8. T. J. Brummel *et al.*, *Cell* **78**, 251 (1994).
9. E. Ruberte, T. Marty, D. Nellen, M. Affolter, K. Basler, *Cell* **80**, 889 (1995).
10. D. Nellen, R. Burke, G. Struhl, K. Basler, *Cell* **85**, 357 (1996).
11. T. Lecuit *et al.*, *Nature* **381**, 387 (1996).
12. R. Burke, K. Basler, *Development* **122**, 2261 (1996).
13. M. A. Singer, A. Penton, V. Twombly, F. M. Hoffmann, W. M. Gelbart, *Development* **124**, 79 (1997).
14. E. Martin-Blanco, J. C. Pastor-Pareja, A. Garcia-Bellido, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7888 (2000).
15. M. G. Ricos, N. Harden, K. P. Sem, L. Lim, W. Chia, *J. Cell Sci.* **112**, 1225 (1999).
16. M. Affolter, D. Nellen, U. Nussbaumer, K. Basler, *Development* **120**, 3105 (1994).
17. E. Moreno, K. Basler, G. Morata, *Nature* **416**, 755 (2002).
18. T. Adachi-Yamada, K. Fujimura-Kamada, Y. Nishida, K. Matsumoto, *Nature* **400**, 166 (1999).
19. T. Adachi-Yamada, M. B. O'Connor, *Dev. Biol.* **251**, 74 (2002).
20. M. Milan, L. Perez, S. M. Cohen, *Dev. Cell* **2**, 797 (2002).
21. J. Rosenblatt, M. C. Raff, L. P. Cramer, *Curr. Biol.* **11**, 1847 (2001).
22. T. Adachi-Yamada, M. B. O'Connor, *J. Biochem.* (*Tokyo*) **136**, 13 (2004).
23. A. J. Ingram, L. James, H. Ly, K. Thai, J. W. Scholey, *Kidney Int.* **58**, 1431 (2000).
24. M. J. Galko, M. A. Krasnow, *PLoS Biol.* **2**, E239 (2004).
25. M. Ramet, R. Lanot, D. Zachary, P. Manfruelli, *Dev. Biol.* **241**, 145 (2002).
26. G. Campbell, A. Tomlinson, *Cell* **96**, 553 (1999).
27. J. R. Howe *et al.*, *Nat. Genet.* **28**, 184 (2001).
28. J. R. Howe *et al.*, *Science* **280**, 1086 (1998).
29. A. P. Haramis *et al.*, *Science* **303**, 1684 (2004).
30. M. G. Sayed *et al.*, *Ann. Surg. Oncol.* **9**, 901 (2002).
31. We thank E. Benecchi for assistance with transmission electron microscopy; M. Schober and J. David Lambert for critical readings of the manuscript; K. Gibson, S. Cherry, and C. Micchelli for helpful discussions; and G. Campbell, T. Kornberg, L. Raftery, K. Basler, R. Padgett, and the Bloomington Drosophila Stock Center for fly stocks. Our sincere apologies to colleagues whose work we were unable to cite owing to space constraints. N.P. is an Investigator of the Howard Hughes Medical Institute and M.C.G. is supported by the Jane Coffin Childs Memorial Fund for Cancer Research.

Supporting Online Material
www.sciencemag.org/cgi/content/full/307/5716/1785/DC1
Materials and Methods
Figs. S1 to S4
References and Notes

1 September 2004; accepted 11 January 2005
10.1126/science.1104751

Extrusion of Cells with Inappropriate Dpp Signaling from *Drosophila* Wing Disc Epithelia

Jie Shen and Christian Dahmann*

Decapentaplegic (Dpp) is a signaling molecule that controls growth and patterning of the developing *Drosophila* wing. Mutant cells lacking Dpp signal transduction have been shown to activate c-Jun amino-terminal kinase (JNK)–dependent apoptosis and to be lost from the wing disc epithelium. These observations have led to the hypothesis that Dpp promotes cell survival by preventing apoptosis. Here, we show that in the absence of JNK-dependent apoptosis, mutant cells lacking Dpp signal transduction can survive; however, they are still lost from the wing disc epithelium. This loss correlates with extensive cytoskeletal changes followed by basal epithelial extrusion. We propose that Dpp promotes cell survival within disc epithelia by affecting cytoskeletal organization.

Signaling by members of the transforming growth factor–β (TGF-β) protein family is critical for epithelial growth and differentiation, and inappropriate signaling is common in cancer (*1*, *2*). Dpp, a TGF-β superfamily member related to bone morphogenetic protein (BMP) 2/4, is required for growth and patterning of the wing primordium (wing disc pouch) in *Drosophila* (*3*). Inappropriately reduced Dpp signaling leads to smaller wing size, activation of the JNK pathway, and apoptosis (*4*, *5*). These and other observations have led to the hypothesis that Dpp acts as a survival factor for wing disc cells by preventing activation of the JNK-dependent apoptotic pathway (*5*–*8*).

