Chapter 13
Drosophila: A Model for Studying Prostaglandin Signaling

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Abstract Prostaglandin (PG) synthesis and signaling are conserved in *Drosophila melanogaster*. PGs are produced downstream of cyclooxygenase or COX enzymes, the targets of nonsteroidal anti-inflammatory drugs. Almost 20 years ago, biochemical studies suggested that *Drosophila* possess COX activity. Recent efforts utilizing a combination of pharmacological and genetic approaches revealed that PGs have critical functions in *Drosophila* oogenesis or follicle development. Pxt was identified as the COX-like enzyme and is required for multiple aspects of female fertility, including temporal regulation of both gene expression and actin cytoskeletal remodeling. Here we review the PG synthesis and signaling machinery, the evidence for PG activity in *Drosophila*, the roles of PGs in flies, primarily focused on oogenic activities, and the conservation of PG function in higher animals. We also point out how studies on PGs in a genetic model system, such as flies, can significantly advance our understanding of the molecular actions of PGs.

Keywords Prostaglandins • *Drosophila* • Oogenesis • Reproduction • Actin cytoskeleton • Fascin • Enabled • Cancer • Gene amplification

Prostaglandins (PGs) are lipid signaling molecules that mediate a wide range of physiological processes including reproduction, cardiovascular function and disease, pain and inflammation, and cancer development and progression (reviewed in [1, 2]). Although PGs were identified more than 60 years ago, the study of PGs in the genetic model system of *Drosophila melanogaster* (hereafter referred to as *Drosophila*) has largely been restricted to the past decade. Here we review (1) the

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PG synthesis and signaling machinery, (2) the evidence for PG signaling in *Drosophila*, (3) the currently known roles of PGs in flies, and (4) the extent to which the roles of PGs in *Drosophila* are conserved across organisms.

13.1 Prostaglandin Synthesis and Signaling

Prostaglandin (PG) synthesis is a multistep processes that begins with the release of arachidonic acid (AA) from the glycerol backbone of membrane phospholipids through the enzymatic activity of phospholipase A_2 (PLA_2). This free AA is then converted into the PG precursor, PGH_2, through the enzymatic activity of cyclooxygenase enzymes (in mammals, COX-1 and COX-2), which are the pharmacological targets of nonsteroidal anti-inflammatory drugs (reviewed in [3–6]). Downstream of COX enzymes, PGH_2 is processed into the biologically active PGs (PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2) and thromboxane (TXA_2) through the activity of specific synthases (PGD_2: H-PGDS, L-PGDS; PGE_2: mPGES-1, mPGES-2, cPGES; $PGF_{2\alpha}$: AKR1B1; PGI_2: PGIS; TXA_2: TXAS) [7]. These bioactive species then go on to serve as autocrine/paracrine signaling molecules.

Although PGs may induce MAPK signaling pathways [8–12], or serve as peroxisome proliferator-activated receptor-gamma (PPARγ) nuclear hormone receptor ligands [13–16] independently of G protein-coupled receptors (GPCRs) (reviewed in [17]), their most widely accepted and best understood mechanism of action is to serve as ligands for specific GPCRs [18]. Each bioactive species of PG can bind to and activate from one to four cognate GPCRs (PGD_2: DP, CRTH2; PGE_2: EP1, EP2, EP3, EP4; $PGF_{2\alpha}$: FP; PGI_2: IP), which elicit their downstream effects through activation of Gα [17, 19] and, in some cases, Gβγ [20].

13.2 Evidence for Prostaglandin Synthesis and Signaling in *Drosophila*

13.2.1 Evidence for COX Activity

PGs are derived from the COX-dependent oxygenation of three long-chain polyunsaturated fatty acids (PUFAs): arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and dihomo-gamma-linolenic acid (DGLA, 20:3n-6). In many organisms, these long-chain PUFAs are acquired through the diet or through the elongation/desaturation of the essential fatty acid, linoleic acid (LA, 18:2n-6). Although some insects are capable of de novo synthesis of LA [21], there is little evidence that this occurs in *Drosophila* [22, 23].

