© 2020. Published by The Company of Biologists Ltd | Development (2020) 147, dev189787. doi:10.1242/dev.189787

RESEARCH ARTICLE

The ETS-transcription factor Pointed is sufficient to regulate the posterior fate of the follicular epithelium

Cody A. Stevens[1], Nicole T. Revaitis[1], Rumkan Caur[2] and Nir Yakoby[1,2,*]

ABSTRACT

The Janus-kinase/signal transducer and activator of transcription (JAK/STAT) pathway regulates the anterior posterior axis of the *Drosophila* follicle cells. In the anterior, it activates the bone morphogenetic protein (BMP) signaling pathway through expression of the BMP ligand *decapentaplegic* (*dpp*). In the posterior, JAK/STAT works with the epidermal growth factor receptor (EGFR) pathway to express the T-box transcription factor *midline* (*mid*). Although MID is necessary for establishing the posterior fate of the egg chamber, we show that it is not sufficient to determine a posterior fate. The ETS-transcription factor pointed (*pnt*) is expressed in an overlapping domain to *mid* in the follicle cells. This study shows that *pnt* is upstream of *mid* and that it is sufficient to induce a posterior fate in the anterior end, which is characterized by the induction of *mid*, the prevention of the stretched cells formation and the abrogation of border cell migration. We demonstrate that the anterior BMP signaling is abolished by PNT through *dpp* repression. However, ectopic DPP cannot rescue the anterior fate formation, suggesting additional targets of PNT participate in the posterior fate determination.

KEY WORDS: EGFR signaling, Anterior-posterior axis coordination, ETS-transcription factor, Cell morphogenesis

INTRODUCTION

Animal development is an intricate process that is spatiotemporally coordinated by several cell signaling pathways that control cellular proliferation, migration and differentiation (Davidson and Erwin, 2006; Housden and Perrimon, 2014; Levine, 2010; Levine and Tjian, 2003). During development, body axes formation evolved in animals through different strategies (Genikhovich and Technau, 2017). In *Drosophila melanogaster*, formation of axes occurs during oogenesis, before egg fertilization (Lynch and Roth, 2011; Moussian and Roth, 2005). Numerous pathways are spatiotemporally coordinated to set the body axes in flies (Deng and Bownes, 1997; Fregoso Lomas et al., 2016; Gonzalez-Reyes and St Johnston, 1998; Moussian and Roth, 2005; Neuman-Silberberg and Schüpbach, 1993; Neuman-Silberberg and Schupbach, 1994; Nilson and Schüpbach, 1998; Twombly et al., 1996; Xi et al., 2003). However, the targets of these pathways that regulate the fates of these axes are still not well understood (Fregoso Lomas et al., 2013).

[1]Center for Computational and Integrative Biology, Rutgers, The State University of NJ, Camden, NJ 08102, USA. [2]Department of Biology, Rutgers, The State University of NJ, Camden, NJ 08102, USA.

*Author for correspondence (yakoby@camden.rutgers.edu)

N.Y., 0000-0002-7959-7588

Handling Editor: Swathi Arur
Received 24 February 2020; Accepted 29 September 2020

The follicle cells, a layer of follicular epithelium surrounding the developing oocyte, are dynamically patterned along the anterior-posterior axis (Bastock and St Johnston, 2008; Berg, 2005; Hinton, 1969; Horne-Badovinac and Bilder, 2005; Ward and Berg, 2005; Yakoby et al., 2008a). Early activation of the Janus-kinase/signal transducer and activator of transcription (JAK/STAT) pathway by the secretion of the ligand Unpaired (UPD) from the polar cells sets a mirror symmetry of two ends and main-body fates of the follicle cells (Fig. 1A) (Gonzalez-Reyes and St Johnston, 1998; Xi et al., 2003). The posterior fate is set by the secretion of the TGF-α-like ligand Gurken (GRK) from around the oocyte nucleus and activation of the epidermal growth factor receptor (EGFR) pathway in the overlaying follicle cells (Gonzalez-Reyes and St Johnston, 1998; Neuman-Silberberg and Schüpbach, 1993; Ray and Schupbach, 1996; Revaitis et al., 2020; Sapir et al., 1998). The anterior end is established by activating the bone morphogenetic protein (BMP) pathway by its ligand Decapentaplegic (DPP), which is induced by the JAK/STAT signaling (Deng and Bownes, 1997; Peri and Roth, 2000; Twombly et al., 1996; Xi et al., 2003; Yakoby et al., 2008b). The anterior and posterior domains are shown in Fig. 1A,B.

The sufficiency of EGFR activation to establish the border between the dorsal-anterior and the main-body follicle cells was initially computationally predicted (Zartman et al., 2011). The Nilson lab found that the EGFR target *midline* (*mid*), the *Drosophila* homolog of Tbx20, sets this boundary (Fregoso Lomas et al., 2013). MID acts to inhibit *broad* (*br*), a transcription factor gene that marks the primordia of the future respiratory dorsal appendages on the mature eggshell (Cheung et al., 2013; Deng and Bownes, 1997; Fregoso Lomas et al., 2013; Fuchs et al., 2012; Pyrowolakis et al., 2017; Tzolovsky et al., 1999). Further investigation showed that the JAK/STAT pathway, together with EGFR, induce *midline* expression by the inhibition of the main body fate determinant *mirror* (MIRR) (Fregoso Lomas et al., 2016; Jordan et al., 2000; Xi et al., 2003). The main body follicle cells are shown in Fig. 1A,B.

The ETS-transcription factor *pointed*-P1 (*pnt*-P1) is expressed dynamically in the follicle cells; in the posterior end during early oogenesis at stage 6 (S6) (the domain is shown in Fig. 1A,B) and later at S10 in the dorsal midline (the domain is shown in Fig. 1C) (Morimoto et al., 1996; Yakoby et al., 2008a). In the dorsal midline, PNT-P1 sets the distance between the two dorsal appendage primordia (Boisclair-Lachance et al., 2009; Deng and Bownes, 1997; Morimoto et al., 1996; Zartman et al., 2009). However, the role of early posterior expression of PNT-P1 is still unknown. Here, we show a new hierarchy in the regulation of the posterior end during early oogenesis. The expression of *midline* is regulated by PNT-P1. Ectopic expression of *pnt*-P1, but not *mid*, is sufficient to repress the early anterior BMP signaling and all associated morphological changes in the anterior domain, which resembles the behavior of cells in the posterior end. Together, we conclude that

