HHS Public Access

Author manuscript

Mech Dev. Author manuscript; available in PMC 2018 December 01.

Published in final edited form as:

Mech Dev. 2017 December ; 148: 56–68. doi:10.1016/j.mod.2017.06.003.

A hormonal cue promotes timely follicle cell migration by modulating transcription profiles

Lathiena Manning[a,b], **Jinal Sheth**[a,1], **Stacey Bridges**[c,1], **Afsoon Saadin**[a], **Kamsi Odinammadu**[a], **Deborah Andrew**[d], **Susan Spencer**[e], **Denise Montell**[f,*], and **Michelle Starz-Gaiano**[a,*]

[a]Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD, United States

[b]UNC Chapel Hill, NC, United States

[c]University of Maryland School of Medicine, Baltimore, MD, United States

[d]Johns Hopkins School of Medicine, Baltimore, MD, United States

[e]St. Louis University, St. Louis, MO, United States

[f]University of Santa Barbara, Santa Barbara, CA, United States

Abstract

Cell migration is essential during animal development. In the *Drosophila* ovary, the steroid hormone ecdysone coordinates nutrient sensing, growth, and the timing of morphogenesis events including border cell migration. To identify downstream effectors of ecdysone signaling, we profiled gene expression in wild-type follicle cells compared to cells expressing a dominant negative Ecdysone receptor or its coactivator Taiman. Of approximately 400 genes that showed differences in expression, we validated 16 candidate genes for expression in border and centripetal cells, and demonstrated that seven responded to ectopic ecdysone activation by changing their transcriptional levels. We found a requirement for seven putative targets in effective cell migration, including two other nuclear hormone receptors, a calcyphosine-encoding gene, and a prolyl hydroxylase. Thus, we identified multiple new genetic regulators modulated at the level of transcription that allow cells to interpret information from the environment and coordinate cell migration in vivo.

Keywords

Collective cell migration; *Drosophila*; Oogenesis; Nuclear hormone signaling

[*]Corresponding authors. denise.montell@lifesci.ucsb.edu (D. Montell), starz@umbc.edu (M. Starz-Gaiano).

[1]These authors contributed equally.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mod.2017.06.003.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript

1. Introduction

Cell migration is an essential process in normal development and disease progression. For animal development to occur properly, cells must move at precisely the right times and to the correct locations. Decades of research have provided a detailed understanding of some of the molecular changes that mechanically allow a cell to translocate: regulators that re-organize the actomyosin cytoskeleton, guidance factors that direct a cell to the right location, and transcriptional programs that promote a cell to become motile (reviewed in (Horwitz and Webb, 2003; Huber et al., 2005; Insall and Machesky, 2009; Janetopoulos and Firtel, 2008; Petrie et al., 2009; Simoes-Costa and Bronner, 2015)). However, many studies have investigated how cells move singly, not how they could act together to coordinate their movements with each other or how they update signaling to reflect ongoing changes in their environment. These are important issues to study as they have implications in normal embryonic development, immune response, and cancer metastasis, among other diseases.

We are using the well-characterized migrations of subsets of cells in the *Drosophila* ovary to investigate complex questions in coordinated cell movements, with a focus on the border cells (reviewed in (Aman and Piotrowski, 2010; Montell et al., 2012; Rorth, 2002; Saadin and Starz-Gaiano, 2016)). This system is advantageous because migrating cells can be observed directly in their normal context through live-imaging of egg chambers, many tools are available to manipulate gene expression, and the genetic regulators of these cells are similar to those in other migratory cell types (Campbell and Casanova, 2016; Hudson and Cooley, 2014; Scarpa and Mayor, 2016). In the ovary, egg chambers are made up of germline cells surrounded by somatic follicle cells, which exist in a single-layer epithelium ((King, 1970) and see Fig. 1A). Multiple follicle cell migrations and cell rearrangements must occur sequentially for normal oogenesis (Berg, 2005; Cetera and Horne-Badovinac, 2015; Wu et al., 2008). In mid-oogenesis, six to eight border cells arise within the follicular epithelium that surrounds the germ line. Border cells form around two anterior polar cells, which are specified earlier, and the two different cell types adhere together to move between the large germline nurse cells and to reach the edge of the oocyte. Soon after border cell migration is completed, about 50 centripetal cells, located along the equator of the egg chamber, migrate internally to cover the anterior side of the oocyte (reviewed in (Dobens and Raftery, 2000; Duhart et al., 2017)). Centripetal cells move like an iris, spreading to close across the oocyte. Both migration events are necessary for proper eggshell formation and development of a viable egg. Border cell specification requires activation of Janus kinase/Signal Transducer and Activator of Transcription (Jak/STAT) signaling, which turns on the transcription factor Slow border cells (Slbo) in the border cells (Saadin and Starz-Gaiano, 2016). *slbo* is required for border cell motility, and it is also expressed in the centripetal cells (Montell et al., 1992), where it represses *cut* expression (Levine et al., 2010). Both cell types require dynamic regulation of cytoskeletal and adhesion molecules, such as myosin, actin, and E-cadherin, for their movements (Edwards and Kiehart, 1996; Montell et al., 2012; Niewiadomska et al., 1999; Tepass et al., 1996).

Additionally, border cell and centripetal cell migration events require steroid hormone signaling to occur properly (Bai et al., 2000; Cherbas et al., 2003; Domanitskaya et al., 2014; Hackney et al., 2007; Jang et al., 2009). In flies, the sole steroid hormone is ecdysone,

which binds the Ecdysone receptor (EcR) and Ultraspiracle (USP) heterodimer (Yao et al., 1993) (together often referred to as EcR, reviewed in (Belles and Piulachs, 2015; Riddiford et al., 1993; Yamanaka et al., 2013)). Upon ligand binding, this complex translocates to the nucleus and regulates transcription along with a number of cofactors. As with steroid hormone signaling in other organisms, ecdysone coordinates growth and developmental timing events in different cell types and tissues. In adult females, ecdysone is produced in egg chambers, and its signaling serves as a checkpoint to permit egg development only when nutritional levels are appropriate (Belles and Piulachs, 2015; Buszczak et al., 1999; Carney and Bender, 2000; Danielsen et al., 2013; Morris and Spradling, 2012; Riddiford et al., 1993; Sieber and Spradling, 2015; Terashima and Bownes, 2005). The titer of hormone produced in egg chambers peaks around stage 9 (Schwartz et al., 1989), and both germline and follicle cells require ecdysone to allow oogenesis to proceed to later, vitellogenic stages (Buszczak et al., 1999). In this way, ecdysone can coordinate the development of different cell types in the ovary. Ecdysone is produced in all follicle cells, but not all cells respond to the signal: reporter assays in mid-oogenesis reveal that the border cells show a particularly strong transcriptional response to this signal, and the centripetal cells also respond (Bai et al., 2000; Cherbas et al., 2003; Domanitskaya et al., 2014; Hackney et al., 2007; Jang et al., 2009). Interestingly, a small number of wild-type follicle cells are sufficient to produce enough hormone to mediate motility in border cells, suggesting the cells can import this signal (Domanitskaya et al., 2014). Details of how the spatio-temporal response is initiated are not clear, although it is partially explained by a negative regulator of EcR signaling called Abrupt (Jang et al., 2009). STAT downregulates the *abrupt* gene, which then allows ecdysone signaling in the border cells and promotes migration via downstream transcriptional targets.

Border cells also require the EcR co-activator *taiman* (*tai*), a protein homologous to human SRC3, for their efficient movement in response to ecdysone (Bai et al., 2000; Jang et al., 2009; McDonald et al., 2003). Disruption of *EcR* or *tai* function results in slow border cell migration and abnormal adhesion of the border/polar cell cluster (Bai et al., 2000). Conversely, early expression of an activated form of *tai* (*tai B*) can cause abnormally early movements of the border cell cluster, but only in combination with ectopically early specification via STAT activation (Jang et al., 2009). Several key ecdysone targets have been identified during metamorphosis (Beckstead et al., 2005; Beckstead et al., 2007), and in ovary (Ables et al., 2015; Ables and Drummond-Barbosa, 2010; Ables et al., 2016; Buszczak et al., 1999; Terashima and Bownes, 2005), but few are known to have roles in cell motility. Furthermore, it is not clear how downstream targets for EcR, Tai, STAT, and Slbo coordinate their activities to result in proper temporal control of border cell movements.

To identify downstream effectors of ecdysone signaling, we performed multiple gene expression analyses on border cells purified from different mutant backgrounds. We identified over 400 genes with altered expression levels in migrating follicle cells when either EcR or Tai function was disrupted by expression of dominant negative proteins. Several of these genes have been previously identified as border cell regulators, but many others are novel candidates. We validated 16 genes by confirming their expression in egg chambers, particularly in border and centripetal cells. We showed that several genes are responsive to Ecdysone hormone, since their expression levels increased in cultured egg

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

chambers when we added exogenous 20-hydroxyecdysone (20-HE), an activated form of the ecdysone hormone. Disruption of seven targets with varied molecular functions each led to defects in cell migration, including other nuclear hormone receptors, a calcyphosine-like gene, and a prolyl hydroxylase. Thus, our approach identified multiple new genetic regulators modulated at the transcriptional level that allow cells to interpret information from the environment and coordinate cell migration in vivo.

2. Results

2.1. A microarray approach to determine downstream targets of Ecdysone receptor and Taiman in motile border cells

To identify key transcriptional programs downstream of steroid hormone signaling in border cells, we genetically blocked this signaling pathway. Using the Gal4-UAS system, we expressed either dominant negative EcR (EcR W650A (Cherbas et al., 2003)) or dominant negative Tai (LXXLL (Bai et al., 2000; Jang et al., 2009)) in the follicle cells using *slbo*-Gal4, which drives expression in the motile border cells and centripetal cells but not polar cells (Rorth et al., 1998). EcR W650A alters a conserved amino acid required for hormone binding, however it maintains Usp binding and dominantly blocks transcription by competing away this required partner (Cherbas et al., 2003). The Tai dominant negative binds the Ecd/Usp heterodimer in a ligand-dependent manner, but does not contain the domains to recruit co-activators, and thus blocks activation (Jang et al., 2009). In addition to driving expression of the dominant negative(s) in the *slbo* pattern, we simultaneously expressed Green fluorescent protein and mouse-CD8 antigen (mCD8-GFP) to use as a molecular tag to purify the motile cells (see Methods and (Wang et al., 2006)). To enrich for earlier stages in oogenesis, we utilized virgin females, which initially harbor mostly egg chambers at stage 9 or younger. As previously reported, overexpression of dominant negative EcR or Tai resulted in incomplete border cell migration in the majority of stage 10 egg chambers (Fig. 1) and incomplete centripetal cell movements (Cherbas et al., 2003; Hackney et al., 2007; Jang et al., 2009).

Next we purified the border cells and centripetal cells expressing EcR W650A or TaiDN, as well as those from control egg chambers (*slbo*-Gal4, UAS-mCD8GFP, UAS-*LacZ*) by dissecting ovaries from young females, dissociating the cells by elastase treatment, and subjecting the cells to magnetic beads linked to CD8-directed antibodies (Wang et al., 2006). After confirming that the majority of cells we isolated were GFP positive, we extracted mRNAs from each cell population, and amplified the resultant cDNAs. We assayed three biological replicates per genotype. cRNAs were generated by in vitro transcription and probed against the Affymetrix Gene Chip and detected probes were evaluated using two types of analysis platforms (MAS 5 and ChipStat, see Methods and (Master et al., 2005; Wang et al., 2006)) to determine differences in gene expression between the mutants and the wild-type control.

