Comment on “Dynamics of Dpp Signaling and Proliferation Control”

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Wartlick et al. (Research Articles, 4 March 2011, p. 1154) reported that growth rates in the Drosophila wing disc correlate with increasing Dpp signaling levels, suggesting that the rate of Dpp increase determines the cell-cycle length. Contradicting their model, we found that cells in which the increase of Dpp signaling levels was genetically abrogated grew at rates comparable to those of wild-type cells.

Patternning and growth of organs are regulated by morphogen gradients. In a recent work, Wartlick et al. (1) analyzed the formation of the anterior-posterior (A-P) Decapentaplegic (Dpp) gradient during Drosophila wing disc development. The authors found that the amplitude and the decay length of the morphogen gradient increase during development.

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Fig. 1. Madbrk clones: Constant dad-GFP levels and wild-type growth rates. The generation of Mad brk mutant clones has been described previously (15). Clones were induced at 36 hours after hatching. Mutant clones are marked by the loss of arm-lacZ (A to O) or by the loss of ubi-GFP (O to F). (A) Examples for posterior dad-GFP gradients of wing discs containing Madbrk mutant clones. (B) dad-GFP levels in medial Mad brk mutant clones (red) and the amplitude of dad-GFP levels at the A-P axis in wild-type discs (black), plotted according to the length of the discs. (C) Same measurements as in (B), but plotted according to the time points when the discs were collected. No trend toward increasing dad-GFP levels in mutant clones was observed (60 hours, 13.0 ± 0.9; 72 hours, 9.7 ± 0.3; 84 hours, 9.3 ± 0.4; 96 hours, 10.9 ± 0.7; 108 hours, 11.1 ± 0.5). (D) Examples for wing discs containing Madbrk/wild-type twin spots at different time points after hatching. Only medial twin spots that did not fuse with neighboring clones (marked with asterisks) were used for the statistical analysis. The medial area is illustrated by the dashed lines. (E) Average size-ratio of the individual Madbrk/wild-type twin spots for the different time points when discs were collected. The difference remains within 20%. (F) Average area of mutant clones and the wild-type twin spots for the different time points. All error bars represent the mean ± SEM.
the target brk. We used the Mad^{d2} allele (10)—which comprises a premature stop codon upstream of the Dpp receptor phosphorylation site and therefore represents a situation in which Dpp responsiveness is completely abrogated (11)—and the amorphic brk^{M68} allele (2).

We first tested whether Dpp signaling levels in Mad^{d2}brk^{M68} mutant cells are indeed constant during development and compared the dynamics of Dpp signaling levels in mutant versus wild-type wing disc cells. We focused our analysis on the medial area (defined as the area within the decay length of dad from the Dpp source), where Dpp signaling levels are high and the increase can be measured accurately (1). Moreover, in the medial area brk is normally repressed by high Dpp signaling levels so that medial Mad^{d2}brk^{M68} clones differ from the wild type only in their lack of Mad. As a transcriptional read-out for Dpp signaling activity, we used a dad-nGFP reporter (12), which contains the same dad enhancer that Wartlick et al. used. We first analyzed dad-nGFP levels in wild-type discs. The increasing levels during development correlate with wing disc size (Fig. 1A-C) and thus support the hypothesis of a dynamic Dpp gradient (1). However, when monitoring dad-GFP in Mad^{d2}brk^{M68} mutant clones, reporter expression remained constant at low levels throughout development (Fig. 1A-C). These findings therefore confirm that Dpp signaling levels do not increase in such clones and validate the use of this tool to test the growth model of Wartlick et al. (1).

Next, we measured the growth rates of medial Mad^{d2}brk^{M68} mutant clones during wing disc development and compared them with those of their wild-type twin spots. Strikingly, Mad^{d2}brk^{M68} mutant clones and wild-type clones grew at the same rate (Fig. 1, D to F), as assessed at the population level (Fig. 1F) and when the ratios of mutant and corresponding wild-type twin spots were compared directly (Fig. 1E). Thus, our experiments reveal that increasing Dpp signaling levels are not necessary for driving cell proliferation. Rather, the results are in agreement with previously suggested models, in which Dpp signaling plays a permissive role in allowing proliferation. The results are in agreement with recently suggested models, in which Dpp signaling plays a permissive role in allowing proliferation by repressing the expression of the growth inhibitor Brk (6, 13, 14). In these models, position-independent growth rates are explained by the integration of complementary signals from additional growth modulators (14).

Taken together, our results confirm the observation that Dpp signaling levels increase during wild-type wing disc development but contradict the model in which this increase is the cause for cell proliferation. It will be interesting to find out why the Dpp signaling activity adopts to the disc size with increasing amplitudes.

References and Notes

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