Development (2020) 147, dev189787. doi:10.1242/dev.189787

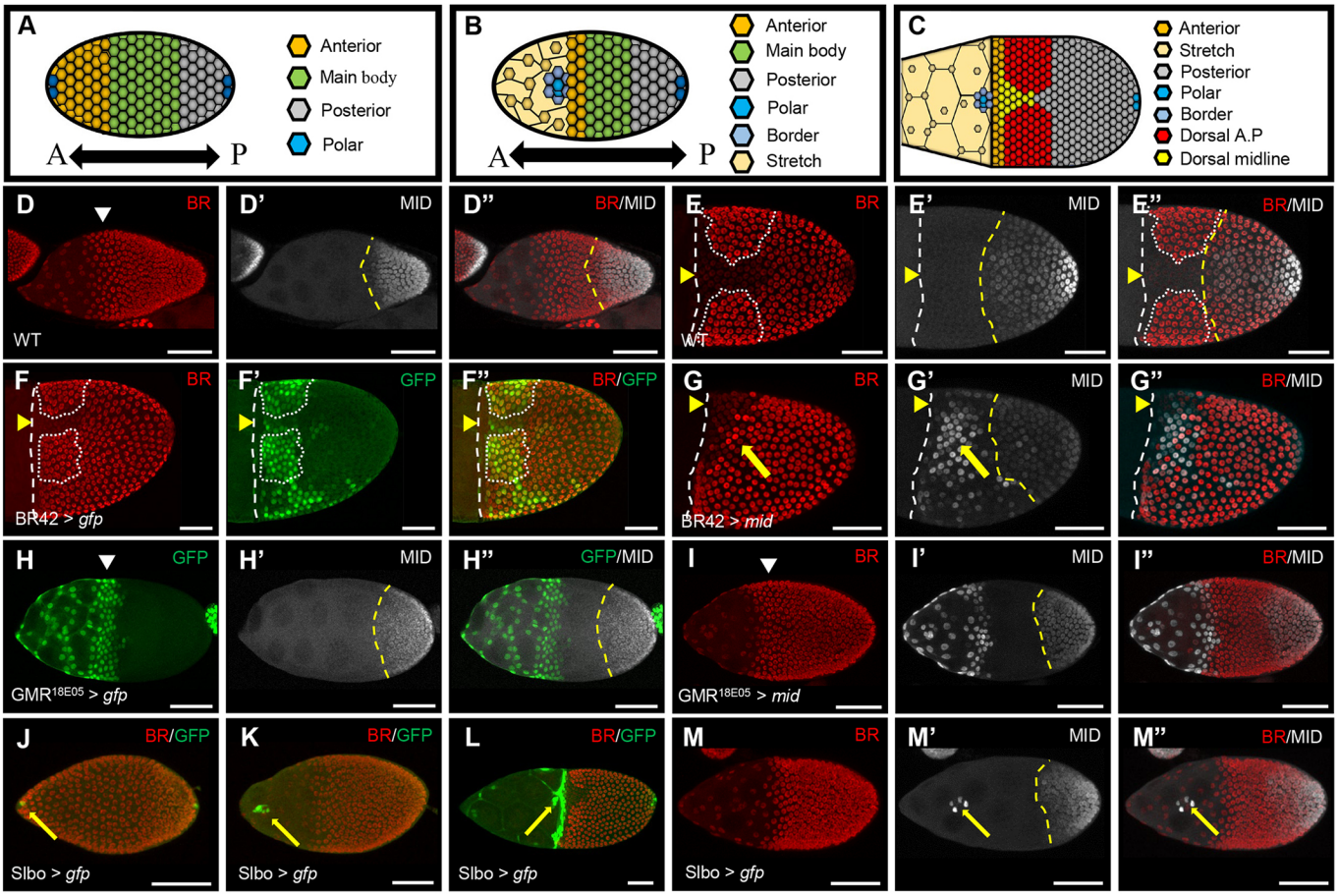

Fig. 1. The *midline* gene is necessary but not sufficient for posterior fate determination. (A-C) Cartoon representation of egg chambers at stage 8 (S8) (A), S9 (B) and S10 (C). The different domains are color coded: anterior domain, main body follicle cells, posterior domain, polar cells, border cells, stretch cells, dorsal A.P (appendage primordia) and dorsal midline. (D-E″) Wild-type expression of *midline* (MID, white) in early S9 (D-D″) (*n*=7) and later at S10B (E-E″) of oogenesis (*n*=10). The pattern of Broad (BR) is used as a spatial reference for the dorsal midline at S10B. (F-F″) The pattern of BR42-GAL4 driver expressing GFP (F′, green) (*n*=4). We focus on the BR domain, which is outlined with a dotted white line. (G-G″) Ectopic expression of MID by the BR42-GAL4 driver (yellow arrow, G′) disrupts BR patterning (G and G″) (*n*=5). (H-H″) The pattern of GMR^{18E05}-GAL4 expressing GFP (H, green) in the anterior domain (*n*=6). (I-I″) Ectopic expression of MID by GMR^{18E05}-GAL4 (*n*=8). (J-L) The pattern of Slbo-GAL4 driver expressing GFP (green) during S8 (J, *n*=6), S9 (K, *n*=5) and S10B (L, *n*=10) of oogenesis. Yellow arrows indicate the migrating border cells. (M-M″) Ectopic expression of MID by the Slbo-GAL4 (M′,M″, yellow arrows) (*n*=8). Yellow dashed line (D′,D″,E′,E″,G′,H′,H″,I′,M′) marks the anterior boundary of MID. Broad (BR, red) is expressed early uniformly in the follicular epithelium (D-D″), and later marks the dorsal appendage primordia (E-E″, white dotted outline). White arrowheads (D,H,I) mark posterior migration of follicular epithelium. (E-G″) White dashed lines mark the anterior boundary of follicular epithelium. Yellow arrowheads mark the dorsal midline. In all images, the anterior is to the left. Scale bars: 50 µm. *n*, the number of images with similar results.

PNT-P1 is sufficient to regulate the posterior fate of the follicular epithelium.

RESULTS

MID is not sufficient to repress the anterior fate of the follicular epithelium

The follicle cells are extensively patterned prior to specifying different domains (Berg, 2005; Niepielko et al., 2014; Revaitis et al., 2017; Yakoby et al., 2008a). This section clarifies some of the domains discussed in this paper. Up to S7 of oogenesis, the anterior, posterior and main body domains are set (Fig. 1A). These domains are marked by the posterior expression of *mid*, the anterior expression of *dpp* and the main body expression of *mirr* (Fregoso Lomas et al., 2013; Jordan et al., 2000; Twombly et al., 1996; Xi et al., 2003). At S9, the follicular epithelium progressively engulfs the growing oocyte, and a subsect of anterior cells differentiate to become the stretched follicle cells, which overlie the nurse cells (Fig. 1B). In addition, the anterior polar cells recruit approximately six neighboring cells, collectively known as the border cells, that together delaminate and migrate through the nurse cells posteriorly towards the oocyte (Fig. 1B). Later, at S10, the oocyte nucleus is at a dorsal anterior position, which designates the dorsal anterior domain (Fig. 1C). This domain comprises the late expression of BR, which marks the primordia of the future dorsal appendages, and PNT, which marks the dorsal midline (Morimoto et al., 1996; Tzolovsky et al., 1999). Here, we focus on three main domains: (1) the anterior domain, which includes the border cells and the stretched follicle cells; (2) the posterior domain; and (3) the dorsal-anterior domain.

As previously reported (Fregoso Lomas et al., 2013), MID patterns the posterior domain of the follicular epithelium (Fig. 1D,E). The pattern extends more anteriorly on the dorsal side to generate the posterior border of the dorsal appendage primordia (Fig. 1E″). In agreement with its role (Fregoso Lomas et al., 2013), using the dorsal anterior driver (BR42-GAL4, Fig. 1F-F″) to ectopically express *mid* was sufficient to repress BR patterning (Fig. 1G-G″). As has previously been reported, both ends of the developing egg chamber maintain an anterior fate in the absence of EGFR signaling

Development (2020) 147, dev189787. doi:10.1242/dev.189787

(Gonzalez-Reyes and St Johnston, 1998; Neuman-Silberberg and Schupbach, 1994; Peri and Roth, 2000; Twombly et al., 1996). Given that *mid* is a target of EGFR signaling, we aimed to determine whether MID is the primary mechanism of EGFR signaling that coverts an anterior to a posterior cell fate.

As mentioned above, the anterior domain of the egg chamber acquires distinct cellular morphologies at S9, including formation of stretched cells and the migration of the border cells (Duhart et al., 2017; Kolahi et al., 2009; Montell et al., 1992). We asked whether MID is sufficient to repress these anterior fate characteristics. Ectopic expression of *mid* in the anterior domain (using GMR^{18E05}-GAL4, Fig. 1H-H″) and in the polar cells (using Slbo-GAL4, Fig. 1J-L) had no impact on the morphogenesis of follicle cells (Fig. 1I-I″,M-M″). We conclude that, although MID is necessary to restrict the posterior boundary of the dorsal appendage primordia, it is not sufficient to set a posterior fate.

PNT regulates the pattern of MID

The ETS-transcription factor *pointed* (PNT-P1) is a regulator of tissue development and is a downstream target of EGFR and JAK/STAT signaling pathways (Gabay et al., 1996; Morimoto et al., 1996; O'Neill et al., 1994; Rebay et al., 2000; Rogers et al., 2017; Wassarman et al., 1995; Xi et al., 2003). Interestingly, the posterior pattern of PNT-P1 fully overlaps the pattern of MID (Fig. S1A,B). As PNT-P1 and MID are targets of EGFR and JAK/STAT signaling pathways (Fregoso Lomas et al., 2016; Xi et al., 2003), we aimed to determine whether PNT regulates MID expression in the follicular epithelium. Looking at 84 independent *pnt* null clones, we observed a cell-autonomous complete loss of MID in 55% of the clones (Fig. 2A-A″, Fig. S2A-A″), and a reduced level of MID in 45% of the clones (Fig. S2B-B″).