2.2. Multiple classes of genes respond to EcR and Tai signaling in the follicle cells

The microarray analyses identified a wide variety of genes that are putatively expressed in the border cells and responsive to ecdysone or *tai* function. Based on genes that are

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

expressed at a lower level in the mutants, we can infer the downstream targets of ecdysone and Tai signaling. 101 total genes were downregulated at least 1.2-fold when EcR signaling was blocked in border cells, suggesting that these genes are normally activated downstream of this pathway (Fig. 2A, and Supplemental Table 1). Conversely, expression levels of 210 genes were increased in the mutants, suggesting EcR signaling normally results in their repression (Fig. 2B). These changes may be direct or indirect. While the fold changes detected are small in many cases, several of the identified genes are known regulators of border cell migration from prior work (e.g., *jaguar* (Geisbrecht and Montell, 2002), *peb* (Melani et al., 2008; Verheyen and Cooley, 1994), *chic* (Verheyen and Cooley, 1994), *dhc64C* (Van de Bor et al., 2011), *actin57B* (Geisbrecht and Montell, 2004; Kim et al., 2011; Somogyi and Rorth, 2004)) and more are enriched in border cells (Table 1 and (Borghese et al., 2006; Wang et al., 2006)), so we believe that many of the identified candidates are likely to be relevant. Notably, 45% (154/337) of the upregulated genes were identified in other, previous microarray analyses of border and centripetal cells, including *argk*, *mfas*, *lola-like*, *18w*, *vrille*, and *ogre* (Borghese et al., 2006; Wang et al., 2006) (Table 1 and Fig. 2C). Moreover, we did not see enrichment of known main body genes, such as *bunched* (Dobens et al., 2005) and *mirror* (Xi et al., 2003). We detected chorion protein encoding genes *Cp15*, *16*, *36*, and *38*, which have been shown to be regulated by Ecdysone receptor (Hackney et al., 2007; Mitsialis and Kafatos, 1985; Shea et al., 1990), further supporting the specificity of our experiment, and our data suggest that several of these are also downstream of Taiman. Less than half of the genes upregulated in response to Ecdysone in follicle cells were previously found by Beckstead and colleagues in their set of over 4000 ecdysone target genes that are altered during metamorphosis (Beckstead et al., 2005), suggesting that our set includes many ovary-specific targets (Fig. 2D). According to our second set of microarray results, Tai dominant negative expression resulted in reduced expression of 79 genes and elevated expression of 128 (Fig. 2A–B and Supplemental Table 2). Many genes had not previously been implicated in cell migration and some were novel, suggesting they may be new potential regulators of border and centripetal cell migration, although some may be expressed later in oogenesis.

As expected, a significant set of putative EcR and Tai target genes overlapped (Fig. 2A–B). Table 1 shows the highest-ranking candidate genes identified by the microarray analyses in both data sets. These genes span a range of gene ontology (GO) categories and no functional categories are particularly enriched; that is, the GO representation by molecular function in the set overall is not significantly different from the proportion of each of the classes as they are represented in the genome (Fig. 2E–F). In terms of biological processes, genes encoding signal peptide processing factors were enriched 44.3-fold ($p < 0.01$) in the list upregulated by EcR, and those encoding vitelline membrane or chorion formation proteins were over-represented by 25-fold ($p < 0.01$) (identified using DAVID (Dennis et al., 2003; Huang da et al., 2009; Sherman et al., 2007)). Interestingly, in the gene ontology molecular function category, extracellular matrix structural constituents were significantly over-represented among the set of genes normally downregulated by ecdysone signaling (increased 8.8 fold, $p = 0.01$), as were histone binding proteins (increased 9.1 fold, $p < 0.01$). Other enriched categories in this set included snoRNA binding and proton-transporting ATPases (15.8 and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

7.2 fold increases, respectively, $p < 0.01$). Notably, of the genes normally downregulated by Tai function, cytoskeletal proteins were overrepresented 16.2-fold ($p < 0.01$).

2.3. Gene products enriched in follicle cells and responsive to steroid hormone

To verify that candidate genes of interest are expressed in motile cells during oogenesis, we performed expression analysis. Cells that express *slbo*-Gal4 include 4–6 border cells in stage 9 and older egg chambers, about 50 centripetally migrating cells in stage 10B and older egg chambers, and 10–30 posterior follicle cells in stage 9 and older egg chambers (the number increases during this period of development). Therefore, we expected to observe gene expression and possibly, but not necessarily, enrichment in one or more of these cell types. We used enhancer traps, GFP-protein traps, and in situ hybridization in egg chambers to examine spatiotemporal gene expression (Fig. 3). Most genes and proteins we examined did show expression in border cells and/or centripetal cells, and could be grouped accordingly. We verified nine genes that are expressed in border cells and other follicle cells, including *Eip75B*, *Neprilysin 2*, *Skp2*, *Gilt1*, *Hr4*, *Scylla*, *ImpL2*, *Fer1HCH*, and *PH4a-EFB,* (Fig. 3A–L, and Supplemental Fig. 1), as well as *Argk* and *mfas*, which were previously reported (Wang et al., 2006). Other genes we examined are expressed broadly in centripetal cells plus other follicle cells, or in the germline, suggesting they may be needed in these cell types or have varied functional roles, including *nahoda*, *Orc3*, *porin*, *CG11378*, *palisade*, *CG14834*, *Yp3* (Fig. 3J–O and Supplemental Fig. 1). Most genes we assayed are putative targets of both EcR and Tai. However, *Hr4* and two known egg-development genes (*palisade, Yp3*) (Andersen and Horne-Badovinac, 2016; Fakhouri et al., 2006) were only identified in the EcR microarrays. In contrast, *Fer1HCH*, *Neprilysin 2*, and *CG14834* were only identified as potential Tai targets.

Next we sought to identify the candidate genes that respond rapidly to changes in hormone levels, as these are most likely to be relevant targets. To do this, we cultured dissected ovaries in media with or without addition of 20-Hydroxyecdysone (20-HE) (Buszczak et al., 1999; King-Jones et al., 2005; Kozlova and Thummel, 2002), which is an active form of ecdysone. We then either examined reporter gene expression or examined mRNA levels by quantitative RNase protection assays (RPA). A striking increase in the *Eip75B* reporter expression domain demonstrated that the culture conditions were appropriate and that this gene is highly responsive to ecdysone signaling in follicle cells (Fig. 4A–B, and see (Buszczak et al., 1999)). For six of eight genes assayed by RPA, we found reproducible increases in mRNA levels 2 h after 20-HE addition (Fig. 4C–D), and no appreciable change in the *rp49* control expression. Other protein-trap reporters expressed in border cells, including Fer1HCH and Skp2, did not change in response to 20-HE addition, but this may be due to saturating expression of GFP (data not shown). A GFP-trap for GILT1, however, did reveal higher border cell expression upon 20HE addition, suggesting the exogenous hormone raised expression levels (Supplemental Fig. 2). Consistent with these experiments, bioinformatic analysis with Jasper (Mathelier et al., 2016; Sandelin et al., 2004) showed that multiple putative target genes have EcR binding sites in regulatory regions (Fig. 4E). These results confirmed that genes identified by the microarray are likely to be true downstream targets of ecdysone signaling.

Author Manuscript

2.4. Border cell migration requires additional inputs from nuclear hormone receptors

To determine if proper border cell migration required any of the EcR or Tai-responsive genes identified, we assayed numerous mutants. We selected high-ranking candidates for which reagents were available. For many genes, we used tissue-specific RNA interference (RNAi) (Dietzl et al., 2007; Perkins et al., 2015) to reduce gene function in the border and centripetal cells using *slbo*-Gal4, or we specifically targeted the border cells by driving expression with the c306-Gal4 line. The c306-Gal4 line turns gene expression on earlier, so is more likely to reduce gene function prior to border cell migration. Out of 19 genes tested by RNAi-mediated knock down specifically in border cells, 11 resulted in border cell migration defects with >10% penetrance (Supplemental Table 3).

Ecdysone induced protein at 75B (Eip75B) is nuclear hormone receptor shown to bind oxygenated heme, and is a known downstream target of ecdysone signaling (Buszczak et al., 1999; Reinking et al., 2005). We found high levels of beta-Galactosidase expression in the border cells specifically in ovaries from females bearing an enhancer trap inserted into the *Eip75B* locus (Supplemental Fig. 1), and protein trap expression in border cells and other follicle cells (Fig. 3A). Gene expression increased upon treatment of ovaries with 20-HE (Fig. 4D). RNAi-based knockdown of this gene resulted in border cell migration delays in about 44% of stage 10 egg chambers (Fig. 5A, C). In addition, homozygous mutants for the viable allele *Eip75* ^{1} had border cell migration delays in 10% of egg chambers (n = 30).

Another nuclear hormone receptor, Hormone receptor 4 (Hr4), also appears to have a role in border cell migration. Hr4 is the homolog of mammalian Germ Cell Nuclear Factor, and is an early response gene in ecdysone signaling (King-Jones et al., 2005; Ou et al., 2011). Although its ligand is unknown, it links growth and timing of maturation in larva (King-Jones et al., 2005; Ou et al., 2011). The Hr4 GFP trap was detected in border cells at the time of their specification and in outer follicle cells including centripetal cells, and *Hr4* RNA showed a similar pattern (Fig. 3B, Supplemental Fig. 1). *Hr4* expression increased in response to exogenous 20-HE (Fig. 4C, D). Disruption of *Hr4* specifically in the border cells led to cell migration defects in about 13% of egg chambers (Fig. 5C). The mutant phenotype was supported by clonal analysis; mutant border cell clusters often were delayed in migration or failed to delaminate on time (Fig. 5B,C). Antibody staining for Hr4 revealed germline expression in very early egg chambers (Fig. 5D), and more localized expression in border cells in stage 8–10 (Fig. 5E,F). These results suggest important roles for additional nuclear hormone receptor signaling downstream of EcR activation.

2.5. A calcyphosine-encoding gene functions in outer border cells to promote migration

The microarrays identified the novel gene *CG10126*, as a putative downstream target of both EcR and Tai. The gene encodes a protein with a predicted calcium-binding domain and an EF Hand domain, and its closest human ortholog is a calcyphosine-like protein called CAPSL (Strausberg et al., 2002). Little is known about the functional role of vertebrate CAPSL protein, although calcyphosine is expressed in vertebrate thyroid and brain tissues (Halleux et al., 1998; Lecocq et al., 1995; Mailleux et al., 1993; Sauter et al., 1995). Loss of function alleles of this gene were not available at the time of our study, but we found that disruption of *CG10126* via either of two different RNAi lines resulted in significant delays

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in border cell migration (Fig. 6B–D). We observed this defect in 25–35% of egg chambers when expressing the dsRNA in all cells of the border cell cluster (using c306-Gal4) or in just the outer cells (with *slbo*-Gal4). We did not observe an obvious defect in centripetal cells in this cross. Knockdown in only the polar cells (with *upd*-Gal4) resulted in a normal phenotype (Fig. 6C). We did not detect differences in expression of the calcium dependent adhesion molecule, E-cadherin, or the organization of the polar cells to the center of the cluster in these mutant egg chambers (Fig. 6A–B). We found that CG10126 protein could be detected in the border cells, especially at the time of their formation (Fig. 6F). These results suggest a potential requirement for this well-conserved gene in border cell migration.

2.6. Prolyl-4-hydroxylase alpha EFB (PH4αEFB) is required in border cells and centripetal cells, and synchronizes cell migration and oocyte growth

PH4αEFB is downstream of both EcR and Tai signaling, according to our microarray data. The *Drosophila* genome encodes nine *prolyl-4-hydroxylase-alpha* genes, classified by expression pattern, and *PH4αEFB* is expressed in several embryonic tissues, including the epidermis and embryonic fat body at late stages (Abrams and Andrew, 2002). Interestingly, knockdown of *PH4αNE2* disrupted cell migration in a metastasis model in flies (Ito et al., 2014) and *PH4αSG1* and *2* are required for maintaining a patent salivary gland lumen (Abrams and Andrew, 2002). While a number of substrates for these enzymes likely exist, they have been shown to hydroxylate proline residues in pro-collagen to stabilize the triple helical structure of collagen (Gorres and Raines, 2010).

