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Ubx dynamically regulates Dpp signaling by repressing Dad expression during copper cell regeneration in the adult *Drosophila* midgut

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ABSTRACT

The gastrointestinal (GI) tract of metazoans is lined by a series of regionally distinct epithelia. To maintain structure and function of the GI tract, regionally diversified differentiation of somatic stem cell (SC) lineages is critical. The adult *Drosophila* midgut provides an accessible model to study SC regulation and specification in a regionally defined manner. SCs of the posterior midgut (PM) have been studied extensively, but the control of SCs in the middle midgut (MM) is less well understood. The MM contains a stomach-like copper cell region (CCR) that is regenerated by gastric stem cells (GSSCs) and contains acid-secreting copper cells (CCs). Bmp-like Decapentaplegic (Dpp) signaling determines the identity of GSSCs, and is required for CC regeneration, yet the precise control of Dpp signaling activity in this lineage remains to be fully established. Here, we show that *Dad*, a negative feedback regulator of Dpp signaling, is dynamically regulated in the GSSC lineage to allow CC differentiation. *Dad* is highly expressed in GSSCs and their first daughter cells, the gastroblasts (GBs), but has to be repressed in differentiating CCs to allow Dpp-mediated differentiation into CCs. We find that the Hox gene *ultrabithorax* (*Ubx*) is required for this regulation. Loss of Ubx prevents *Dad* repression in the CCR, resulting in defective CC regeneration. Our study highlights the need for dynamic control of Dpp signaling activity in the differentiation of the GSSC lineage and identifies Ubx as a critical regulator of this process.

1. Introduction

Stem cell (SC) proliferation, differentiation, and maintenance have to be precisely controlled to maintain long-term tissue homeostasis. This is particularly relevant in barrier epithelia, including the intestine, stomach, and skin, that are continuously exposed to environmental challenges (Barker et al., 2010). In the gastrointestinal (GI) tract, intestinal stem cell (ISC) populations not only have to ensure accurate regenerative responses to tissue damage, but have to also maintain the diversity of the regionally defined epithelia with distinct function and morphology (such as the esophagus, stomach, and intestine, Barker et al., 2010; Buchon et al., 2013b; Li et al., 2016; Marianes and Spradling, 2013; Tasnim et al., 2016).

The adult *Drosophila* midgut has emerged as an important model to study somatic stem cell biology (Biteau et al., 2011; Buchon et al., 2013a; Buchon and Osman, 2015; Jiang and Edgar, 2011; Lemaitre and Miguel-Aliaga, 2013; Xu et al., 2016). ISCs can be found in all three regions of the midgut: anterior midgut (AM), middle midgut (MM), and posterior midgut (PM), and the SC lineages of the PM and MM regions have been characterized in detail (Biteau et al., 2011; Hou, 2010; Strand and Micchelli, 2011). Detailed molecular characterization of stem cells in 10–14 subdivided regions of the gut has further highlighted the diverse nature of the GI stem cell population, although mechanisms that maintain this diversity remain largely unexplored (Buchon et al., 2013b; Dutta et al., 2015; Marianes and Spradling, 2013).

ISCs in the PM are characterized by the expression of *escargot, esg,* and *Delta, Dl.* During regenerative episodes, these cells undergo asymmetric divisions to give rise to a new ISC and a precursor cell, an enteroblast (EB, esg+/Dl−), which can further differentiate into either an enterocyte (EC, pdm1+) or an enteroendocrine cell (EE, prospero+) (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006, 2007). The MM contains a stomach-like copper cell region (CCR, (Dubreuil, 2004)), which is regenerated by gastric stem cells (GSSC). GSSCs, which also express esg, generate three differentiated cell types: acid-producing copper cells (CCs, Cut+/Labial+), interstitial cells (ISs, Cut-/weak Labial+), and enteroendocrine cells (EEs, prospero+) (Fig. 1A, Strand and Micchelli, 2011). GSSCs are mostly quiescent under homeostatic conditions, but can be stimulated to proliferate by