The presence of long-chain PUFAs and their biological significance in *Drosophila* remains unclear. Early studies indicated that AA is not present in the fly, but its

precursor, LA, is [23–25]. Interestingly, Shen et al. found that when 22-carbon PUFAs are supplied in the diet these lipids are readily converted to 20-carbon PUFAs [25]. Thus, flies possess the machinery to utilize very long chain PUFAs. Other more recent studies, conducted using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), suggest that 20-carbon PUFAs are present in *Drosophila*. Using LC-MS/MS to analyze the fatty acid content of membrane phospholipids in both whole adults and isolated adult testes, Steinhauer et al. reported the presence of numerous phospholipid species containing AA in a wild-type fly strain, ranging from 0.2 to 1.5 % of the total phospholipid class [26]. Other groups also suggest 20-carbon PUFAs may be present in *Drosophila* [27–29]. Together these studies support the idea that the lipid precursors for PG synthesis are present at low levels in *Drosophila*.

In 1986, Pages et al. found that *Drosophila* extracts incubated with AA can generate PGE_2, PGF_1, and $PGF_{2\alpha}$, as detected by gas chromatography–mass spectrometry. Furthermore, endogenous PGE_2 was detected in untreated extracts using high performance liquid chromatography–radioimmunoassay [24]. These data were the first to suggest that a COX-like activity may be conserved in *Drosophila*.

Given the finding by Pages et al. [24] and the highly conserved roles of PGs in female reproduction [30, 31], we hypothesized that if PG synthesis and signaling were conserved in *Drosophila* it would regulate oogenesis or follicle development. Initially, we took advantage of the ability of mid-oogenesis stage 10B (S10B) follicles or eggs to mature in in vitro culture to ask whether COX enzyme activity was required to facilitate late-stage oogenesis [32]. These studies demonstrated that COX-1-like activity is required for follicle maturation as COX-1 inhibitors, but not COX-2 selective inhibitors, block follicle maturation in a dose-dependent manner [32]. Importantly, this COX inhibitor-dependent block in development is rescued by concomitant treatment with exogenous PGH_2, $PGF_{2\alpha}$, or fluprostenol, a stabilized $PGF_{2\alpha}$ analogue. These studies revealed that both COX-like activity and PGs are required for late-stage follicle development in *Drosophila* [32].

13.2.2 Identification the COX-Like Enzyme Pxt

The results of our pharmacological experiments [32], in combination with the previous findings of Pages et al. [24], strongly suggested the presence of a COX-like enzyme in *Drosophila*. BLAST analysis [33] revealed *Drosophila* Pxt as a candidate COX-like enzyme [32]. Sequence alignment using MUltiple Sequence Comparison by Log-Expectation (MUSCLE) [34, 35] reveals that Pxt is 26.76 % identical to ovine COX-1 and that a number of key residues [36] are conserved between COX-1 and Pxt. Most notably, the three critical residues for heme coordination in the peroxidase active site of COX-1 (Gln203, His207, and His388) are conserved in Pxt (Gln396, His402, and His590). Interestingly, Pxt possesses a candidate COX catalytic residue (Pxt Tyr564 vs. COX-1 Tyr385), although there is no

Table 13.1 Putative *Drosophila* homologues of prostaglandin (PG) synthesis and signaling proteins defined by BLASTp

PG pathway component	Function	Putative *Drosophila* homologue	Expression level during mid-to-late oogenesis[b]
COX	COX1-like	Pxt	High
	COX-like?	CG4009	Low, except high during S12
		CG10211	Below detection
PGD_2 synthases	H-PGDS	Gsts1	Low
	L-PGDS	n/a	n/a
PGE_2 synthases	mPGES1	Mgst1	Medium
		CG33178	Below detection
	mPGES2	Su(P) (CG4086)	Low
	cPGES	CG16817	High
		CG9267	Medium
$PGF_{2\alpha}$ synthases	AKR1C3	CG6084[a]	High
	AKR1B1		
PGI_2 synthase	PGIS	n/a	n/a
TXA_2 synthase	TBXAS	n/a	n/a
PG-like receptor	GPCR	CG7497	Below detection
15d-PGDH	Degrades PGs	Pdh	Below detection
		CG4086	Medium

[a]Indicates existence of numerous other similar proteins in *Drosophila*; however, CG6084 exhibits the highest homology

[b]Expression during oogenesis determined by microarray analysis of staged wild-type follicles [37]

clear conservation of the residues that have been shown to be critical for substrate binding through mutagenesis studies performed on mammalian COX enzymes. Additionally, the residue that is the target of aspirin-mediated acetylation (Ser530) is not clearly conserved.