Disruptions in *PH4αEFB* by various means led to abnormal border cell migration, abnormal centripetal cell migration, and sometimes egg chamber growth defects (Fig. 7). We used a combination of mutant alleles and transgenes to explore these phenotypes. Knockdown of gene function in border cells resulted in incomplete border cell migration in about 10% of egg chambers (Supplemental Table 3). However, analysis of combinations of four different insertional alleles and a deficiency revealed several types of phenotypes that suggested border cell migration was not well synchronized with the rest of egg development when *PH4αEFB* was broadly disrupted. Specifically, we observed three classes of outcomes: incomplete border cell migration at late stage 9 or stage 10 (Fig. 7B–E), poor oocyte growth but border cells at the expected location of the oocyte border (Fig. 7F), and border cell migration that appeared to be asynchronous with the rest of egg development. In this latter case, the border cells appear to have migrated early as their movements were complete when the oocyte was still relatively small, and they appeared abnormally ahead of the flattening of the outer "stretch" follicle cells (Fig. 7G). *PH4αEFB*^{e02827} homozygotes had the strongest phenotypes, with about 6% of egg chambers with asynchronous migration and 12% with late migration (Fig. 7E, I). While this penetrance is low, asynchronous/early migration is essentially never observed in healthy, wild-type egg chambers. Other allelic combinations had variable degrees of similar defects (Fig. 7B–I), indicating that disruption of this gene is the cause.

Interestingly, *PH4αEFB* also functions in the centripetal cells. Knockdown of *PH4αEFB* in centripetal cells using *slbo*-Gal4 and either of two RNAi lines led to a delay in these cells' ability to complete their migration, comparable to the phenotype observed with expression

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

of EcR W650A in these cells in late stage 10B (Fig. 7J–L). In stage 11 controls, 75% of egg chambers had no visible gaps in the centripetal cells stretched over the oocyte, as visualized by beta-Catenin expression and DAPI (n = 12). In contrast, 64% of slbo-Gal4, *PH4αEFB* RNAi egg chambers had small holes in the extent of their coverage, or they spread unevenly toward one side (n = 16 for TRiP HMS00567 and n = 15 for TRiP HMS00835). Border cell migration was not affected in this genotype, possibly because the levels of the protein are already high at the time of RNAi knockdown by *slbo*-Gal4. Consistent with this idea, antibody staining for PH4αEFB in wild type egg chambers reveals that the protein is highly expressed in the border cells by stage 9 and is detected in some centripetal cells in stages 10 and later (Fig. 7H, Supplemental Fig. 2), but it is not highly expressed in posterior follicle cells. Some protein is also detected in the germline, especially near the anterior of the oocyte at stage 10 and in the nurse cell cytoplasm (Fig. 7H, Supplemental Fig. 2). We have not ruled out the possibility that the protein may also be expressed in other tissues that could indirectly affect our phenotypes. The protein expression pattern in ovary, though, is consistent with this gene being a downstream target of ecdysone signaling, and for its requirement in coordinating migration timing with other aspects of egg development.

3. Discussion

Through microarray analysis, we have identified a number of new potential regulators of collective cell migration. Prior studies had demonstrated that the transcriptional regulators EcR and Tai are required in border cells after motile cell specification occurs through JAK/STAT/Slbo activation(Bai et al., 2000; Cherbas et al., 2003; Hackney et al., 2007; Jang et al., 2009). A role for transcriptional regulation during cell migration is underappreciated, aside from the transcriptional programs required for cell fate, possibly because many cell migration events have been studied in vitro and signaling regulation on the protein level is often sufficient for accurate movements (Ables et al., 2016; Riddiford et al., 1993). In vivo, however, transcriptional control adds a layer of adaptability in responding to environmental and growth cues, so it is likely to be essential within migrating cells during development.

Ecdysone, like other steroid hormones, coordinates developmental timing cues, and in *Drosophila* it links nutritional status and egg development (Belles and Piulachs, 2015; Danielsen et al., 2013; Sieber and Spradling, 2015). In this way, the hormone can act to integrate environmental information with developmental programs. Gene ontology analysis suggests that EcR signaling may specifically increase signal peptide processing factors and downregulate extracellular matrix, which provides a potential mechanism to change the local environment. Tai is the *Drosophila* homolog of SRC-3, which acts as a transcriptional cofactor through the LXXLL domain (Bai et al., 2000). SRC-3 is upregulated in breast cancer and some other cancers (Gojis et al., 2010), and promotes cell migration in human ovarian cancer (Yoshida et al., 2005). In mammals, SRC-3 partners with the estrogen receptor, but it may have other partners (Ma et al., 2011).

Our study showed a requirement for two additional nuclear hormone receptors in efficient border cell migration: Eip75B and Hr4. Eip75B is able to bind to heme and may regulate oxygen sensing (Reinking et al., 2005). In vitro, egg chambers require oxygen to develop, so this protein may be important for promoting oogenesis when oxygen levels are appropriate.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

It is not yet clear what ligands bind to Hr4, but they may provide an additional checkpoint signal. Hr4 has been characterized as a transcriptional repressor (King-Jones et al., 2005; Ou et al., 2011). Interestingly, nine of the genes down regulated by Hr4 also appear to be repressed by Tai according to our analysis, which suggests these two regulators could act as partners during border cell migration. Recent studies have also linked Tai with Yorkie signaling (Zhang et al., 2015), which is also active in border cell migration (Lin et al., 2014; Lucas et al., 2013), so it will be interesting to elucidate all of the pathways in which Tai acts.

Among positively regulated genes, we identified several new players in cell migration. *CG10126* encodes a protein with high similarity to the mammalian calcyphosine family, including an EF-hand domain and a calcium binding domain, and we detected its expression in border cells. RNAi knockdown suggests that this gene is required autonomously for border cell migration, but loss of function mutant alleles will be necessary to confirm this. It will be interesting to determine how this protein functions in cell migration, particularly since it is absent from mouse, and little is known of its human homologs. Additional uncharacterized steroid-responsive genes from our results could be interesting areas for future investigation.

We determined a requirement for the prolyl hydroxylase PH4αEFB in coordinating the timing of border and centripetal cell migration with other events in egg development, such as oocyte growth. Insertional mutants for *PH4αEFB* seemed to affect multiple aspects of egg chamber development, even when *in trans* to a deficiency for this locus. Thus, clonal analysis of a strong loss-of-function allele will be needed to understand in which cells this gene acts. Nevertheless, our RNAi results imply a cell-autonomous requirement for this gene in both border and centripetal cells. While PH4αEFB likely has many enzymatic targets, we postulate that its post-translational modification and strengthening of collagen and extracellular matrix (ECM) components could explain the phenotypes observed. ECM is critical for shaping the egg as it develops, and a basement membrane surrounds the follicle cell epithelium. It is possible that mutations that disrupt collagen deposition could lead to the unusual early border cell migration phenotype we observed, since it could reduce the force needed for the cluster to delaminate or detach from the ECM. Along these lines, it is intriguing that a major category of genes downregulated by EcR were in the class “structural constituents of the extracellular matrix”. While the ECM is a strong and stable structure, it is also continuously remodeled, so transcriptional and post-translational regulation of these factors may significantly change its tensile strength and other structural properties, which could dramatically affect cell movements.

A number of genes identified in the microarray do not appear to be required individually for border cell migration, as knockdown by RNAi or loss-of-function mutations did not show defects in this process. This may point to functional redundancy of many components or a general robustness built into the signaling system to preserve its function. Other targets may be coordinately expressed but not required. We also tested 14 negatively regulated candidate genes by overexpression in border and centripetal cells, and did not observe noticeable cell migration defects (Supplemental Table 4). This could be because some genes do not have to be quickly shut down, or they may also be regulated post-transcriptionally. Nevertheless, in some cases, rapid transcriptional downregulation may be a response that is needed for

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

movement. For example, our analysis suggests that Tai may function to downregulate transcription of cytoskeletal proteins, including actin and Rac, which may allow a motile cell to change shape more quickly.

Overall, our study suggests that cells migrating in vivo can interpret developmental and environmental cues, such as hormones, nutrients, and growth factors, and respond by regulating their transcriptional profile. This response includes upregulation of signaling proteins and modifying or downregulating extracellular matrix, which could feed-forward to modify the local environment. It is likely that these kinds of responses may be conserved in a number of developmentally-regulated cell migration events.

4. Materials and methods

4.1. Fly stocks

Flies were cultured under standard conditions unless otherwise noted. *slbo*-Gal4, UAS-mCD8-GFP/CyO virgin females were crossed in bottles to: UAS-DN EcR-W650A Tp1–9 (II) for the dominant negative Ecdysone receptor genotype; *dp*, *tai*^{61G1}, PZ6356, FRT40/CyO; UAS-nls-DNtai (LXXLL)-GFP for the dominant negative Tai genotype; and *dp*, PZ6356, FRT40/CyO; UAS-LacZ as the wild-type control genotype. Virgin straight-winged progeny were collected and aged at 18 °C for 2–7 days then mated with males of the same genotype and fattened overnight on yeasted vials at 29 °C before dissection. For expression analysis, GFP-Protein Trap lines, originated from (Buszczak et al., 2007; Morin et al., 2001), were obtained through the Bloomington *Drosophila* Stock Center (Indiana University) or the Fly Trap collection (Cooley Laboratory, Yale University). For RNAi experiments, virgin females were collected from: *slbo*-Gal4, UAS-mCD8-GFP/CyO (for border and centripetal cell expression starting at stage 9), c306-Gal4 (for earlier anterior follicle cell expression), or *upd*-Gal4 (for polar cell expression). For nuclear hormone receptor mutants, the following alleles were examined: *Eip75B* ^{1}/TM6B (Bialecki et al., 2002) and *Hr4*^{1}/FM7c (King-Jones et al., 2005). The *Hr4*^{1} allele was recombined onto a chromosome with FRT19A, and negatively marked clones were induced by heat shock using hs-FLP and ubi-GFP as previously described (Bai et al., 2000). Egg chambers were scored 8 days after heat shock. The RNAi lines are listed in the figure legends or supplemental data Table 3 and were obtained from the Vienna Stock Center (VDRC) or the Transgenic RNAi Project (TRiP). Allelic information for *PH4αEFB* insertional mutants is indicated in the figures

4.2. Cell isolation, RNA purification, microarray hybridization

Ovaries from genotypes described were dissected in Grace's medium (Invitrogen) with 10% normal goat serum. After 10 ovaries were dissected, they were transferred to ice, and the total dissection time was limited to a maximum of 2 h. Following dissection, 100 ovary pairs for each genotype were washed in Cell Dissociation buffer (Sigma C-1544) and treated with elastase (Sigma E-0127) at 4 mg/ml in dissociation buffer with stirring and inverting for 5 min. 0.5 ml of dissection media was added to each and the supernatants were transferred to a new tube. Dissociated cells were spun at 1000 g for 5 min at 4 °C. Pelleted cells were resuspended in dissection media for viewing or buffer for sorting. Dissociated cells were purified with magnetic beads coupled to anti-mouse CD8 antibody (Miltenyi Biotec 494-01)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

according to manufacturer's instructions. After purification, an aliquot was removed to verify successful enrichment of GFP positive cells. Total RNA was purified from cells using RNeasy (Qiagen) in triplicate for each genotype. 100 ng of each RNA was used to make cRNA probes following the Affymetrix Small Sample Target Labeling Assay Version II protocol. Briefly, first strand cDNA synthesis was carried out using oligo-dT primer and the Invitrogen SuperScriptII kit. Second strand synthesis used Invitrogen *E. coli* DNA ligase, *E. coli* DNA polI and RNaseH. Double stranded cDNA was ethanol-precipitated and added to IVT reactions using the Ambion MEGAscript T7 kit. cRNA was purified on RNeasy columns (Qiagen) then first strand synthesis was repeated using random primers and second strand synthesis followed using T7-Oligo(dT) primers. IVT reactions and biotin labeling were performed using the ENZO BioArray High Yield RNA Transcript Labeling kit. Labeled cRNA targets were cleaned up on RNeasy columns, fragmented and hybridized to Affymetrix *Drosophila* Genome1 Array GeneChips. Processing and detection were performed as suggested by Affymetrix using an Affymetrix GeneArray Scanner 2500A. Each experiment was repeated with three independent biological samples.

