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dBre1/dSet1-dependent pathway for histone H3K4 trimethylation has essential roles in controlling germline stem cell maintenance and germ cell differentiation in the *Drosophila* ovary

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ABSTRACT

The *Drosophila* ovarian germline stem cells (GSCs) constantly experience self-renewal and differentiation, ensuring the female fertility throughout life. The balance between GSC self-renewal and differentiation is exquisitely regulated by the stem cell niche, the stem cells themselves and systemic factors. Increasing evidence has shown that the GSC regulation also involves epigenetic mechanisms including chromatin remodeling and histone modification. Here, we find that dBre1, an E3 ubiquitin ligase, functions in controlling GSC self-renewal and germ cell differentiation via distinct mechanisms. Removal or knock down of dBre1 function in the germline or somatic niche cell lineage leads to a gradual GSC loss and disruption of H3K4 trimethylation in the *Drosophila* ovary. Further studies suggest that the defective GSC maintenance is attributable to compromised BMP signaling emitted from the stem cell niche and impaired adhesion of GSCs to their niche. On the other hand, *dBre1-RNAi* expression in escort cells causes a loss of H3K4 trimethylation and accumulation of spectrosome-containing single germ cells in the germarium. Reducing *dpp* or *dally* levels suppresses the germ cell differentiation defects, indicating that dBre1 limits BMP signaling activities for the differentiation control. Strikingly, all phenotypes observed in *dBre1* mutant ovaries can be mimicked by RNAi-based reduced expression of dSet1, a *Drosophila* H3K4 trimethylase. Moreover, genetic studies favor that *dBre1* interacts with *dSet1* in controlling GSC maintenance and germ cell differentiation. Taken together, we identify a dBre1/dSet1-dependent pathway for the H3K4 methylation involved in the cell fate regulation in the *Drosophila* ovary.

Introduction

Renewal and homeostasis of many adult tissues including hematopoietic system, skin and gut are mainly dependent on stem cells that continuously experience self-renewal and directed differentiation throughout the life of an animal. The adult stem cells are often anchored in specialized microenvironments called niches, and exquisitely regulated by local signals from the niche, intrinsic factors in the stem cells, and systemic factors such as insulin and the steroid hormone (Ables and Drummond-Barbosa, 2010; Hsu and Drummond-Barbosa, 2009, 2011; König et al., 2011; LaFever and Drummond-Barbosa, 2005; Wong et al., 2005). Unraveling how this regulation occurs will gain more insights into the fundamental biological mechanisms governing the tissue maintenance and regeneration.

The *Drosophila* ovary provides an in vivo model system for studying adult stem cell behavior and regulation. Each ovariole, a basic structural unit of the ovaries, consists of the anteriorly located germarium and a string of progressively matured egg chambers. At the anterior tip of each germarium, two to three germline stem cells (GSCs) form a single GSC unit with a number of somatic niche cells such as terminal filament cells (TFs) and cap cells, closely apposed to the stem cells (Fig. 1A) (Lin, 2002; Spradling et al., 2001, 2008). GSCs constantly undergo the asymmetric division by which one daughter cell remaining in contact with the GSC niche retains stem cell identity, whereas the other is displaced away from the niche, acquiring cystoblast (CB) fate. The CBs further divide with incomplete cytokinesis to consecutively produce 2-cell, 4-cell, 8-cell and 16-cell germline cysts (Spradling, 1993). GSCs and the differentiating descendant cells are covered by escort cells (ECs), a group of somatic cells lying adjacent to cap cells (Decotto and Spradling, 2005). While TFs and cap cells constitute the GSC niche, ECs have recently been defined as the germ cell differentiation niche (Kirilly et al., 2011).

