Development 135, 839-847 (2008) doi:10.1242/dev.017590

Drosophila Pxt: a cyclooxygenase-like facilitator of follicle maturation

Tina L. Tootle and Allan C. Spradling*

Prostaglandins are local transient hormones that mediate a wide variety of biological events, including reproduction. The study of prostaglandin biology in a genetically tractable invertebrate model organism has been limited by the lack of clearly identified prostaglandin-mediated biological processes and prostaglandin metabolic genes, particularly analogs of cyclooxygenase (COX), the rate-limiting step in vertebrate prostaglandin synthesis. Here, we present pharmacological data that *Drosophila* ovarian follicle maturation requires COX-like activity and genetic evidence that this activity is supplied in vivo by the *Drosophila* peroxidase Pxt. *pxt* mutant females are sterile, and maturing follicles show defects in actin filament formation, nurse cell membrane stability and border cell migration. Maturation of *pxt* follicles in vitro is stimulated by prostaglandin treatment and fertility is restored in vivo to *pxt* mutants by expressing mammalian Cox1 protein. Our experiments suggest that prostaglandins promote *Drosophila* follicle maturation, in part by modulating the actin cytoskeleton, and establish *Drosophila* oogenesis as a model for understanding these critical biological regulators.

KEY WORDS: Prostaglandin, Cyclooxygenase, Actin, Ovarian follicle, Oogenesis

INTRODUCTION

Prostaglandins (PGs) are locally synthesized and locally acting hormones that mediate a wide variety of biological activities, ranging from inflammation and pain to reproduction and cancer (Funk, 2001). Cells generate PGs from arachidonic acid via the action of cyclooxygenase (COX) enzymes, which, by two distinct catalytic activities, cyclooxygenation and peroxidation, generate the PG intermediate, PGH_2. The two subclasses of vertebrate COX enzymes, Cox1 and Cox2, differ in their structure, susceptibility to inhibitors and biological function. Specific PG synthases act on PGH_2 to synthesize additional PGs, including PGD_2, PGE_2 and PGF_{2a}. PGs are ligands for G-protein-coupled receptors (GPCRs) and possibly for nuclear hormone receptors, thereby regulating critical signaling cascades (Bos et al., 2004; Gilmour and Mitchell, 2001). However, the specific downstream events mediated by PG signaling remain unclear. Many pharmaceuticals have been designed to block COX enzyme catalytic activity and, therefore, PG synthesis (Funk, 2001; Taketo, 1998).

In a variety of organisms, prostaglandins regulate female reproduction, including follicle maturation, ovulation, fertilization, maintenance of pregnancy, and induction of labor (Langenbach et al., 1999; Loftin et al., 2002). Cox2 knockout mice are defective in all aspects of female reproduction (Lim et al., 1997), while loss of Cox1 blocks labor. PGs and PG synthetic activity has also been found in many invertebrates (Stanley, 2006; Stanley-Samuelson and Pedibhotla, 1996), including *Drosophila* (Pages et al., 1986). PG levels in these animals frequently rise upon mating, while exogenous PGs stimulate both egg development and laying (Stanley, 2006; Stanley-Samuelson and Pedibhotla, 1996). However, the molecular mechanisms of PG signaling during oogenesis have not been established.

Howard Hughes Medical Institute, Carnegie Institution, Department of Embryology, 3520 San Martin Drive, Baltimore, MD 21218, USA.

*Author for correspondence (e-mail: spradling@ciwemb.edu)

Accepted 17 December 2007

The *Drosophila* ovary is composed of ovarioles containing sequentially maturing follicles termed egg chambers (Spradling, 1993). New follicles are continuously produced from stem cells in the anterior region or germarium. Groups of 16 interconnected sibling germline cells differentiate into 15 nurse cells and an oocyte before being surrounded by epithelial cells to form follicles. Subsequently, follicles mature through 14 morphological stages (S1-14), during which they increase greatly in size (S1-10), take up yolk proteins from the hemolymph (S8-10), and dump the nurse cell contents into the oocyte (S11). Follicles subsequently undergo a complex process of maturation that involves the programmed migration of specific subpopulations of follicle cells, including the border cells, centripetal cells and dorsal appendage cells that secrete and shape distinct parts of the protective eggshell.

We have used pharmacology and genetics to explore the roles of PGs during *Drosophila* oogenesis. By using an in vitro egg maturation assay, in which S10B egg chambers mature to S14s in culture, we find that *Drosophila* egg maturation requires a Cox1-like activity. Genetic studies of the COX-like peroxidase Pxt indicate that it functions upstream of PGs to regulate actin during nurse cell dumping. These studies provide the first evidence that PGs mediate *Drosophila* oogenesis, making this a valuable system with which to elucidate the molecular mechanisms by which prostaglandin signaling acts during reproduction.

MATERIALS AND METHODS

Fly strains

Fly stocks were maintained at 20-25°C on standard cornmeal-agar-yeast food. Wet yeast, dry active yeast reconstituted to a thin paste with water, was provided for all ovary analyses including IVEM, immunofluorescence and in situ hybridization. y^1 w^1 was used as the control in all experiments. EY03052 (Bellen et al., 2004) was obtained from the Bloomington Stock center and f01000 (Thibault et al., 2004) was obtained from the Harvard Exelixis collection.

In vitro egg maturation (IVEM)

S10B follicles were hand dissected in room temperature freshly made IVEM media composed of Grace's medium (BioWhittaker, Cambrex) with heat-inactivated 10% fetal bovine serum (Invitrogen) and 1× penicillin/streptomycin glutamine (100×, Gibco). Dissected S10Bs were transferred

to fresh IVEM media while dissection continued and were then transferred into 24-well tissue culture plates with 1 ml of IVEM media plus COX inhibitors and PGs as indicated and allowed to develop. For each experiment, controls were treated with the same volume of ethanol and/or DMSO used for the experimental conditions. S10Bs were allowed to develop for 10 or more hours and then stages were scored. Those chambers not undergoing nurse cell dumping (s10-11) were scored as stalled, while those mostly through completely dumped were scored as developed. Each experiment was performed at least in duplicate and standard deviations are shown, except that standard error is presented for experiments with three or more replicates. COX inhibitors were obtained from Cayman Chemical, except indomethacin (Sigma). U44069, the stabilized form of PGH_2, Fluprostenol and PGF_{2a} were from Cayman Chemical. All other prostaglandins were from Sigma. Live time-lapse movies of S10Bs were generated using a Zeiss Stereolumar, images were taken every 10 minutes for 10 hours.

Fertility counts

Fertility was determined by mating a known number of females with y^1w^1 males, in the case of the *pxt* alleles, and heterozygous sibling males for the rescue experiments. The flies were allowed to mate for 3-4 days and then sequentially transferred to new vials over a period of 2-3 weeks. All transfers were kept and the progeny were counted 20 days after mating.

Transgenics

Full-length *pxt* cDNA, LD43174, and mouse Cox1 cDNA, B0811H12, were cloned into the Gateway entry vector (Invitrogen), and swapped into a pUASp vector (a kind gift from Terence Murphy), thereby placing the cDNA expression under the control of the yeast upstream activating sequence (UAS) modified to enhance germline expression. Mouse Cox1 was also cloned by the same means without the starting ATG and placed into the pUASp Venus vector (kind gift from Terence Murphy), generating an N-terminal Venus fusion. P-element transformation was performed by Genetic Services (Cambridge, MA). Multiple insertion lines were generated for each element. Chromosomal locations of the P-elements were determined by standard crosses. Insertions on the 2nd chromosome were crossed to pxt^{f01000} to obtain a stock of UASp/balancer;f01000/balancer. These flies were crossed to hsGAL4 or c587;Sco/CyO;f01000/balancer to generate GAL4;UASp;f01000/f01000. hsGAL4 flies were incubated at 37°C (air) for 1 hour, on three consecutive days. A known number of females were then allowed to lay eggs in a new vial over the next 4 days. Fertility/female was assessed by counting the number of adults eclosing by day 19.