4.3. Bioinformatic analysis

Gene expression analysis was performed as previously described using Affymetrix Microarray Suite MAS 5.0 (Affymetrix) and ChipStat, which detects significant gene expression changes even when fold change is small (Master et al., 2005; Wang et al., 2006). Briefly, ChipStat utilizes the number of individual probe pairs that differ between conditions (certainty of $p < 0.05$) to determine positives, even if the fold-change is very small, for at least 6 of 14 probe pairs. MAS 5.0 performs 9 possible pairwise comparisons and ranks significance of overall fold changes. Prior studies with these methods had a false positive rate of <1.5% (Master et al., 2005; Wang et al., 2006). Other studies also support utilizing multiple analysis methods, for example (Dalman et al., 2012). Gene ontology categories were determined using GeneSpring 7.3 (Silicon genetics) or DAVID (Dennis et al., 2003). Binding site identification was determined using JASPAR (Mathelier et al., 2016; Sandelin et al., 2004). Flybase was used to determine genomic information, including gene structure and location of Affymetrix probes (Gramates et al., 2017).

4.4. RNase protection assays

Probes for RNase protection assays were amplified by PCR (see supplemental methods for primer information), labeled with ^{32}P and quantified on a scintillation counter. Ovaries were dissected from either Canton S or GFP-trap GILT1 females ($GILT1^{CC00817}$) (Kelso et al., 2004) that were kept on yeasted vials overnight. Ovaries were cultured for 2 h in Schneider's media (Invitrogen) supplemented with 10% Fetal Bovine Serum and DMSO in the presence or absence of 5 μM of 20-Hydroxyecdsone (Sigma H5142). Total RNA from ovaries was purified using RNeasy (Qiagen). RNase protection assays were performed as previously described (Gilman, 2001).

4.5. Expression analyses

Females were fattened overnight on yeasted vials, then ovaries were dissected and fixed. In situ hybridizations were performed as previously described in (Wang et al., 2006; Zimmerman et al., 2013). Antibody stainings were performed in NP40 buffer as described in

(McDonald and Montell, 2005), but some antibodies were pre-absorbed on ovaries prior to being used in the staining reaction. Antibodies used were: mouse anti-Armadillo (beta-catenin, N27A1, DSHB) diluted 1:25, mouse anti-Fas3 (Fas3, DSHB) diluted 1:10, rabbit anti-GFP diluted 1:1000 (Invitrogen), rabbit anti-Dhr4 diluted 1:50 ((King-Jones et al., 2005) a gift from King-Jones and Thummel), rabbit anti-CG10126 preabsorbed on ovaries at 1:100 and used diluted to 1:500 for staining (Susan Spencer), and guinea pig anti-PH4αEFB preadsorbed for 2 h at 1:200 and used at 1:2000 (Deborah Andrew). Secondary antibodies used anti-mouse or anti-rabbit conjugated to Alexa 488 or 568 diluted 1:400 (Invitrogen). DAPI (Sigma) 1:1000 was used to mark nuclei. Images were acquired using a Zeiss AxioImager microscope using ApoTome optical sectioning and processed through AxioVision, except Fig. 7I–K, which were obtained and rendered on a Leica SP5 laser scanning confocal microscope.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Bloomington *Drosophila* Stock Center for the RNAi and other transgenic flies, the Vienna *Drosophila* RNAi Center and Harvard TRiP Stock Center for RNAi fly lines, Flybase for genetic and genomic information, and Developmental Studies Hybridoma Bank, K. King-Jones and C. Thummel for antibodies. We thank L. Chodish and the JHMI Microarray core facility for helpful discussions about data analysis. We acknowledge Yvonne Puplampu-Dove and Christy Taylor for help with experiments. We are grateful to Mallika Bhattacharya and Tagide deCarvalho in the Keith Porter Imaging Facility for assistance with imaging the centripetal cells. This project was supported in part by the National Institutes of Health grant R01 013899 (to DA), NIH grant R01 GM73164 (to DM), the National Science Foundation Award IOS-1054422 (to MSG) and Basil O'Connor Starter Scholar Award #5-FY11-477 from the March of Dimes (to MSG).

References

Ables ET, Drummond-Barbosa D. The steroid hormone ecdysone functions with intrinsic chromatin remodeling factors to control female germline stem cells in *Drosophila*. Cell Stem Cell. 2010; 7:581–592. [PubMed: 21040900]

Ables ET, Bois KE, Garcia CA, Drummond-Barbosa D. Ecdysone response gene E78 controls ovarian germline stem cell niche formation and follicle survival in *Drosophila*. Dev Biol. 2015; 400:33–42. [PubMed: 25624267]

Ables, ET., Hwang, GH., Finger, DS., Hinnant, TD., Drummond-Barbosa, D. A Genetic Mosaic Screen Reveals Ecdysone-Responsive Genes Regulating *Drosophila* Oogenesis. Vol. 6. G3; Bethesda: 2016. p. 2629-2642.

Abrams EW, Andrew DJ. Prolyl 4-hydroxylase alpha-related proteins in *Drosophila melanogaster*: tissue-specific embryonic expression of the 99F8-9 cluster. Mech Dev. 2002; 112:165–171. [PubMed: 11850189]

Aman A, Piotrowski T. Cell migration during morphogenesis. Dev Biol. 2010; 341:20–33. [PubMed: 19914236]

Andersen D, Horne-Badovinac S. Influence of ovarian muscle contraction and oocyte growth on egg chamber elongation in *Drosophila*. Development. 2016; 143:1375–1387. [PubMed: 26952985]

Arbeitman MN, Fleming AA, Siegal ML, Null BH, Baker BS. A genomic analysis of *Drosophila* somatic sexual differentiation and its regulation. Development. 2004; 131:2007–2021. [PubMed: 15056610]

Bai J, Uehara Y, Montell DJ. Regulation of invasive cell behavior by taiman, a *Drosophila* protein related to AIB1, a steroid receptor coactivator amplified in breast cancer. Cell. 2000; 103:1047–1058. [PubMed: 11163181]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Beckstead RB, Lam G, Thummel CS. The genomic response to 20-hydroxyecdysone at the onset of *Drosophila* metamorphosis. Genome Biol. 2005; 6:R99. [PubMed: 16356271]

Beckstead RB, Lam G, Thummel CS. Specific transcriptional responses to juvenile hormone and ecdysone in *Drosophila*. Insect Biochem Mol Biol. 2007; 37:570–578. [PubMed: 17517334]

Belles X, Piulachs MD. Ecdysone signalling and ovarian development in insects: from stem cells to ovarian follicle formation. Biochim Biophys Acta. 2015; 1849:181–186. [PubMed: 24939835]

Berg CA. The *Drosophila* shell game: patterning genes and morphological change. Trends Genet. 2005; 21:346–355. [PubMed: 15922834]

Bialecki M, Shilton A, Fichtenberg C, Segraves WA, Thummel CS. Loss of the ecdysteroid-inducible E75A orphan nuclear receptor uncouples molting from metamorphosis in *Drosophila*. Dev. Cell. 2002; 3:209–220.

Borghese L, Fletcher G, Mathieu J, Atzberger A, Eades WC, Cagan RL, Rorth P. Systematic analysis of the transcriptional switch inducing migration of border cells. Dev Cell. 2006; 10:497–508. [PubMed: 16580994]

Buszczak M, Freeman MR, Carlson JR, Bender M, Cooley L, Segraves WA. Ecdysone response genes govern egg chamber development during mid-oogenesis in *Drosophila*. Development. 1999; 126:4581–4589. [PubMed: 10498692]

Buszczak M, Paterno S, Lighthouse D, Bachman J, Planck J, Owen S, Skora AD, Nystul TG, Ohlstein B, Allen A, Wilhelm JE, Murphy TD, Levis RW, Matunis E, Srivali N, Hoskins RA, Spradling AC. The carnegie protein trap library: a versatile tool for *Drosophila* developmental studies. Genetics. 2007; 175:1505–1531. [PubMed: 17194782]

Campbell K, Casanova J. A common framework for EMT and collective cell migration. Development. 2016; 143:4291–4300. [PubMed: 27899506]

Carney GE, Bender M. The *Drosophila* ecdysone receptor (EcR) gene is required maternally for normal oogenesis. Genetics. 2000; 154:1203–1211. [PubMed: 10757764]

Cetera M, Horne-Badovinac S. Round and round gets you somewhere: collective cell migration and planar polarity in elongating *Drosophila* egg chambers. Curr Opin Genet Dev. 2015; 32:10–15. [PubMed: 25677931]

Cherbas L, Hu X, Zhimulev I, Belyaeva E, Cherbas P. EcR isoforms in *Drosophila*: testing tissue-specific requirements by targeted blockade and rescue. Development. 2003; 130:271–284. [PubMed: 12466195]

Dalman MR, Deeter A, Nimishakavi G, Duan ZH. Fold change and p-value cutoffs significantly alter microarray interpretations. BMC Bioinf. 2012; 13(Suppl 2):S11.

Danielsen ET, Moeller ME, Rewitz KF. Nutrient signaling and developmental timing of maturation. Curr Top Dev Biol. 2013; 105:37–67. [PubMed: 23962838]

Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: database for annotation, visualization, and integrated discovery. Genome Biol. 2003; 4:P3. [PubMed: 12734009]

Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblauer S, Couto A, Marra V, Keleman K, Dickson BJ. A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. Nature. 2007; 448:151–156. [PubMed: 17625558]

Dobens LL, Raftery LA. Integration of epithelial patterning and morphogenesis in *Drosophila* ovarian follicle cells. Dev Dyn. 2000; 218:80–93. [PubMed: 10822261]

Dobens L, Jaeger A, Peterson JS, Raftery LA. Bunched sets a boundary for Notch signaling to pattern anterior eggshell structures during *Drosophila* oogenesis. Dev Biol. 2005; 287:425–437. [PubMed: 16223477]

Domanitskaya E, Anllo L, Schupbach T. Phantom, a cytochrome P450 enzyme essential for ecdysone biosynthesis, plays a critical role in the control of border cell migration in *Drosophila*. Dev Biol. 2014; 386:408–418. [PubMed: 24373956]

Duhart JC, Parsons TT, Raftery LA. The repertoire of epithelial morphogenesis on display: progressive elaboration of *Drosophila* egg structure. Mech Dev. 2017; 148:18–39. [PubMed: 28433748]

Edwards KA, Kiehart DP. *Drosophila* nonmuscle myosin II has multiple essential roles in imaginal disc and egg chamber morphogenesis. Development. 1996; 122:1499–1511. [PubMed: 8625837]

Fakhouri M, Elalayli M, Sherling D, Hall JD, Miller E, Sun X, Wells L, LeMosy EK. Minor proteins and enzymes of the *Drosophila* eggshell matrix. Dev Biol. 2006; 293:127–141. [PubMed: 16515779]

Figueroa-Clarevega A, Bilder D. Malignant *Drosophila* tumors interrupt insulin signaling to induce cachexia-like wasting. Dev. Cell. 2015; 33:47–55.

