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EXTRA VIEW

Dual role of BMP signaling in the regulation of *Drosophila* intestinal stem cell self-renewal

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ABSTRACT

Many adult organs including *Drosophila* adult midguts rely on resident stem cells to replenish damaged cells during tissue homeostasis and regeneration. Previous studies have shown that, upon injury, intestinal stem cells (ISCs) in the midguts can increase proliferation and lineage differentiation to meet the demand for tissue repair. Our recent study has demonstrated that, in response to certain injury, midguts can expand ISC population size as an additional regenerative mechanism. We found that injury elicited by bleomycin feeding or bacterial infection increased the production of two BMP ligands (Dpp and Gbb) in enterocytes (ECs), leading to elevated BMP signaling in progenitor cells that drove an expansion of ISCs by promoting their symmetric self-renewing division. Interestingly, we also found that BMP signaling in ECs inhibits the production of Dpp and Gbb, and that this negative feedback mechanism is required to reset ISC pool size to the homeostatic state. Our findings suggest that BMP signaling exerts two opposing influences on stem cell activity depending on where it acts: BMP signaling in progenitor cells promotes ISC self-renewal while BMP signaling in ECs restricts ISC self-renewal by preventing excessive production of BMP ligands. Our results further suggest that transient expansion of ISC population in conjunction with increasing ISC proliferation provides a more effective strategy for tissue regeneration.

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Drosophila adult midguts contain intestinal stem cells (ISCs) that can self-renew and undergo lineage differentiation to produce two types of mature cells: enterocytes (ECs) and enteroendocrine cells (EEs).[1,2] In the EC lineage, an ISC can undergo asymmetric cell division to produce one daughter cell that retains ISC fate (self-renewal) and another daughter cell that adopts enteroblast (EB) fate. Notch (N) signaling restricts ISC self-renewal by promoting its differentiation into EB.[1,2] Asymmetric cell division that produce an ISC/EB pair accounts for the majority of ISC divisions under homeostatic conditions; however, at low frequency, an ISC can undergo symmetric self-renewing division to produce two ISCs or symmetric differentiation division to produce two EBs.[3-6]

The balance between ISC self-renewal and differentiation appears to be regulated by both cell intrinsic and extrinsic mechanisms that influence N signaling.[7] One study showed that the Par complex and integrins direct the apical basal division of ISCs, resulting in asymmetric segregation of aPKC into the apical daughter cells, and that aPKC promotes the EB fate through N signaling.[4] Another study showed that Sara endosomes are asymmetrically segregated into the EB-fated ISC daughters, and that the N ligand Delta (Dl) is associated with the Sara endosomes and rapidly degraded by lysosomes, which may contribute to the biased N pathway activation in EBs.[8] However, perturbation of either aPKC or Sara function only modestly altered the balance between ISC self-renewal and differentiation, raising a possibility for the involvement of additional mechanism(s). Indeed, our previous study provided evidence that EC-derived Dpp and Gbb generate a "basal high" and "apical low" BMP activity gradient so that after an apical-basal ISC division, the basally localized daughter cell transduces higher levels of BMP signaling than the apical one (Fig. 1A).[3] We further showed that BMP signaling

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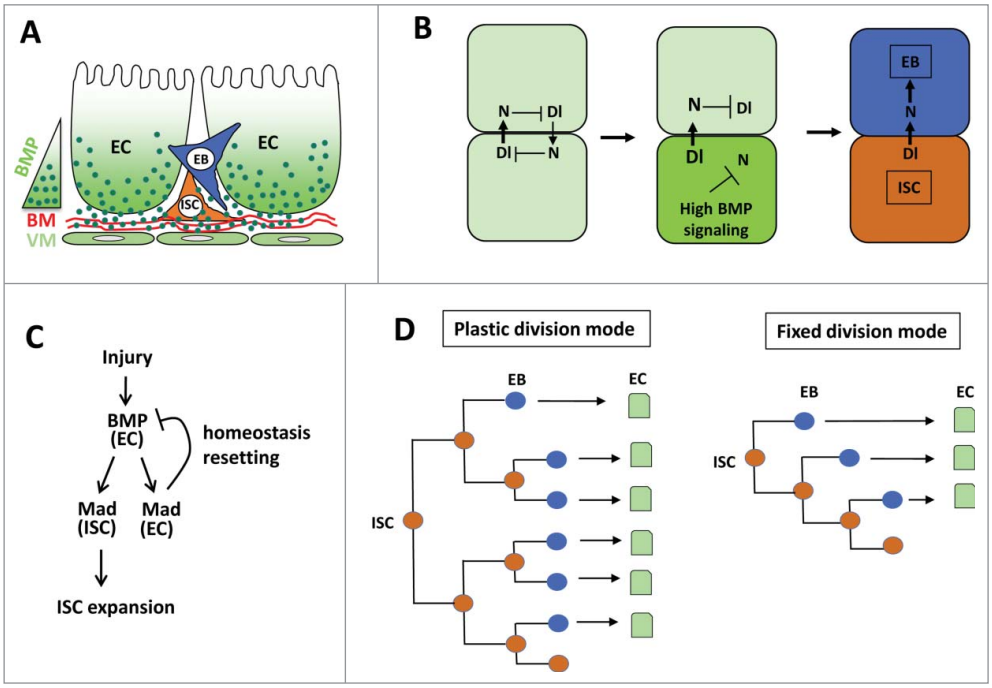