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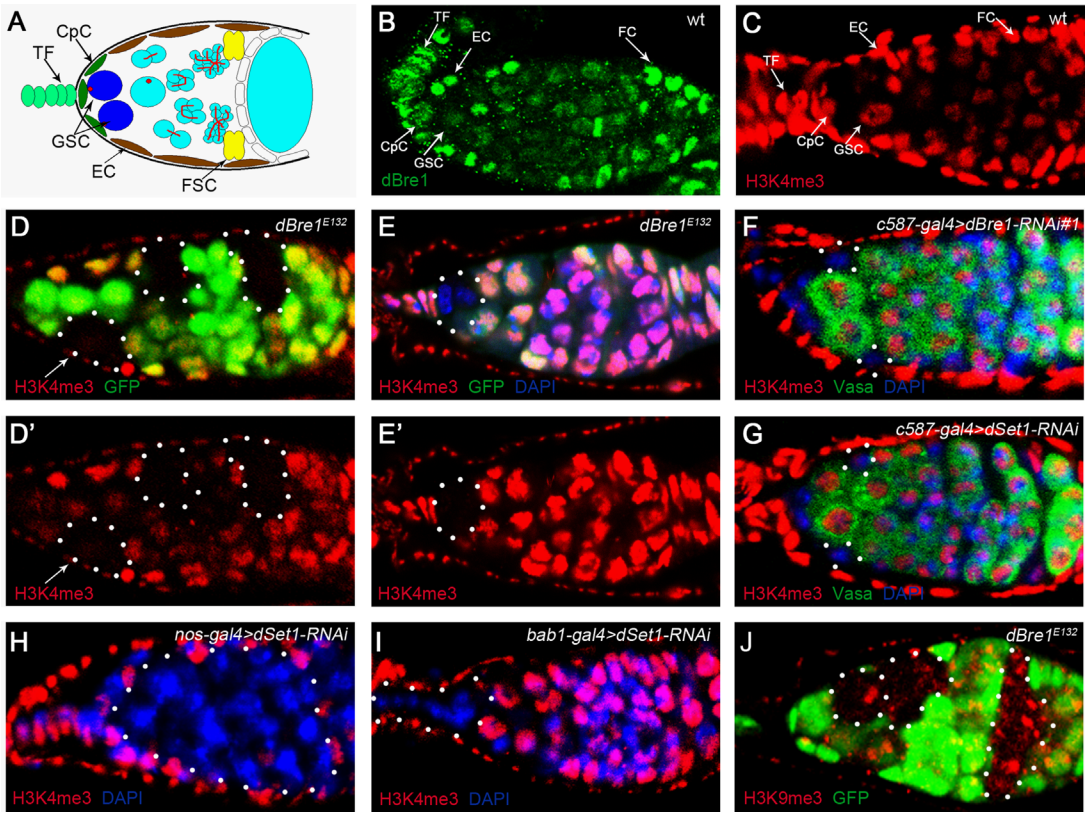

Fig. 1. The bulk histone H3K4 trimethylation in the *Drosophila* ovary is dependent on dBre1 and dSet1: (A) a schematic diagram of wild type germarium with different cell types: germline stem cells (GSCs) (dark blue) and surrounding somatic cells including terminal filaments (TFs) (light green), cap cells (CpCs) (dark green), escort cells (ECs) (brown) and follicle stem cells (FSCs) (yellow). (B–I) Wild type control (B, C) and mutant germaria carrying clones homozygous for $dBre1^{E132}$ (D and E) or expressing *dBre1-RNAi#1* (F) or *dSet1-RNAi* (G–I) under the control of specific *gal4* driver stained for dBre1 (B) or trimethylated H3K4 (C–I). (B) Expression of *dBre1* is evident in almost all cells of germarium, predominantly in TFs, CpCs, ECs and follicle cells (FCs). (C) H3K4me3 staining is ubiquitously present in all cell types. (D and E) H3K4 trimethylation is barely detectable in the germline clones (D, D′) or CpC clones (E, E′) homozygous for $dBre1^{E132}$. Note that the clones are outlined with white dots; arrow indicates GSC. (F–I) Loss of H3K4 trimethylation is evident in the ECs expressing a *dBre1-RNAi#1* (F) or *dSet1-RNAi* (G) transgene. Meantime, similar results are observed in the germ cells (H) or the TFs and CpCs (I) in which *dSet1* is knocked down using the RNAi-based approach. Note that RNAi-expressing cells are marked with broken lines. (J) As a control, H3K9me3 staining is present in the germline clones (broken lines) homozygous for $dBre1^{E132}$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