RT-PCR

Total RNA was isolated from fattened ovaries (15-20 pairs of ovaries) or whole flies (15-20 females) using either TriZOL reagent (Invitrogen) or Qiagen RNeasy kit following the manufacturers' directions. RNA was treated overnight at room temperature with 2U RNase-free DNase (Ambion). Concentration was quantified using UV spectrometry. RNA (1 μg) was used to generate cDNA via the Reverse Transcription System (Promega) using random primers and following manufacturer's directions. PCR was then performed using 2 μl or 1/10th of the RT reaction with primers to *pxt* and *rps17*, as the control. The following *pxt* primers were used: to generate a ~750 bp fragment starting from the ATG, 5′pxt rt ATGAGTCGCATTTTATTT and 3′ pxt rt TGTGGTTCTGGATTATTG; to generate a ~550 bp fragment, 5′ pxt 901 GATCGTCCTCATCCTAAGT and 3′ pxt 1390/1450 3′ TGGTTTGTTCGGCCATCT; and to generate the control ~250 bp fragment of *rps17*, 5′ rps17 CGAACCAAGACGGTGAAGAAG and 3′ rps17 CCTGCAACTTGATGGAGATACC. An MJ Research PTC-100 Programmable Thermal Controller was used to amplify 50 μl reactions with GoTaq Flexi DNA polymerase and 2.5 mM $MgCl_2$ with the following program: 2 minutes at 95°C, 30 cycles of 30 seconds at 95°C, 30 seconds at 42°C, 1 minute at 72°C, followed by 5 minutes at 72°C. Products were resolved on 0.8% agarose LE gels (Roche) in 1×TAE buffer with 0.25 μg/μl ethidium bromide and imaged with BioRad Gel Doc XR Scanner and Quantity One software (V4.5.2). Experiments were carried out at least in triplicate and representative data are shown. Real time quantitative RT-PCR reactions were performed on ovary RT reactions on an Opticon Monitor 2 (MJ Research) using a 50 μl reaction comprising 2 μl RT and 0.5 μl of a 7.5× SYBR Green stock (Molecular Probes). The program used was 2 minutes at 95°C, 35 cycles of 30 seconds at 95°C, 30 seconds at 42°C, 1 minute at 72°C, followed by 5 minutes at 72°, and a melting curve of each sample was determined. Results were analyzed using the Opticon Monitor software. Transcripts were expressed relative to the level of the control *rps17* gene and normalized to the control ovaries. Each reaction was carried out in triplicate on at least two separate RNA samples.

In situ hybridization

Whole-mount in situ hybridization was performed as previously described (Lecuyer et al., 2008) using an antisense RNA probe to full-length *pxt* cDNA generated by in vitro T7 transcription from *Hin*dIII-digested LD43174.

Immunofluorescence

Whole-mount samples were fixed with 4% paraformaldehyde for 15 minutes and processed using standard procedures (Cox and Spradling, 2003). The following antisera from the Developmental Studies Hybridoma Bank were used: mouse anti-Hts (1B1, 1:20) and mouse anti-Fas3 (7G10, 1:50). Goat anti-mouse conjugated to Alexa 488 (Molecular Probes) was used at 1:2000. Rhodamine phalloidin (1:200; Invitrogen) was added to both the primary and secondary antibody incubations. To visualize DNA, DAPI (1 μg/ ml) was added to the final wash before mounting the samples in Vectashield (Vector Labs).

Microscopy

DIC imaging was performed on a Zeiss Axiophot microscope with a Qimaging RETIGA 1300 camera and QCapture software. Time-lapse microscopy was performed on Zeiss Stereolumar microscope and acquired with AxioVision software. Static confocal images were taken with either a 20× (NA 0.7) or a 63× (NA 1.32) PlanApo lens on laser-scanning confocal microscope (SP2 or SP5; Leica). All confocal images are projected *z*-stacks.

RESULTS

Cox1-like activity is required in late oogenesis

Drosophila S10B egg chambers can reliably complete development in vitro (Fig. 1A-C), termed in vitro egg maturation (IVEM, Fig. 1D, Movie 1), a property they share with mammalian follicles (Sirois et al., 1992). We took advantage of this capability to investigate whether a *Drosophila* COX-like protein functions during late oogenesis by asking whether COX inhibitors interfere with in vitro follicle maturation. The effect of each inhibitor on follicle development was tested at multiple concentrations and the IC_{50}, the concentration at which half of the egg chambers do not mature in vitro, was determined (Table 1 and supplementary material Fig. S1). We define not maturing or 'stalled' as incomplete nurse cell dumping and breakdown, morphologically resembling stages 10-11. Nurse cell dumping is a prerequisite for normal maturation, and, when disrupted, it leads to smaller oocytes and abnormal dorsal appendages. Several Cox1-specific inhibitors block egg chamber maturation in a dose-dependent manner (Table 1 and Fig. 1D-I), but most Cox2 inhibitors have little or no affect on maturation (Table 1). NS-398, a preferential Cox2 inhibitor, is an exception, as it blocks maturation but at a higher concentration known to inhibit Cox1 function (Table 1, Fig. 1J). The IC_{50} concentrations we observed are slightly higher than what is seen in cell culture experiments, most probably owing to lower drug penetration into a multicellular tissue. Thus, pharmacological inhibition of Cox1 activity disrupts egg maturation (Fig. 1I,J; Movie 2).

Exogenous PGs restore egg chamber development

If the COX inhibitor-induced block in egg maturation is due to the loss of PG synthesis then addition of exogenous PGs should restore development. A complicating factor is that PGs are locally acting, anionic lipids and therefore do not survive long in an aqueous environment. To circumvent this problem, stabilized analogs of PGs

