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SHORT COMMUNICATION

Spatiotemporal patterning of polyamines in *Drosophila* development

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Abstract While several studies have implicated polyamines (PAs) in development, little research has been done in genetically tractable model systems like *Drosophila*. Here, we integrate transcriptional and metabolic data across *Drosophila* development, and are the first to show temporal, stage-specific regulation of PA accumulation in embryonic trachea and eye discs using immunohistochemistry. Understanding the regulation driving this accumulation can provide insight into PA metabolism and transport. Our findings suggest that *Drosophila* has great potential for investigating PAs in developmental biology.

Keywords Spermidine · Spermine · Trachea · Eye imaginal disc · Embryogenesis · Polyamine metabolism · *Drosophila*

Abbreviations

PA	Polyamine
Put	Putrescine
Spd	Spermidine
Spm	Spermine
Dpp	Decapentaplegic
Hh	Hedgehog
Wg	Wingless
EGFR	Epidermal growth factor receptor
ODC	Ornithine decarboxylase
SRM	Spermine synthase
SMS	Spermidine synthase
Arg	Arginase
SamDC	S-adenosylmethionine decarboxylase
SamS	S-adenosylmethionine synthetase
DHPS	Deoxyhypusine synthase
DOHH	Deoxyhypusine hydroxylase
SMO	Spermine oxidase
PAO	Polyamine oxidase
SAT1/2	Diamine acetyltransferase 1/2
AcCoA	Acetyl coenzyme A
OAZ/Oda	Antizyme
PCA	Principle component analysis
Btl	Breathless
E-Cad	E-cadherin
MF	Morphogenetic furrow

Introduction

Polyamines (PAs)—putrescine, spermidine, and spermine—are ubiquitous organic molecules influencing multiple cellular processes, including gene regulation, signal transduction, and ion channel gating (Igarashi and Kashiwagi 2010; Pegg and Casero 2011; Chowhan and Singh 2013). In particular, PAs have been implicated in the regulation of a variety of developmental processes. In retina development of rabbits, chicks, and rats, PAs were found to be spatially localized (De Mello et al. 1976; Ientile et al. 1986; Taibi et al. 1994, 1995; Withrow et al. 2002). In rabbits, PA depletion disrupts cone differentiation (Withrow et al. 2002). Polyamines also function in the growth and

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differentiation of the mammary epithelium (Rillema et al. 1977; Oka et al. 1991; Murakami et al. 2009). Dynamic levels of PAs in embryogenesis of chicks and frogs have linked them to embryonic gastrulation (Löwkvist et al. 1980, 1985; Rosander et al. 1995; Shinga et al. 1996; Shibata et al. 1998). Various enzymes within the PA metabolic pathway have been found to be required for embryonic viability in mice (Pendeville et al. 2001; Nishimura et al. 2012).

In *Drosophila melanogaster,* dynamic PA levels have been observed throughout development, with increased PA levels detected during periods of rapid growth (Dion and Herbst 1967, 1970; Herbst and Dion 1970; Hamana et al. 1989; Callaerts et al. 1992). Interestingly, spermidine increases the net rate of RNA synthesis in larvae (Byus and Herbst 1976a), suggesting a possible mechanism of broad transcriptome regulation. Although several studies have investigated specific PA metabolism enzymatic activities during development (Byus and Herbst 1976b; Birnbaum and Gilbert 1990), there has not been a comprehensive analysis. Outside of development, *Drosophila* has been used as a pharmacological model for polyamine-conjugate chemotherapeutics (Tsen et al. 2008; Chelouah et al. 2011), for mechanistic studies in signaling pathways (Stark et al. 2011), and in the context of aging and autophagy (Minois et al. 2012; Gupta et al. 2013; Sigrist et al. 2014). We have previously shown that polyamines are required for long-term growth of *Drosophila* cell lines in a chemically defined medium (Burnette et al. 2014). In the current work, we combine integrative bioinformatics analysis of transcriptional data with an immunohistochemical investigation of spermidine and spermine (Spd/Spm) levels during development. We identify novel dynamic spatiotemporal PA accumulation profiles in both the larval eye imaginal disc and the embryonic trachea. To our knowledge, this is the first report of dynamic, spatially restricted accumulation of PAs during specific stages of organogenesis in *Drosophila.*