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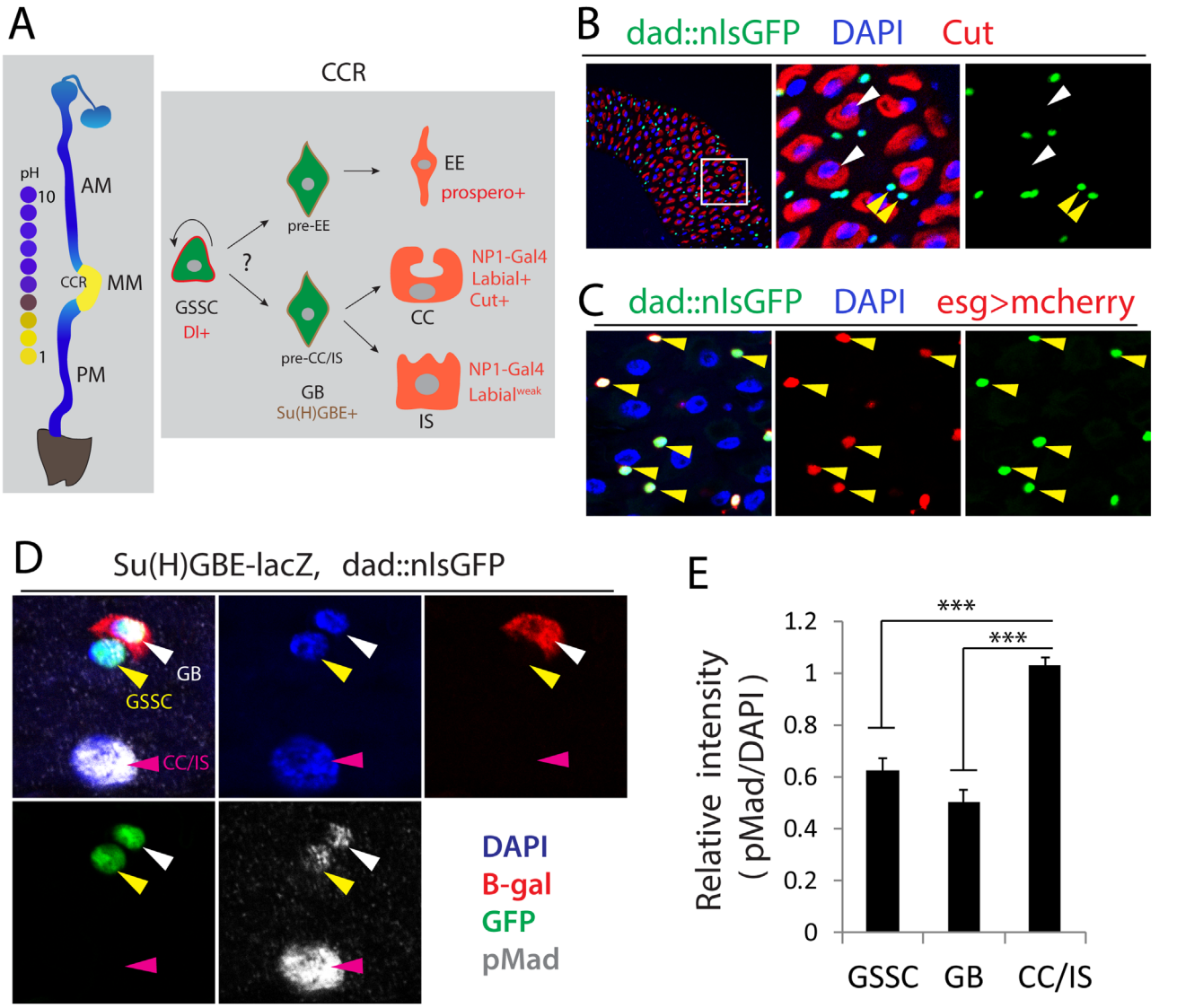

Fig. 1. Dad expression and pMad staining in the CCR. (A) Left: Schematic of Drosophila midgut compartments with pH indicator. AM, anterior midgut; MM, middle midgut; CCR, copper cell region; PM, posterior midgut. Right: cell lineage in the CCR with markers and drivers indicated. The questionmark indicates that there is no experimental evidence about the existence of pre-committed GBs, and we speculate this model based on recent studies on stem cell lineage in the posterior midgut. GSSC, gastric stem cell; GB, gastroblast; EE, enteroendocrine cell; CC, copper cell; IS, interstitial cell. (B) Dad::nlsGFP (green) is expressed in small diploid cells (yellow arrowheads), not in Cut+ (red) CCs (white arrowheads). (C) Dad::nlsGFP (green) expressing cells are positive for esg > mcherry (red, esgGal4, uas-mcherry; esg is a marker for GSSC and GB). (D) One of the Dad::nlsGFP+ (green) doublet cells is Su(H)GBE-lacZ (red) positive in the CCR. pMad (white) antibody staining is positive for all cell types, but shows a higher level in the polyploidy CC/IS. (E) Quantification of pMad intensity relative to DAPI intensity from (D). Note that pMad intensity is significantly higher in CCs/ISs compared to GSSCs or GBs. N=11 guts from three biological replicates (36 GSSCs, 33 GBs, 80 CCs/ISs). Averages and SEM are shown. One-way ANOVA with post-hoc *t*-test was performed,*** $p < 0.001$.

stress (such as heat-shock). This activation of GSSCs seems to be mediated primarily by epidermal growth factor (EGF) signaling (Strand and Micchelli, 2011, 2013). Recent studies have refined our understanding of ISC lineage and suggest that two types of differentiated cells (ECs and EEs) are generated from pre-committed ISCs, and not from a common enteroblasts (EBs) (Beehler-Evans and Micchelli, 2015; Biteau and Jasper, 2014; Guo and Ohlstein, 2015; Wang et al., 2015; Zeng and Hou, 2015). These studies have focused on the stem cell lineage in the PM, and there is no published evidence for or against this model in the middle midgut yet. Based on the similarities of these lineages, it can be speculated that the same model applies in this region (Fig. 1A, Li and Jasper, 2016).

To date, numerous signaling pathways have been reported to regulate ISC function in the PM, and recent studies have begun to explore in detail how the integration of these pathways influences proliferation and differentiation of ISCs (Biteau et al., 2011; Buchon et al., 2013a; Buchon and Osman, 2015; Deng et al., 2015; Guo and Ohlstein, 2015; Jiang and Edgar, 2011; Lemaitre and Miguel-Aliaga, 2013; Meng and Biteau, 2015). The regulation of GSSC proliferation and differentiation in the CCR, in turn, is still relatively poorly understood. Studies from others and us have recently shown that signaling by Decapentaplegic (Dpp) is required for CC regeneration in the adult CCR (Guo et al., 2013; Li et al., 2013a), while Dl/Notch signaling between GSSCs and gastroblasts (GBs) helps determine specification of GSSC daughter cells (Wang et al., 2014), similar to the regulation of ISC differentiation in the PM (Ohlstein and Spradling, 2007).

Dpp is a homologue of bone morphogenetic protein (BMP), and controls a number of vital events during development (Peterson and O'Connor, 2014). Canonically, Dpp signals through the BMP Type I receptor Thickveins (Tkv), the Type II receptor Punt, and the