Although the sequence homology between Pxt and COX-1 enzymes is not particularly striking, genetic loss of Pxt phenocopies the effects of COX inhibition. Specifically, similar to wild-type follicles treated with COX inhibitors, *pxt* mutant S10B follicles fail to complete maturation in vitro and exogenous PGs can restore development [32]. Additionally, *pxt* mutants are female sterile, and this sterility can be rescued by germline expression of mouse COX-1 [32]. Together, these data suggest that Pxt is the *Drosophila* COX-like enzyme and that both COX-like activity and PG signaling are required for *Drosophila* follicle maturation.

In addition to Pxt, *Drosophila* possesses putative homologues of other PG synthesis and signaling components (identified by BLAST [33]). Table 13.1 summarizes these candidates and their level of expression during mid-to-late oogenesis, as revealed by our microarray analysis [37].

13.3 The Roles of Prostaglandins in *Drosophila*

Given the widely conserved roles of PGs in reproduction [30, 31] and our finding that both COX activity and Pxt, the COX-like enzyme, mediate *Drosophila* follicle development [32], we have continued to exploit the system of *Drosophila* oogenesis to discover the specific activities of PGs. Thus, here we primarily discuss the oogenic roles of PGs. Additionally, we briefly discuss functions of PGs in *Drosophila* other than female reproduction.

13.3.1 Oogenic Roles of Prostaglandins

The *Drosophila* ovary is comprised of about 15 or 16 ovarioles or chains of sequentially developing follicles. Each follicle passes through 14 well-characterized, morphologically defined stages of development, termed Stages 1–14 (S1–S14) [38]. The germarium, a specialized structure housing both the germline and somatic follicle cell stem cells, is located at the anterior tip of each ovariole. Each developing follicle is a self-contained unit consisting of 16 interconnected germline-derived cells (15 nurse cells and a single oocyte) and approximately 1000 somatic epithelial cells, termed follicle cells.

Pxt, and thus PG signaling, are required for multiple aspects of *Drosophila* oogenesis [32]. Loss of Pxt results in age-dependent defects in follicle packaging/fusion, nurse cell cortical actin integrity during mid-to-late oogenesis (see following), border cell migration during Stage 9 (S9), germline stem cell division, and ovulation [32]. Here we focus on the characterized roles of PG signaling in eggshell formation [37] and temporal regulation of actin remodeling [32, 39, 40].

13.3.1.1 Prostaglandins Regulate Eggshell Formation

Pxt is required for the coordination of eggshell gene expression throughout the end of *Drosophila* oogenesis [37]. The somatic follicle cells secrete the eggshell. The *Drosophila* eggshell consists of five structural layers, which are sequentially synthesized in the following order: the vitelline membrane, the wax layer, and the chorion, which consists of the inner chorion layer, the endochorion, and the exochorion (Fig. 13.1a, d). Proper eggshell assembly requires tight temporal regulation of gene expression for the eggshell structural components. As such, the expression of vitelline membrane components begins during Stage 8 (S8), peaks during S10, and ceases by the end of S11, whereas the expression of chorion components occurs in three distinct phases, spanning from S10 to S14: early, middle, and late [41].

In *pxt* mutants, the expression of many vitelline membrane genes is prolonged while chorion gene expression is severely disrupted. The onset of expression of some chorion genes is early, although the expression of other chorion genes is