Results

To characterize PA regulation in development, we used publicly available expression data across 30 stages of *Drosophila* development (Graveley et al. 2011). Hierarchical clustering and principal component analysis (PCA) of putative *Drosophila* PA metabolism genes resulted in four clusters correlated with the enzymatic flow of PA metabolism: upstream biosynthesis (red), downstream biosynthesis (green), interconversion (blue), and inhibition (yellow) (Fig. 1a, b). To link regulatory clusters to metabolite accumulation, in vivo PA levels were extracted from previous studies quantifying developmental organismal levels (Dion and Herbst 1970; Callaerts et al. 1992). Regulatory clusters align to predict some basic dynamics of PA accumulation through development (Fig. 1c). Importantly, large shifts in transcriptional and metabolic profiles coincide with significant developmental events: the shift from proliferation to differentiation during embryonic development occurs as the interconversion cluster reaches a diminished quasi-steady state (Fig. 1c, highlighted gray); the shift away from exponential growth in preparation for pupariation coincides with an increase in the inhibition cluster and subsequent decreases in PA levels (Fig. 1c).

Analysis of Spd/Spm in developing embryos revealed PA accumulation within the lumen of tubes formed by tracheal cells, visualized with expression of actin-GFP under control of *breathless* (Fig. 2c, d). The embryonic trachea is an excellent model for tubulogenesis (Swanson and Beitel 2006; Affolter and Caussinus 2008), and develops in the latter half of embryogenesis by invagination of epithelial cells from the outer wall of the organism to form precursor sacs (Fig. 2a, b; Schottenfeld et al. 2010). Breathless (*btl)*-expressing tracheal cells migrate and intercalate toward sources of Branchless, and tubes are formed by cellular secretion and arrangement around a chitinous matrix that causes luminal expansion to the final diameter (Tonning et al. 2005). Specifically, PAs are accumulated in the lumen of the developing embryonic trachea with the same dynamics as the luminal chitin-binding protein Gasp (Fig. 2c, d).

Along with embryogenesis, we also discovered spatial accumulation of polyamines during morphogenesis of the eye imaginal disc. Spd/Spm is accumulated along the morphogenetic furrow, visualized with E-cadherin::GFP fusion protein (Fig. 2g–j). In *Drosophila,* the eye–antennal imaginal disc is the precursor structure to the adult compound eye and antenna (Fig. 2e, f), and its transformation from an undifferentiated epithelial sac to a patterned organ of photoreceptor complexes called ommatidia has long been used to study cell proliferation, differentiation and patterning (Kumar 2011). Prior to differentiation, a wave of mitosis determines the size of the eye and the number of ommatidia. Following this wave, the morphogenetic furrow initiates at the posterior end of the disc, and rapidly differentiates the epithelia into light receptors and support cells comprising the ommatidia (Tsachaki and Sprecher 2012). Specifically, PAs localize to the morphogenetic furrow during retinal patterning of the eye imaginal disc (Fig. 2g–j). Interestingly, the PA pattern is initially intense and widespread (Fig. 2g, h), but becomes progressively more confined to the furrow (Fig. 2i, j). Further, Gasp was found to accumulate in the same pattern along the morphogenetic furrow in eye discs (results not shown).