To test this hypothesis, we analyzed the ability of cells mutant for the *Drosophila* gene *thickveins* (*tkv*), which encodes a receptor essential for Dpp signal transduction (*3*), and the gene *basket* (*bsk*), which encodes JNK (*9*), to survive within the developing wing disc pouch. Marked "twinspots" composed of sibling double-mutant *tkv bsk* clones of cells and wild-type clones of cells were generated within the same wing disc by Flp-mediated mitotic recombination (*10*). The ratio of *tkv*$^-$ *bsk*$^-$ clones to sibling wild-type clones was determined and is referred to as the frequency of *tkv*$^-$ *bsk*$^-$ clone recovery. When clones were induced in first instar larvae, the frequency of *tkv*$^-$ *bsk*$^-$ clone recovery in the wing disc pouch of late-third instar larvae was only 24% (n = 187), consistent with previous observations (*6*). Apoptosis was blocked in the mutant clones (fig. S1). *bsk*$^-$ clones were recovered at high frequency (97%, n = 100). The low frequency of *tkv*$^-$ *bsk*$^-$ clone recovery suggests that the loss of cells lacking Dpp signal transduction from the wing disc pouch is largely independent of JNK-mediated apoptosis (supporting online material text). Thus, Dpp must use additional mechanisms to prevent loss of cells from the wing disc pouch.

To elucidate these mechanisms, we generated *tkv*$^-$ *bsk*$^-$ clones in first instar larvae and analyzed their morphology in the wing disc pouch of late-third instar larvae. In the pseudostratified epithelium (Fig. 1A), *bsk*$^-$ clones displayed a normal shape (Fig. 1B). In contrast, *tkv*$^-$ *bsk*$^-$ clones were shorter along their apical-basal axis, had lost contact to the apical epithelial surface (Fig. 1C), and formed cyst-like structures with the apical cell membranes facing the center of the clone instead of the disc lumen (Fig. 1D). Furthermore, *tkv*$^-$ *bsk*$^-$ cells lost E-cadherin–based junctions to heterozygous neighboring cells and made E-cadherin–based junctions to other *tkv*$^-$ *bsk*$^-$ cells within the same clone (Fig. 1E). Clones mutant for both *mothers against dpp* (*mad*), which encodes a transcription factor essential for Dpp signal transduction (*3*), and *bsk* formed cyst-like structures similar to *tkv*$^-$ *bsk*$^-$ clones (fig. S2).

Epithelial cell shape is largely determined by the cytoskeleton. Cell shape changes leading to the formation of cyst-like structures could thus be due to cytoskeletal organization defects in *tkv*$^-$ *bsk*$^-$ cells. F-actin was enriched at the center of *tkv*$^-$ *bsk*$^-$ clones (Fig. 1F), whereas a dense apical network of microtubules, present in wild-type cells (*11*), was markedly reduced (Fig. 1, G and H). Basal microtubules appeared normal in *tkv*$^-$ *bsk*$^-$ cells (Fig. 1G). Both actin and microtubule cytoskeletons were normal in *bsk*$^-$ clones (Fig. 1B) (*12*). These data indicate that Dpp signaling is required to maintain normal cytoskeletal organization in wing disc pouch cells. Furthermore, the presence of an apical microtubule network correlated with Dpp signaling activity along the anterior-posterior axis of wild-type wing discs (fig. S3), suggesting that Dpp signaling also plays a role in determining position-specific aspects of the microtubule cytoskeleton.

To test whether the loss of *tkv*$^-$ *bsk*$^-$ clones was through the formation of the cyst-like structures, we generated *tkv*$^-$ *bsk*$^-$ clones at different times during development and determined the number of cyst-like structures and the frequency of *tkv*$^-$ *bsk*$^-$ clone recovery in late-third instar larvae (Fig. 2A). Twenty-four hours after clone induction, 99% of *tkv*$^-$ *bsk*$^-$ clones were recovered (n = 93) (Fig. 2B). Cells within clones still had contact to the apical epithelial surface, appeared to make normal E-cadherin–based junctions with

Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany.