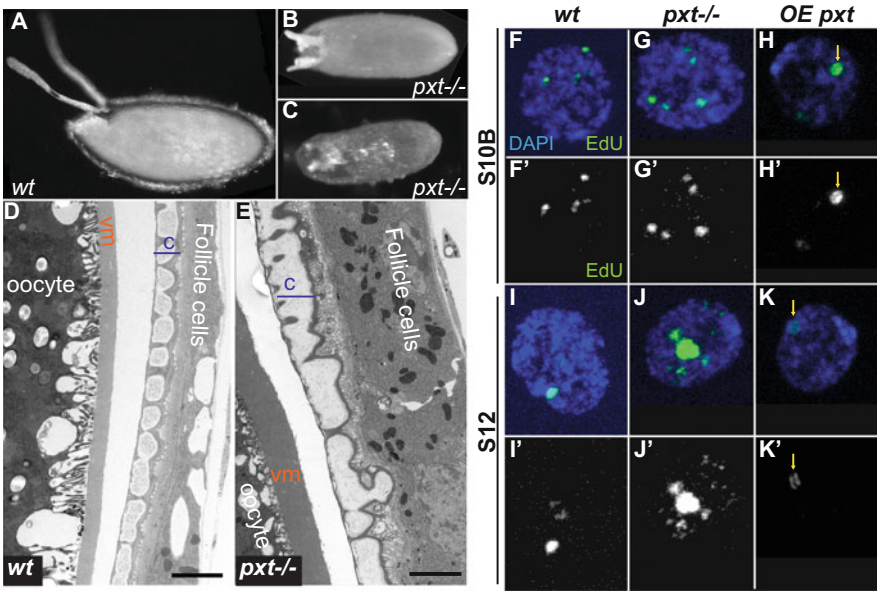

Fig. 13.1 Loss of Pxt in the soma results in defects in eggshell gene expression and eggshell formation. (**a**, **b**) Images of laid eggs of the indicated genotypes. (**d**, **e**) Transmission electron micrographs (Michael Sepanski) of the eggshell of S14 follicles (*c* chorion, *vm* vitelline membrane). *Bars* 5 μm. (**f**–**k**′) Confocal images of follicle cell nuclei from specified stages and genotypes labeled with DAPI (*blue* in **f**–**k**) to mark the nucleus and EdU (*green* in **f**–**k**; *white* in **f**′–**k**′) to label the number and size of gene amplification sites. Loss of Pxt results in short eggs with defective eggshells (**b**, **c** compared to **a**). In *pxt* mutants, the vitelline membrane is produced and fuses prematurely (not shown), and the chorion of S14 follicles exhibits structural defects (**e** compared to **d**). These eggshell defects are likely caused by the altered temporal regulation of eggshell gene expression during mid-to-late oogenesis (not shown), which may be caused by the altered gene amplification observed. When Pxt is lost, S10B follicle cells exhibit an increase in the number of sites of gene amplification observed by EdU labeling (**g**–**g**′ compared to **f**–**f**′), and during S12, when most of the amplification has ceased in wild-type follicles (**i**–**i**′), multiple and larger sites of amplification are observed in *pxt* mutants (**j**–**j**′). Conversely, overexpression of Pxt results in decreased sites of amplification and an increase in the rate of elongation as separated replication forks, indicated by double-bar EdU structures, are often observed (**h**–**h**′ and **k**–**k**′, *yellow arrows*)

delayed in *pxt* mutants [37]. Additionally, the expression of some chorion genes persists longer in *pxt* mutants than in wild-type [37]. These defects in temporal regulation of gene expression ultimately lead to numerous eggshell abnormalities, including a loss of vitelline membrane integrity and altered chorion production, resulting in short, uneven dorsal appendages and chorion patterning defects [37] (Fig. 13.1b, c compared to Fig. 13.1a, e compared to Fig. 13.1d). Although Pxt is required in the germline for nurse cell actin remodeling and nurse cell dumping (see following) [32], it is required in the soma (follicle cells) for temporal coordination of eggshell gene expression [37].

One means by which PG signaling could regulate the timing of eggshell gene expression is by affecting gene amplification of eggshell gene clusters. To promote

the proper formation of the eggshell, the appropriate eggshell genes must be rapidly transcribed at high rates during a strict temporal window. The eggshell-encoding genes are organized into a few clusters throughout the *Drosophila* genome. These gene clusters undergo gene amplification [42]. During gene amplification, particular regions of the genome undergo multiple rounds of replication to increase the DNA copy number of those regions. In nondividing cells, such as the *Drosophila* follicle cells after S6, gene amplification can be visualized as spots of nucleotide analogue, such as EdU incorporation. The size of the EdU spot generally corresponds to the amount gene amplification. There are six characterized sites of gene amplification in the *Drosophila* follicle cells [43]. During S10B–S11, gene amplification is initiating and all the sites of amplification are visible (Fig. 13.1f–f′); by S12 it has shifted to elongation and only a subset of the sites are visible (Fig. 13.1i–i′) [44].

Both loss of and overexpression of Pxt affect gene amplification during follicle development. Follicles from *pxt* mutants exhibit an increase in both the visible number and size of amplification sites during both S10B and S12 (Fig. 13.1g–g′, j-j' compared to Fig. 13.1f–f′', i–i′, respectively). Conversely, ubiquitous expression of either Pxt or mouse COX-1 results in a reduction in the level of amplification as decreased EdU incorporation is observed during both S10B and S12 (Fig. 13.1h–h′, k–k′ compared to Fig. 13.1f–f′, i–i′, respectively). Additionally, double-bar EdU spots, indicative of replication forks [44], are easily observed in follicles overexpressing Pxt or mouse COX-1, suggesting increased elongation. These data indicate the Pxt and PG signaling regulate the sites, level, and extent of elongation of gene amplification (Tootle, Williams, and Spradling, unpublished observations).