Flickinger TW, Salz HK. The *Drosophila* sex determination gene snf encodes a nuclear protein with sequence and functional similarity to the mammalian U1A snRNP protein. Genes Dev. 1994; 8:914–925. [PubMed: 7926776]

Geisbrecht ER, Montell DJ. Myosin VI is required for E-cadherin-mediated border cell migration. Nat Cell Biol. 2002; 4:616–620. [PubMed: 12134162]

Geisbrecht ER, Montell DJ. A role for *Drosophila* IAP1-mediated caspase inhibition in Rac-dependent cell migration. Cell. 2004; 118:111–125. [PubMed: 15242648]

Gilman M. Ribonuclease protection assay. Curr Protoc Mol Biol. 2001; 24(II):4.7.1–4.78 4.7. [PubMed: 18265211]

Gojis O, Rudraraju B, Gudi M, Hogben K, Sousha S, Coombes RC, Cleator S, Palmieri C. The role of SRC-3 in human breast cancer. Nat Rev Clin Oncol. 2010; 7:83–89. [PubMed: 20027190]

Gorres KL, Raines RT. Prolyl 4-hydroxylase. Crit Rev Biochem Mol Biol. 2010; 45:106–124. [PubMed: 20199358]

Gramates LS, Marygold SJ, Santos GD, Urbano JM, Antonazzo G, Matthews BB, Rey AJ, Tabone CJ, Crosby MA, Emmert DB, Falls K, Goodman JL, Hu Y, Ponting L, Schroeder AJ, Strelets VB, Thurmond J, Zhou P. the FlyBase C. FlyBase at 25: looking to the future. Nucleic Acids Res. 2017; 45:D663–D671. [PubMed: 27799470]

Hackney JF, Pucci C, Naes E, Dobens L. Ras signaling modulates activity of the ecdysone receptor EcR during cell migration in the *Drosophila* ovary. Dev Dyn. 2007; 236:1213–1226. [PubMed: 17436275]

Halleux P, Schurmans S, Schiffman SN, Lecocq R, Conreur JL, Dumont J, Vanderhaeghen JJ. Calcium binding protein calcyphosine in dog central astrocytes and ependymal cells and in peripheral neurons. J Chem Neuroanat. 1998; 15:239–250. [PubMed: 9860089]

Horwitz R, Webb D. Cell migration. Curr Biol. 2003; 13:R756–R759. [PubMed: 14521851]

Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009; 4:44–57. [PubMed: 19131956]

Huber MA, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol. 2005; 17:548–558. [PubMed: 16098727]

Hudson AM, Cooley L. Methods for studying oogenesis. Methods. 2014; 68:207–217. [PubMed: 24440745]

Insall RH, Machesky LM. Actin dynamics at the leading edge: from simple machinery to complex networks. Dev Cell. 2009; 17:310–322. [PubMed: 19758556]

Ito S, Ueda T, Ueno A, Nakagawa H, Taniguchi H, Kayukawa N, Miki T. A genetic screen in *Drosophila* for regulators of human prostate cancer progression. Biochem Biophys Res Commun. 2014; 451:548–555. [PubMed: 25117438]

Janetopoulos C, Firtel RA. Directional sensing during chemotaxis. FEBS Lett. 2008; 582:2075–2085. [PubMed: 18452713]

Jang AC, Chang YC, Bai J, Montell D. Border-cell migration requires integration of spatial and temporal signals by the BTB protein abrupt. Nat Cell Biol. 2009; 11:569–579. [PubMed: 19350016]

Kelso RJ, Buszczak M, Quinones AT, Castiblanco C, Mazzalupo S, Cooley L. Flytrap, a database documenting a GFP protein-trap insertion screen in *Drosophila melanogaster*. Nucleic Acids Res. 2004; 32:D418–D420. [PubMed: 14681446]

Kim JC, Orr-Weaver TL. Analysis of a *Drosophila* amplicon in follicle cells highlights the diversity of metazoan replication origins. Proc Natl Acad Sci U S A. 2011; 108:16681–16686. [PubMed: 21933960]

Kim JH, Cho A, Yin H, Schafer DA, Mouneimne G, Simpson KJ, Nguyen KV, Brugge JS, Montell DJ. Psidin, a conserved protein that regulates protrusion dynamics and cell migration. Genes Dev. 2011; 25:730–741. [PubMed: 21406550]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

King, RC. Ovarian Development in *Drosophila melanogaster*. Academic Press; New York: 1970.

King-Jones K, Charles JP, Lam G, Thummel CS. The ecdysone-induced DHR4 orphan nuclear receptor coordinates growth and maturation in *Drosophila*. Cell. 2005; 121:773–784. [PubMed: 15935763]

Kozlova T, Thummel CS. Spatial patterns of ecdysteroid receptor activation during the onset of *Drosophila* metamorphosis. Development. 2002; 129:1739–1750. [PubMed: 11923209]

Krauchunas AR, Horner VL, Wolfner MF. Protein phosphorylation changes reveal new candidates in the regulation of egg activation and early embryogenesis in *D. melanogaster*. Dev Biol. 2012; 370:125–134. [PubMed: 22884528]

Kwon Y, Song W, Droujinine IA, Hu Y, Asara JM, Perrimon N. Systemic organ wasting induced by localized expression of the secreted insulin/IGF antagonist ImpL2. Dev Cell. 2015; 33:36–46. [PubMed: 25850671]

Lecocq R, Lamy F, Erneux C, Dumont JE. Rapid purification and identification of calcyphosine, a Ca(2+)-binding protein phosphorylated by protein kinase A. Biochem J. 1995; 306(Pt 1):147–151. [PubMed: 7864802]

Levine B, Hackney JF, Bergen A, Dobens L 3rd, Truesdale A, Dobens L. Opposing interactions between *Drosophila* cut and the C/EBP encoded by slow border cells direct apical constriction and epithelial invagination. Dev Biol. 2010; 344:196–209. [PubMed: 20450903]

Lin TH, Yeh TH, Wang TW, Yu JY. The Hippo pathway controls border cell migration through distinct mechanisms in outer border cells and polar cells of the *Drosophila* ovary. Genetics. 2014; 198:1087–1099. [PubMed: 25161211]

Lucas EP, Khanal I, Gaspar P, Fletcher GC, Polesello C, Tapon N, Thompson BJ. The Hippo pathway polarizes the actin cytoskeleton during collective migration of *Drosophila* border cells. J Cell Biol. 2013; 201:875–885. [PubMed: 23733343]

Ma G, Ren Y, Wang K, He J. SRC-3 has a role in cancer other than as a nuclear receptor coactivator. Int J Biol Sci. 2011; 7:664–672. [PubMed: 21647249]

Mailleux P, Halleux P, Verslijpe M, Segers V, Vanderhaeghen JJ. Neuronal localization in the rat brain of the messenger RNA encoding calcyphosine, a new calcium-binding protein. Neurosci Lett. 1993; 153:125–130. [PubMed: 8327186]

Master SR, Stoddard AJ, Bailey LC, Pan TC, Dugan KD, Chodosh LA. Genomic analysis of early murine mammary gland development using novel probe-level algorithms. Genome Biol. 2005; 6:R20. [PubMed: 15693949]

Mathelier A, Fornes O, Arenillas DJ, Chen CY, Denay G, Lee J, Shi W, Shyr C, Tan G, Worsley-Hunt R, Zhang AW, Parcy F, Lenhard B, Sandelin A, Wasserman WW. JASPAR 2016: a major expansion and update of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 2016; 44:D110–D115. [PubMed: 26531826]

McDonald JA, Montell DJ. Analysis of cell migration using *Drosophila* as a model system. Methods Mol Biol. 2005; 294:175–202. [PubMed: 15576913]

McDonald JA, Pinheiro EM, Montell DJ. PVF1, a PDGF/VEGF homolog, is sufficient to guide border cells and interacts genetically with Taiman. Development. 2003; 130:3469–3478. [PubMed: 12810594]

Melani M, Simpson KJ, Brugge JS, Montell D. Regulation of cell adhesion and collective cell migration by hindsight and its human homolog RREB1. Curr Biol. 2008; 18:532–537. [PubMed: 18394891]

Mitsialis SA, Kafatos FC. Regulatory elements controlling chorion gene expression are conserved between flies and moths. Nature. 1985; 317:453–456. [PubMed: 2413366]

Montell DJ, Rorth P, Spradling AC. Slow border cells, a locus required for a developmentally regulated cell migration during oogenesis, encodes *Drosophila* C/EBP. Cell. 1992; 71:51–62. [PubMed: 1394432]

Montell DJ, Yoon WH, Starz-Gaiano M. Group choreography: mechanisms orchestrating the collective movement of border cells. Nat Rev Mol Cell Biol. 2012; 13:631–645. [PubMed: 23000794]

Morin X, Daneman R, Zavortink M, Chia W. A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila*. Proc Natl Acad Sci U S A. 2001; 98:15050–15055. [PubMed: 11742088]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Morris LX, Spradling AC. Steroid signaling within *Drosophila* ovarian epithelial cells sex-specifically modulates early germ cell development and meiotic entry. PLoS One. 2012; 7:e46109. [PubMed: 23056242]

Niewiadomska P, Godt D, Tepass U. DE-Cadherin is required for intercellular motility during *Drosophila* oogenesis. J Cell Biol. 1999; 144:533–547. [PubMed: 9971747]

Ou Q, Magico A, King-Jones K. Nuclear receptor DHR4 controls the timing of steroid hormone pulses during *Drosophila* development. PLoS Biol. 2011; 9:e1001160. [PubMed: 21980261]

Perkins LA, Holderbaum L, Tao R, Hu Y, Sopko R, McCall K, Yang-Zhou D, Flockhart I, Binari R, Shim HS, Miller A, Housden A, Foos M, Randkelv S, Kelley C, Namgyal P, Villalta C, Liu LP, Jiang X, Huan-Huan Q, Wang X, Fujiyama A, Toyoda A, Ayers K, Blum A, Czech B, Neumuller R, Yan D, Cavallaro A, Hibbard K, Hall D, Cooley L, Hannon GJ, Lehmann R, Parks A, Mohr SE, Ueda R, Kondo S, Ni JQ, Perrimon N. The transgenic RNAi project at Harvard Medical School: resources and validation. Genetics. 2015; 201:843–852. [PubMed: 26320097]

Petrie RJ, Doyle AD, Yamada KM. Random versus directionally persistent cell migration. Nat Rev Mol Cell Biol. 2009; 10:538–549. [PubMed: 19603038]

Reinking J, Lam MM, Pardee K, Sampson HM, Liu S, Yang P, Williams S, White W, Lajoie G, Edwards A, Krause HM. The *Drosophila* nuclear receptor e75 contains heme and is gas responsive. Cell. 2005; 122:195–207. [PubMed: 16051145]

Riddiford, LM., Bate, M., Martinez-Arias, A. The Development of *Drosophila*. Cold Spring Harbor Laboratory; New York: 1993. p. 899-940.

Rorth P. Initiating and guiding migration: lessons from border cells. Trends Cell Biol. 2002; 12:325–331. [PubMed: 12185849]

Rorth P, Szabo K, Bailey A, Laverty T, Rehm J, Rubin GM, Weigmann K, Milan M, Benes V, Ansorge W, Cohen SM. Systematic gain-of-function genetics in *Drosophila*. Development. 1998; 125:1049–1057. [PubMed: 9463351]

Saadin A, Starz-Gaiano M. Circuitous genetic regulation governs a straightforward cell migration. Trends Genet. 2016; 32:660–673. [PubMed: 27600524]

Saleem S, Schwedes CC, Ellis LL, Grady ST, Adams RL, Johnson N, Whittington JR, Carney GE. *Drosophila melanogaster* p24 trafficking proteins have vital roles in development and reproduction. Mech Dev. 2012; 129:177–191. [PubMed: 22554671]

Sandelin A, Alkema W, Engstrom P, Wasserman WW, Lenhard B. JASPAR: an open-access database for eukaryotic transcription factor binding profiles. Nucleic Acids Res. 2004; 32:D91–D94. [PubMed: 14681366]

Sauter A, Staudenmann W, Hughes GJ, Heizmann CW. A novel EF-hand Ca(2+)-binding protein from abdominal muscle of crustaceans with similarity to calcyphosine from dog thyroidea. Eur J Biochem. 1995; 227:97–101. [PubMed: 7851448]

Scarpa E, Mayor R. Collective cell migration in development. J Cell Biol. 2016; 212:143–155. [PubMed: 26783298]

Schwartz MB, Kelly TJ, Woods CW, Imberski RB. Ecdysteroid fluctuations in adult *Drosophila melanogaster* caused by elimination of pupal reserves and synthesis by early vitellogenic ovarian follicles. Insect Biochem. 1989; 19:243–249.