*To whom correspondence should be addressed. E-mail: dahmann@mpi-cbg.de

neighboring cells (Fig. 2C), and had undetectable levels of Dpp signal transduction (fig. S4). Induction of *tkv*⁻ *bsk*⁻ clones earlier in development resulted in the recovery of fewer clones (Fig. 2B). The *tkv*⁻ *bsk*⁻ clones that were recovered in the wing disc pouch of late-third instar larvae had formed cyst-like structures and were on average extruded farther to the basal side of the epithelium (Fig. 2, B and C). At 96 hours after clone induction, only 20% of the clones were recovered (n = 44), the majority of which had formed cyst-like structures and had been extruded from the basal side of the epithelium (Fig. 2, B to D). This suggests that the low frequency of *tkv*⁻ *bsk*⁻ clone recovery is due to the formation of cyst-like structures followed by extrusion from the basal side of the epithelium.

Some *tkv*⁻ *bsk*⁻ clones were observed in adult wings as extruded cyst-like structures located between the dorsal and ventral wing surfaces (Fig. 2, E to H), consistent with previous results (*8*, *13*). These *tkv*⁻ *bsk*⁻ cells displayed hairs, characteristic structures of the adult epithelium, indicating that the extruded cells were alive and had undergone differentiation. Thus, in the absence of JNK, cells unable to transduce Dpp form cyst-like structures, are extruded from the wing disc epithelium, and can survive.

Our results suggest that Dpp signaling is involved in regulating cytoskeletal organization and is critical for normal cell morphology and integrity of wing disc epithelia. These functions of Dpp signaling are consistent with its role in embryonic dorsal closure and pupal thorax closure (*14*, *15*). Hence, the regulation of cytoskeletal organization may be a more general function of Dpp not restricted to wing disc epithelia.

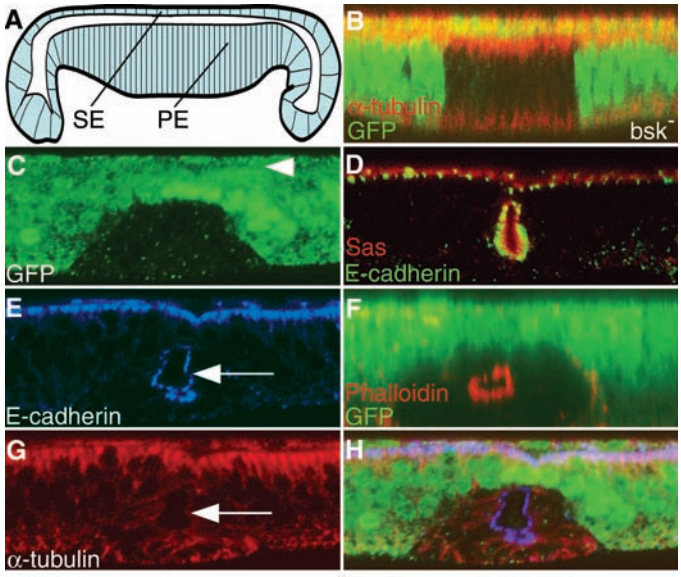

Fig. 1. Epithelial integrity requires Dpp signaling. (**A**) In wing discs, a monolayer of pseudostratified (PE) and squamous (SE) epithelial cells is arranged in a sac-like structure with the apical membranes facing a lumen. [(B) to (H)] Optical cross sections of wing discs. (**B**) *bsk*⁻ clones, labeled by the absence of green fluorescent protein (GFP) (green), have normal shape and α-tubulin staining (red). (**C**) *tkv*⁻ *bsk*⁻ clones (no GFP) are shorter and have lost contact from the apical epithelial surface (arrowhead). (**D**) Apical membranes of *tkv*⁻ *bsk*⁻ cells, as identified by Stranded at second (Sas) (red), face the center of the clone. E-cadherin staining is shown in green. (**E**) *tkv*⁻ *bsk*⁻ cells form E-cadherin–based junctions with one another in the center of the clone (arrow). (**F**) F-actin, as identified by phalloidin staining (red), is increased in the center of *tkv*⁻ *bsk*⁻ clones (no GFP). (**G**) The apical microtubule network, as visualized by α-tubulin staining, is highly reduced in *tkv*⁻ *bsk*⁻ clones (arrow). (**H**) Merge of panels (C), (E), and (G).