13.3.1.2 Prostaglandins Regulate Actin Cytoskeletal Remodeling

Actin cytoskeletal remodeling has critical roles in *Drosophila* follicle development and female fertility [45]. Here we specifically focus on those events occurring during mid-to-late oogenesis (S9–S14).

During S9 (Fig. 13.2a), the developing follicle undertakes numerous processes that are essential for female fertility, including yolk uptake [46, 47], slow cytoplasmic streaming [48, 49], and border cell migration [50]. Aside from cortical actin deposits underlying the nurse cell membranes, the cytoplasm of the germline-derived nurse cells is largely devoid of actin filament bundles through the end of S10A (Fig. 13.2b–b′).

Loss of Pxt results in aberrant, early actin remodeling during S9. Specifically, the majority of *pxt* mutant follicles exhibit early actin filament or aggregate formation in the posterior nurse cells (Fig. 13.2c–c′) [40]. Wild-type follicles exhibit similar actin structures at a low frequency. Studies using immunofluorescence reveal that the actin elongation factor Enabled (Ena), the sole *Drosophila* Ena/VASP family member, preferentially localizes to the early actin structures in *pxt* mutants. Subsequent genetic interaction studies show that reduced Ena levels rescue the aberrant early actin remodeling when Pxt is lost [40]. Notably, Ena expression is not affected in *pxt* mutants. These data lead to the model that, during S9, PGs elicits a