Shea MJ, King DL, Conboy MJ, Mariani BD, Kafatos FC. Proteins that bind to *Drosophila* chorion cis-regulatory elements: a new C2H2 zinc finger protein and a C2C2 steroid receptor-like component. Genes Dev. 1990; 4:1128–1140. [PubMed: 2120114]

Sherman BT, Huang da W, Tan Q, Guo Y, Bour S, Liu D, Stephens R, Baseler MW, Lane HC, Lempicki RA. DAVID knowledgebase: a gene-centered database integrating heterogeneous gene annotation resources to facilitate high-throughput gene functional analysis. BMC Bioinf. 2007; 8:426.

Sieber MH, Spradling AC. Steroid signaling establishes a female metabolic state and regulates SREBP to control oocyte lipid accumulation. Curr Biol. 2015; 25:993–1004. [PubMed: 25802149]

Simoes-Costa M, Bronner ME. Establishing neural crest identity: a gene regulatory recipe. Development. 2015; 142:242–257. [PubMed: 25564621]

Somogyi K, Rorth P. Evidence for tension-based regulation of *Drosophila* MAL and SRF during invasive cell migration. Dev. Cell. 2004; 7:85–93.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, Wagner L, Shenmen CM, Schuler GD, Altschul SF, Zeeberg B, Buetow KH, Schaefer CF, Bhat NK, Hopkins RF, Jordan H, Moore T, Max SI, Wang J, Hsieh F, Diatchenko L, Marusina K, Farmer AA, Rubin GM, Hong L, Stapleton M, Soares MB, Bonaldo MF, Casavant TL, Scheetz TE, Brownstein MJ, Usdin TB, Toshiyuki S, Carninci P, Prange C, Raha SS, Loquellano NA, Peters GJ, Abramson RD, Mullahy SJ, Bosak SA, McEwan PJ, McKernan KJ, Malek JA, Gunaratne PH, Richards S, Worley KC, Hale S, Garcia AM, Gay LJ, Hulyk SW, Villalon DK, Muzny DM, Sodergren EJ, Lu X, Gibbs RA, Fahey J, Helton E, Ketteman M, Madan A, Rodrigues S, Sanchez A, Whiting M, Madan A, Young AC, Shevchenko Y, Bouffard GG, Blakesley RW, Touchman JW, Green ED, Dickson MC, Rodriguez AC, Grimwood J, Schmutz J, Myers RM, Butterfield YS, Krzywinski MI, Skalska U, Smailus DE, Schnerch A, Schein JE, Jones SJ, Marra MA. Mammalian Gene Collection Program T. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci U S A. 2002; 99:16899–16903. [PubMed: 12477932]

Tepass U, Gruszynski-DeFeo E, Haag TA, Omatyar L, Torok T, Hartenstein V. Shotgun encodes *Drosophila* E-cadherin and is preferentially required during cell rearrangement in the neurectoderm and other morphogenetically active epithelia. Genes Dev. 1996; 10:672–685. [PubMed: 8598295]

Terashima J, Bownes M. A microarray analysis of genes involved in relating egg production to nutritional intake in *Drosophila melanogaster*. Cell Death Differ. 2005; 12:429–440. [PubMed: 15776001]

Van de Bor V, Zimniak G, Cerezo D, Schaub S, Noselli S. Asymmetric localisation of cytokine mRNA is essential for JAK/STAT activation during cell invasiveness. Development. 2011; 138:1383–1393. [PubMed: 21350010]

Verheyen EM, Cooley L. Profilin mutations disrupt multiple actin-dependent processes during *Drosophila* development. Development. 1994; 120:717–728. [PubMed: 7600952]

Wang X, Bo J, Bridges T, Dugan KD, Pan TC, Chodosh LA, Montell DJ. Analysis of cell migration using whole-genome expression profiling of migratory cells in the *Drosophila* ovary. Dev. Cell. 2006; 10:483–495.

Wu X, Tanwar PS, Raftery LA. *Drosophila* follicle cells: morphogenesis in an eggshell. Semin Cell Dev Biol. 2008; 19:271–282. [PubMed: 18304845]

Xi R, McGregor JR, Harrison DA. A gradient of JAK pathway activity patterns the anterior-posterior axis of the follicular epithelium. Dev Cell. 2003; 4:167–177. [PubMed: 12586061]

Yamanaka N, Rewitz KF, O'Connor MB. Ecdysone control of developmental transitions: lessons from *Drosophila* research. Annu Rev Entomol. 2013; 58:497–516. [PubMed: 23072462]

Yao TP, Forman BM, Jiang Z, Cherbas L, Chen JD, McKeown M, Cherbas P, Evans RM. Functional ecdysone receptor is the product of EcR and Ultraspiracle genes. Nature. 1993; 366:476–479. [PubMed: 8247157]

Yoshida H, Liu J, Samuel S, Cheng W, Rosen D, Naora H. Steroid receptor coactivator-3, a homolog of Taiman that controls cell migration in the *Drosophila* ovary, regulates migration of human ovarian cancer cells. Mol Cell Endocrinol. 2005; 245:77–85. [PubMed: 16298470]

Zhang C, Robinson BS, Xu W, Yang L, Yao B, Zhao H, Byun PK, Jin P, Veraksa A, Moberg KH. The ecdysone receptor coactivator Taiman links Yorkie to transcriptional control of germline stem cell factors in somatic tissue. Dev Cell. 2015; 34:168–180. [PubMed: 26143992]

Zimmerman SG, Peters NC, Altaras AE, Berg CA. Optimized RNA ISH, RNA FISH and protein-RNA double labeling (IF/FISH) in *Drosophila* ovaries. Nat Protoc. 2013; 8:2158–2179. [PubMed: 24113787]

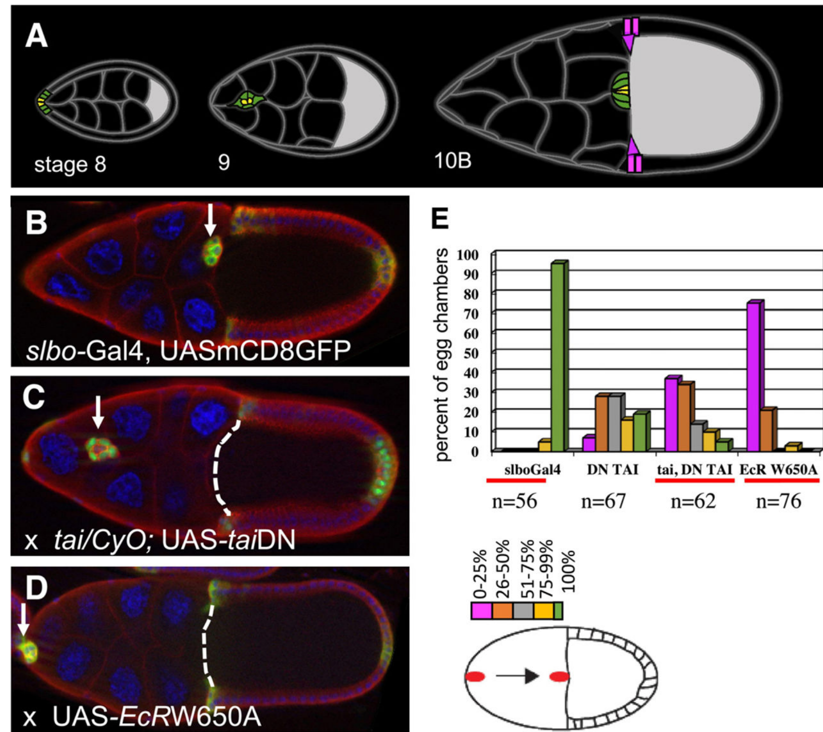


Fig. 1.

Ecdysone signaling is required for collective cell migration in fly egg chambers. A. Cartoon depicting stages 8–10 of oogenesis *Drosophila* oogenesis. Anterior is to the left. All egg chamber images were acquired as optical sections. Large germline cells, the nurse cells (outlined) and oocyte (gray), are surrounded by a single layer epithelium of follicle cells. At stage 8 border cells (green) are specified next to the anterior polar cells (yellow). At stage 9, polar cells are carried between nurse cells by the motile border cells. At stage 10B, the border cells have reached the oocyte and the centripetal cells (magenta) are moving interiorly toward them to cover the anterior border of the oocyte. B. A control egg chamber with wild-type border cell migration. Membrane-tethered GFP (green) is expressed in the border cells, centripetal cells, and a few posterior follicle cells, under the control of *slbo*-Gal4. Beta-catenin staining (ARM, red) marks the periphery of follicle cells and is enriched in the border cells. At stage 10, border cells are normally at the oocyte border (arrow). C. When *taiman* function is disrupted through *slbo*-Gal4 driven dominant negative expression in a tai^{61G1} heterozygous mutant background, border cell migration is delayed. In this stage 10 egg chamber, border cells (arrow) have reached about 30% of the migratory distance to the oocyte border (dashed line). D. When Ecdysone receptor function is disrupted through *slbo*-Gal4 driven dominant negative expression, border cell migration is delayed. In this stage 10 egg chamber, border cells (arrow) have not moved any of the migratory distance to the oocyte border (dashed line). E. Quantification of the penetrance of the border cell migration defects caused by dominant negative Tai, dominant negative Tai plus a heterozygous mutant allele of *tai*, or dominant negative Ecdysone receptor expression. The three genotypes indicated by the red underlines were chosen for microarray analysis since they had the strongest effects. Mutant phenotype was scored by the distance border cells had

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

moved by stage 10, as depicted in the lower schematic. n refers to the number of egg chambers scored.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

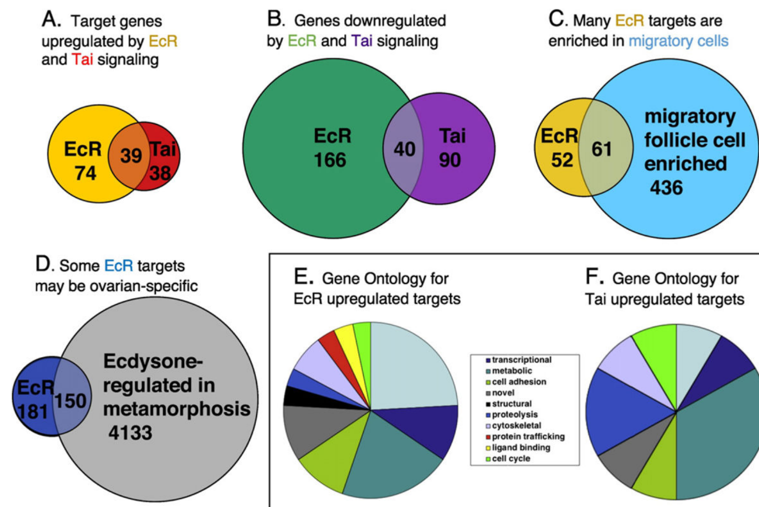


Fig. 2.