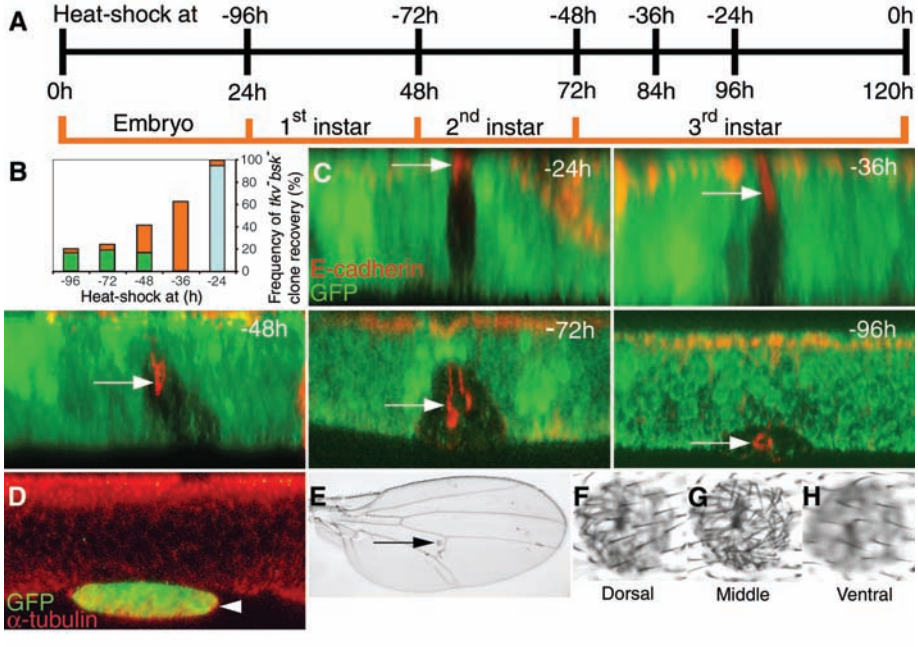

Fig. 2. Time course of extrusion of *tkv*⁻ *bsk*⁻ clones. (**A**) Timeline indicating how long before analysis larvae were heat shocked to induce clones. (**B**) Frequency of *tkv*⁻ *bsk*⁻ clone recovery expressed as a percentage. For each time point, the colors indicate the fraction of *tkv*⁻ *bsk*⁻ clones either with normal morphology (blue), constricted apically but still in contact with the apical epithelial surface (orange), or forming cyst-like structures that have lost contact from the apical epithelial surface (green). (**C**) Optical cross sections of wing discs with *tkv*⁻ *bsk*⁻ clones (no GFP, lack of green). Arrows point to E-cadherin staining (red) in mutant clones. Clones were induced at the indicated times before analysis. (**D**) Optical cross section of a wing disc with positively marked *tkv*⁻ *bsk*⁻ clones (arrowhead; GFP, green) induced 96 hours before analysis and stained for α-tubulin (red). (**E**) *tkv*⁻ *bsk*⁻ clones form cyst-like structures in the adult wing (arrow). [(F) to (H)] Higher magnification of (**F**) dorsal, (**G**) middle, and (**H**) ventral views of the wing area marked by the arrow in (E).

References and Notes

1. Y. Shi, J. Massague, *Cell* **113**, 685 (2003).
2. P. M. Siegel, J. Massague, *Nature Rev. Cancer* **3**, 807 (2003).
3. L. A. Raftery, D. J. Sutherland, *Dev. Biol.* **210**, 251 (1999).
4. P. J. Bryant, *Dev. Biol.* **128**, 386 (1988).
5. T. Adachi-Yamada, K. Fujimura-Kamada, Y. Nishida, K. Matsumoto, *Nature* **400**, 166 (1999).
6. T. Adachi-Yamada, M. B. O'Connor, *Dev. Biol.* **251**, 74 (2002).
7. E. Moreno, K. Basler, G. Morata, *Nature* **416**, 755 (2002).
8. M. Milan, L. Perez, S. M. Cohen, *Dev. Cell* **2**, 797 (2002).
9. J. R. Riesgo-Escovar, M. Jenni, A. Fritz, E. Hafen, *Genes Dev.* **10**, 2759 (1996).
10. Materials and methods are available as supporting material on *Science* Online.
11. S. Eaton, R. Wepf, K. Simons, *J. Cell Biol.* **135**, 1277 (1996).
12. J. Shen, C. Dahmann, data not shown.
13. N. Azpiazu, G. Morata, *Development* **127**, 2685 (2000).
14. M. G. Ricos, N. Harden, K. P. Sem, L. Lim, W. Chia, *J. Cell Sci.* **112**, 1225 (1999).
15. E. Martin-Blanco, J. C. Pastor-Pareja, A. Garcia-Bellido, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7888 (2000).

16. We thank T. Adachi-Yamada and K. Basler for fly stocks; K. Basler, S. Eaton, T. Hyman, K. Simons, and H. Thompson for comments on the manuscript; and M. Gibson and N. Perrimon for exchanging manuscripts before publication. This work was supported by the Max Planck Society.

Supporting Online Material

www.sciencemag.org/cgi/content/full/307/5716/1789/DC1
Materials and Methods
SOM Text
Figs. S1 to S4
References

2 September 2004; accepted 11 January 2005
10.1126/science.1104784