Figure 1. Injury stimulated and self-restrained BMP signaling regulates stem cell pool size by antagonizing N. (A) Dpp and Gbb are produced by ECs. Basal secretion coupled with basement membrane (BM) trapping sets up an apical-basal BMP activity gradient consisting of Dpp-Gbb heterodimers. (B) After an apical basal division, the two ISC daughter cells are initially equivalent with respect to N signaling. However, the basally localized cell transduces higher level of BMP signaling activity than the apical one to inhibit N and upregulate Dl. The initial small difference in N pathway activity is amplified by feedback mechanisms, leading to unidirectional N signaling and bistable cell choice with the basal cell sending the N signal and becoming ISC whereas the apical one transducing N signal and differentiating into EB. (C) Injury stimulates the production of BMP in ECs that activates Mad in progenitor cells to promote symmetric self-renewal division, leading to an expansion of ISC pool size. Elevated BMP signaling in ECs inhibits ligand production to reset ISC homeostasis. (D) Transient expansion of ISC by increasing the symmetric self-renewing division followed by excessive symmetric differentiation division (plastic division mode) produces more new cells in given rounds of division compared with the condition where ISCs undergo strictly asymmetric division (fixed division mode). BM, basement membrane; VM, visceral muscle. Adapted from Tian et al.[15]

promotes ISC fate by antagonizing N-mediated differentiation in the basally-localized ISC daughter cells (Fig. 1B).[3] As such, loss of BMP signaling resulted in ectopic N pathway activity in the basally-localized ISC daughter cells, which drove their precocious differentiation into EBs, leading to stem cell depletion. On the other hand, ectopic BMP signaling inhibited ISC-to-EB differentiation, leading to the formation of large clusters of stem cell like cells called "ISC tumors".[3] Hence, EC-derived BMP ligands serve as niche signals to promote ISC self-renewal. The profound loss and gain of ISC phenotypes associated with the perturbation of BMP signaling argue that this cell extrinsic mechanism plays a critical role in controlling the balance between ISC self-renewal and differentiation.

Under homeostatic conditions, *Drosophila* midguts turn over slowly and ISCs remain relatively quiescent. In response to injury caused by genetic cell ablation, chemical feeding, or bacterial infection, the damaged guts speed up ISC division and lineage differentiation to effectively replace damaged cells.[9-14] Several signaling pathways including the JAK-STAT, EGFR, Hippo, Hedgehog, BMP and Wnt pathways have been implicated in the control of stem cell proliferation and lineage differentiation.[7] Whether midguts also change the division mode of ISCs to increase their population size in favor of regeneration has remained largely unexplored. Our recent study revealed that midguts injured by bleomycin feeding or bacterial infection not only increased ISC proliferation but also transiently expanded stem cell population size as a part of their regenerative program.[15]

Upon injury elicited by bleomycin feeding, the expression of both *dpp* and *gbb* was upregulated in ECs whereas BMP signaling, as revealed by phospho-Mad (pMad) staining, was elevated in both progenitor cells and ECs through paracrine and autocrine signaling, respectively (Fig. 1C).[15] Bleomycin-induced upregulation of BMP signaling in progenitor cells correlated with increased ISC (marked by Dl expression) number.