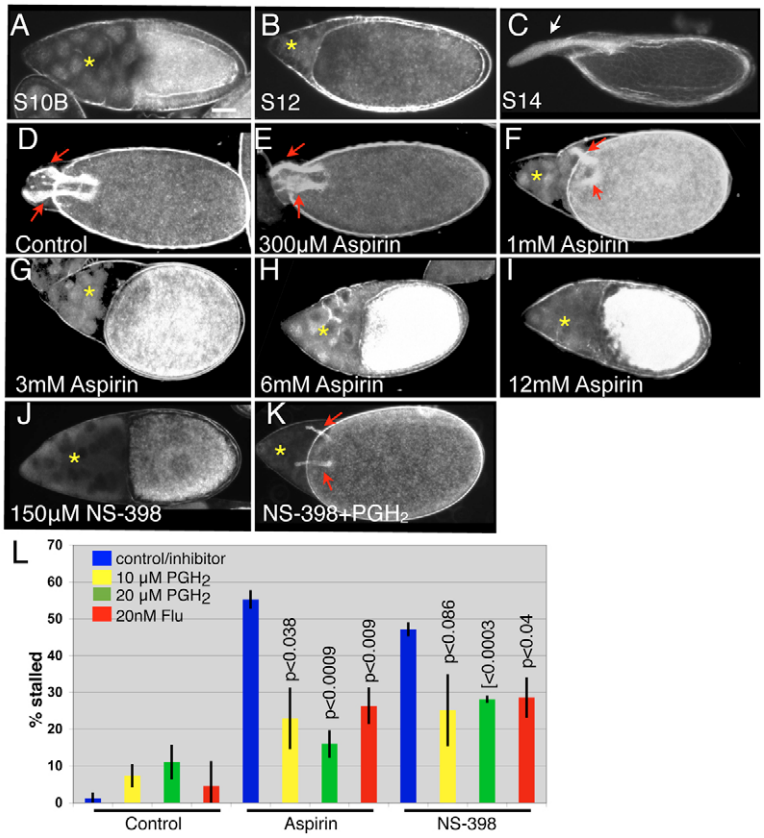

Fig. 1. Stage 10B egg chamber maturation in vitro is influenced by Cox1 inhibitors and exogenous PGs. (**A**) Normal egg chamber at stage 10B, (**B**) stage 12 or (**C**) stage 14. (**D-K**) Extent of development characteristic of stage 10B egg chambers matured in vitro and treated as indicated. (D) Control, develops to stage 14 with only slight reduction in dorsal appendages; (E-I) aspirin blocks maturation in a dose-dependent manner. (J) 150 μM NS-398 blocks in vitro development; (K) partial rescue of developmental block induced by 150 μM NS-398 by PGH_2 at 20 μM. Anterior is towards the left in all images. Asterisks indicate nurse cell region; arrows indicate dorsal appendages. (**L**) Effects of Cox1 inhibitors and exogenous PGs on egg chamber development in vitro. Scale bar: 50 μm. 'Stalled' was defined as incomplete nurse cell breakdown in these experiments. *t*-tests were performed comparing drug treated (blue bar) to drug plus prostaglandin (yellow, green or red). Flu, fluprostenol.

were tested. Addition of a stabilized form of the PG intermediate, PGH_2, which in mammals can serve as a substrate for endogenous PG synthases, partially rescues the aspirin- or NS-398-mediated block to egg maturation (Fig. 1K,L). The incomplete development of PGH_2-rescued egg chambers may be due to the uniform application of the PG, as endogenous PGs are likely to be produced in a spatially and temporally restricted manner.

PGH_2 can be converted by specific PG synthases into the individual classes of PGs that activate distinct signaling cascades. To determine which specific PG(s) mediate egg chamber development we examined the exogenous effects of the individual PGs. Most exogenous PGs, except PGH_2 (the precursor PG) and $PGF_{2\alpha}$, inhibit egg chamber development after stage 12 (see Fig. S2 in the supplementary material). This suggests that during S10-14 egg chamber development PGH_2 is processed into $PGF_{2\alpha}$, and not the other PGs, to facilitate maturation. Consequently, we tested whether exogenous $PGF_{2\alpha}$ can rescue COX inhibition. Both $PGF_{2\alpha}$ (not shown) and its stabilized analog Fluprostenol (Flu) significantly rescue the aspirin or NS-398-induced block in maturation (Fig. 1L). We conclude that COX inhibitors block cultured *Drosophila* egg chamber development at expected concentrations and this inhibition is significantly overcome by supplying exogenous PGs. These observations strongly suggest that a COX-like enzyme and PGs play a physiological role during late follicle development in *Drosophila*.

Identifying a COX-like enzyme in *Drosophila*

We used BLAST analysis to identify the *pxt* gene (AY119616) as a candidate *Drosophila* COX. Mammalian COX enzymes are thought to have evolved from heme-dependent peroxidases such as myeloperoxidase by acquiring a cyclooxygenase activity center (Garavito and Mulichak, 2003; Picot et al., 1994). *Drosophila* Pxt is such a heme peroxidase, with the conserved catalytic residues needed for heme coordination. Within the 362 amino acids of the homology region, Pxt is 38% similar and 24% identical to vertebrate Cox1 enzymes, the closest such match among *Drosophila* proteins. Like mammalian COX enzymes (Otto et al., 1993), Pxt is predicted to be membrane bound, as the first 18 amino acids encode a signal peptide, and to be glycosylated (PROSITE analysis) (Hulo et al., 2006).

Mutations of *pxt* have been recovered in large insertional mutagenesis screens that allow the function of this gene to be probed. We studied two such alleles: a P-element insertion EY03052 and a Piggybac insertion f01000 (Fig. 2A). The $pxt^{EY03052}$ insertion is located 17 bp upstream of the predicted 5′ UTR, while pxt^{f01000} contains an insertion within the 5′ UTR, 38 bp upstream of the starting methionine. Reverse transcriptase-PCR (RT-PCR and qRT-PCR) indicates that both insertions substantially reduce the level of *pxt* RNA in the ovary (Fig. 2B) and whole fly (data not shown). However, whole-mount in situ hybridization (see Fig. S3 in the supplementary material) indicates that $pxt^{EY03052}$ exhibits some mislocalized and misregulated *pxt* expression, while pxt^{f01000} exhibits almost a complete loss of expression.

Like genetic loss of mammalian COX enzymes (Lim et al., 1997; Stanley, 2006; Stanley-Samuelson and Pedibhotla, 1996), loss of *pxt* affects female fertility. Homozygous pxt^{f01000} females are sterile, while the fertility of $pxt^{EY03052}$ females drops with age more rapidly than normal (Fig. 2C). *pxt* mutant follicles frequently show defects in nurse cell dumping (Fig. 2D) that resemble wild-type chambers developed in vitro in the presence of COX inhibitors (Fig. 1F). In 4-day-old pxt^{f01000}females, 93% of S14 follicles are short in length and failed to undergo complete nurse cell dumping (n=131). $pxt^{EY03052}$ and $pxt^{EY03052}/pxt^{f01000}$ mutant follicles display these same phenotypes but with lower penetrance and less severity (data not

Table 1. Cox1 inhibitors block egg chamber development

	Drug	Known Cox1 IC_{50}	Known Cox2 IC_{50}	Egg maturation IC_{50}
Cox1 inhibitors	Aspirin	0.75 mM	1.25 mM	1.5 mM
	Indomethacin	0.1 μM	6 μM	11 μM
	Valeryl Salicylate	0.8 mM	15 mM	1.2 mM
	Resveratol	Cyclo 15 μM, Per 3.7 μM	NA	256 μM
	SC-560	9 nM	6.3 μM	>1 M
Cox2 inhibitors	APHS	17 μM	0.8 μM	>1 M
	NS-398	75 μM	0.15-1.77 μM	164 μM
	SC-58125	>10.5 μM	70 nM	232 μM
	Valdecoxib	26.1 μM	0.87 μM	>1 M
	CAY10404	>500 μM	<1nM	>1 M

Egg maturation IC_{50} concentrations were calculated by performing a dose curve and using the equation from the resulting line (see Fig. S1 in the supplementary material). Inhibitors with little to no effect on egg maturation are indicated as having an IC_{50} greater than 1 M. Known IC_{50} values come from the data supplied by Cayman Chemical, from where the reagents were purchased.
Cyclo, cyclooxygenase; Per, peroxidase.

shown), in agreement with the molecular data suggesting that $pxt^{EY03052}$ is a weaker allele. Thus, both genetic loss of *pxt* and COX inhibition cause a block in nurse cell dumping.

pxt is dynamically expressed during oogenesis

Whole-mount in situ hybridization was used to determine the spatial and temporal patterns of *pxt* expression, and therefore the likely sites of PG synthesis, during oogenesis. *pxt* RNA is present at low levels in the germarium and early follicles (Fig. 2E). Expression begins to increase in S6 in both somatic and germline cells, becomes progressively stronger during vitellogenesis (Fig. 2E-G, see Fig. S3A in the supplementary material), and is highly expressed in germ cells (Fig. 2F,G) and somatic cells (outlined in Fig. 2G) from S10 throughout the remainder of oogenesis (Fig. 2H,I). A subset of follicle cells, termed border cells (BC), migrates from the anterior of the egg chamber to the nurse cell/oocyte boundary during S9 and exhibit a high level of *pxt* expression (Fig. 2F,F′, arrow). The high levels of *pxt* expression in late-stage follicles are consistent with Pxt being the enzyme susceptible to inhibition by mammalian Cox1 inhibitors during in vitro follicle development.

pxt mutants are rescued by *pxt* cDNA

To verify that the sterility of pxt^{f01000} females and their egg chamber defects are caused by disruption of *pxt*, we performed rescue experiments. Expression of a full-length *pxt* cDNA under the control of the UASp promoter (which allows expression in the germline) using heat-shock GAL4 (HS-GAL4) beginning in adulthood, restored fertility at an average level of 18 progeny/female over the 4 days following heat shock treatment (Fig. 3A). For comparison, *pxt* heterozygous females produced 26 progeny/female in a similar period, while unrescued mutants produced none. Such rescue proves that the pxt^{f01000} phenotype is due to the loss of *pxt* function, and that the RB isoform alone can provide substantial gene function without being developmentally regulated. When *pxt* cDNA was expressed using the c587 somatic cell driver, fertility was also restored but to a lesser extent, 5.4 progeny per female in the same interval. Thus, somatic expression of *pxt* is sufficient to rescue fertility but expression in both germ cells and somatic cells driven by HS-GAL4 is more efficacious. Fertility is also restored by precise excisions of either element (data not shown).