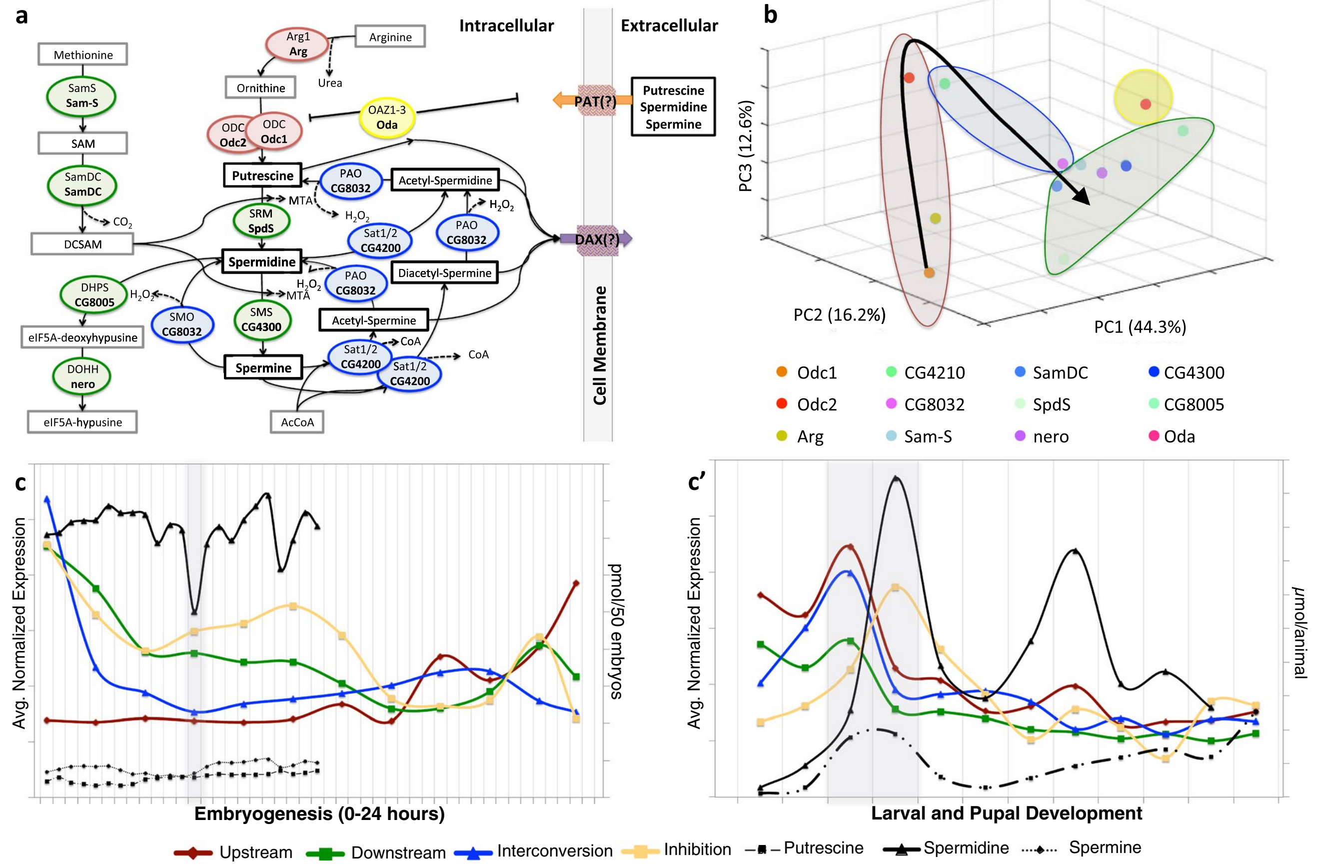

Fig. 1 **a** Polyamine metabolic pathway, adapted (Minois et al. 2011). Metabolites in *white boxes*, enzymes in *ovals*—human gene *top*, *Drosophila* ortholog *bottom* (*bold*). Enzymes are *color-coded* to indicate observed regulatory clusters. **b** Principle component analysis (PCA) of PA genes across development reveals four clusters that follow the regulatory logic of the metabolic pathway (*black arrow*): upstream (*red*) and downstream (*blue*) biosynthesis, interconversion (*blue*), and inhibition (*yellow*). **c**, **d** Normalized gene cluster dynamics and measured PA accumulation profiles (*left* and *right* axes, respectively) across stages of *Drosophila* embryonic (*top*) and larval (*bottom*) development (gaps represent data gaps from the literature) (Graveley et al. 2011; Dion and Herbst 1970; Callaerts et al. 1992). Average cluster dynamics align to predict basic dynamics of PA accumulation through development (color figure online)