As expected, ectopic expression of *pnt*-P1 in dorsal anterior domain disrupts the BR pattern and expands the MID domain anteriorly (Fig. 2B-B″ compared with Fig. 1E-E″). Noticeably, MID did not expand to the entire dorsal anterior domain in this background (Fig. 2B′-B″). The absence of MID can be explained by the expression of MIRR in this domain, which represses *mid* (Fregoso Lomas et al., 2013). Further support for this observation is found in the dorsal midline; although PNT is naturally expressed in this domain at S10 of oogenesis, MID is still absent (Fregoso Lomas et al., 2013; Morimoto et al., 1996) (Fig. 1E′, Fig. S1B). To avoid the inhibition, we used anterior GAL4 drivers that are expressed outside of the endogenous MIRR domain (Fig. 1H,J-L). Indeed, ectopic expression of *pnt*-P1 in the anterior domain, as well as in the polar cells, induced MID expression cell-autonomously (Fig. 2C-D″). Hence, PNT is sufficient to induce *mid* expression.

The *pnt* gene has two isoforms, *pnt*-P1 and *pnt*-P2 (Klambt, 1993; Scholz et al., 1993). As mentioned above, PNT-P1 represses

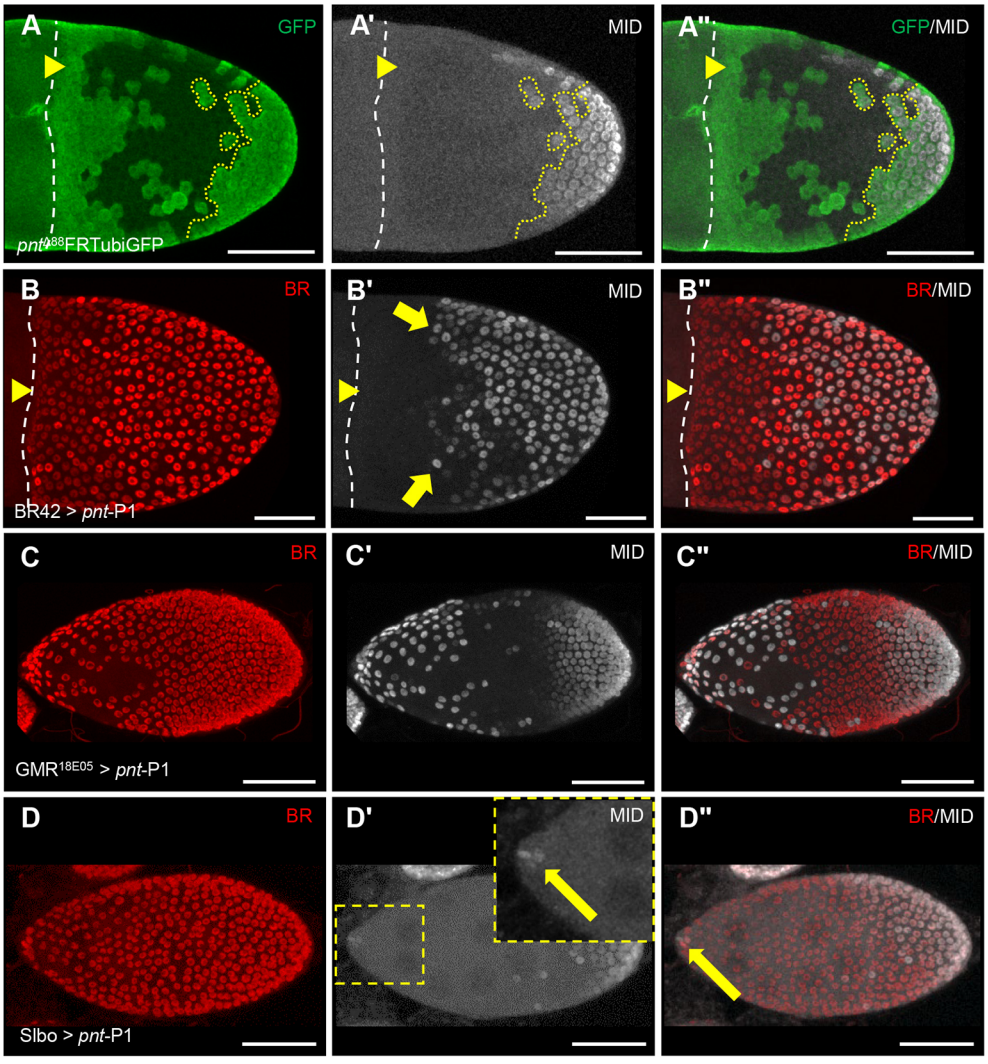

Fig. 2. Pointed is an upstream regulator of *midline* in the follicular epithelium. (A-A″) Null clones of *pointed* ($pnt^{\Delta 88}$) negatively marked by the loss of GFP (A). The clonal boundary is indicated by a yellow dotted line exhibiting cell-autonomous loss of MID (A′,A″) (*n*=46). (B-B″) Ectopic expression of *pnt*-P1 by the BR42-GAL4 driver exhibiting ectopic MID (B′) and loss of BR (B), and merge (B″) (*n*=4). Yellow arrows in B′ mark the ectopic MID. (C-C″) Ectopic expression of *pnt*-P1 in the anterior domain using the GMR^{18E05}-GAL4 exhibiting ectopic MID (C′). BR is used as a spatial marker (C) and merge (C″) (*n*=11). (D-D″) Ectopic expression of *pnt*-P1 in the border cells exhibiting ectopic MID (D′). BR is used as a spatial marker (D) and merge (D″) (*n*=12). Yellow arrow (D′, inset) marks ectopic MID (D′,D″). Yellow arrowheads (A-B″) mark the dorsal midline. White dashed lines mark the anterior boundary of follicular epithelium. In all images, anterior is to the left. Scale bars: 50 µm. *n*, the number of images with similar results.

Development (2020) 147, dev189787. doi:10.1242/dev.189787

the late *br* to set the dorsal midline (Deng and Bownes, 1997). We tested whether ectopic expression of *pnt*-P2 will impact the BR and MID patterning. Ectopic expression of *pnt*-P2 in the dorsal anterior domain did not change the BR patterning (compare Fig. S3A,B with Fig. 1D,E). In addition, ectopic expression of *pnt*-P2 in the anterior domain did not induce MID and the development of egg chambers continued normally (Fig. S3A′,B′). This lack of impact on BR patterning is in agreement with the normal expression pattern of *pnt*-P2 in this domain at S10B of oogenesis (Morimoto et al., 1996). We conclude that PNT-P1 is the isoform that activates *mid*.

Ectopic PNT-P1 represses BMP signaling

It was previously reported that the activation of EGFR in the anterior domain represses BMP signaling (Revaitis et al., 2017) (Fig. 3B,B″ compared with A,A″). The expression of a constitutively activated EGFR (ca*Egfr*) in the anterior domain not only abrogated BMP signaling, it also induced MID (Fig. 3A′ compared with B′, and A″ compared with B″). Next, we tested whether PNT-P1, as a target of EGFR, is sufficient to mediate this function. Ectopic expression of *pnt*-P1 in the anterior domain abolished BMP signaling and induced MID (Fig. 3C-C″). To discern between PNT and MID activities, we ectopically expressed MID in the same domain and observed no impact on BMP signaling (Fig. 3D-D″). These results are consistent with the previous observations where the ectopic expression of *mid* in the anterior domain had no observable effect on the development of egg chambers (Fig. 1I-I″,M-M″), whereas ectopic expression of *pnt*-P1 in all anterior cells resulted in abolishing BMP signaling and terminating the development of egg chambers at S9. We conclude, PNT-P1 is sufficient to repress BMP signaling in the anterior domain.