Ecdysone receptor (EcR) and Taiman (Tai) regulate overlapping sets of genes in motile follicle cells. A–D. Venn diagrams show the numbers of genes identified in each genotype and the overlap between positive targets of EcR and Tai signaling (A), negative targets of EcR and Tai signaling (B), positive targets in EcR signaling and genes previously identified as enriched in migratory cells (Borghese et al., 2006; Wang et al., 2006) (C), and positive targets of EcR signaling in border cells compared to those genes previously identified as ecdysone target genes during metamorphosis (Beckstead et al., 2005) (D). E. Pie graphs depict functional gene ontology representations for all genes identified as positive targets of EcR signaling (left) or positive targets of Tai signaling (right).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

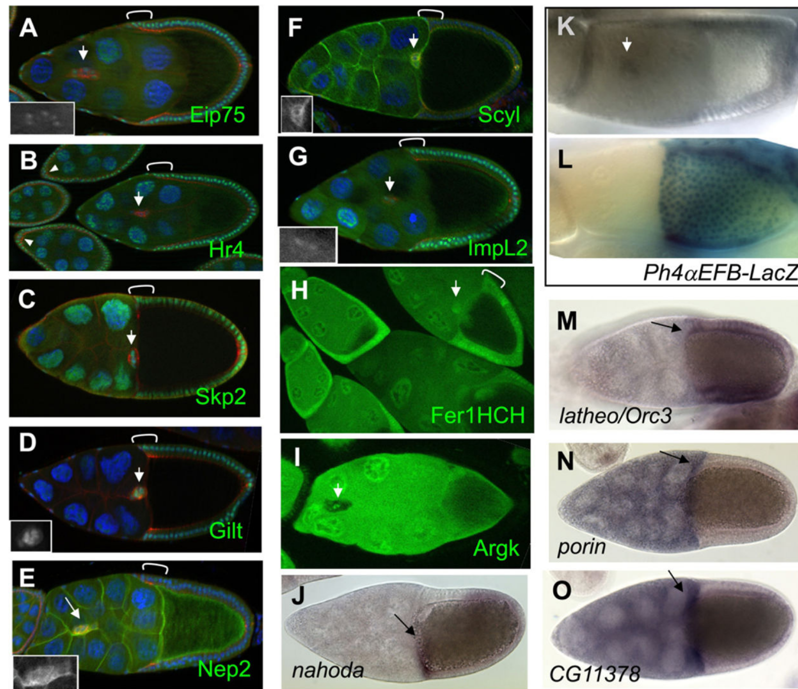
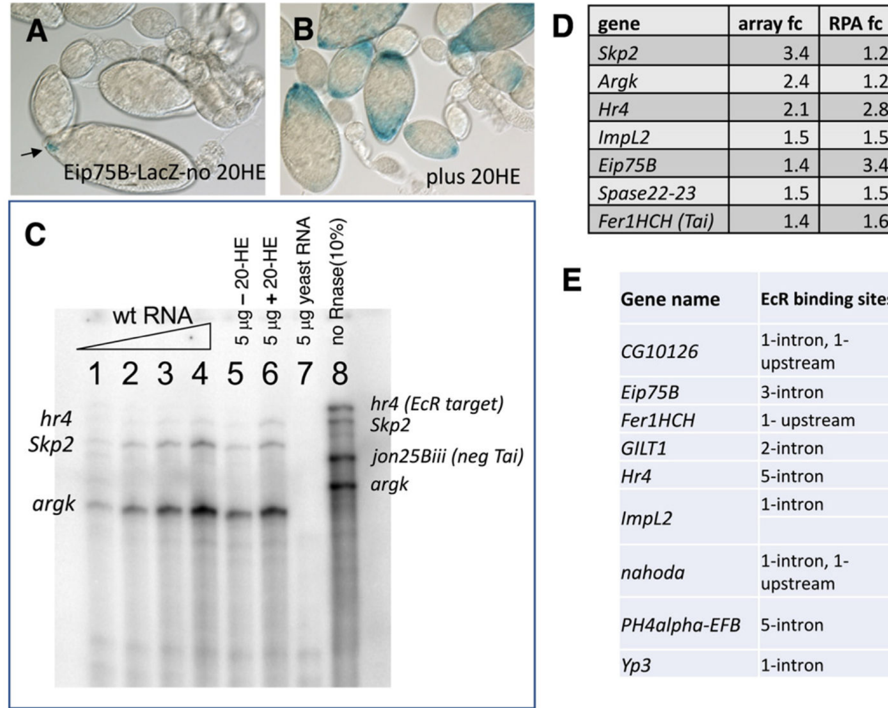


Fig. 3.

Expression patterns of candidate EcR and Tai target genes. A–I. GFP-protein splice traps report expression in border cells, centripetal cells, and other ovarian cells at stages 9 and 10. Border cells are indicated with an arrow and centripetal cells with a bracket. Arm expression (red) is observed in most follicle cells and enriched in border cells. Insets show grayscale expression of GFP protein identified near the arrow. Arrowheads in (B) indicate newly specified border cells in earlier egg chambers. K–L. *LacZ*-enhancer traps report expression for *PH4aEFB*, found in border cells (arrow in K), centripetal cells, and main body follicle cells (L). J, M–O In situ hybridizations show mRNA expression of indicated target genes in egg chambers; black arrows indicate centripetal cells.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript



gene	array fc	RPA fc
Skp2	3.4	1.2
Argk	2.4	1.2
Hr4	2.1	2.8
ImpL2	1.5	1.5
Eip75B	1.4	3.4
Spase22-23	1.5	1.5
Fer1HCH (Tai)	1.4	1.6

Gene name	EcR binding sites
CG10126	1-intron, 1-upstream
Eip75B	3-intron
Fer1HCH	1- upstream
GILT1	2-intron
Hr4	5-intron
ImpL2	1-intron
nahoda	1-intron, 1-upstream
PH4alpha-EFB	5-intron
Yp3	1-intron

Fig. 4.

Expression of identified genes increase in response to exogenous 20-Hydroxyecdysone added to ovary. A. Cultured egg chambers stained to reveal expression of an *Eip75B* reporter gene (*LacZ*). Some expression can be detected in the border cells at the anterior tip at stage 9 (blue, arrow). B. *Eip75B* reporter gene expression is expanded along the anterior follicle cells and in some posterior cells upon culturing egg chambers with 20-Hydroxyecdysone (20HE). C. RNase protection assays of RNA from freshly dissected egg chambers (lanes 1–4 in increasing amounts) or those in culture without (5) or with addition of 20HE (6). Lane 7 is a yeast RNA control and lane 8 is a no-RNase control that shows the unprotected labeled RNA sizes for *hr4*, *Skp2*, *jon25Bii*, and *Argk*. Protected bands for *hr4*, *Skp2*, and *Argk* suggest that expression of these genes is increased upon addition of 20HE. D. Quantification of fold changes (fc) of selected targets in microarray analysis and RPA assays. At least 2 replicates were performed and data is averaged for RPA results. E. Predicted binding sites for the EcR complex in the regulatory regions of putative downstream target genes.

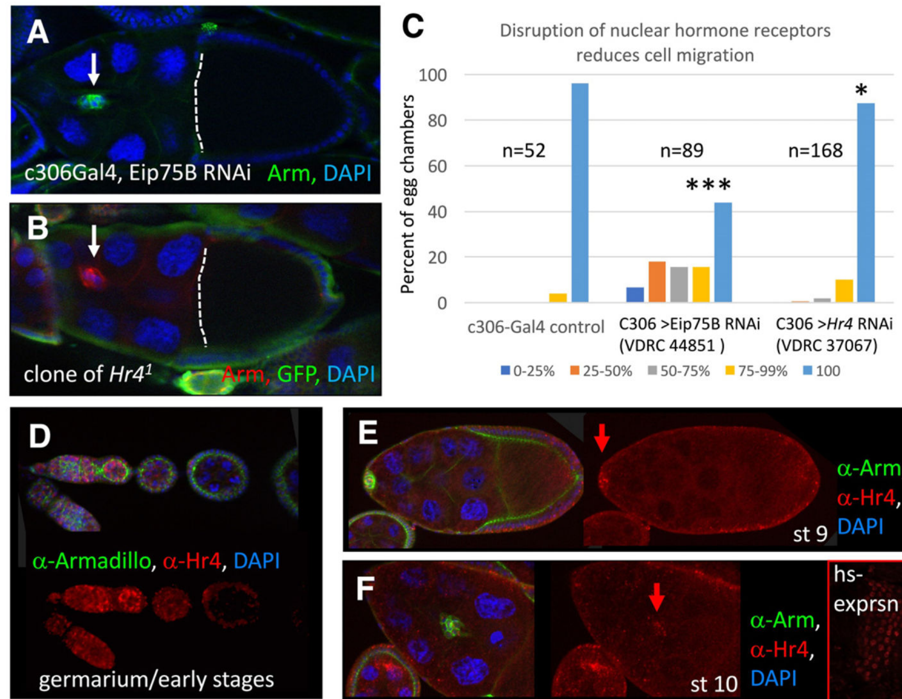


Fig. 5.

E75B and Hr4 nuclear hormone receptors are required for proper border cell migration. A. RNAi-mediated reduction of Eip75B (line VDRC 44851) in border cells driven by an anterior-follicle cell driver (c306) results in a failure of cell migration (arrow) to the oocyte (dashed line). Border cells are labeled by Arm expression (green). B. Border cells clonal for a homozygous mutation in *Hr4* (*Hr4*^{1}) results in a failure of cell migration (arrow) to the oocyte (dashed line). Border cells are GFP-negative and marked by Arm expression (red). Clonal events were assayed 8 days after induction. C. Quantification of the percentage of egg chambers with indicated border cell migration distances in a control genotype, or when *Eip75B* or *Hr4* were disrupted in border cells by c306-driven RNAi. Statistical significance was measured by chi square tests with Yates correction comparing the mutant phenotype proportion versus control: *** represents $p < 0.0001$ and * represents $p < 0.05$. D. Expression of Hr4 protein (red) in early egg chambers by antibody staining. Protein is high in germline and follicle cells early, but refines to mainly follicle cell expression around stage 5. E. Hr4 protein expression (red) in later stage egg chambers. Protein is enriched in border cells as they are forming (arrow) and is detected in other follicle cells and cytoplasmically in the germline. F. At late stage 9, Hr4 protein is higher in migrating border cells than in neighboring nurse cells. Heat shock mediated overexpression in ovary results in high levels of nuclear staining in outer follicle cell at stage 9/10, indicating the specificity of the antibody and availability of a ligand (right inset).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

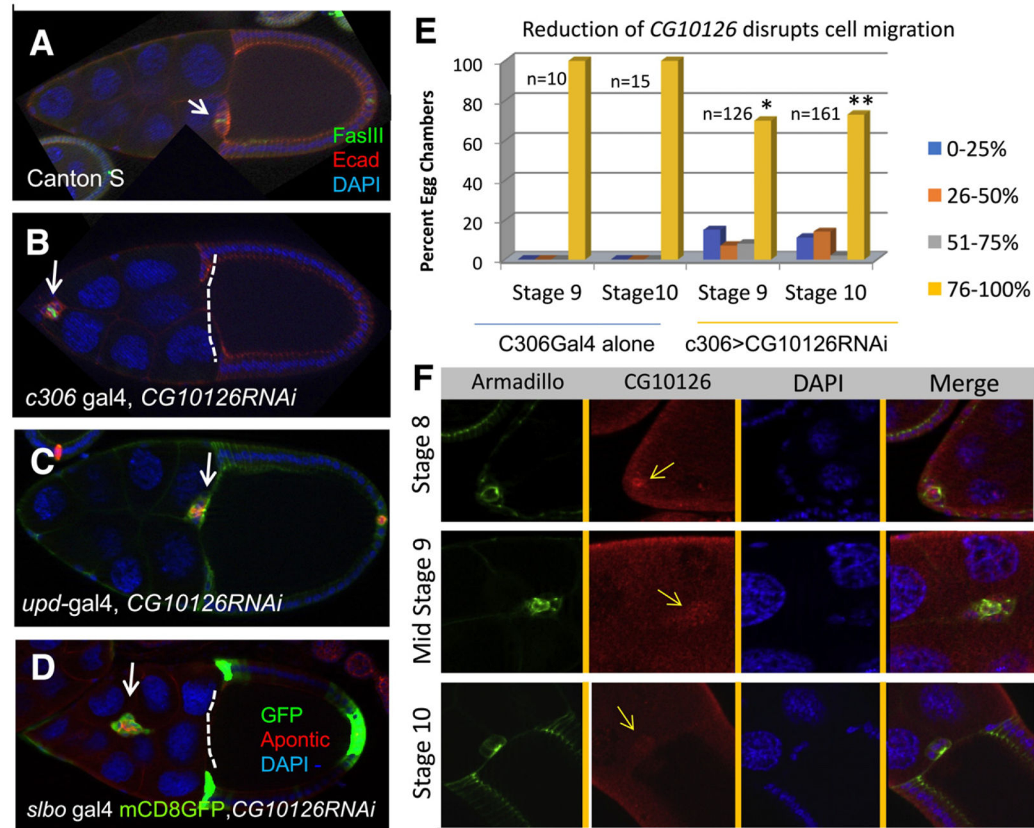


Fig. 6.