It is known that the GSC niche plays an instructive role in maintaining self-renewal of the stem cells by preventing their differentiation. Cap cells in the niche produce BMP-like signal molecule Decapentaplegic (Dpp) for activating BMP signaling pathway in GSCs to prevent differentiation via silencing expression of differentiation-promoting genes such as *bag-of-marbles* (*bam*) (Chen and McKearin, 2003a, 2003b; McKearin and Ohlstein, 1995; Rojas-Ríos et al., 2012; Song et al., 2004; Xie and Spradling, 1998). The niche-controlled GSC self-renewal is largely dependent on both niche maintenance and signal output from the niche in the adult ovary. In the case of niche size control, Notch signaling has been shown to be required for maintaining cap cells and thus GSCs in adulthood (Hsu and Drummond-Barbosa, 2011; Song et al., 2007). In parallel, JAK/STAT signaling pathway positively regulates *dpp* expression in cap cells, thereby determining the signal output level for the control of GSC self-renewal (Lopez-Onieva et al., 2008; Wang et al., 2008a). Meanwhile, regulation of BMP signal output can occur in the level of morphogen diffusion that is modulated by *dally*, a glypican-encoding gene. By repressing *dally* transcription, EGFR pathway in ECs acts to limit BMP signaling activities to GSCs in the anterior tip of the germarium (Guo and Wang, 2009; Hayashi et al., 2009; Liu et al., 2010; Schulz et al., 2002). Remarkably, the range of BMP signaling activity precisely determines the balance between GSC self-renewal and differentiation, as exemplified by the differential response of GSCs vs. CBs one cell diameter away from the stem cells to BMP signals (Eliazer et al., 2011; Guo and Wang, 2009; Harris et al., 2011; Hayashi et al., 2009; Liu et al., 2010; Wang et al., 2008b, 2011; Xia et al., 2010, 2012). Besides those extrinsic factors from the niche, intrinsic ones in the stem cells required for responding to niche-derived BMP signals are also essential for GSC maintenance (Chen et al., 2009, 2010; Jiang et al., 2008; Sun et al., 2010; Xi et al., 2005). In addition, DE-cadherin-mediated adhesion between cap cells and GSCs is required for anchoring GSCs in the niche, thus contributing to continuous self-renewal of GSCs (Song et al., 2002).

Like genetic factors, epigenetic mechanisms involving chromatin remodeling and histone modification are equally important for adult stem cell regulation. Increasing evidence has demonstrated that the control of GSC maintenance and differentiation in the *Drosophila* ovary requires epigenetic contributions (Buszczak et al., 2009; Eliazer et al., 2011; Maines et al., 2007; Wang et al., 2011; Xi and Xie, 2005). In one hand, chromatin remodeling factors such as ISWI and Stonewall are essential for maintaining GSC self-renewal cell-autonomously in a BMP/Bam-dependent or -independent manner respectively. On the other hand, Lsd1, a H3K4 demethylase in the *Drosophila* ovary, has recently been shown to promote the germ cell differentiation non-autonomously presumably through repressing *dpp* expression. A more recent study identified *Drosophila* histone H3K9 trimethylase Eggless (Egg) as an essential regulator controlling GSC self-renewal and differentiation in the ovaries. Although GSC regulation at epigenetic level is evident, the underlying mechanisms remain to be further explored.

Drosophila Bre1 (*dBre1*) encodes an E3 ubiquitin ligase required for the monoubiquitination of histone H2B both in vitro and