The anterior domain of the egg chamber is patterned by BMP signaling, as evident by the defects in eggshell morphologies upon perturbations in this pathway (Chen and Schüpbach, 2006; Marmion et al., 2013; Marmion and Yakoby, 2018; Peri and Roth, 2000; Twombly et al., 1996). Additionally, BMP signaling is necessary for anterior follicle cell flattening and stretching (Brigaud et al., 2015). Thus, we aimed to understand the role PNT-P1 has on the regulation of BMP signaling. Ectopic expression of *pnt*-P1 in the anterior domain terminated egg chamber development at S9, which is similar to consequences of ectopic expression of ca*Egfr* in this domain (Revaitis et al., 2017). To circumvent lethality, we used the GMR^{43H01}-GAL4 driver to limit the expression of PNT-P1 to a region of the anterior follicle cells, including the border cells and a subset of posterior follicle cells (Revaitis et al., 2017) (Fig. 4A-A″,B-B″).

It has previously been reported that *dpp* is ectopically expressed in the posterior end of an EGFR signaling mutant (Peri and Roth, 2000). As ectopic ca*Egfr* represses anterior BMP signaling (Revaitis et al., 2017), we wanted to understand whether PNT-P1

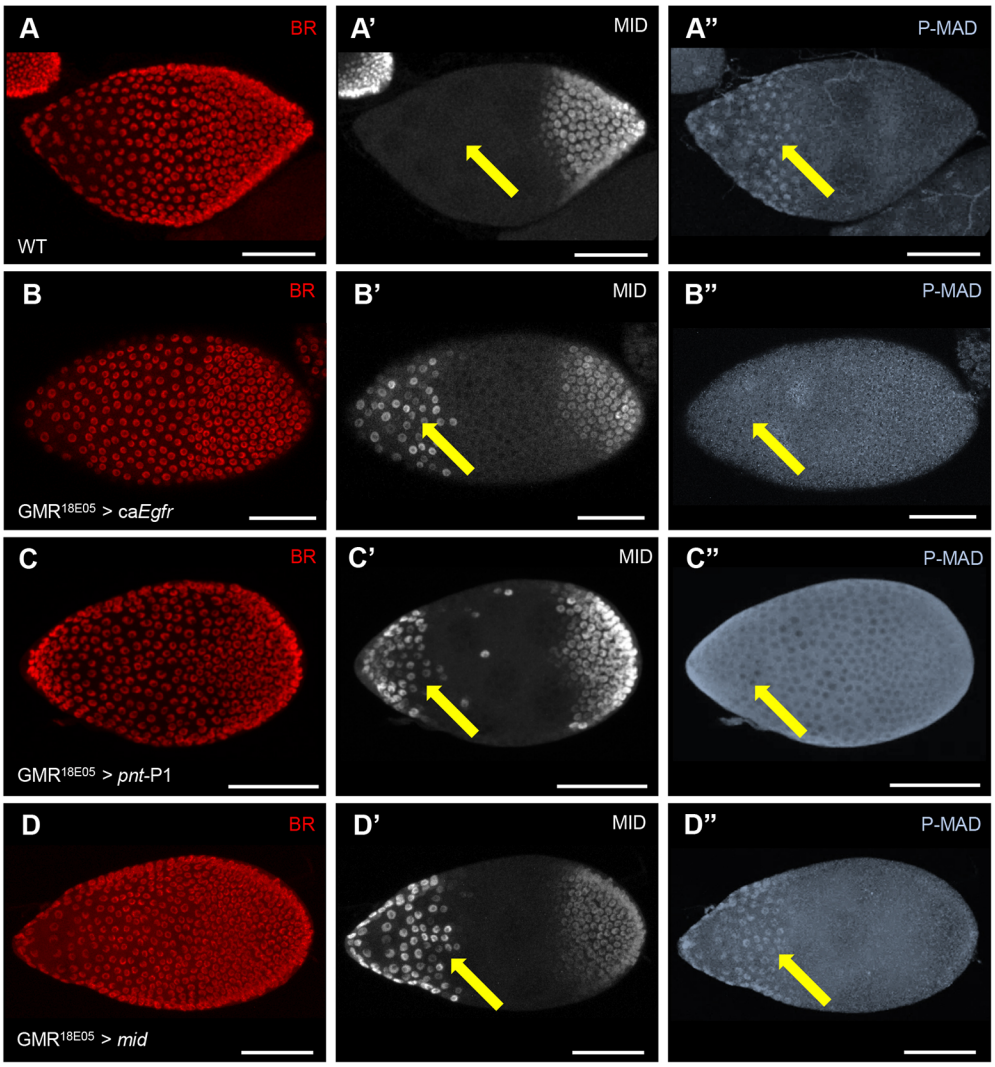

Fig. 3. Ectopic *pointed* represses BMP signaling. (A-A″) Wild-type expression of BR (A), MID (A′) and P-MAD pattern (A″) at early stage 9 of oogenesis. Yellow arrows in A′ and A″ indicate the lack of anterior MID and presence of P-MAD, respectively (*n*=6). (B-B″) Using the GMR^{180E5}-GAL4 driver to ectopically express a constitutively active EGFR (ca*Egfr*) in the anterior epithelium at early stage 9 of oogenesis. BR (B), gain of MID (B′) and loss of P-MAD (B″) are observed, yellow arrows (*n*=5). (C-C″) Using the GMR^{180E5}-GAL4 driver to ectopically express *pnt*-P1 in the anterior epithelium. BR (C), gain of MID expression (C′) and loss of P-MAD (C″) are observed, marked by a yellow arrow (*n*=11). (D-D″) Using the GMR^{18E05}-GAL4 driver to ectopically express MID in the anterior epithelium. BR (D), gain of MID expression (D′) and intact P-MAD pattern are observed, marked by a yellow arrow (*n*=13). In all images, BR marks the follicular epithelium. In all images, the anterior of the egg chamber is to the left. Scale bars: 50 µm. *n*, number of images with similar results.

