Comment on "Dynamics of Dpp Signaling and Proliferation Control"

Gerald Schwank,* Schu-Fee Yang, Simon Restrepo, Konrad Basler†

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.

Patterning 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 and that the gradient scales with tissue size. Because cell proliferation is spatially uniform, and the relative position of a cell within the disc does not change, their finding implies that all cells sense the same relative increase of Dpp levels during wing disc growth. This relative increase of Dpp signaling levels was found to be about 50% during each cell cycle. Wartlick *et al*. thus proposed a model in which the temporal increase of Dpp signaling controls growth: The cell cycle length is determined by how fast a 50% increase of cellular Dpp signal is reached. Their model therefore enables them to explain how position-independent growth rates can be achieved by the Dpp morphogen gradient. However, it is difficult to reconcile this model with previously published data. Several independent studies had demonstrated that Dpp plays a permissive role in growth regulation, allowing proliferation by suppressing the expression of the growth repressor Brk (*2*–*4*). Moreover, it has been demonstrated that wing discs mutant for hypomorphic *dpp* and *brk* alleles overgrow (*5*, *6*), and recently Hamaratoglu *et al*. showed that the Dpp signaling gradient does not scale to the same extent throughout the wing disc (*7*). Here, we reassessed the growth model by generating clones in which the Dpp signaling pathway was fully abrogated. Our results demonstrate that Dpp signaling levels in such clones did not increase, yet growth was normal. These findings therefore clearly imply that an increase in Dpp signaling is not required to drive cell proliferation.

In *Drosophila*, Dpp signaling is mediated by the sole Smad protein Mad (*8*). Upon phosphorylation via the typeI-typeII Dpp receptor complex, Mad binds to Medea and translocates to the nucleus to regulate target gene expression (*8*, *9*). Whereas some target genes are activated by Dpp signaling, others are repressed (*3*). One such repressed target is *brk*, which encodes a transcriptional repressor that is a key mediator for Dpp-dependent patterning and growth (*2*, *4*, *5*). To create a situation where Dpp signaling levels are constant, we fixed Dpp signaling genetically by removing the transcription factor *Mad* as well as

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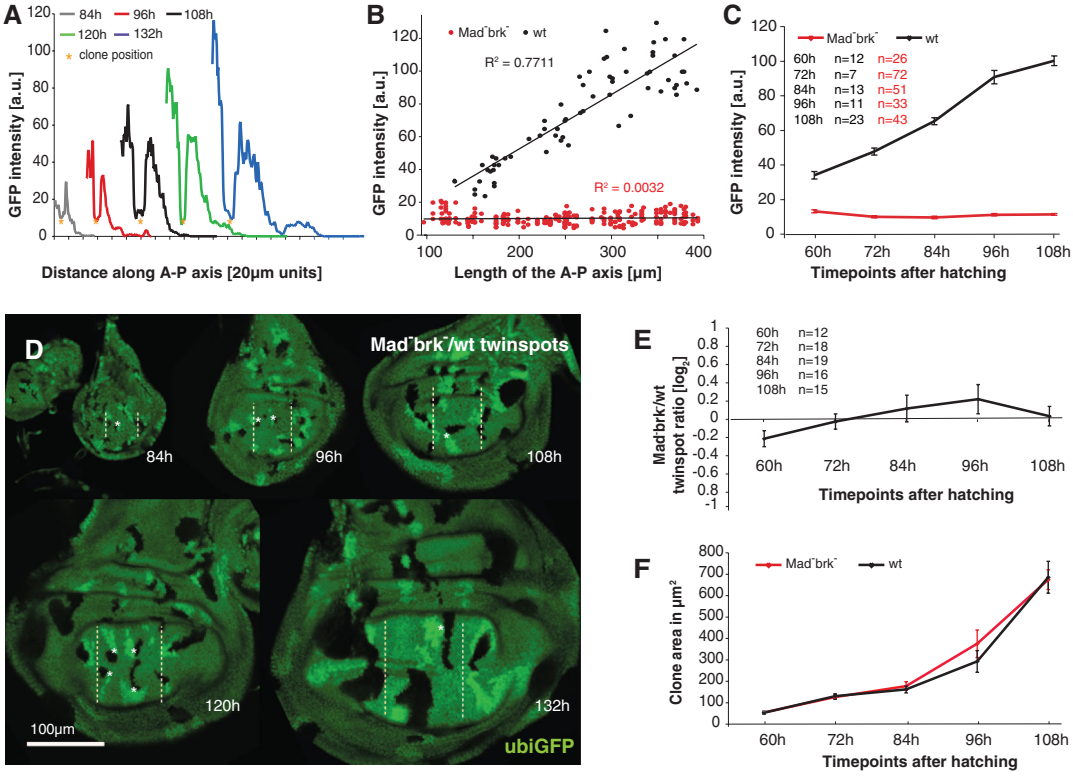

Fig. 1. *Mad*^{12}*brk*^{M68} clones: Constant *dad-GFP* levels and wild-type growth rates. The generation of *Mad brk* mutant clones has been described previously (*15*). Clones were induced at 36 hours after hatching. Mutant clones are marked by the loss of *arm-lacZ* (A to C) or by the loss of *ubi-nGFP* (D to F). (**A**) Examples for posterior *dad-GFP* gradients of wing discs containing *Mad*^{12}*brk*^{M68} 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 *Mad*^{12}*brk*^{M68}/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 *Mad*^{12}*brk*^{M68}/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^{12} 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 $Mad^{12}brk^{M68}$ 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 $Mad^{12}brk^{M68}$ 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 $Mad^{12}brk^{M68}$ 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 $Mad^{12}brk^{M68}$ mutant clones during wing disc development and compared them with those of their wild-type twin spots. Strikingly, $Mad^{12}brk^{M68}$ 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 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

1. O. Wartlick *et al*., *Science* **331**, 1154 (2011).
2. A. Jaźwińska, N. Kirov, E. Wieschaus, S. Roth, C. Rushlow, *Cell* **96**, 563 (1999).
3. T. Marty, B. Müller, K. Basler, M. Affolter, *Nat. Cell Biol.* **2**, 745 (2000).
4. M. Minami, N. Kinoshita, Y. Kamoshida, H. Tanimoto, T. Tabata, *Nature* **398**, 242 (1999).
5. G. Campbell, A. Tomlinson, *Cell* **96**, 553 (1999).
6. G. Schwank, S. Restrepo, K. Basler, *Development* **135**, 4003 (2008).
7. F. Hamaratoglu, A. M. de Lachapelle, G. Pyrowolakis, S. Bergmann, M. Affolter, *PLoS Biol* **9**, e1001182 (2011).
8. J. Kim, K. Johnson, H. J. Chen, S. Carroll, A. Laughon, *Nature* **388**, 304 (1997).
9. J. B. Hudson, S. D. Podos, K. Keith, S. L. Simpson, E. L. Ferguson, *Development* **125**, 1407 (1998).
10. J. J. Sekelsky, S. J. Newfeld, L. A. Raftery, E. H. Chartoff, W. M. Gelbart, *Genetics* **139**, 1347 (1995).
11. E. Eivers *et al*., *PLoS ONE* **4**, e6543 (2009).
12. N. Ninov *et al*., *Curr. Biol.* **20**, 513 (2010).
13. F. A. Martín, A. Pérez-Garijo, E. Moreno, G. Morata, *Development* **131**, 4921 (2004).
14. G. Schwank *et al*., *Dev. Cell* **20**, 123 (2011).
15. G. Schwank *et al*., *PLoS Biol.* **9**, e1001111 (2011).

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