Furthermore, two-color lineage tracing experiments revealed that bleomycin induced the expansion of ISCs by switching their division mode from asymmetric division to symmetric self-renewing division, which was largely inhibited in *dpp* heterozygous midguts,[15] suggesting that elevated BMP production is critical for ISC expansion. In addition, inhibition of BMP signaling activity in ISCs blocked bleomycin-stimulated symmetric self-renewing division and ISC expansion; as a consequence, midgut regeneration was severely compromised.[15]

Using various reporters, several labs observed *dpp* expression in a number of cell types including ECs,[3,16] visceral muscles (VM) that surround the gut epithelium,[17-19] and trachea cells that reach the intestinal cells through the visceral muscles.[20] In addition, it was shown that bleomycin also increased *dpp* expression in the VM[17] whereas bacterial infection recruited *dpp*-expressing haemocytes to the midgut.[21] However, we found that depletion of Dpp from either VM cells, trachea cells, or haemocytes by tissue specific RNAi did not affect bleomycin-induced ISC expansion.[15] By contrast, depletion of Dpp and Gbb from ECs diminished bleomycin-induced ISC expansion, suggesting that injury-stimulated BMP production from ECs drives ISC expansion.[15] Our previous study showed that the BMP activity responsible for driving ISC self-renewal depends on both Dpp and Gbb that appear to form a heterodimer.[3] In the midguts, Gbb is produced exclusively in ECs,[3,19] and its basal enrichment depends on EC-produced Dpp because both Dpp homodimer and Dpp-Gbb heterodimer are preferentially secreted on the basal side of the ECs whereas Gbb alone does not exhibit a directional secretion.[3] In addition, Dpp-Gbb heterodimer is trapped on the basement membrane (BM) via physical interaction between Dpp and the type IV collagens enriched on the BM, which contributes to formation of a steep BMP activity gradient.[3] Therefore, EC-derived Dpp not only provides a source of BMP but also promotes basal secretion of Gbb, which is critical for the generation of basally enriched BMP activity composed of Dpp-Gbb heterodimers. This may explain why Dpp produced from other cells cannot functionally replace Dpp produced in ECs in the regulation of ISC self-renewal. In support of this notion, we found that a combined overexpression of Gbb and Dpp but not overexpression of Dpp or Gbb alone in visceral muscle cells could induce ectopic ISC formation (our unpublished observation). How Dpp-Gbb heterodimers are secreted basally remains an important and unsolved question. Two GEF/Rab GTPase pairs, Crag/Rab10 and Stratum/Rab8, as well as the phosphoinositide PI(4,5) P2 and the protease-like protein Scarface, control the basal secretion of basement membrane proteins in *Drosophila* epithelial cells.[22-26] Therefore, it would be interesting to determine whether these molecules are also involved in directing the basal secretion of Dpp-Gbb heterodimers.

Injury-stimulated ISC expansion is transient in nature. For example, ISC number increased significantly after a 24-hour feeding with bleomycin; however, after a 24-hour recovery following the injury, ISC poor size returned to its homeostatic level.[15] The expansion and contraction of ISC population during tissue repair correlated with the dynamic change in BMP ligand production: the expression of both *dpp* and *gbb* increased after the 24-hour bleomycin feeding but dropped below the basal level after the 24-hour recovery.[15] Monitoring *dpp* expression using *dpp-Gal4* driven GFP (*dpp>GFP*) and pMad staining simultaneously revealed that BMP ligand production and pathway activity were very heterogeneous in ECs of bleomycin-fed guts with high pMad signals correlated with low levels of *dp*p expression. The heterogeneous pattern of *dpp>GFP* expression and pMad staining became more severe over time so that after 48-hour treatment with bleomycin, many cells exhibited high levels of pMad staining but no *dpp>GFP* expression while others exhibit *dpp>GFP* expression but no detectable pMad staining. The reciprocal pattern of *dpp>GFP* expression and pMad signal suggests that BMP signaling in ECs may inhibit the production of its own ligands. Indeed, experiments involving both gain and loss of BMP signaling activity in ECs confirmed that *dpp* and *gbb* expression is negatively regulated by BMP pathway activity.[15] Importantly, blocking this autoinhibition by dampening BMP signaling in ECs sustained BMP production after injury and prevented the decline of ISC number during the recovery period,[15] suggesting that BMP autoinhibition provides a mechanism to reset the homeostatic state of ISC pool size (Fig. 1C). We found that the expression of *dpp* and *gbb* dropped well below the basal levels during the recovery period, which could result in a transient reduction in BMP signaling in progenitor cells, and as a consequence, a reduction in ISC self-renewing capacity. Indeed, two color lineage tracing

experiments indicated a transient increase in symmetric differentiation division during the recovery period, which led to the production of more EBs at the expanse of ISCs.[15]