Development (2020) 147, dev189787. doi:10.1242/dev.189787

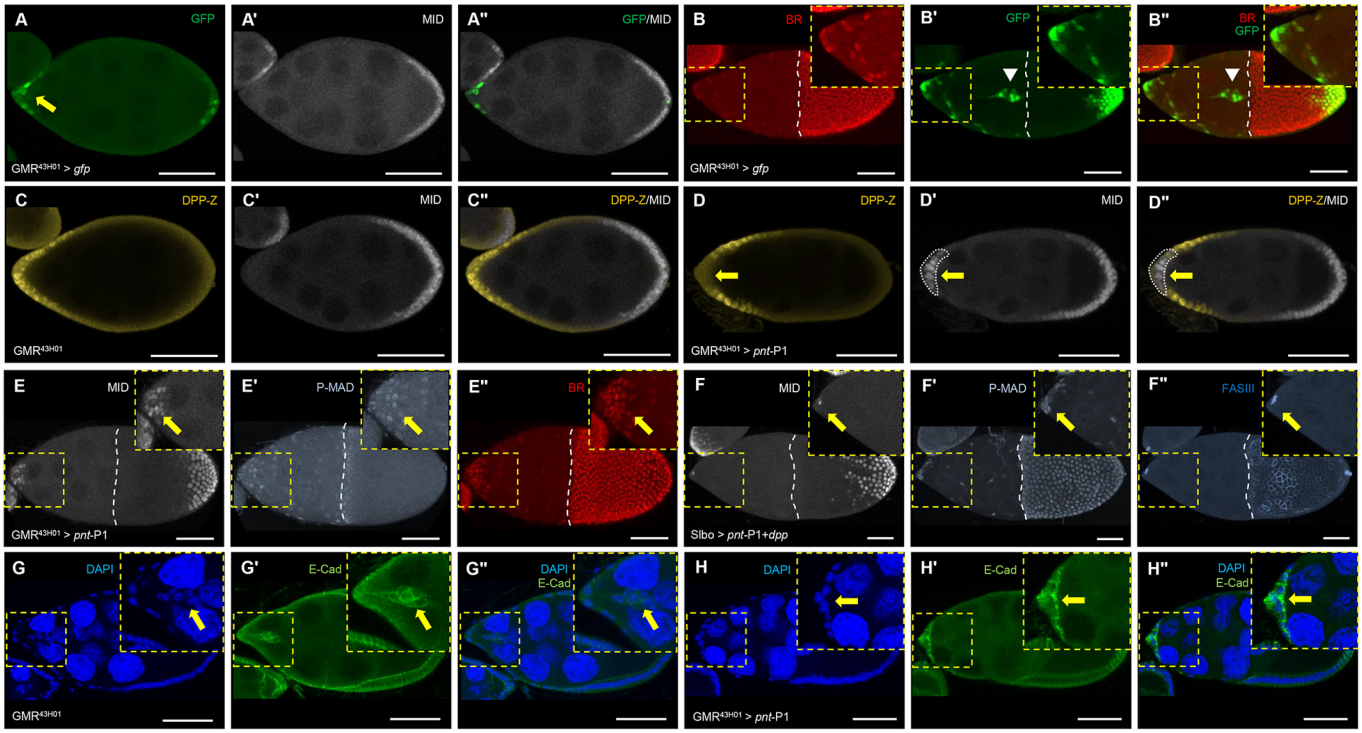

Fig. 4. Pointed represses *dpp* and anterior cell morphogenesis independent of BMP signaling. (A-A″) GMR^{43H01}-GAL4 driving expression of GFP at S9 egg chamber, yellow arrow (A) marks anterior GFP (*n*=6). (A′) The pattern of MID. (A″) Merged image. (B-B″) GMR^{43H01}-GAL4 driving expression of GFP at S10 (*n*=10). (B) Broad, (B′) GFP, (B″) merge. Expression is observed in some of the anterior stretch follicle cells, migrating border cell cluster and posterior cells (B′,B″, white arrowheads). We focus on the anterior and border cells (insets). (C-C″) The pattern of a LacZ-DPP reporter (DPP-Z) in the GMR^{43H01}-GAL4 driver background cross-section of early S9 egg chamber (C), MID pattern (C′) and merged image (C″) (*n*=6). (D-D″) GMR^{43H01}-GAL4 driver expressing *pnt*-P1 in the anterior resulting in ectopic expression of MID (D′, dotted white lines, yellow arrows) and loss of DPP-Z (D,D″ merge, yellow arrows) (*n*=11). (E-E″) Ectopic expression of *pnt*-P1 using the GMR^{43H01}-GAL4 driver in anterior stretch cells induced MID (E, yellow arrow, inset) and contained P-MAD (E′, yellow arrow, inset). These cells did not stretch and remained clustered in the anterior, marked by BR (E″, yellow arrow, inset) (*n*=5). (F-F″) Slbo-GAL4 driving expression of both *pnt*-P1 and *dpp*. (F) MID expression observed in anterior polar cells (yellow arrow, inset). (F′) P-MAD was present in these cells (yellow arrow, inset). (F″) FASIII marks polar cells failing to migrate posteriorly (yellow arrow, inset) (*n*=6). (G-G″) GMR^{43H01}-GAL4 exhibiting cell nuclei marked by DAPI (G) and cell boundaries marked by E-cadherin (E-Cad) (G′), insets showcase migrating border cells (G-G″, yellow arrows) (*n*=6). (H-H″) Ectopic expression of *pnt*-P1 in anterior stretch cells. (H) DAPI marks cell nuclei, (H′) E-Cad marks cell boundaries. Insets showcase accumulation of E-Cad and loss of border cell migration (H-H″, yellow arrows) (*n*=7). Broad (BR) in B,E″ marks follicular epithelium. White dashed lines (E-F″) mark the anterior boundary of follicular epithelium. In all images, the anterior of the egg chamber is to the left. For A-D,G-H, cross-sections are shown. Scale bars: 50 µm. *n*, the number of images with similar results.

represses BMP signaling through *dpp* repression. Using the *dpp*-βGal reporter (DPP-Z) to mark the *dpp* expression domain (Fig. 4C-C″), we ectopically expressed *pnt*-P1 in a small region of anterior cells (Fig. 4D-D″) and monitored changes in the pattern of DPP-Z. As expected, MID was induced in cells ectopically expressing *pnt*-P1 (Fig. 4D′). Interestingly, we found a cell-autonomous loss of DPP-Z in these cells (Fig. 4D-D″). As DPP is a diffusible ligand, we next asked whether surrounding unaffected cells can provide DPP to activate signaling in the cells that do not express *dpp*. In cells expressing *pnt*-P1, marked by ectopic MID (Fig. 4E), we detected BMP activation (Fig. 4E′).

Ectopic PNT represses anterior morphogenesis independently of BMP signaling

As discussed above, while BMP signaling was detected in cells expressing *pnt*-P1 (Fig. 4E′), these cells still remained in the anterior and failed to migrate as border cells (Fig. 4E″). It is possible that the amount of DPP from neighboring cells was not sufficient to activate BMP signaling to a level that induces border cells migration. Therefore, we aimed to determine whether increasing the levels of DPP in these cells would rescue their mobility. Using the Slbo-GAL4 driver, we ectopically expressed both *pnt*-P1 and *dpp*. As expected, MID was detected in the polar cells (Fig. 4F). In addition, BMP signaling was activated in these cells (Fig. 4F′). At the same time, these cells remained in the anterior domain (compare Fig. 4F″ with Fig. 1J-L). Interestingly, BMP signaling is a known repressor of *mid* (Fregoso Lomas et al., 2016); however, the anterior BMP signaling could not overcome the induction of *mid* by the ectopic PNT-P1 (Fig. 4F). We conclude that PNT-P1 induction of *mid* abrogates the repression of *mid* by BMP signaling. Furthermore, ectopic expression of *dpp* cannot rescue migration of border cells in the presence of PNT-P1.

As explained above, border cells migration and stretching of cells over the nurse cells are hallmarks of the anterior follicle cells at S9 of oogenesis (Kolahi et al., 2009; Van Buskirk and Schüpbach, 1999). The expression of *pnt*-P1 stopped the migration of border cells and stretching of the anterior cells, even though BMP signaling was restored. The observed cell clumping in the anterior domain is mirrored in mutant backgrounds of the transcriptional inhibitor *yan*, whereas loss of *yan* resulted in an accumulation of E-cadherin (E-Cad) and revocation of migration of border cells (Schober et al., 2005). Here, we ectopically expressed *pnt*-P1 in the future border cells, and in agreement with *yan* perturbations, observed a loss of border cell migration and the accumulation of E-Cad (compare Fig. 4G′ with H′). As PNT-P1 and YAN compete on the same DNA-binding motifs (Wei et al., 2010), we proposed that the

Development (2020) 147, dev189787. doi:10.1242/dev.189787

ectopic PNT-P1 could be outcompeting YAN DNA binding, resulting in the accumulation of E-Cad.

The disruption of EGFR signaling allows for the ectopic expression of *dpp* in the posterior end (Peri and Roth, 2000). We tested whether loss of PNT is sufficient to allow *dpp* expression in the posterior domain. We could not detect DPP-Z expression in large posterior clones null for *pnt* (Fig. S4A-A‴). In addition, in these clones, we could not detect activation of BMP signaling (Fig. S4B-B″). We suggest that, in this background, EGFR is still activated; therefore, factors other than PNT-P1 are likely being induced by EGFR signaling to repress posterior *dpp* expression. We conclude that PNT-P1 is sufficient to repress the anterior *dpp* but it is not necessary to repress posterior *dpp*. Future studies will focus on finding other targets of EGFR that also control posterior *dpp* expression.