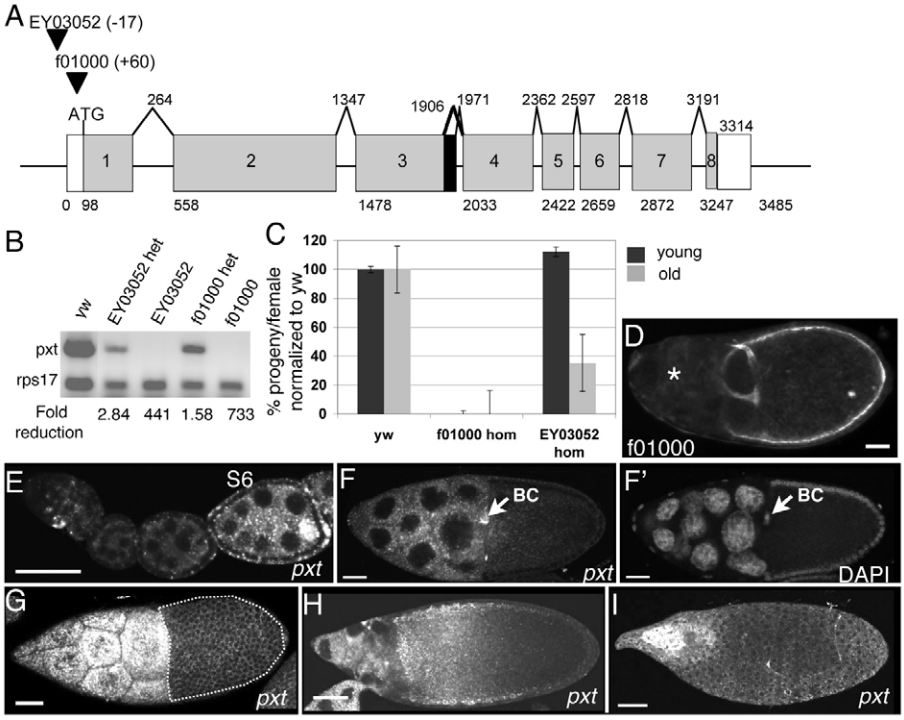

Fig. 2. *pxt* is expressed throughout oogenesis and required for egg maturation. (**A**) Schematic of the *pxt* gene, showing the 5′ and 3′UTR as white boxes, the exons as gray boxes, the alternative splice exon as a black box, the introns as lines and the insertions as black triangles. (**B**) RT-PCR and qRT-PCR (numbers below) showing *pxt* expression in whole ovaries from the indicated genotypes. (**C**) Female fertility of *pxt* genotypes relative to wild type 2-5 days (dark gray) or >15 days (light gray) after eclosion. (**D**) pxt^{f01000} egg chamber showing a nurse cell dumping defect (asterisk marks nurse cells). (**E-I**) Whole-mount in situ hybridization using a *pxt* probe. (E) *pxt* is expressed weakly early in oogenesis, with an upregulation of expression beginning at S6. (F,G) *pxt* is expressed in both the germ and somatic cells, including border cell expression (F,F′, arrows) and mainbody follicle cells (outlined in G). (H,I) Expression remains strong throughout the rest of oogenesis. The probe concentration is higher in E than in F-I. F′ shows DAPI. Scale bar: 50 μm.

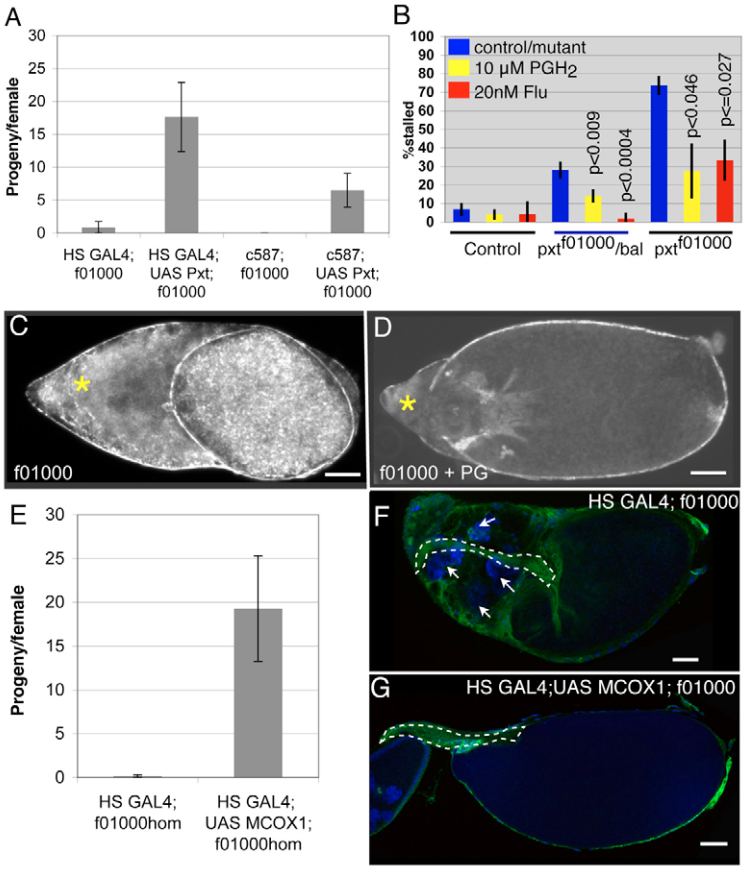

Fig. 3. *pxt* mutants are rescued by *pxt* cDNA, exogenous PGs and mouse Cox1. (**A**) Fertility of pxt^{f01000} mutant females (over a 4-day period) is rescued by expression of a UASp-pxt^{+} transgene driven by heat shock-GAL4 (HS GAL4) or the somatic ovarian driver c587. (**B**) Rescue of pxt^{f01000} developmental arrest in vitro by added PGH_2 (10 μM) or fluprostenol (Flu) (20 nM). *t*-tests were performed comparing drug treated (blue) with drug plus prostaglandin (yellow or red). DIC image of a pxt^{f01000} stage 10B egg chamber developed in vitro without PG (**C**) showing residual nurse cells (*) or with added 60 μM PGH_2 showing rescue (**D**). (**E**) Fertility of pxt^{f01000} mutant females (over 4 days) is rescued by expression of a UASp-MCOX1 transgene driven by heat shock-GAL4 (HS GAL4). (**F**,**G**) MCOX1 expression (G) rescues nurse cell dumping (arrows indicate nurse cell nuclei) and dorsal appendage (outlined) formation. Projections of confocal sections of S14s from HS GAL4; Sco/CyO; f01000 (F) and HS GAL4; UAS MCOX1/balancer; f01000 (G). Green is 1B1 (marking membrane) and blue is DAPI. Scale bar: 50 μm.