Discussion

While no previous research has demonstrated a link between PAs and tubulogenesis, yeast cells with depleted amines had severely affected intercellular chitin structures (Balasundaram et al. 1991). Because *Drosophila* tracheal tube maturation depends on cellular secretion and arrangement around a chitinous matrix, the increased level of PAs observed in the tracheal lumen correlates with chitin and extracellular matrix deposition. In chicks, PAs are necessary to sustain tissue proliferation in the retina, and are dynamically accumulated during normal development (Taibi et al. 1994, 1995). Our observation of dynamic PA accumulation within differentiating cells along the morphogenetic furrow (Fig. 2h, k) suggests that these roles for PAs in eye development are likely conserved between *Drosophila* and mammalian retinal development (De Mello et al. 1976; Ientile et al. 1986; Taibi et al. 1994, 1995; Withrow et al. 2002). Further, MAPK signaling, known to be important for eye differentiation (Kumar et al. 1998), was recently linked to polyamine metabolism in *Drosophila* (Stark et al. 2011), providing a putative function for PAs in this organ.

A possible mechanism for PA regulation of development is through the production of hydrogen peroxide, a reactive oxygen species (ROS). ROS play important roles in cellular function (Finkel 1998; Kamata and Hirata 1999), with excessive ROS production being linked to various diseases and aging (Dröge 2002). In neural physiology in particular, ROS have been shown to be differentially accumulated and tied to proliferation and neurogenesis (Olguín-Albuerne and Morán 2015; Wei et al. 2015). ROS have also been implicated in signal transduction, activating or inactivating tyrosine kinases and acting as intracellular second messengers (Kamata and Hirata 1999; Morey et al. 2001; Le Belle et al. 2011). In *Drosophila*, cellular redox state was shown