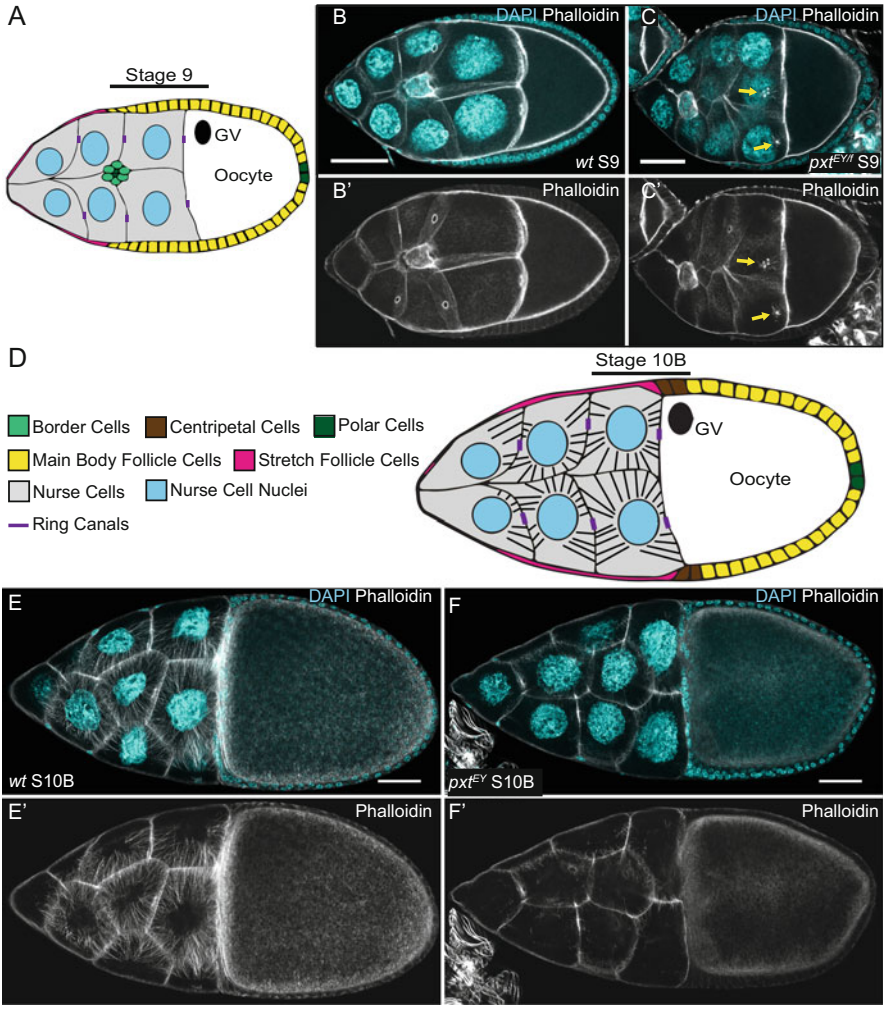

Fig. 13.2 Actin remodeling during *Drosophila* oogenesis requires the *Drosophila* COX-like enzyme, Pxt. (**a**) Schematic detailing the cellular composition of a S9 follicle (*GV* germinal vesicle). (**b**–**b**′, **d**–**f**′) Maximum projections of three confocal slices of follicles, staged as indicated, taken at 20×. (**c**–**c**′) Single confocal slice of S9 follicle, taken at 20×. Anterior is to the *left*. F-actin (phalloidin) *white*, DNA (DAPI) *cyan*. (**b**–**b**′, **e**–**e**′) Wild type *wt* (*yw*). (**c**–**c**′, **f**–**f**′) $pxt^{EY03052}$ mutant, pxt^{EY}. (**d**) Schematic detailing the cellular composition of a S10B follicle (*GV* germinal vesicle). S9 and S10B follicles consist of 16 germline-derived cells [1 oocyte (*white*) and 15 nurse cells (*gray*)] that are surrounded by a somatic epithelium (**a**, **c**). During S9, the nurse cell cytoplasm is largely devoid of actin filament structures aside from the cortical actin meshwork underlying the membranes (**b**–**b**′). *pxt* mutants exhibit a range of actin remodeling defects during S9, including cortical actin breakdown (not shown) and early actin remodeling, resulting in extensive early actin filaments and actin aggregate structures (*yellow arrows*). (**c**–**c**′) During S10B, wild-type follicles rapidly undergo actin remodeling to generate a network of parallel actin filament bundles extending from the nurse cell membranes toward the nuclei (**e**–**e**′). *pxt* mutants exhibit a range of actin remodeling defects at S10B, ranging from mild defects in the number and distribution of actin filament bundles to a near complete loss of actin filament bundles (**f**–**f**′). Images are representative, taken from multiple experiments. *Scale Bars* 50 μm

signaling cascade that normally inhibits Ena localization or activity to temporally restrict actin remodeling.

During S10B (Fig. 13.2d), the actin cytoskeleton within the nurse cells undergoes a rapid remodeling resulting in increased cortical actin deposition and the formation of a cage-like network of parallel actin filament bundles extending from the nurse cell membranes inward, toward the nurse cell nuclei (Fig. 13.2e–e′) [51, 52]. This dramatic actin remodeling is required to provide the contractile force necessary for the rapid transfer of nurse cell cytoplasm (nurse cell dumping) into the growing oocyte at S11 [53], while preventing the nurse cell nuclei from obstructing the ring canals, the specialized cytoplasmic bridges through which the nurse cell cytoplasm must flow [54, 55]. Importantly, nurse cell dumping, and thus actin remodeling, are required for female fertility. By S12, the nurse cells have completely transferred their cytoplasmic contents to the elongated oocyte, retaining only their nuclei, which will persist through S13. The mature follicle (S14) consists of only the oocyte, somatic follicle cells, and fully formed eggshell structures.

Initial studies revealed that numerous actin cytoskeletal defects are observed in *pxt* mutant S10B follicles. In contrast to wild-type follicles, *pxt* mutant follicles exhibit a significant reduction, if not complete loss, in the ability to form cytoplasmic actin filament bundles during S10B (Fig. 13.2f–f′) [32]. Additionally, nurse cell cortical actin levels are highly reduced in *pxt* mutants. As a result, *pxt* mutant follicles fail to generate the contractile force necessary for nurse cell dumping during S11 and exhibit multinucleate nurse cells resulting from cortical actin and nurse cell membrane breakdown [32, 39]. Expression of mouse COX-1 is able to almost completely suppress all the actin remodeling defects observed in *pxt* mutants [32]. Similarly, COX inhibitor treatment of cultured follicles results in the formation of large actin aggregates within the nurse cells and a reduction in nurse cell cortical actin deposits. Importantly, exogenous PGH_2 suppresses these COX inhibitor-induced actin cytoskeletal defects [32]. Together, these data suggest that both the COX-like activity of Pxt and PG signaling are required for appropriate actin cytoskeletal remodeling during S10B.