PGs act downstream of Pxt

If Pxt acts as a Cox1 enzyme, then the effects of pxt^{f01000} mutation on follicle development might largely be due to reduced PG production. To test this possibility, we first examined the ability of *pxt* mutant S10B follicles to develop in vitro. Approximately 75% of pxt^{f01000} stage 10B follicles fail to mature in culture, compared with ~7% of wild-type controls (Fig. 3B,C). (This differs slightly from our previous measurement of 93% with arrested dumping in vivo because only completely normal-looking S10B follicles were selected for in vitro development.) Addition of either 10 μM PGH_2 or 10 nM Fluprostenol (Flu), the stabilized analog of $PGF_{2\alpha}$, greatly improves development (Fig. 3B). In the presence of these PGs, ~70% of the follicles undergo nurse cell dumping (Fig. 3D). Therefore, Pxt functions in a process required for egg chamber maturation that can be rescued by exogenous PGs, strongly supporting the idea that Pxt acts as a *Drosophila* COX enzyme to synthesize PGs.

Mouse Cox1 can substitute for Pxt

If Pxt functions as a Cox1 enzyme to generate PGs, then it might be possible to rescue *pxt* function using a mammalian COX protein. Consequently, we also generated transgenic lines expressing mouse Cox1 full-length cDNA (MCOX1, NM008969) under the control of the UASp promoter. Inducing MCOX1 expression using heat-shock GAL4 in adult pxt^{f01000} females restores fertility to a level of 19 progeny/female (Fig. 3E), which is as effective as rescue with *Drosophila* Pxt under the same conditions (Fig. 3A). The S14 defects observed in *pxt* mutants are suppressed by MCOX1 expression, as nurse cell dumping is completed (Fig. 3G, compare with 3F). Thus, Pxt is homologous to and can be functionally replaced by mouse Cox1; *pxt* mutants and COX inhibitors block egg maturation, and development can be rescued by exogenous PGs. These observations strongly argue that *Drosophila* Pxt functions as a *Drosophila* COX during follicle maturation and acts by generating PGs.

Actin fiber formation is decreased in *pxt* mutants

We examined the actin cytoskeleton of S10B pxt egg chambers to investigate how loss of *pxt* and PG production interferes with nurse cell dumping. At the onset of nurse cell dumping, the regulated formation of non-contractile actin bundles in S10 nurse cells tethers the nurse cell nuclei and prevents them from plugging up the oocyte ring canals (Robinson and Cooley, 1997). Moreover, non-muscle myosin II-mediated contraction of nurse cell subcortical actin provides the contractile force behind dumping and egg chamber elongation (Wheatley et al., 1995; Edwards and Kiehart, 1996). pxt^{f01000} mutant follicles contained significantly fewer actin bundles in the nurse cell cytoplasm during S10 compared with wild type (Fig. 4B,B′, compare with 4A,A′). We did not observe nurse cell nuclei in the ring canals of mutant follicles, suggesting that *pxt* mutation disrupts the actin-based contraction that generates the force for dumping. In support of this, subcortical actin levels are strongly decreased in the mutant follicles (Fig. 4E,E′, compare with 4D,D′). The loss of both subcortical actin and cytoplasmic actin bundles in *pxt* mutants is likely to be the underlying cause of the nurse cell dumping defects in the mutant.

The actin defects observed in pxt^{f01000} females are almost completely suppressed by MCOX1 expression (Fig. 4A,C). Robust actin bundles (Fig. 4C,C′) and subcortical actin (Fig, 4F,F′) form in S10B nurse cells in the rescued mutants. The ability of mouse Cox1 to efficiently substitute for Pxt during oogenesis strongly argues that production of PGs or other Cox1 products are essential for actin-

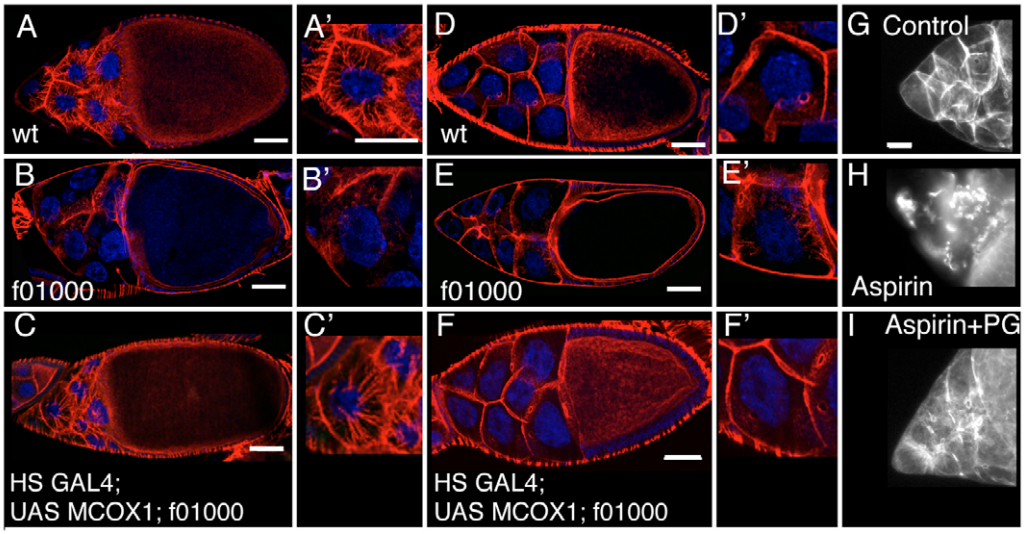

Fig. 4. Loss of *pxt* and COX inhibitors disrupt actin bundles required for nurse cell dumping. (**A-F′**) Actin bundles in wild-type nurse cells in cytoplasmic (A,A′) and subcortical regions (D,D′) are greatly reduced in $pxt^{-/-}$ mutants (B,B′ and E,E′, respectively), and restored by expression of MCOX1 under the control of HS GAL4 (C,C′ and F,F′). (**G-I**) Actin bundles in stage 10B egg chambers developed in vitro until stage 11 in the absence (G) or presence (H) of 1.5 mM aspirin, or with aspirin and 60 μM PGH_2 (I). The presence of aspirin disrupts subcortical actin and causes actin aggregation in dumping nurse cells (H), which is partially reversed by PG addition (I). A-F are projections of several confocal sections. A′-F′ are zoomed images of one nurse cell from the adjacent image. Red is phalloidin and blue is DAPI. (E-G) Epifluorescent images. Scale bars: 50 μm.

based contraction and actin bundle formation during nurse cell dumping. However, the mechanism(s) by which, either directly or indirectly, COX activity is required remains to be determined.

