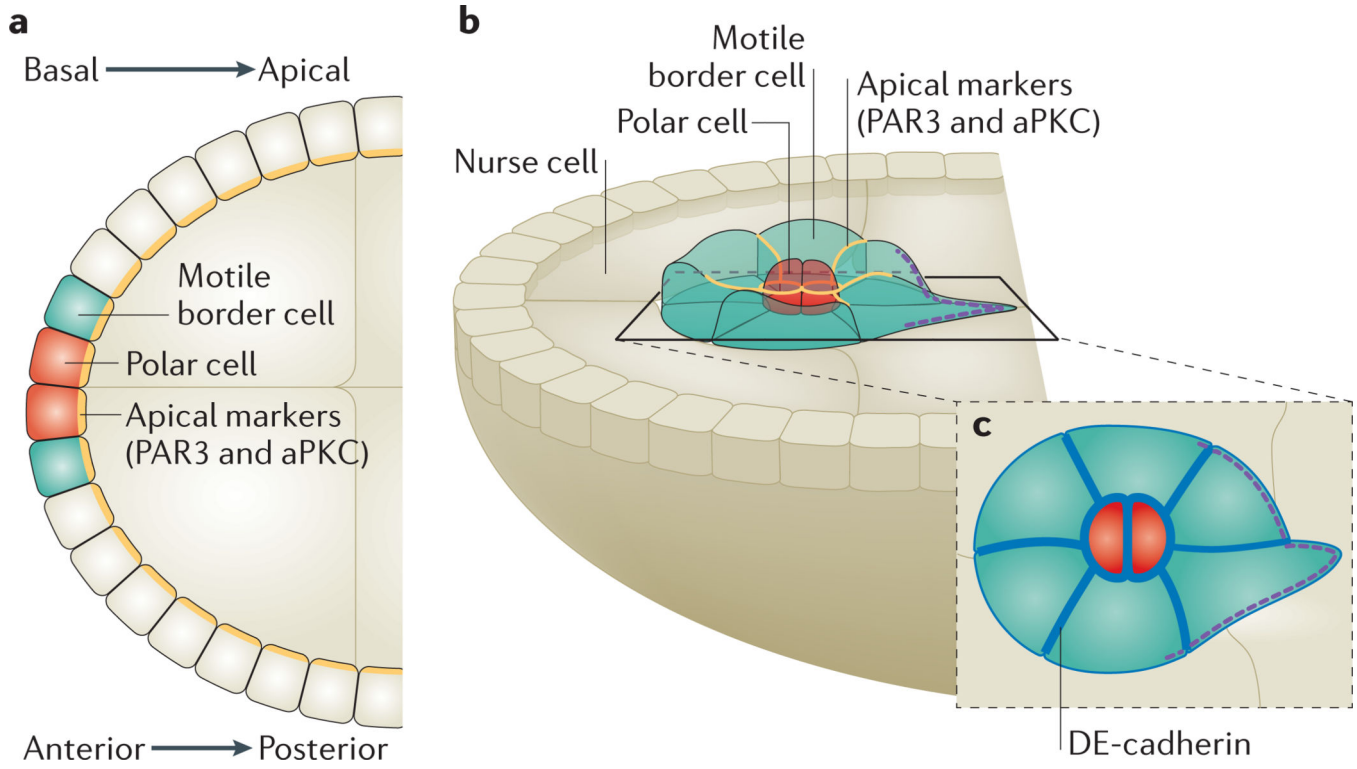

Figure 3. Migrating border cells are polarized along three axes of polarity

a | Before the onset of migration, at stage 8, border cells (shown in green) surround the two anterior polar cells (shown in red) in the follicular epithelium. At this stage, all follicle cells are polarized, as shown by apical localization of markers such as PAR3 (partitioning defective 3) and aPKC (atypical protein kinase C; shown in yellow). **b** | As migration begins, border cells move towards the oocyte and adopt a leading edge (purple dots) and lagging edge (to the left) asymmetry. At the same time, border cells maintain apical markers (shown in yellow) orthogonally to the direction of border cell movement. **c** | A slice through the migrating border cell cluster shown in (**b**) reveals inside–outside polarity. The central non-motile polar cells are indicated in red. *Drosophila melanogaster* cadherin (DE-cadherin; shown in blue) levels are high at border cell–border cell and border cell–polar cell interfaces and lower at border cell–nurse cell interfaces. In this section, the leading edge (purple dots) is to the right and the apical–basal axis is perpendicular to the plane of the page.

Table 1

Techniques used to decipher the molecular control of border cell migration

Experimental strategy	Genes identified	Advantages	Disadvantages
Loss-of-function screen for viable homozygous mutant adults	*slbo*, *apt*, *Pvf1* (Refs 24,54,159)	Unbiased, relatively easy to identify mutant gene if transposon is the mutagen, enhancer traps reveal gene expression patterns	Will miss homozygous lethal mutants unless there is a hypomorphic allele
Loss-of-function screen using analysis in mosaic clones	*Stat92E*, *tai*, *par1* *psidin*[36, 38–40,52,69,73,106,145,160,166]	Unbiased, can identify homozygous lethal mutations, indicates in which cells a gene is required	Gene identification is typically more difficult and time consuming, community resources make the identification of mutants easier[167]
Loss-of-function screen for enhancers or suppressors of mutants	*Pvf1* (Refs 71,91)	Can identify additional components in a pathway of interest, can uncover mutations that have weak effects individually	May identify components that interact very indirectly, many components will be missed
Gain-of-function screens, in which genes are specifically overexpressed in cells of interest	Genes encoding EGFR ligands, *Pvf1* *ab*[70,91–94,168–170]	May identify genes that are missed in loss-of-function screens, such as genes that function redundantly	Potentially misleading because some genes that do not normally have a role can cause a phenotype when overexpressed
Candidate testing	RAC, *shg*, *jar*, genes encoding RABs, *ELMO*, *mir-279* (Refs 53,98,128,157)	Can be the fastest way to an important result, can reveal novel or cell type-specific functions for known proteins	Biased, less opportunity for an unexpected discovery, does not always yield a phenotype
Imaging techniques	*par1*[106], *Notch*[25], *RAC*[129]	Higher spatial and temporal resolution than fixed tissue analysis, can distinguish different migration phenotypes that look the same in fixed tissue (for example, revealed role of *par1* in protrusion dynamics, *Notch* in detachment and RAC in cell–cell coordination)	Technically challenging, specialized equipment and expertise required
Mathematical modelling	*Stat92E*, *slbo*, *apt*, *mir-279* (Refs 53,54,82,171,172)	Illustrates how complex networks of components might work together, generates predictions that can be tested, can reveal that components are missing	Indicates if a particular set of components and relationships can produce a particular result, not whether it really does