Although PGs have been implicated in regulating the actin cytoskeleton in other systems, the molecular mechanisms by which this occurs remain largely unknown [56–62]. Given our finding that PGs are required for the dynamic remodeling events occurring during *Drosophila* S10B [32] and the well-established roles of numerous actin-binding proteins in this process [45], we reasoned that we could exploit *Drosophila* genetics and our ability to mature S10B in culture to identify the actin-binding proteins whose activity is regulated by PG signaling [32, 63].

A pharmaco-interaction screen was performed to identify downstream targets of PG signaling based on their ability to dominantly modify follicle sensitivity to the effects of COX inhibition. Subsequently, using a combination of phenotypic analyses, pharmaco-genetic interactions with additional COX inhibitors, and genetic interactions, the actin bundling protein, Fascin was validated as a novel downstream target of PG signaling [39]. Indeed, overexpression of Fascin in the germline significantly restores cortical actin integrity and actin bundle formation in *pxt* mutants. Additionally, this screen uncovered an allele-dependent interaction that

suggests Ena may be a downstream target of PG signaling (Spracklen, Meyer, and Tootle, unpublished observation). Supporting this idea, Ena localization to the sites of actin remodeling during S10B is reduced in *pxt* mutants [40]. As Ena [40] and Fascin [39] expression are unaffected by loss of Pxt, PGs likely regulate these actin-binding proteins via posttranslational mechanisms.

PGs are critical in regulating the actin cytoskeleton during *Drosophila* oogenesis. During S9, Pxt leads to the production of PGs that block Ena localization and activity to preclude aberrant actin remodeling [40]. Pharmacological data suggest PGE_2-dependent signaling restricts actin remodeling [32]. Conversely, during S10B, Pxt-dependent generation of PGs, likely $PGF_{2\alpha}$ [32], mediates actin remodeling by positively regulating Ena [40] and Fascin [39]. In future, it will be critical to define the molecular mechanisms and the signal transduction cascades by which PGs regulate the identified targets Fascin and Ena and to identify other novel downstream effectors of PG signaling.

13.3.2 Other Roles of Prostaglandins in Drosophila

The studies of the roles of PGs in *Drosophila* have largely been limited to oogenesis, but PGs are likely to have additional functions in this organism. Indeed, *pxt* mutant flies are sickly; they exhibit reduced viability, developmental delays, reduced lifespan, motility defects, and abnormal fluid retention. Furthermore, COX inhibitor studies have implicated PGs in Fascin-dependent neural morphogenesis and branching [64]. Additionally, it has recently been observed that loss of Pxt results in sperm individualization defects (Josefa Steinhauer, personal communication); notably, these defects may be caused by altered actin dynamics [65]. Thus, *Drosophila* provides a rich system to elucidate both functions and molecular mechanisms of PG action in a variety of contexts.

13.4 Conservation of Prostaglandin Function

13.4.1 Follicle Development

The female reproductive functions of PG signaling are highly conserved. PGs regulate egg development and ovulation from insects [31] to mammals [30]. Indeed, PG synthesis inhibitors cause fertility defects in women, likely the result of altered follicle maturation and ovulation [66–68]. Interestingly, COX2-dependent production of PGE_2 and $PGF_{2\alpha}$ are implicated in mediating follicle development in mammals [69–71], whereas COX-1-dependent $PGF_{2\alpha}$ mediates it in zebrafish [72], silkmoths [73], and *Drosophila* [32]. It is important to determine if the molecular targets of these PG signaling pathways are conserved across organisms.

13.4.2 Gene Amplification

Both PGs [74–81] and gene amplification [82, 83] are implicated in driving cancer development and progression and are independently associated with poor patient prognosis. We speculate that one means by which PGs may contribute to cancer is by modulating gene amplification. In breast cancer, when the oncogene HER-2/neu is highly expressed it is often caused by gene amplification [84]. Subbaramaiah et al. found that the majority of HER-2/neu-positive tumor samples tested exhibit high COX-2 levels [33]. It will be interesting to determine if there is mechanistic association between PG signaling and gene amplification in cancer.

