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The Drosophila BMPRII, wishful thinking, is required for eggshell patterning

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ABSTRACT

The *Drosophila* eggshell is an elaborate structure that is derived from a monolayer of follicular epithelium surrounding the developing oocyte within the female ovary. The bone morphogenetic protein (BMP) signaling pathway is essential for controlling the patterning and morphogenesis of the eggshell. During oogenesis, the roles of patterning and morphogenesis by the BMP type I receptor *thickveins* (*tkv*) have been studied extensively. However, signaling through this pathway requires both type I and II receptors, and the latter has yet to be established in oogenesis. We focus on *wishful thinking* (*wit*), the *Drosophila* homolog to the mammalian BMP type II receptor, BMPRII. We found that *wit* is expressed dynamically in the FCs of *D. melanogaster* in an evolutionary conserved pattern. The expression patterns are highly correlated with the dynamics of the BMP signaling, which is consistent with our finding that *wit* is a target of BMP signaling. Furthermore, we established that WIT is necessary for BMP signaling, and loss of WIT is associated with cell autonomous loss of BMP responses. Of importance, we demonstrated that perturbations in WIT led to changes in eggshell morphologies in domains that are patterned by BMP signaling. Previous studies have shown a role for WIT in BMP signaling during neurogenesis; however, our results reveal a role for WIT in epithelial cells' development.

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Introduction

In *Drosophila melanogaster*, the eggshell is derived from the follicle cells (FCs), a two-dimensional layer of epithelial tissue surrounding the developing oocyte (Hinton and Service, 1969; Horne-Badovinac and Bilder, 2005; Spradling, 1993). Two main signaling pathways are responsible for eggshell patterning, the epidermal growth factor receptor (EGFR) and the bone morphogenetic protein (BMP) (Berg, 2005; Deng and Bownes, 1997; Dobens and Raftery, 2000; Neuman-Silberberg and Schupbach, 1993, 1994; Peri and Roth, 2000; Queenan et al., 1997b; Twombly et al., 1996). Structures of the eggshell, such as the respiratory dorsal appendages (DAs) and the operculum, are sensitive to changes in the levels of BMP signaling (Berg, 2008; James and Berg, 2003; Ward and Berg, 2005; Ward et al., 2006). Thus, the eggshell is an excellent system to study how pathway components contribute to the levels of signaling (Dequier et al., 2001; Dobens and Raftery, 2000; Peri and Roth, 2000; Shravage et al., 2007; Twombly et al., 1996; Yakoby et al., 2008a).

In the FCs, BMP signaling is dynamic. Early signaling is restricted to a band of anterior FCs, reflecting the anteriorly emanating BMP-2/4-like ligand Decapentaplegic (DPP) that signals through a uniformly expressed type I BMP receptor Thickveins (Tkv) (Deng and Bownes, 1997; Dobens et al., 1997; Jekely and Rorth, 2003; Mantrova et al., 1999; Peri and Roth, 2000; Twombly et al., 1996). Later, the DPP ligand signals in two dorsolateral patches on either side of the dorsal midline, which reflects the late pattern of *tkv* (Lembong et al., 2009; Mantrova et al., 1999; Yakoby et al., 2008b). The role of *tkv* in guiding the levels and patterns of BMP signaling has been extensively studied in numerous tissues (Crickmore and Mann, 2006; Lecuit and Cohen, 1998; Lembong et al., 2009; Niepielko et al., 2011, 2012; Shravage et al., 2007; Vuilleumier et al., 2010; Xia et al., 2010; Yakoby et al., 2008b). However, BMP signals by binding to a complex of type I and type II BMP receptors (Affolter and Basler, 2007; Araujo et al., 2011; Baker and Harland, 1997; Massague et al., 2000; Parker et al., 2004; Wu and Hill, 2009).

While *wit* is expressed in numerous tissues during fly development (Marques et al., 2002), it is surprising that so far the function of WIT has been limited to neurons. In this study, we report that the type II BMP receptor *wishful thinking* (*wit*) is expressed in a pattern that follows the dynamics of BMP signaling

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in the FCs. We found that WIT is regulated by BMP signaling cell autonomously. Furthermore, WIT is required for BMP signaling, and the disruption of WIT is associated with patterning defects and morphological deformities of the eggshell. Collectively, our data suggests that during oogenesis WIT acts as a type II receptor to regulate FC patterning and morphogenesis of the eggshell.

Materials and methods

Fly stocks

The following stocks were used in this study: wild-type *D. melanogaster* (OreR), *D. simulans* and *D. sechellia* (a gift from D. Stern), E4-Gal4 and CY2-Gal4 (Queenan et al., 1997a), UAS-*dpp* (a gift from T. Schüpbach), e22c-Gal4 (Bloomington Stock Center), and UAS-*dad* and DadZ (Tsuneizumi et al., 1997). The FLP/FRT mitotic recombination system (Duffy et al., 1998; Xu and Rubin, 1993) was used to generate clones of mutant follicle cells, marked by the absence of GFP. Analysis of BMP input was conducted with the Mad^{12} allele: FRT^{40A} Mad^{12}/FRT^{40A} ubiGFP;GR1-Gal4 UAS-FLP (a gift from R. Padgett). The role of WIT in BMP signaling was conducted with the Wit^{G15} allele: FRT^{79} Wit^{G15}/FRT^{79} ubiGFP;e22c-Gal4 UAS-FLP (a gift from M. O'Connor). Depletion of *wit* was conducted with an RNAi stock from the TRiP RNAi collection at Harvard, JF01969, and included a UAS-*dicer2*. Flies were grown on cornmeal agar; all crosses were completed at 22 °C.