DISCUSSION

The anterior-posterior patterning of the follicular epithelium is an intricate process that sets the initial boundaries of the egg chamber. Here, we investigated the role of PNT-P1 as a regulator of the posterior end, and demonstrate that this ETS-transcription factor is an upstream regulator of MID. Interestingly, MID is either completely or partially lost in posterior clones null for *pnt*. These results are consistent with the reported regulation of MID and PNT. Specifically, in a clonal analysis of cells expressing an amorphic version of either Stat92E (STAT) or Hopscotch (HOP), both of which are essential for JAK/STAT signaling, MID was completely lost or only reduced (Fregoso Lomas et al., 2016). In addition, similar perturbations in JAK/STAT signaling led to the complete loss of PNT (Xi et al., 2003). Taken together, the complete loss of PNT and the loss/reduction of MID in JAK/STAT perturbations further support that PNT is an upstream regulator of MID, as shown is our experiments. We reason that *mid* is expressed very early in oogenesis, hence the complete or partial loss of MID in our experiments could reflect a degradation process of MID that was induced earlier to the formation of the clone. Of note, our results are in agreement with the regulation of *mid* by PNT in the developing *Drosophila* cardiac cells (Schwarz et al., 2018).

The BMP signaling pathway has multiple components necessary for signaling, including ligands, receptors and intracellular components (Chen and Schüpbach, 2006; Dobens and Raftery, 1998, 2000; Marmion et al., 2013; Marmion and Yakoby, 2018; Yakoby et al., 2008a,b). It has previously been reported that *dpp* is ectopically expressed in the posterior end of an EGFR signaling mutant (Peri and Roth, 2000). Our results demonstrate that ectopic expression of *pnt* in the anterior domain is sufficient to repress *dpp* expression cell-autonomously. In addition, restricting the number of anterior cells expressing *pnt* can rescue the activation of BMP signaling by the emanating DPP from surrounding unaffected cells. These results indicated that all other BMP pathway components remain intact in the presence of PNT-P1. Our findings further support our previous prediction that the repression of anterior BMP signaling upon ectopic EGFR activation is due to *dpp* repression (Revaitis et al., 2017).

In the anterior domain, the stretched cells grow to engulf the nurse cells for them to go through apoptosis after releasing their contents into the developing oocyte (Timmons et al., 2016). This process is terminated by the ectopic expression of *pnt*-P1 in the anterior cells. This suggests that PNT-P1 can block the communication between the anterior follicle cells (stretched cells) and the germ-line nurse cells. As the nurse cells may participate in the anterior fate determination, such as stretch cell formation, the absence of nurse cells in the posterior end may prevent a 'true' anterior from forming in a *pnt* null background (Fig. S4). Further support for the suggested role of PNT-P1 in cell movement is found by the natural expression of *pnt*-P1 in the dorsal midline (Morimoto et al., 1996). These cells do not migrate, whereas their neighboring cells, the dorsolateral appendage primordia, migrate anteriorly to form the tube-like dorsal appendages (Ward and Berg, 2005). Interestingly in a background of cells expressing MAE, an inhibitor of PNT, the dorsal midline cells move and become part of a single wide dorsal appendage (Yamada et al., 2003). Taken together, we suggest that the expression of PNT anchors groups of cells in tissues.

MATERIALS AND METHODS

Flies and reagents

All flies were raised on standard cornmeal agar and kept at room temperature, unless specified in heat shock treatment. The fly strains used in this study were obtained from the following sources: wild-type *D. melanogaster* (25211), UAS-*pnt*-P1 (869), GMR^{43H01}-GAL4 (47931), PNT-GFP (42680) and UAS-*pnt*-P2 (399) were obtained from the Bloomington Drosophila Stock Center. BR-42;*tub*Gal80ts, FRT82B*pnt*Δ88 and *e22cflp*;FRT82B*ubi*GFP were a gift from S. Shvartsman (Princeton University, NJ, USA). UAS-*mid* was a gift from L. Nilson (McGill University, Montreal, Canada). Slbo-Gal4 was a gift from D. Harrison (University of Kentucky, KY, USA). UAS-ca*Egfr* was a gift from T. Schüpbach (Princeton University, NJ, USA). UAS-GFP*nls* was a gift from J. Posakony (University of California San Diego, CA, USA) and UAS-*dpp* a gift from S. Newfeld (Arizona State University, AZ, USA). The p*tub*Gal80ts;GMR^{18E05}-GAL4 and the DPP-Z reporter were used here (Revaitis et al., 2017).

Immunostaining

Immunohistochemistry analysis was carried out on 2-7 days old flies raised on active yeast for 24 h at room temperature (23°C) prior to dissection. Ovaries were dissected in 1 ml Schneider's media and fixed in a 4% paraformaldehyde/heptane/0.2% Triton X-100 in PBS (PBST) solution for 20 min. Samples were rinsed three times, 5 min each time, in 0.2% PBST solution, then permeabilized in 1% PBST solution for 1 h. Samples were rinsed once in 0.2% PBST then blocked in 0.2% PBST with 1% bovine serum albumin (BSA) solution for 1 h. Samples were incubated overnight at 4°C in primary antibody cocktail with 0.2% PBST and 1% BSA. After incubation, samples were washed three times for 20 min each in 0.2% PBST, then secondary antibody cocktail was added with 0.2% PBST and 1% BSA, and incubated for 1 h protected from light at room temperature. Samples were then washed three times for 20 min each time in 0.2% PBST and mounted in Fluoromount-G mounting media. Primary antibody concentrations used were mouse anti-Broad (1:250; DSHB), sheep anti-GFP (1:1000, BioRad), rabbit anti-phosphorylated-Smad (1:3600; a gift from E. Laufer, Columbia University, NY, USA; Yakoby et al., 2008b), rabbit anti-β-galactosidase (1:1000; Invitrogen), guinea pig anti-MID (1:1000; a gift from L. Nilson), mouse anti-Fasciclin III (1:250; DSHB), rabbit anti-YAN (1:250, a gift from S. Shvartsman) and rat anti-DCAD2 (1:50; DSHB). Secondary antibodies used were Alexa Fluor 488 donkey anti-mouse, Alexa Fluor 488 donkey anti-sheep, Alexa Fluor 568 donkey anti-rabbit, Alexa Fluor 568 donkey anti-mouse, Alexa Fluor 633 goat anti-guinea pig and Alexa Flour 568 goat anti-rat (1:1250; Invitrogen). DAPI was used for nuclear staining (84 ng/ml; Thermo Fisher). Samples were imaged on Leica SP8 confocal microscope with 20× objective. Images were processed using FIJI (Fiji is Just ImageJ) software (Schindelin et al., 2012).

Heat-shock induction of transgenic lines

Fly lines containing a temperature-sensitive GAL80 repressive element were raised on active yeast for 3 days at 28°C prior to dissection to alleviate GAL80 and drive ectopic gene expression.

Generation of negatively marked clones

The FLP/FRT recombinant technique (Xu and Rubin, 1993) was used to generate loss-of-function null clones marked by the absence of GFP (*ubi*-GFP). The *e22c*flp;FRT82B-*ubi*GFP line was crossed to the FRT82B *pnt*$^{\Delta 88}$ line (*e22c*>flp; FRT82B *pnt*$^{\Delta 88}$/FRT82B *ubi*-GFP) to generate mutant clones null for both *pnt*-P1 and *pnt*-P2 isoforms marked with the absence of GFP.

Quantification and statistical analysis

All images were obtained with equal confocal microscopy wavelength settings among images using the same channel. In *pnt* null clonal experiments, boundaries of loss of PNT were drawn according to loss of observable expression of GFP. FIJI software was used for all images for correct orientation and leveling of brightness and contrast. In all images, *n*-value represents number of egg chambers observed with a similar phenotypic profile.

Acknowledgements

We thank Laura Nilson for the *midline* antibody and the UAS-*mid* fly lines. We also thank Doug Harrison for the Slbo-Gal4 fly line, and Stanislav Shvartsman for the BR42-Gal4 and *pnt*$^{\Delta 88}$FRT fly lines. We acknowledge the Bloomington Drosophila Stock Center for the fly stocks. We are also grateful to members of the Yakoby Lab for many fruitful discussions.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.A.S., N.Y.; Validation: C.A.S.; Formal analysis: C.A.S.; Investigation: C.A.S., R.C.; Resources: N.T.R.; Writing - original draft: C.A.S., N.Y.; Writing - review & editing: C.A.S., N.T.R., N.Y.; Supervision: N.Y.; Project administration: N.Y.; Funding acquisition: N.Y.