To verify that prostaglandins play a role in modulating actin during nurse cell dumping, we cultured S10B follicles with or without COX inhibitors, allowed them to mature to stage 12, and examined actin structures by phalloidin staining (Fig. 4G-I). (Prolonged incubation in vitro interferes with normal fixation of the large S10B cytoplasmic actin fibers, so we focused on subcortical actin.) COX inhibition, either with aspirin or NS-398, results in a loss of nurse cell subcortical actin and causes large actin aggregates to form (Fig. 4H, compare with G, and data not shown). Addition of exogenous PGH_2 results in a more normal pattern of actin organization (Fig. 4I). These data argue that PGs rather than some other product of the Cox1-like activity of Pxt are needed for normal actin structures during s10-11.

pxt mutants impair membrane stability and border cell migration

We also used *pxt* mutants to look for possible roles of PGs during the earlier stages of oogenesis. *Drosophila* ovarioles normally contain 6-7 egg chambers that regularly increase in developmental age (Fig. 5A). By contrast, ovarioles from *pxt* mutant females typically contain only 3-5 egg chambers (Fig. 5B), and are deficient in maturing follicles (pxt^{f01000} contain 80% fewer S10s/ovariole; see Fig. S4 in the supplementary material). This is probably due to follicle instability because 25% of pxt^{f01000} ovarioles contain a fusion of two adjacent egg chambers and in 58% of ovarioles there is at least one degenerating follicle (see Fig. S5 in the supplementary material). pxt^{f01000} nurse cell plasma membranes are unstable and frequently break down to produce multinucleate nurse cells (Fig. 5C, nuclei are outlined in white, compare with S8 in Fig. 5A), in which an actin-rich aggregate containing ring canal remnants remains (arrows in Fig. 5C). The multinucleate cells are probably caused by membrane breakdown and not cytokinesis defects, because this phenotype is not seen in the early stages and begins to arise around S8. The membrane breakdown, fusion and degeneration defects increase with age, because in 25-day-old *pxt* females, the ovarioles are reduced to a single fused structure (Fig. 5D).

Another striking defect was observed during border cell migration, a process requiring dynamic cytoskeletal and adhesion changes (Montell, 2003). In both homozygotes and transheterozygotes of $pxt^{EY03052}$ and pxt^{f01000}, more than 50% of S9-10 border cell clusters to not complete migration normally. Some mutant cells reach the oocyte, whereas others form a trail of lagging cells along the migration path (outlined in Fig. 5E, compare with Fig. 5A, outlined and labeled BC).

Intriguingly, *pxt* mutant phenotypes within an egg chamber usually decrease in severity along the anteroposterior (AP) axis. Membrane breakdown preferentially occur in anterior nurse cells (outlined in yellow in Fig. 5F). Conversely, posterior S10B nurse cells tend to retain more normal actin structures, including membrane and filaments (outlined in white in Fig. 5F). Interestingly, these observations suggest the possibility that a posterior to anterior gradient of PGs, or a downstream target of PGs, influences factors that regulate nurse cell dumping, membrane stability, and cell migration within the ovarian follicle.

DISCUSSION

A Cox1-like activity is required for *Drosophila* ovarian follicle maturation

PGs play important and diverse roles in insects and other invertebrates, such as regulating immune responses and reproduction, including oogenesis (Stanley, 2006; Stanley-Samuelson and Pedibhotla, 1996). Biochemical studies suggest that invertebrate PGs are produced in a similar manner to those in vertebrates (Stanley, 2000), and mammalian COX inhibitors have been shown to block insect PG synthetic activity in tissue extracts (Buyukguzel et al., 2002; Stanley-Samuelson and Ogg, 1994). However, the genes encoding the proteins that mediate invertebrate

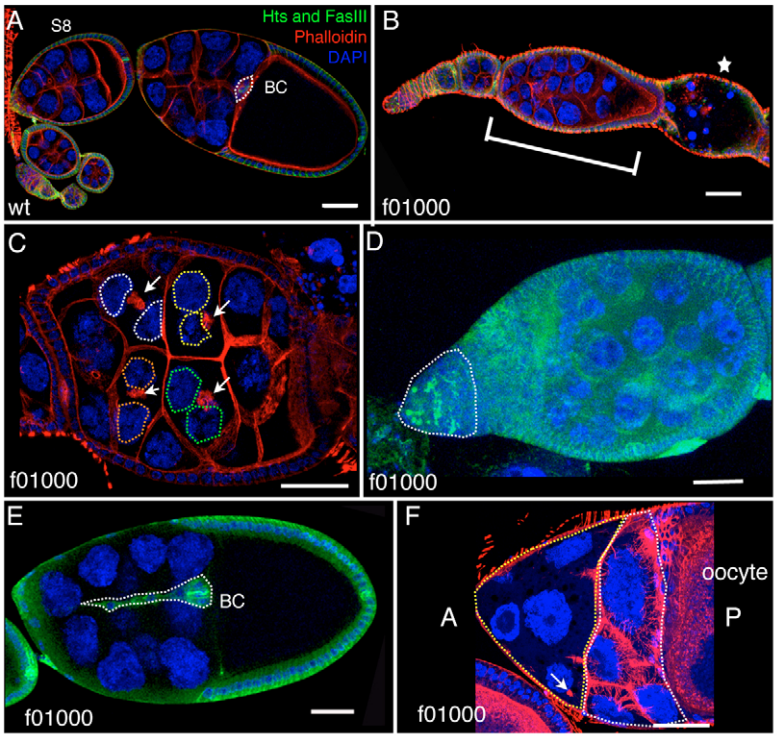

Fig. 5. *pxt* mutants exhibit actin-related defects throughout oogenesis. (**A**) Normal ovariole and (**B**) shortened *pxt* ovariole with missing stages, fused chambers (bracket) and degenerating chambers (star). (**C**) Multi-nucleate nurse cells in *pxt* egg chambers as a result of membrane breakdown. Nurse cell nuclei are outlined and arrows indicate actin-rich aggregates containing residual ring canals, compare with S8 in A. (**D**) Loss of follicular separation and germarium defects in older pxt mutant females. Broken line indicates germarium-stage cysts. (**E**) Defective border cell migration due to lagging cells (outlined, compare with 'BC' in A). (**F**) Nurse cells further away from the oocyte have membrane instability (outlined in yellow) and actin aggregates (arrow), whereas those closer to the oocyte have more normal actin filament formation (outlined in white). (A-F) Projections of confocal sections. Green is Hts (1B1) and Fas3 (7G10); red is phalloidin and blue is DAPI. A, anterior; P, posterior. Scale bars: 50 μm.

PG production have not been previously identified. This has slowed attempts to understand the detailed functions of PGs in *Drosophila* and precluded the use of genetics to address many important issues about PG signaling. We find that *Drosophila* S10B-14 follicle development requires a Cox1-like activity that can be satisfied by exogenous PGs, including $PGF_{2\alpha}$. Thus, follicle maturation provides a phenotypic focus for further genetics studies of PG action in *Drosophila*.

The Pxt peroxidase functions as a *Drosophila* COX enzyme

Using pharmacology, sequence homology and developmental genetics, we have identified Pxt as a candidate COX enzyme. Pxt is a heme peroxidase, and like mammalian COX enzymes, is predicted to be glycosylated and membrane bound. Despite only moderate homology to known COX enzymes, genetic loss of *pxt* resembles the effects of COX inhibitors on follicle development in vitro. Exogenous PGs can overcome the block in development caused by COX inhibitors and by *pxt* mutation. Therefore, PGs act downstream or in parallel to Pxt, supporting the idea that Pxt is responsible for the synthesis of PGs. Furthermore, we find that expression of mouse Cox1 in pxt^{f01000} mutant adults fully rescues female fertility and follicle development. The fact that mouse Cox1 can functionally replace Pxt during *Drosophila* oogenesis strongly argues that Pxt is a *Drosophila* COX enzyme.

Although *Drosophila* COX and mammalian COX enzymes appear to have diverged over time, they are likely to have the same basic three-dimensional structure within the conserved domain (Garavito and Mulichak, 2003; Picot et al., 1994). The critical residues (Gln203, His207, His388) for heme coordination necessary for the peroxidase activity are conserved in Pxt (Gln396, His402, His590). The conservation is too weak to predict the existence of the hydrophobic substrate-binding channel within the cyclooxygenase active site and the substrate binding residues Arg120 and Tyr355 are not clearly conserved. However, Pxt does contain a candidate Tyr385 (Pxt Tyr564), the residue at the active site that acquires an activating electron from the peroxidase part of the enzyme and is required for the cyclooxygenase activity. This suggests that there may be substantial differences in the structure of Pxt and Cox1, despite the ability of Cox1 to substitute functionally for Pxt.