Inhibition of BMP ligand production by EC BMP signaling is also essential for maintaining ISC homeostasis. Previous studies revealed that inhibition of BMP signaling in ECs resulted in ISC overproliferation in part due to an increased production of Upd3 and elevated JAK-STAT pathway activity.[3,20] Consistent with this, hypomorphic BMP pathway mutant clones (e.g. *mad*^{1,2}) not only grew larger than control clones but also stimulated the proliferation of neighboring wild type ISCs.[3] In addition, *mad*^{1,2} clones were also associated with ectopic ISCs both inside and outside clones (our unpublished observation), implying that mutant clones with defect in BMP pathway activity produced a diffusive signal that promotes ISC self-renewal. Indeed, knockdown of Mad or the BMP pathway receptors Tkv and Put in ECs resulted in elevated expression of both *dpp* and *gbb*, enhanced BMP pathway activity in progenitor cells, and expansion of ISC number accompanied by increased mitotic index.[15] Importantly, knockdown of Dpp/Gbb in Mad-depleted ECs or in *mad*$^{1-2}$ mutant clones attenuated the increase in ISC number and mitotic index (our unpublished observation),[15] suggesting that elevated BMP production in BMP signaling deficient ECs is responsible for the observed ISC expansion. In keeping with the notion that BMP signaling in ECs inhibits BMP ligand production, excessive activation of the BMP pathway in ECs by overexpression of Tkv suppressed bleomycin-stimulated ISC expansion due to blunted *dpp* and *gbb* expression.[15] Taken together, these observations suggest that BMP signaling in ECs plays a negative role in the regulation of ISC self-renewal by restricting BMP ligand production. This negative feedback mechanism safeguards the homeostatic state of ISC population by preventing excessive BMP production and ectopic ISC formation during normal gut homeostasis and by downsizing the transiently-expanded ISC population to the homeostatic level after tissue repair. Hence, BMP signaling plays a dual role in the regulation of ISC self-renewal and exerts two opposing influences on stem cell activity depending on where it acts: BMP signaling in progenitor cells promotes ISC self-renewal while BMP signaling in ECs restricts it by preventing excessive production of BMP ligands. Interestingly, Wingless (Wg) signaling also plays an analogous dual role in the regulation of ISC proliferation: Wg signaling in progenitor cells promotes ISC proliferation in response to injury whereas Wg signaling in ECs inhibits ISC proliferation indirectly by restricting the production of JAK-STAT pathway ligands.[27,28]

What could be the advantage of having transient expansion of ISC pool as part of the regenerative program? In *Drosophila* midguts, ISC is the only cell type that can undergo mitotic division. Indeed, increasing the proliferation rate of ISC appears to be a universal response in all the injury models studied so far although the underlying mechanisms could vary.[7] However, injury-induced ISC expansion were only observed in association with specific tissue damage. For example, both DSS and bleomycin feeding can stimulate ISC proliferation but only bleomycin but not DSS can increase BMP production to stimulate ISC expansion.[15] Because bleomycin feeding resulted in EC loss whereas DSS did not, there could be a more urgent need for a rapid production of new cells to replenish the lost ECs in bleomycin-fed guts. As shown in Figure 1D, a transient expansion of ISC pool size by increasing the symmetric self-renewing division followed by excessive symmetric differentiation division can produce more new cells after fixed rounds of cell division in comparison to the condition where ISCs undergo strictly asymmetric division.

How BMP expression is stimulated upon injury remains to be determined. Bleomycin feeding causes EC damage whereas DSS only disrupts the basement membrane without causing EC loss.[9] Perhaps damaged ECs could activate a "stress signal" to stimulate BMP expression. How does BMP signaling negatively regulate the expression of BMP ligand? Mad could directly bind to the regulatory regions of *dpp* and *gbb* to inhibit their expression. Alternatively, Mad could act indirectly by activating a repressor(s) that inhibits *dpp* and *gbb* expression. Identification and characterization of the auto-inhibitory elements from the regulatory regions of *dpp* and *gbb* should provide an answer. How BMP activity gradient is generated and how it antagonizes N signaling to promote ISC self-renewal remain to be explored. The observation that BMP pathway deficient progenitor cells exhibited N pathway activity in the absence of detectable Dl expression implies that loss of BMP signaling in ISC daughter cells may unleash ligand-independent N pathway activity. Further genetic and biochemical

studies are needed to delineate the precise mechanism by which BMP inhibits N signaling. It will also be interesting to determine how ISC daughter cells integrate extrinsic signals such as BMP with intrinsic mechanisms such as asymmetric inheritance of aPKC to influence N signaling and the choice between self-renewal and differentiation. Finally, the regenerative mechanism involving transient expansion of stem cell pool size as a result of dynamic change in niche signal production in response to injury is likely to be utilized in other systems. Indeed, it has been implicated that injury-stimulated upregulation of Wnt signaling may transiently expand satellite stem cell pool size to enhance muscle regeneration.[29] Future study is required to elucidate whether this mechanism is more broadly utilized in other regenerative paradigms.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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