13.4.3 Actin Cytoskeletal Remodeling

Numerous in vitro studies have implicated PG signaling in regulating the actin cytoskeleton. However, such studies have provided limited insight into the underlying mechanisms of PG action. Multiple studies indicate that PGs induce changes in cytoplasmic actin bundles by cAMP-dependent mechanisms. Indeed, PGE_2- and PGI_2-induced actin stress fiber disassembly in human pulmonary artery endothelial cells occurs via cAMP-dependent kinase (PKA) and nucleotide exchange proteins directly activated by cAMP (Epac1)/Ras-related protein 1 (Rap1)-dependent activation of Rac [57]. In human umbilical vein endothelial cells, TXA_2 inhibits, while PGE_2 promotes, $\alpha_v\beta_3$-dependent cell adhesion and cell spreading by both PKA-dependent Rac activation and Rac-independent activities [59]. PGE_2 mediates actin stress fiber disassembly in human aortic smooth muscle cells by PKA-dependent decreases in focal adhesion kinase (FAK) phosphorylation [58]. PGs can also modulate the actin cytoskeleton via Rho GTPases. Specifically, PGE_2 promotes actin stress fiber assembly in rat IMCD cells [85], and $PGF_{2\alpha}$ mediates filopodia retraction and actin stress fiber assembly in 293-EBNA cells via Rho activation [85, 86].

PGs also regulate actin cytoskeletal dynamics in vivo to control platelet activation and aggregation [87]. The major prostanoid produced in platelets is TXA_2, which serves as a potent activator of platelet aggregation [88]. Conversely, PGI_2 [89], PGE_1 [90], and PGD_2 [91] inhibit platelet aggregation, and PGE_2 may both potentiate [90, 92] and inhibit platelet aggregation [93, 94]. The main activity of these prostanoids is to regulate vasodilator-stimulated phosphoprotein (VASP), a member of the Ena/VASP family of actin elongation factors. VASP is activated by TXA_2 and is inhibited through cAMP/cGMP-dependent phosphorylation downstream of PGI_2 and PGE_1 [95, 96]. The opposing actions of distinct prostanoids in regulating VASP activity is strikingly similar to our findings on PG regulation of Ena, the sole *Drosophila* Ena/VASP family member. Indeed, we find that PG signaling inhibits Ena during S9, but likely promotes Ena activity during S10B [40].

Another context in which PGs likely regulate actin cytoskeletal dynamics is in cancer. Cytoskeletal remodeling is necessary for proliferation, adhesion, and migration—cellular properties altered in cancer [97]. Remodeling is achieved through the coordinated activities of actin-binding proteins, the misregulation of which is associated with cancer development and progression [98–104]. Given that actin remodeling is aberrant in cancer, PGs regulate actin cytoskeletal remodeling in multiple contexts, and PGs play critical roles in cancer development and progression [74–81], it is tempting to speculate that PG signaling controls actin-binding protein localization/activity by posttranslation mechanisms to drive tumorigenic and metastatic events. This idea is particularly appealing as our studies in *Drosophila* identified Fascin [39] and Ena [40], the homologues of known cancer-associated actin-binding proteins, as downstream effectors of PG-dependent actin cytoskeletal remodeling.

Fascin is required for the generation of filopodia [105, 106] and invadopodia [98, 107], structures required for cancer invasion and metastasis [108, 109]. Indeed, high fascin-1 levels correlate with highly aggressive cancers in patients [108, 110–114]. Notably, high PG levels are similarly associated with poor patient prognosis [74–77, 79]. Together these data lead us to postulate that PG signaling posttranslationally regulates Fascin to drive cancer aggressiveness.

Our data also implicate Ena/VASP family members as targets of PG signaling during *Drosophila* oogenesis [40]. Alternatively spliced invasive isoforms of mammalian Ena (Mena), which is the closest homologue to *Drosophila* Ena [115], are upregulated as tumors become more advanced and strongly correlate with increased invasiveness and metastatic potential [116, 117]. Given that PGs regulate another Ena/VASP family member, VASP, in platelets [87] and our data that Ena activity is tightly controlled by PGs in *Drosophila* [40], it seems likely that PG signaling is a conserved means of modulating this family of actin-binding proteins.

13.5 Concluding Remarks

The study of PG signaling in *Drosophila* is in its infancy, yet it has already provided key insights into how PGs regulate actin cytoskeletal remodeling. By taking advantage of the genetic tools in *Drosophila*, further insights into the developmental and homeostatic roles of PGs and their downstream signaling cascades can be uncovered. Thus, this system is poised to truly advance our understanding of PG signaling. In the future, it will be important to determine the extent to which the means of PG signaling in *Drosophila* are conserved in higher organisms, both during normal physiology, such as reproduction and tissue homeostasis, and disease, such as cardiovascular diseases and cancer.

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