Wit reporters

Genomic fragments from the *wit* locus were amplified by PCR and cloned into a pCR8/GW/TOPO entry vector by TOPO-cloning and subsequently into pattBGWhZn by Gateway cloning reaction (Invitrogen). The vector pattBGWhZn is a derivative of pUASTattB (Bischof et al., 2007) and contains an *attR1-ccdB-CmR-attR2* cassette followed by a *hsp*70 minimal promoter and a *lacZ* reporter encoding a nuclear version of ß-Galactosidase. All reporter constructs were inserted into the chromosomal position 68A4 of the attP2-line by PhiC31/attB-mediated integration (Groth et al., 2004).

In situ and immunofluorescence hybridizations, microscopy

The expression pattern of *wit* in the FCs was previously described (Yakoby et al., 2008a). The *wit* gene was cloned from a cDNA library generated with the Stratagene cDNA Synthesis Kit. Forward primer: CAAGTATCCCGCACCACTTT. Reverse primer: CCATCATSCGATCRTCGT. *In situ* hybridization was performed as previously described (Wang et al., 2006), but without the RNase digestion step (Yakoby et al., 2008a). Dissection and fixation was conducted as reported elsewhere (Pacquelet and Rorth, 2005). Primary antibodies: mouse anti-Wit (23C7; 1:500, DSHB), rabbit anti-phosphorylated-Smad1/5/8 (1:3000, a generous gift from D. Vasiliauskas, S. Morton, T. Jessell and E. Laufer), mouse anti-betagalactosidase (1:1000, Promega), rabbit anti-betagalactosidase (1:1000, Invitrogen) and sheep anti-GFP (1:2000, Biogenesis). Secondary antibodies: Alexa Fluor (1:1000, Molecular Probes), and DAPI (1:10000). Images were captured with a Leica DM3000 Compound Microscope or a Leica SP5 Confocal microscope (Leica). Scanning electron microscopy was conducted with a Leo 1450EP SEM. Images were processed with ImageJ (Rasband, 1997–2009) and Gimp (GNU Image Manipulation Program, 1995–2008). To evaluate the levels of BR reduction in cells null for *wit*, a line plot of Broad was traced using ImageJ across several wild type cells as well as clones null for *wit*. The average gray value was taken for wild type cells as well as the clones in the same image. Percent reduction for each egg chamber was calculated as 1 − (clone/WT).

Results

The dynamic pattern of wit/WIT is regulated by BMP signaling

Previously, we reported that *wishful thinking* (*wit*) is patterned in the FCs of *D. melanogaster* (Yakoby et al., 2008a). Initially,

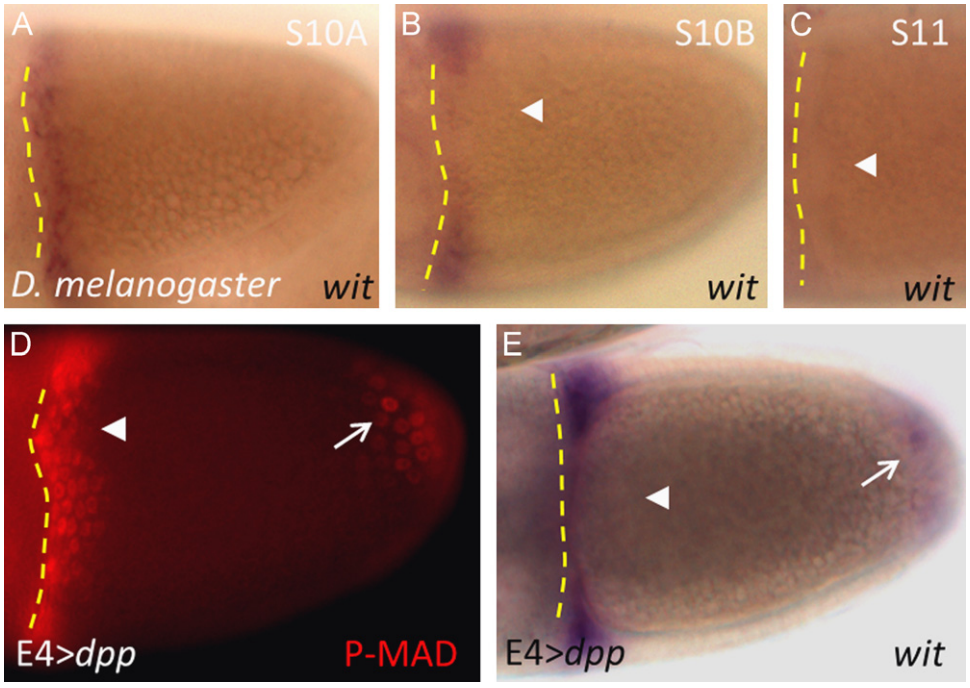

Fig. 1. The expression of *wit* is dynamic. (A–C) The *wit* transcript is initially expressed in an anterior band of cells at stage 10A (S10A), then clears from the dorsal side (S10B), and is gone by S11. Yellow broken line denotes the anterior domain of the oocyte associated FCs. Arrowhead denotes the dorsal side. (D) Ectopic expression of DPP in the posterior end of the FCs (arrow) induces BMP signaling, detected by P-MAD (red). (E) *wit* is expressed in the posterior FCs of this genetic background.