The limited previous studies of Pxt structure and expression have suggested alternative functions for the protein. Distant homologies exist with peroxinectin (17-31% identity), a crayfish cell-adhesion peroxidase (Altschul et al., 1997; Vazquez et al., 2002). Pxt has also been suggested, by homology to a putative *Aedes aegypti* chorion peroxidase, to be the peroxidase responsible for eggshell hardening, a process caused by the formation of dityrosine crosslinks (Li et al., 2004; Petri et al., 1976). Although our more detailed expression data and the phenotypes of *pxt* mutants indicate that Pxt functions as a COX enzyme and is required during oogenesis prior to eggshell formation, we cannot rule out additional functions for the protein. Studies of purified Pxt will be required to address its full range of biochemical and enzymatic properties.

PGs appear to play a conserved role in follicle maturation and ovulation

Both pharmacological and genetic studies show that PGs are required for mammalian follicles to mature normally and undergo ovulation. Cox2 knockout mice are defective in both follicle maturation and ovulation (Loftin et al., 2002). In rats, inhibition of PG synthesis results in mistargeted and incomplete follicle rupture. The small percentage of follicles that do ovulate fail to be fertilized, most probably owing to impaired oocyte maturation (Lim et al., 1997). COX inhibitors, such as NSAIDs, cause reversible female infertility and may act in a similar manner (Akil et al., 1996; Pall et al., 2001; Smith et al., 1996). Like many mammals, *Drosophila* females also ovulate only one mature oocyte at a time, most probably by inducing contraction of the muscles surrounding just

one of the more than 30 ovarioles that make up the two ovaries. In addition to their defects in follicle maturation, *pxt* mutants ovulate only rarely (see Fig. S4 in the supplementary material; T.L.T. and A.C.S., unpublished). Thus, the roles of PGs during follicle development and ovulation may be conserved between *Drosophila* and mammals.

Each cycle, the single mammalian follicle that will be ovulated, the dominant follicle, accumulates high levels of smooth muscle myosin and actin which contributes to the contractile force needed for ovulation (Gougeon, 1996). These changes in actin organization are hypothesized to be downstream of PG signaling. $PGF_{2\alpha}$, the PG that had the most effect on *Drosophila* IVEM, is intimately associated with muscle contractions in mammals (Funk, 2001; Langenbach et al., 1999). Consequently, our finding that Pxt and PGs affect some oogenic processes that are modulated by actin and myosin, including border cell migration and nurse cell dumping, represents a potential parallel between the role of PGs in *Drosophila* and mammalian oogenesis.

Even the induction of PG-dependent maturation may occur in a similar manner. In mammals, PG synthesis is hormonally upregulated during the 10 hours preceding ovulation (Duffy and Stouffer, 2001; Murdoch et al., 1993; Sirois et al., 1992), and thereafter mediates the terminal differentiation of follicles (El-Nefiawy et al., 2005). Similarly, we find that *pxt* levels are upregulated during stages 9-10, and that COX activity, Pxt and PGs are subsequently required to complete the last 10-15 hours of *Drosophila* follicular development. Interestingly, the steroid hormone ecdysone is known to regulate the S8 oogenic checkpoint, which commits egg chambers to finish developing into mature follicles (Buszczak et al., 1999). *Drosophila pxt* expression and PG synthesis may be upregulated by ecdysone at the onset of S8 as part of the maturation program.

Pxt and PGs likely affect multiple pathways

Many PGs serve as short-range hormones that act as ligands for G protein-coupled receptors (GPCRs). In mammals there are eight receptors with distinct specificities for the active PGs, and each receptor favors the initiation of a specific signaling cascade (Bos et al., 2004). Such GPCR signaling can secondarily modulate additional signaling pathways. We have found that $PGF_{2\alpha}$ mediates *Drosophila* egg maturation in vitro. Mammalian $PGF_{2\alpha}$ acts as a ligand not only for the F receptor (FP), but also for two E receptors (EP_1 and EP_3), thereby increasing the possible signaling outcomes. One known downstream target is protein kinase A (PKA), which can then activate multiple MAPKs (Bos et al., 2004). It should be possible in the future to discern which effects of Pxt are exerted autonomously, and which are downstream of intercellular signals.

Despite a possible plethora of mechanisms, PGs have been frequently found ultimately to affect muscle contraction and ovulation, or to modulate the actin cytoskeleton in mammals. Within many cultured mammalian cell types, PGs cause changes in the organization, stability and polymerization of actin that influence membrane permeability and cell motility (Banan et al., 2000; Sheng et al., 2001). PG action appears to be rapid, suggesting that sophisticated feedback circuits may be involved. For example, reduction in the level of actin microfilaments has been reported to stimulate PG synthesis and release (Wang and Hatton, 2006). Thus, PGs may facilitate fine-tuning of the actin cytoskeleton in a rapid time frame.

Our studies show that Pxt and PGs can affect the actin cytoskeleton in *Drosophila*. Actin filaments are greatly reduced in *pxt* mutant stage 10B follicles, which phenotypically resemble follicles lacking major components of the actin cytoskeleton (Robinson and Cooley, 1997). PG-mediated regulation of actin dynamics is currently not well understood, particularly when intercellular interactions are involved. *Drosophila* oogenesis provides an attractive system to elucidate how PGs modulate actin in the context of developing tissues.

A PG signaling gradient?

Our data suggests that disruption of PG signaling has a graded effect within the developing follicle. The actin-related defects in *pxt* mutants are more prevalent at the anterior of the egg chamber, including nurse cell membrane loss and accumulation of actin puncta. Conversely, actin bundles are more likely to form in a normal fashion in the nurse cells closest to the oocyte. A PG signaling gradient might be relevant to many incompletely understood biological differences that exist along the AP follicle axis, such as the gradient of nurse cell ploidy, the temporal gradient of vitelline membrane formation within the oocyte, the directional migration signal for border cells and the programmed reorganization of the oocyte cytoskeleton in mid-oogenesis that underlies patterning. Ultimately, by using *Drosophila* genetics, it may be possible to better understand how PGs act, providing new insights and therapeutic targets for the widely conserved biological processes they influence.

The authors thank the members of the Spradling, Farber and Yanowitz laboratories for critiques of the manuscript, the Spradling and Farber laboratories for helpful discussions, Vanessa Damm for assistance with FISH, Sheryl Southard and Julio Castaneda for their rotation work using IVEM, Mahmud Siddiqi for assistance with time-lapse imaging, and Rafael Villagaray for assistance with Quicktime movie generation. A.C.S. is an Investigator of the Howard Hughes Medical Institute. T.L.T. is supported by NIH 5F32CA119676.

Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/135/5/839/DC1

References

Akil, M., Amos, R. S. and Stewart, P. (1996). Infertility may sometimes be associated with NSAID consumption. *Br. J. Rheumatol.* **35**, 76-78.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389-3402.

Banan, A., Smith, G. S., Kokoska, E. R. and Miller, T. A. (2000). Role of actin cytoskeleton in prostaglandin-induced protection against ethanol in an intestinal epithelial cell line. *J. Surg. Res.* **88**, 104-113.

Bellen, H. J., Levis, R. W., Liao, G., He, Y., Carlson, J. W., Tsang, G., Evans-Holm, M., Hiesinger, P. R., Schulze, K. L., Rubin, G. M. et al. (2004). The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. *Genetics* **167**, 761-781.

Bos, C. L., Richel, D. J., Ritsema, T., Peppelenbosch, M. P. and Versteeg, H. H. (2004). Prostanoids and prostanoid receptors in signal transduction. *Int. J. Biochem. Cell Biol.* **36**, 1187-1205.