ab, *abrupt; apt*, *apontic*; EGFR, epidermal growth factor receptor; *ELMO*, *Engulfment and cell motility; jar*, *jaguar; mir-279*, *microRNA-279; par1*, *partitioning defective 1; psidin*, *phagocyte signalling impaired; Pvf1*, *PDGF and VEGF related factor 1; shg*, *shotgun; slbo*, *slow border cells; Stat92E*, *Signal transducer and activator of transcription protein at 92E; tai*, *taiman*.

Table 2

Cytoskeletal regulators involved in border cell migration

Gene name	Protein function	Border cell function	Refs
Act5C	Actin monomer	Overexpression rescues low RAC activity	74,89,158
α *-cat*	Part of the cadherin complex, actin binding	When fused to DE-cadherin, α-catenin fully rescues *arm* mutant clones	113
arm (which encodes β-catenin)	Part of the cadherin complex, cadherin binding	Loss causes incomplete migration	113,157
cpb	Actin filament capping	Overexpression leads to early arrival at oocyte	150
Cdc42	GTPase activity, cell polarity, actin polymerization	Loss causes mild cluster disassembly	77
cher (which encodes Filamin)	Actin binding and crosslinking	Loss causes incomplete migration	139,173
chic (which encodes Profilin)	Actin monomer binding	Loss causes incomplete migration	158
cindr	Endophilin binding, endocytosis	Loss causes mild delay in migration	98
Cortactin	Actin binding	Loss causes mild delay in migration	98,152
dia	Actin binding, RHO binding	Constitutively active DIA blocks migration	143
ELMO	RAC exchange factor	Loss results in incomplete migration	33,158
ena	Barbed end F-actin binding	Loss or gain impedes migration	150
jar (which encodes myosin VI)	Actin binding, motor protein	Loss causes incomplete migration	157,174
kst	β-heavy-spectrin	Loss causes delayed migration and trailing border cells	175
mim	I-bar family	Loss causes delayed migration and poor direction sensing	98
mbc	RAC exchange factor, DOCK180	Loss causes incomplete migration	33,94,158
Mbs	Myosin phosphatase	Loss enhances *par1* phenotype	107
psidin	Actin binding	Loss results in incomplete migration	160
Rac1	GTPase activity	Dominant-negative causes little to no migration	128–130,158
Rho1	GTPase activity	Dominant-negative causes cluster disassembly	77,128
rok	Protein Ser/Thr kinase	Dominant-negative causes incomplete migration and detachment defects	107,146
shg (which encodes DE-cadherin)	Homophilic cell adhesion, ARM binding	Loss or gain prevents migration	23,74,113,157,176
ssh	Ser/Thr/Tyr phosphatase, Cofilin activator	Loss enhances *tsr* phenotype	160
sqh (which encodes non-muscle myosin II)	Myosin II light chain	Loss results in cells remaining at anterior tip, interacts genetically with PAR1	107,154,156
spri	Adaptor protein	Redundant with Cbl, but incomplete migration with PVR or EGFR overexpression	97,177
th (which encodes DIAP1)	Interacts with RAC and Profilin to regulate actin dynamics	Overexpression suppresses migration, defects caused by blocking RAC	158
Tm1	F-actin binding	Loss results in incomplete border cell migration	160
tsr (which encodes Cofilin)	Actin binding, F-actin severing	Loss results in incomplete migration and excess F-actin	145

Gene name	Protein function	Border cell function	Refs
twf	Protein tyrosine kinase	Loss results in incomplete migration and increased F-actin levels	146,178
zip	Myosin II heavy chain	Dominant-negative results in detachment defects	107

Act5C, actin 5C; α=cat, α-catenin; arm, armadillo; cher, cheerio; chic, chickadee; *cindr, CIN85 and CD2AP orthologue; cpb, capping protein beta; dia, diaphanous*; DE-cadherin, *Drosophila melanogaster* epithelial cadherin; DIAP1, *D. melanogaster* Inhibitor of apoptosis 1; EGFR, epidermal growth factor receptor; ELMO, Engulfment and cell motility; *ena, enabled*; F-actin, filamentous actin; *jar, jaguar; kst, karst; mbc, myoblast city; Mbs, myosin-binding subunit; mim, missing in metastasis; par1, partitioning defective 1; psidin, phagocyte signalling impaired*; PVR, PDGF-and VEGF-receptor related; *rok, RHO-kinase; shg, shotgun; spri, sprint; sqh, spaghetti squash; ssh, slingshot; th, thread; Tm1, Tropomyosin 1; tsr, twinstar; twf, twinfilin; zip, zipper*.