Funding

C.A.S. and N.T.R. were partially supported by the Center for Computational and Integrative Biology, Rutgers-Camden, by the National Institute of General Medical Sciences of the National Institutes of Health (2R15GM101597-02 to N.Y.), and by the National Science Foundation (IOS-1926802 to N.Y.). Deposited in PMC for release after 12 months.

Supplementary information

Supplementary information available online at
https://dev.biologists.org/lookup/doi/10.1242/dev.189787.supplemental

References

Bastock, R. and St Johnston, D. (2008). *Drosophila* oogenesis. *Curr. Biol.* **18**, R1082-R1087. doi:10.1016/j.cub.2008.09.011

Berg, C. A. (2005). The *Drosophila* shell game: patterning genes and morphological change. *Trends Genet.* **21**, 346-355. doi:10.1016/j.tig.2005.04.010

Boisclair Lachance, J.-F., Fregoso-Lomas, M., Eleiche, A., Bouchard Kerr, P. and Nilson, L. A. (2009). Graded Egfr activity patterns the *Drosophila* eggshell independently of autocrine feedback. *Development* **136**, 2893-2902. doi:10.1242/dev.036103

Brigaud, I., Duteyrat, J.-L., Chlasta, J., Le Bail, S., Couderc, J.-L. and Grammont, M. (2015). Transforming growth factor β/activin signalling induces epithelial cell flattening during *Drosophila* oogenesis. *Biol. Open* **4**, 345-354. doi:10.1242/bio.201410785

Chen, Y. and Schüpbach, T. (2006). The role of brinker in eggshell patterning. *Mech. Dev.* **123**, 395-406. doi:10.1016/j.mod.2006.03.007

Cheung, L. S., Simakov, D. S. A., Fuchs, A., Pyrowolakis, G. and Shvartsman, S. Y. (2013). Dynamic model for the coordination of two enhancers of broad by EGFR signaling. *Proc. Natl. Acad. Sci. USA* **110**, 17939-17944. doi:10.1073/pnas.1304753110

Davidson, E. H. and Erwin, D. H. (2006). Gene regulatory networks and the evolution of animal body plans. *Science* **311**, 796-800. doi:10.1126/science.1113832

Deng, W. M. and Bownes, M. (1997). Two signalling pathways specify localised expression of the broad-complex in *Drosophila* eggshell patterning and morphogenesis. *Development* **124**, 4639-4647.

Dobens, L. L. and Raftery, L. A. (1998). *Drosophila* oogenesis: a model system to understand TGF-β/Dpp directed cell morphogenesis. *Ann. N. Y. Acad. Sci.* **857**, 245-247. doi:10.1111/j.1749-6632.1998.tb10123.x

Dobens, L. L. and Raftery, L. A. (2000). Integration of epithelial patterning and morphogenesis in *Drosophila* ovarian follicle cells. *Dev. Dyn.* **218**, 80-93. doi:10.1002/(SICI)1097-0177(200005)218:1<80::AID-DVDY7>3.0.CO;2-8

Duhart, J. C., Parsons, T. T. and Raftery, L. A. (2017). The repertoire of epithelial morphogenesis on display: Progressive elaboration of *Drosophila* egg structure. *Mech. Dev.* **148**, 18-39. doi:10.1016/j.mod.2017.04.002

Fregoso Lomas, M., Hails, F., Boisclair Lachance, J.-F. and Nilson, L. A. (2013). Response to the dorsal anterior gradient of EGFR signaling in *Drosophila* oogenesis is prepatterned by earlier posterior EGFR activation. *Cell Rep.* **4**, 791-802. doi:10.1016/j.celrep.2013.07.038

Fregoso Lomas, M., De Vito, S., Boisclair Lachance, J.-F., Houde, J. and Nilson, L. A. (2016). Determination of EGFR signaling output by opposing gradients of BMP and JAK/STAT activity. *Curr. Biol.* **26**, 2572-2582. doi:10.1016/j.cub.2016.07.073

Fuchs, A., Cheung, L. S., Charbonnier, E., Shvartsman, S. Y. and Pyrowolakis, G. (2012). Transcriptional interpretation of the EGF receptor signaling gradient. *Proc. Natl. Acad. Sci. USA* **109**, 1572-1577. doi:10.1073/pnas.1115190109

Gabay, L., Scholz, H., Golembo, M., Klaes, A., Shilo, B. Z. and Klämbt, C. (1996). EGF receptor signaling induces pointed P1 transcription and inactivates Yan protein in the *Drosophila* embryonic ventral ectoderm. *Development* **122**, 3355-3362.

Genikhovich, G. and Technau, U. (2017). On the evolution of bilaterality. *Development* **144**, 3392-3404. doi:10.1242/dev.141507

Gonzalez-Reyes, A. and St Johnston, D. (1998). The Drosophila AP axis is polarised by the cadherin-mediated positioning of the oocyte. *Development* **125**, 3635-3644.

Hinton, H. E. (1969). Respiratory systems of insect egg shells. *Annu. Rev. Entomol.* **14**, 343-368. doi:10.1146/annurev.en.14.010169.002015

Horne-Badovinac, S. and Bilder, D. (2005). Mass transit: epithelial morphogenesis in the *Drosophila* egg chamber. *Dev. Dyn.* **232**, 559-574. doi:10.1002/dvdy.20286

Housden, B. E. and Perrimon, N. (2014). Spatial and temporal organization of signaling pathways. *Trends Biochem. Sci.* **39**, 457-464. doi:10.1016/j.tibs.2014.07.008

Jordan, K. C., Clegg, N. J., Blasi, J. A., Morimoto, A. M., Sen, J., Stein, D., McNeill, H., Deng, W.-M., Tworoger, M. and Ruohola-Baker, H. (2000). The homeobox gene mirror links EGF signalling to embryonic dorso-ventral axis formation through notch activation. *Nat. Genet.* **24**, 429-433. doi:10.1038/74294

Klämbt, C. (1993). The Drosophila gene pointed encodes two ETS-like proteins which are involved in the development of the midline glial cells. *Development* **117**, 163-176.

Kolahi, K. S., White, P. F., Shreter, D. M., Classen, A.-K., Bilder, D. and Mofrad, M. R. K. (2009). Quantitative analysis of epithelial morphogenesis in *Drosophila* oogenesis: New insights based on morphometric analysis and mechanical modeling. *Dev. Biol.* **331**, 129-139. doi:10.1016/j.ydbio.2009.04.028

Levine, M. (2010). Transcriptional enhancers in animal development and evolution. *Curr. Biol.* **20**, R754-R763. doi:10.1016/j.cub.2010.06.070

Levine, M. and Tjian, R. (2003). Transcription regulation and animal diversity. *Nature* **424**, 147-151. doi:10.1038/nature01763

Lynch, J. A. and Roth, S. (2011). The evolution of dorsal-ventral patterning mechanisms in insects. *Genes Dev.* **25**, 107-118. doi:10.1101/gad.2010711

Marmion, R. A. and Yakoby, N. (2018). In locus analysis of patterning evolution of the BMP type II receptor wishful thinking. *Development* **145**, dev161083. doi:10.1242/dev.161083

Marmion, R. A., Jevtic, M., Springhorn, A., Pyrowolakis, G. and Yakoby, N. (2013). The Drosophila BMPRII, wishful thinking, is required for eggshell patterning. *Dev. Biol.* **375**, 45-53. doi:10.1016/j.ydbio.2012.12.011

Montell, D. J., Rorth, P. and Spradling, A. C. (1992). slow border cells, a locus required for a developmentally regulated cell migration during oogenesis, encodes Drosophila CEBP. *Cell* **71**, 51-62. doi:10.1016/0092-8674(92)90265-E

Morimoto, A. M., Jordan, K. C., Tietze, K., Britton, J. S., O'Neill, E. M. and Ruohola-Baker, H. (1996). Pointed, an ETS domain transcription factor, negatively regulates the EGF receptor pathway in Drosophila oogenesis. *Development* **122**, 3745-3754.