Buszczak, M., Freeman, M. R., Carlson, J. R., Bender, M., Cooley, L. and Segraves, W. A. (1999). Ecdysone response genes govern egg chamber development during mid-oogenesis in Drosophila. *Development* **126**, 4581-4589.

Buyukguzel, K., Tunaz, H., Putnam, S. M. and Stanley, D. (2002). Prostaglandin biosynthesis by midgut tissue isolated from the tobacco hornworm, Manduca sexta. *Insect Biochem. Mol. Biol.* **32**, 435-443.

Cox, R. T. and Spradling, A. C. (2003). A Balbiani body and the fusome mediate mitochondrial inheritance during Drosophila oogenesis. *Development* **130**, 1579-1590.

Duffy, D. M. and Stouffer, R. L. (2001). The ovulatory gonadotrophin surge stimulates cyclooxygenase expression and prostaglandin production by the monkey follicle. *Mol. Hum. Reprod.* **7**, 731-739.

Edwards, K. A. and Kiehart, D. P. (1996). Drosophila nonmuscle myosin II has multiple essential roles in imaginal disc and egg chamber morphogenesis. *Development* **122**, 1499-1511.

El-Nefiawy, N., Abdel-Hakim, K. and Kanayama, N. (2005). The selective prostaglandin EP4 agonist, APS-999 Na, induces follicular growth and maturation in the rat ovary. *Eur. J. Endocrinol.* **152**, 315-323.

Funk, C. D. (2001). Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* **294**, 1871-1875.
Garavito, R. M. and Mulichak, A. M. (2003). The structure of mammalian cyclooxygenases. *Annu. Rev. Biophys. Biomol. Struct.* **32**, 183-206.
Gilmour, R. S. and Mitchell, M. D. (2001). Nuclear lipid signaling: novel role of eicosanoids. *Exp. Biol. Med. Maywood* **226**, 1-4.
Gougeon, A. (1996). Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr. Rev.* **17**, 121-155.
Hulo, N., Bairoch, A., Bulliard, V., Cerutti, L., De Castro, E., Langendijk-Genevaux, P. S., Pagni, M. and Sigrist, C. J. (2006). The PROSITE database. *Nucleic Acids Res.* **34**, D227-D230.
Langenbach, R., Loftin, C., Lee, C. and Tiano, H. (1999). Cyclooxygenase knockout mice: models for elucidating isoform-specific functions. *Biochem. Pharmacol.* **58**, 1237-1246.
Lecuyer, E., Parthasarathy, N. and Krause, H. M. (2008). Fluorescent in situ hybridization protocols in Drosophila embryos and tissues. In *Drosophila: Methods and Protocols* (ed. C. Dahmann). Totowa, NJ: Humana Press (in press).
Li, J., Kim, S. R. and Li, J. (2004). Molecular characterization of a novel peroxidase involved in Aedes aegypti chorion protein crosslinking. *Insect Biochem. Mol. Biol.* **34**, 1195-1203.
Lim, H., Paria, B. C., Das, S. K., Dinchuk, J. E., Langenbach, R., Trzaskos, J. M. and Dey, S. K. (1997). Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* **91**, 197-208.
Loftin, C. D., Tiano, H. F. and Langenbach, R. (2002). Phenotypes of the COX-deficient mice indicate physiological and pathophysiological roles for COX-1 and COX-2. *Prostaglandins Other Lipid Mediat.* **68-69**, 177-185.
Montell, D. J. (2003). Border-cell migration: the race is on. *Nat. Rev. Mol. Cell Biol.* **4**, 13-24.
Murdoch, W. J., Hansen, T. R. and McPherson, L. A. (1993). A review – role of eicosanoids in vertebrate ovulation. *Prostaglandins* **46**, 85-115.
Otto, J. C., DeWitt, D. L. and Smith, W. L. (1993). N-glycosylation of prostaglandin endoperoxide synthases-1 and -2 and their orientations in the endoplasmic reticulum. *J. Biol. Chem.* **268**, 18234-18242.
Pages, M., Rosello, J., Casas, J., Gelpi, E., Gualde, N. and Rigaud, M. (1986). Cyclooxygenase and lipoxygenase-like activity in Drosophila melanogaster. *Prostaglandins* **32**, 729-740.
Pall, M., Friden, B. E. and Brannstrom, M. (2001). Induction of delayed follicular rupture in the human by the selective COX-2 inhibitor rofecoxib: a randomized double-blind study. *Hum. Reprod.* **16**, 1323-1328.
Petri, W. H., Wyman, A. R. and Kafatos, F. C. (1976). Specific protein synthesis in cellular differentiation. III. The eggshell proteins of Drosophila melanogaster and their program of synthesis. *Dev. Biol.* **49**, 185-199.
Picot, D., Loll, P. J. and Garavito, R. M. (1994). The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature* **367**, 243-249.
Robinson, D. N. and Cooley, L. (1997). Genetic analysis of the actin cytoskeleton in the Drosophila ovary. *Annu. Rev. Cell Dev. Biol.* **13**, 147-170.
Sheng, H., Shao, J., Washington, M. K. and DuBois, R. N. (2001). Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. *J. Biol. Chem.* **276**, 18075-18081.
Sirois, J., Simmons, D. L. and Richards, J. S. (1992). Hormonal regulation of messenger ribonucleic acid encoding a novel isoform of prostaglandin endoperoxide H synthase in rat preovulatory follicles. Induction in vivo and in vitro. *J. Biol. Chem.* **267**, 11586-11592.
Smith, G., Roberts, R., Hall, C. and Nuki, G. (1996). Reversible ovulatory failure associated with the development of luteinized unruptured follicles in women with inflammatory arthritis taking non-steroidal anti-inflammatory drugs. *Br. J. Rheumatol.* **35**, 458-462.
Spradling, A. C. (1993). Developmental genetics of oogenesis. In *The Development of Drosophila melanogaster* (ed. B. Martinez-Arias), pp. 1-70. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
Stanley, D. W. (2000). *Eicosanoids in Invertebrate Signal Transduction Systems*. Princeton: Princeton University Press.
Stanley, D. (2006). Prostaglandins and other eicosanoids in insects: biological significance. *Annu. Rev. Entomol.* **51**, 25-44.
Stanley-Samuelson, D. W. and Ogg, C. L. (1994). Prostaglandin biosynthesis by fat body from the tobacco hornworm, Manduca sexta. *Insect Biochem. Mol. Biol.* **24**, 481-491.
Stanley-Samuelson, D. W. and Pedibhotla, V. K. (1996). What can we learn from prostaglandins and related eicosanoids in insects? *Insect Biochem. Mol. Biol.* **26**, 223-234.
Taketo, M. M. (1998). Cyclooxygenase-2 inhibitors in tumorigenesis (part I). *J. Natl. Cancer Inst.* **90**, 1529-1536.
Thibault, S. T., Singer, M. A., Miyazaki, W. Y., Milash, B., Dompe, N. A., Singh, C. M., Buchholz, R., Demsky, M., Fawcett, R., Francis-Lang, H. L. et al. (2004). A complementary transposon tool kit for Drosophila melanogaster using P and piggyBac. *Nat. Genet.* **36**, 283-287.
Vazquez, M., Rodriguez, R. and Zurita, M. (2002). A new peroxinectin-like gene preferentially expressed during oogenesis and early embryogenesis in Drosophila melanogaster. *Dev. Genes Evol.* **212**, 526-529.
Wang, Y. F. and Hatton, G. I. (2006). Mechanisms underlying oxytocin-induced excitation of supraoptic neurons: prostaglandin mediation of actin polymerization. *J. Neurophysiol.* **95**, 3933-3947.
Wheatley, S., Kulkarni, S. and Karess, R. (1995). Drosophila nonmuscle myosin II is required for rapid cytoplasmic transport during oogenesis and for axial nuclear migration in early embryos. *Development* **121**, 1937-1946.