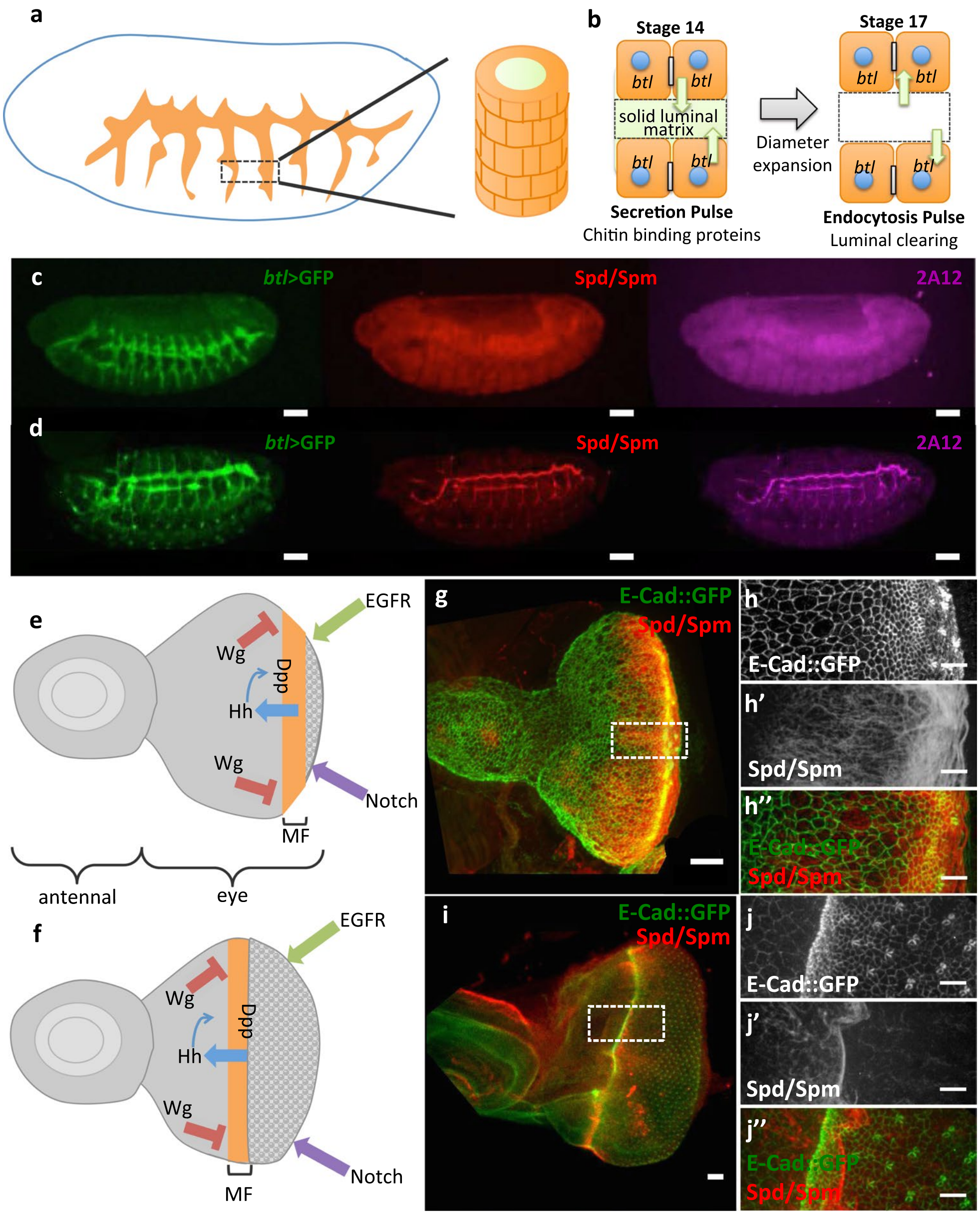

Fig. 2 **a** The embryonic trachea is a branched tubular organ that develops in the latter half of embryogenesis (Swanson and Beitel 2006; Affolter and Caussinus 2008). **b** Tube maturation depends on the formation of a chitinous matrix (shown in *green*) that expands the lumen diameter. This matrix is generated when *btl*-expressing tracheal cells (*orange*) experience a "secretion pulse," inducing luminal deposition of chitin-binding proteins that expand the tube diameter (Tonning et al. 2005). A subsequent endocytosis pulse rapidly clears the luminal matrix. Figure adapted (Tsarouhas et al. 2007; Förster et al. 2010; Maruyama and Andrew 2012). **c**, **d** Analysis of PA accumulation in developing embryos revealed PA deposition within the lumen of expanding trachea. Polyamines are accumulated with the same dynamics of 2A12 (*magenta*), a luminal marker for Gasp, a chitin-binding protein (Tiklová et al. 2013) (*scale bars* 50 µm). **e**, **f** Differentiation of the larval eye–antennal imaginal disc is driven by the morphogenetic furrow (*orange*), which initiates at the posterior end of the disc and proceeds to the anterior side. The progression of the morphogenetic furrow is dependent on well-characterized morphogens including Decapentaplegic (*Dpp*) and Hedgehog (*Hh*) (*blue*) as well as and Wingless (*Wg*) (*red*), Notch (*purple*), and Epidermal growth factor receptor (*EGFR*) (*green*) (Greenwood and Struhl 1999; Curtiss and Mlodzik 2000; Kumar and Moses 2001; Moon et al. 2006; Firth et al. 2010). Figure adapted (Kumar and Moses 2001; Doroquez and Rebay 2006). **g**–**i** PAs (*red*) are accumulated along the morphogenetic furrow in eye discs expressing E-cadherin::GFP (*green*, apical cell boundaries), with differentiated ommatidia present to the posterior (**g**, **h**, day 4; **i**, **j**, day 6 after egg laying). The PA pattern is initially more widespread (**g**, **h**), and becomes progressively more confined to the furrow (**i**, **j**). *Scale bars* 20 µm (**g**, **i**), 5 µm (**h**–**j**) (color figure online)

to specifically modulate MAPK signaling (Morey et al. 2001). Thus, polyamines may exert their effects by perturbing cellular ROS.

Here, we demonstrate that the developmental stage-specific regulation of the PA metabolic pathway results in dynamic accumulation of PAs in particular organs during development. We have discovered novel, spatiotemporal accumulation of PA metabolites in developing embryonic trachea and differentiating eye imaginal discs. While future research will be required to elucidate specific developmental roles of PAs in these organs, this study highlights the utility of *Drosophila* as a powerful model organism for investigating PA biology. Further, the observation of spatiotemporal accumulation of PA levels as measured by immunohistochemistry can lead to novel assays for studying PA transport.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflicts of interest.

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