Moussian, B. and Roth, S. (2005). Dorsoventral axis formation in the *Drosophila* embryo—shaping and transducing a morphogen gradient. *Curr. Biol.* **15**, R887-R899. doi:10.1016/j.cub.2005.10.026

Neuman-Silberberg, F. S. and Schüpbach, T. (1993). The *Drosophila* dorsoventral patterning gene gurken produces a dorsally localized RNA and encodes a TGFα-like protein. *Cell* **75**, 165-174. doi:10.1016/S0092-8674(05)80093-5

Neuman-Silberberg, F. S. and Schupbach, T. (1994). Dorsoventral axis formation in Drosophila depends on the correct dosage of the gene gurken. *Development* **120**, 2457-2463.

Niepielko, M. G., Marmion, R. A., Kim, K., Luor, D., Ray, C. and Yakoby, N. (2014). Chorion patterning: a window into gene regulation and Drosophila species' relatedness. *Mol. Biol. Evol.* **31**, 154-164. doi:10.1093/molbev/mst186

Nilson, L. A. and Schüpbach, T. (1998). 7 EGF receptor signaling in Drosophila oogenesis. *Curr. Top. Dev. Biol.* **44**, 203-243. doi:10.1016/S0070-2153(08)60471-8

O'Neill, E. M., Rebay, I., Tjian, R. and Rubin, G. M. (1994). The activities of two Ets-related transcription factors required for *Drosophila* eye development are modulated by the Ras/MAPK pathway. *Cell* **78**, 137-147. doi:10.1016/0092-8674(94)90580-0

Peri, F. and Roth, S. (2000). Combined activities of Gurken and decapentaplegic specify dorsal chorion structures of the Drosophila egg. *Development* **127**, 841-850.

Pyrowolakis, G., Veikkolainen, V., Yakoby, N. and Shvartsman, S. Y. (2017). Gene regulation during Drosophila eggshell patterning. *Proc. Natl. Acad. Sci. USA* **114**, 5808-5813. doi:10.1073/pnas.1610619114

Ray, R. P. and Schupbach, T. (1996). Intercellular signaling and the polarization of body axes during *Drosophila* oogenesis. *Genes Dev.* **10**, 1711-1723. doi:10.1101/gad.10.14.1711

Rebay, I., Chen, F., Hsiao, F., Kolodziej, P. A., Kuang, B. H., Laverty, T., Suh, C., Voas, M., Williams, A. and Rubin, G. M. (2000). A genetic screen for novel components of the Ras/Mitogen-activated protein kinase signaling pathway that interact with the yan gene of Drosophila identifies split ends, a new RNA recognition motif-containing protein. *Genetics* **154**, 695-712.

Revaitis, N. T., Marmion, R. A., Farhat, M., Ekiz, V., Wang, W. and Yakoby, N. (2017). Simple expression domains are regulated by discrete CRMs During *Drosophila* Oogenesis. *G3* **7**, 2705-2718. doi:10.1534/g3.117.043810

Revaitis, N. T., Niepielko, M. G., Marmion, R. A., Klein, E. A., Piccoli, B. and Yakoby, N. (2020). Quantitative analyses of EGFR localization and trafficking dynamics in the follicular epithelium. *Development* **147**:dev183210. doi:10.1242/dev.183210

Rogers, W. A., Goyal, Y., Yamaya, K., Shvartsman, S. Y. and Levine, M. S. (2017). Uncoupling neurogenic gene networks in the Drosophila embryo. *Genes Dev.* **31**, 634-638. doi:10.1101/gad.297150.117

Sapir, A., Schweitzer, R. and Shilo, B. Z. (1998). Sequential activation of the EGF receptor pathway during Drosophila oogenesis establishes the dorsoventral axis. *Development* **125**, 191-200.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B. et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676-682. doi:10.1038/nmeth.2019

Schober, M., Rebay, I. and Perrimon, N. (2005). Function of the ETS transcription factor Yan in border cell migration. *Development* **132**, 3493-3504. doi:10.1242/dev.01911

Scholz, H., Deatrick, J., Klaes, A. and Klämbt, C. (1993). Genetic dissection of pointed, a Drosophila gene encoding two ETS-related proteins. *Genetics* **135**, 455-468.

Schwarz, B., Hollfelder, D., Scharf, K., Hartmann, L. and Reim, I. (2018). Diversification of heart progenitor cells by EGF signaling and differential modulation of ETS protein activity. *eLife* **7**, e32847. doi:10.7554/eLife.32847

Timmons, A. K., Mondragon, A. A., Schenkel, C. E., Yalonetskaya, A., Taylor, J. D., Moynihan, K. E., Etchegaray, J. I., Meehan, T. L. and McCall, K. (2016). Phagocytosis genes nonautonomously promote developmental cell death in the Drosophila ovary. *Proc. Natl. Acad. Sci. USA* **113**, E1246-E1255. doi:10.1073/pnas.1522830113

Twombly, V., Blackman, R. K., Jin, H., Graff, J. M., Padgett, R. W. and Gelbart, W. M. (1996). The TGF-beta signaling pathway is essential for Drosophila oogenesis. *Development* **122**, 1555-1565.

Tzolovsky, G., Deng, W. M., Schlitt, T. and Bownes, M. (1999). The function of the broad-complex during Drosophila melanogaster oogenesis. *Genetics* **153**, 1371-1383.

Van Buskirk, C. and Schüpbach, T. (1999). Versatility in signalling: multiple responses to EGF receptor activation during *Drosophila* oogenesis. *Trends Cell Biol.* **9**, 1-4. doi:10.1016/S0962-8924(98)01413-5

Ward, E. J. and Berg, C. A. (2005). Juxtaposition between two cell types is necessary for dorsal appendage tube formation. *Mech. Dev.* **122**, 241-255. doi:10.1016/j.mod.2004.10.006

Wassarman, D. A., Therrien, M. and Rubin, G. M. (1995). The Ras signaling pathway in Drosophila. *Curr. Opin. Genet. Dev.* **5**, 44-50. doi:10.1016/S0959-437X(95)90052-7

Wei, G.-H., Badis, G., Berger, M. F., Kivioja, T., Palin, K., Enge, M., Bonke, M., Jolma, A., Varjosalo, M., Gehrke, A. R. et al. (2010). Genome-wide analysis of ETS-family DNA-binding in vitro and in vivo. *EMBO J.* **29**, 2147-2160. doi:10.1038/emboj.2010.106

Xi, R., McGregor, J. R. and Harrison, D. A. (2003). A gradient of JAK pathway activity patterns the anterior-posterior axis of the follicular epithelium. *Dev. Cell* **4**, 167-177. doi:10.1016/S1534-5807(02)00412-4

Xu, T. and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* **117**, 1223-1237.

Yakoby, N., Bristow, C. A., Gong, D., Schafer, X., Lembong, J., Zartman, J. J., Halfon, M. S., Schüpbach, T. and Shvartsman, S. Y. (2008a). A combinatorial code for pattern formation in Drosophila oogenesis. *Dev. Cell* **15**, 725-737. doi:10.1016/j.devcel.2008.09.008

Yakoby, N., Lembong, J., Schupbach, T. and Shvartsman, S. Y. (2008b). Drosophila eggshell is patterned by sequential action of feedforward and feedback loops. *Development* **135**, 343-351. doi:10.1242/dev.008920

Yamada, T., Okabe, M. and Hiromi, Y. (2003). EDL/MAE regulates EGF-mediated induction by antagonizing Ets transcription factor pointed. *Development* **130**, 4085-4096. doi:10.1242/dev.00624

Zartman, J. J., Kanodia, J. S., Cheung, L. S. and Shvartsman, S. Y. (2009). Feedback control of the EGFR signaling gradient: superposition of domain-splitting events in *Drosophila* oogenesis. *Development* **136**, 2903-2911. doi:10.1242/dev.039545

Zartman, J. J., Cheung, L. S., Niepielko, M. G., Bonini, C., Haley, B., Yakoby, N. and Shvartsman, S. Y. (2011). Pattern formation by a moving morphogen source. *Phys. Biol.* **8**, 045003. doi:10.1088/1478-3975/8/4/045003