Functional requirement for a calcyphosine-like protein in border cells. A. A stage 10 egg chamber from a Canton S female shows wild type border cell migration, and antibody staining shows expression of Fas3 (green) at the polar cell junction and E-cadherin (red) highly enriched in the periphery of follicle cells. B. RNAi-mediated reduction of *CG10126* (line VDRC 44104) in border cells driven by an anterior-follicle cell driver (c306) results in a failure of border cell migration (arrow) to the oocyte (dashed line). Border cells are labeled by E-cad (red) and polar cells are marked by Fas3 expression (green). C. Wild-type border cell migration (arrow) when *CG10126* is reduced in polar cells (*upd*-Gal4 × line VDRC 44104). Border cells are labeled by E-cad (green) and polar cells are marked by Fas3 expression (red). D. Abnormal border cell migration (arrow) when *CG10126* is reduced in outer border cells (*slbo*-Gal4, UAS-GFP x line VDRC 44104) E. Quantification of the percentage of egg chambers with indicated border cell migration distances in a control genotype or when *CG10126* was disrupted in border cells by c306-driven RNAi. Line VDRC 44103 gave similar results. Statistical significance comparing the proportion of mutant phenotype versus the same-stage control was measured by Fisher's exact test: * represents $p = 0.03$ and ** represents $p = 0.006$. F. Expression of CG10126 protein (red) in egg chambers by antibody staining. At stage 8 (top panels), protein is enriched in newly-specified border cells (arrow, marked by high Arm expression), and the protein remains detectable in border cells at stage 9 (middle row) and stage 10 (bottom panels).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

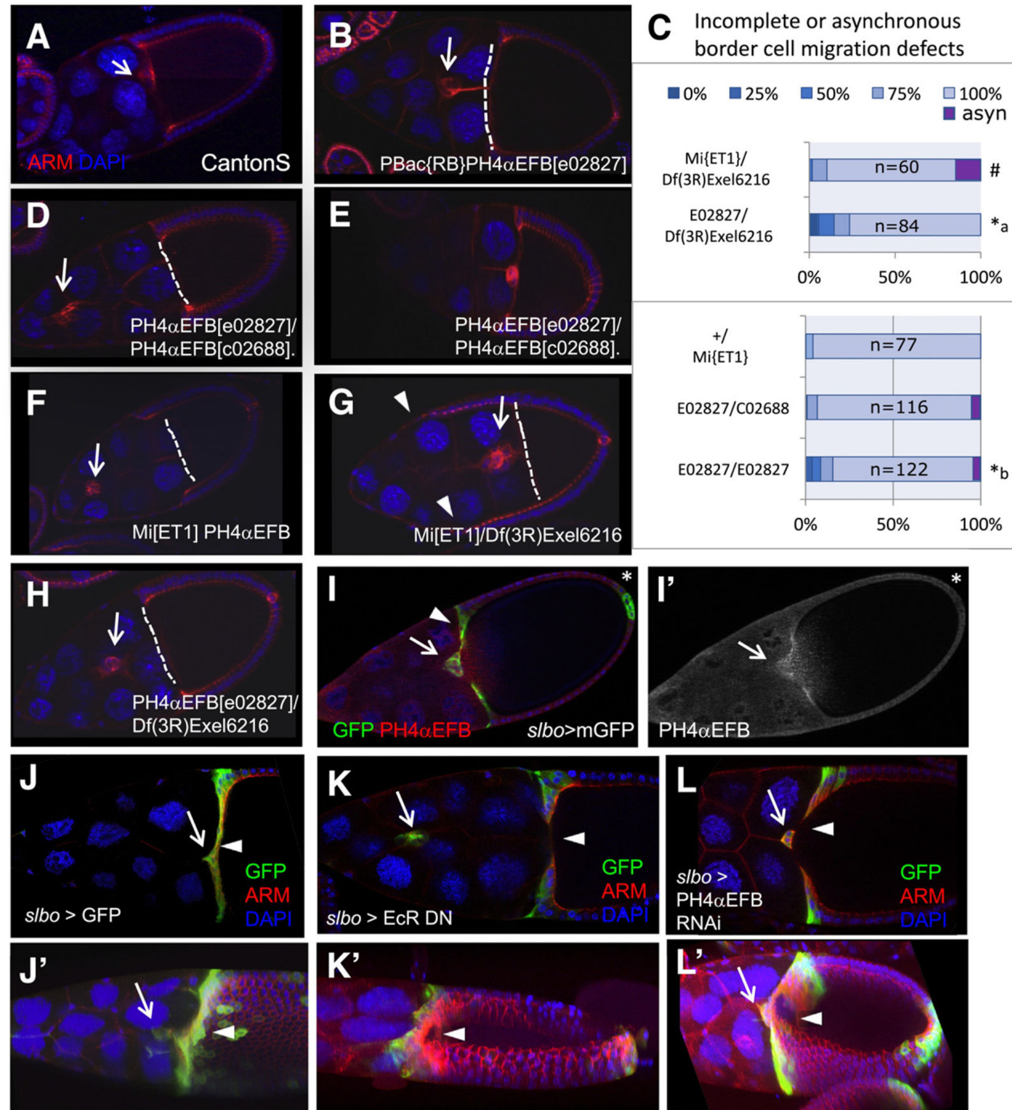


Fig. 7.

Prolyl-hydroxylase alpha EFB is required for border cell migration and may coordinate it with oocyte growth. A. A stage 10 egg chamber from a Canton S female shows wild type border cell migration, and antibody staining shows enriched Arm (red) in border cells (arrow) and at the periphery of other follicle cells. B, D–G. Different viable allelic combinations of *PH4αEFB* insertional mutations *in trans* to each other or over a deficiency removing the gene (genotypes indicated on the figure) have egg chambers in which the border cells (arrow) have not migrated to the oocyte border (dashed line) by late stage 9 or 10. C. Quantification of the percentage of egg chambers with indicated border cell migration distances, or migration that was asynchronous with the rest of oocyte development (asyn), in a control genotype and different allelic combination of *PH4αEFB* mutants. Statistical significance comparing the proportion of mutant phenotype versus the same-stage control was measured by Fisher's exact test: # represents $p = 0.007$ for early migration; *a represents $p = 0.01$ and *b represents $p = 0.04$ for late migration phenotypes. E. Some

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

PH4αEFB allelic combinations, such as *PH4aEFB*^{e02827}/*PH4aEFB*^{c02688}, result in poor oocyte growth, in which the oocyte is <50% of the egg chamber length by stage 10. G. Some *PH4αEFB* mutant egg chambers, such as one from *PH4aEFB*^{MiET1}/Deficiency, show asynchronous border cell migration, in which the border cells (arrow) migrate well ahead of the region of columnar follicle cells (arrowheads), and the oocyte takes up less of the egg chamber's length than normal by stage 9. I. A stage 10 egg chamber from a *slbo*-Gal4, UAS-mCD8-GFP female stained with an antibody against PH4αEFB shows that the protein is concentrated in the border cells (arrow), some centripetal cells (triangle), and is detected nearby in the oocyte and nurse cells. Although GFP is expressed in posterior follicle cells, PH4αEFB is not highly localized there (asterisk). I′. Grayscale image shows PH4αEFB protein expression alone from the egg chamber in (I). J–L. Reduced expression of *PH4αEFB* in centripetal cells using *slbo*-Gal4, UAS-RNAi results in delayed centripetal cell migration. J. In *slbo*-Gal4, UAS-GFP control egg chambers at late stage 10B, border cell migration is complete (arrow) and the centripetal cells have often spread inwards to cover the center anterior portion of the oocyte (arrowhead). This is more apparent viewed as a maximum projection of a confocal series, with the stack of images slightly tipped so anterior is directed below the plane (J′). K. In *slbo*-Gal4, UAS-EcR W650A egg chambers at late stage 10B, border cell migration is incomplete (arrow) and the centripetal cells have not spread inwards to the center, and instead appear bunched together, creating a gap (arrowhead) over the oocyte. In the maximum projection, a hole is evident (arrowhead in K′). L. In *slbo*-Gal4, *PH4αEFB* RNAi (line TRiP HMS00835) egg chambers at late stage 10B, border cell migration is complete (arrow) but the centripetal cells have not reached the center, and instead appear stretched but maintaining connections to the outer surface, which creates a gap (arrowhead) over the oocyte. In the maximum projection, a hole is evident (arrowhead in L′).

Author Manuscript

Table 1

Putative downstream target genes for both EcR and Tai signaling.

Shared target genes for EcR and Tai signaling, ranked by fold change in EcR (E) dominant negative expressing follicle cells compared to control. Genes identified in prior microarray studies as enriched in migratory cells (mig) or downstream targets of Slbo (slbo) are indicated based on references: (Wang et al., 2006) and (Borghese et al., 2006). Additional data supporting a role in oogenesis is indicated under “data”.

Fold change (E)	Name	Prior bc array	Data
3.42	*Skp2*		Figs. 3, 4
2.87	*midline fasciclin (mfas)*	slbo (Borghese, Wang)	(Arbeitman et al., 2004)
2.57	*CG13992*	slbo (Borghese, Wang)	(Fakhouri et al., 2006)
2.54	*prolyl-4-hydroxylase-alpha EFB (PH4aEFB)*	slbo (Wang)	Figs. 3, 7, Suppl. Fig. 3
2.36	*Arginine kinase (Argk)*	mig (Borghese, Wang)	Figs 3, 4
1.83	*latheo/Orc3*	slbo (Wang)	Fig. 3
1.83	*Cp7Fb*	slbo (Borghese, Wang)	
1.68	*CG33099*	slbo (Wang)	
1.62	*porin*		Fig. 3
1.61	*Dynein heavy chain 64C (Dhc64C)*	slbo (Wang)	(Van de Bor et al., 2011)
1.55	*eclair*		(Saleem et al., 2012)
1.53	*yellow-k*		
1.51	*RpS6*		
1.51	*Ecdysone-inducible gene L2 (ImpL2)*		Fig. 3, Suppl. Fig. 1, (Figueroa-Clarevega and Bilder, 2015; Kwon et al., 2015)
1.51	*CG14309*	slbo (Wang)	(Krauchunas et al., 2012)
1.48	*CG11378*	slbo (Borghese, Wang)	
1.48	*Spase22–23*		Suppl. Fig. 1
1.43	*nahoda*		Fig. 3
1.42	*betaTub97F*		
1.41	*Ecdysone-induced protein 75B (Eip75B)*		Figs 3, 5, Suppl. Fig. 1, (Buszczak et al., 1999)
1.37	*CG15168*		
1.37	*CG17124*	slbo (Wang)	
1.35	*Sulfotransferase 4 (St4)*	slbo (Wang)	
1.33	*nemy*		
1.33	*sans fille (snf)*		(Flickinger and Salz, 1994)
1.33	*p24-1*		(Saleem et al., 2012)
1.33	*Phosphorylase kinase (PhKgamma)*		
1.32	*RpS9*		
1.31	*Hexosaminidase 2 (Hexo2)*		
1.30	*CG10217*		
1.30	*Hsp22*		
1.29	*Glutathione S transferase D4*	mig (Borghese), slbo (Wang)	
1.28	*CG16956*	mig (Borghese), slbo (Wang)	(Kim and Orr-Weaver, 2011)
1.25	*CG7512*	mig (Borghese)	

Fold change (E)	Name	Prior bc array	Data
1.24	*CG10126*		Fig. 6

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript