Small Peptides Switch the Transcriptional Activity of Shavenbaby During Drosophila Embryogenesis

T. Kondo, *et al.*

Science **329**, 336 (2010);
DOI: 10.1126/science.1188158

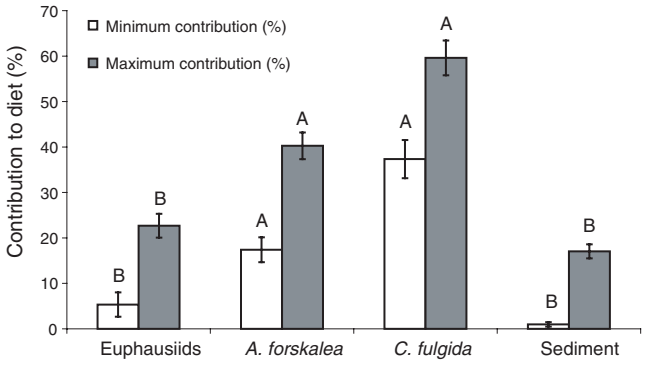

Fig. 3. Isotope analyses showing maximum and minimum contribution of euphausiids, jellyfish, and sediment to the diet of *S. bibarbatus*, as determined by a four-endpoint Isosource model (based on carbon and nitrogen) (*19*). Bars denote mean ± SEM. Bars with identical letters were not significantly different from each other at an α level of 5% (Tukey test, $P < 0.05$; *A. forskalea*, $n = 11$; euphausiids, $n = 6$; *C. fulgida* $n = 25$; mud, $n = 5$ samples; *S. bibarbatus*, $n = 41$).

and ecological adaptations, is already playing a critical role in the ecosystem off Namibia, and it is likely to continue to do so into the future.

References and Notes

1. M. E. Carr, *Deep Sea Res. Part II Top. Stud. Oceanogr.* **49**, 59 (2001).
2. C. P. Lynam *et al.*, *Curr. Biol.* **16**, 1976 (2006).
3. P. Cury, L. Shannon, *Prog. Oceanogr.* **60**, 223 (2004).
4. A. Bakun, *Science* **247**, 198 (1990).
5. C. D. van der Lingen *et al.*, in *Benguela: Predicting a Large Marine Ecosystem. Large Marine Ecosystems 14*, L. V. Shannon, G. Hempel, P. Malanotte-Rizzoli, C. L. Moloney, J. Woods, Eds. (Elsevier, Amsterdam, 2006), pp. 147–184.
6. A. J. Richardson, A. Bakun, G. C. Hays, M. J. Gibbons, *Trends Ecol. Evol.* **24**, 312 (2009).
7. J. Yamamoto *et al.*, *Mar. Biol.* **153**, 311 (2008).
8. R. J. M. Crawford, L. V. Shannon, D. E. Pollock, *Oceanogr. Mar. Biol. Ann. Rev.* **25**, 353 (1987).
9. D. J. Boyer, I. Hampton, *S. Afr. J. Mar. Sci.* **23**, 5 (2001).
10. V. Brüchert, B. Currie, K. R. Peard, *Prog. Oceanogr.* **83**, 169 (2009).
11. V. Brüchert *et al.*, in *Past and Present Water Column Anoxia*, L. N. Neretin, Ed. (Springer, Netherlands, 2006), pp. 161–194.
12. G. Lavik *et al.*, *Nature* **457**, 581 (2009).
13. H. N. Schulz *et al.*, *Science* **284**, 493 (1999).
14. S. J. Weeks, B. Currie, A. Bakun, *Nature* **415**, 493 (2002).
15. S. J. Weeks, B. Currie, A. Bakun, K. R. Peard, *Deep Sea Res. Part I Oceanogr. Res. Pap.* **51**, 153 (2004).
16. J. Rogers, J. M. Bremner, *Oceanogr. Mar. Biol.* **29**, 1 (1991).
17. A. Bakun, S. J. Weeks, *Ecol. Lett.* **7**, 1015 (2004).
18. P. Fréon, M. Barange, J. Arístegui, *Prog. Oceanogr.* **83**, 1 (2009).
19. Materials and methods are available as supporting material on *Science* Online.
20. M. J. Gibbons *et al.*, *S. Afr. J. Mar. Sci.* **22**, 1 (2000).
21. T. Bagarinao, *Aquat. Toxicol.* **24**, 21 (1992).
22. F. J. Millero, T. Plese, M. Fernandez, *Limnol. Oceanogr.* **33**, 269 (1988).
23. T. Bagarinao, R. D. Vetter, *J. Comp. Physiol. B* **160**, 519 (1990).
24. R. Vaquer-Sunyer, C. M. Duarte, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 15452 (2008).
25. P. Domenici, C. Lefrançois, A. Shingles, *Philos. Trans. R. Soc. London Ser. B* **362**, 2105 (2007).
26. N. Matikainen, M. Vornanen, *J. Exp. Biol.* **167**, 203 (1992).
27. S. Kaartvedt, A. Røstad, T. A. Klevjer, *Mar. Ecol. Prog. Ser.* **390**, 237 (2009).
28. E. S. Todd, *Copeia* **2**, 374 (1976).
29. H. Pang, K. N. Bitar, *Am. J. Physiol. Cell Physiol.* **289**, C982 (2005).
30. D. Pauly, V. Christensen, V. J. Dalsgaard, R. Froese, F. Torres Jr., *Science* **279**, 860 (1998).
31. J. J. Heymans, L. J. Shannon, A. Jarre, *Ecol. Model.* **172**, 175 (2004).
32. P. M. S. Monteiro, A. K. van der Plas, J.-L. Melice, P. Florenchie, *Deep Sea Res. Part I Oceanogr. Res. Pap.* **55**, 435 (2008).
33. H. Hamukuaya, M. J. O'Toole, P. M. J. Woodhead, *S. Afr. J. Mar. Sci.* **19**, 57 (1998).
34. R. J. Diaz, R. Rosenberg, *Science* **321**, 926 (2008).
35. C. L. Prosser, F. A. Brown, *Comparative Animal Physiology* (Saunders, Philadelphia, 1961).

36. We thank the crew of the *G. O. Sars*; F. Midtøy for assistance; and P. Ellitson, M. Hordnes, R. Jones, R. Amundsen and the rest of the scientific crew. We thank the National Research Foundation of South Africa, the Research Council of Norway, and our home institutions for funding and support. We thank BENEFIT (Benguela Environment Fisheries Interaction and Training), S. Sundby, D. C. Boyer, J. Otto Krakstad, and the crew of the research vessel *Dr. Fridtjof Nansen* for support with earlier goby cruises, laying the basis for the present study. We thank K. Helge Jensen for statistical support. We appreciate the comments on this manuscript by J. Giske, C. Jørgensen, M. P. Heino, and the anonymous reviewers. Care and handling of experimental animals were performed in accordance with institutional guidelines. J.A.W.S. was a postdoctoral researcher funded by the Natural Sciences and Engineering Reserach Council of Canada at the time when the research was conducted.

Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5989/333/DC1
Materials and Methods
Figs. S1 to S10
Tables S1 and S2
References

9 April 2010; accepted 4 June 2010
10.1126/science.1190708

Small Peptides Switch the Transcriptional Activity of Shavenbaby During *Drosophila* Embryogenesis

T. Kondo,[1,2]*† S. Plaza,[3,4]* J. Zanet,[3,4] E. Benrabah,[3,4] P. Valenti,[3,4] Y. Hashimoto,[1,6]† S. Kobayashi,[1,5] F. Payre,[3,4]‡ Y. Kageyama[1,6]‡

A substantial proportion of eukaryotic transcripts are considered to be noncoding RNAs because they contain only short open reading frames (sORFs). Recent findings suggest, however, that some sORFs encode small bioactive peptides. Here, we show that peptides of 11 to 32 amino acids encoded by the *polished rice* (*pri*) sORF gene control epidermal differentiation in *Drosophila* by modifying the transcription factor Shavenbaby (Svb). Pri peptides trigger the amino-terminal truncation of the Svb protein, which converts Svb from a repressor to an activator. Our results demonstrate that during *Drosophila* embryogenesis, Pri sORF peptides provide a strict temporal control to the transcriptional program of epidermal morphogenesis.

Studies of eukaryotic genomes have revealed that a large proportion of genomic DNA produces atypical long transcripts, the functions of which are controversial (*1*–*4*). These transcripts contain only short open reading frames (sORFs, <100 codons) and thus are generally considered to be non-protein-coding RNAs (ncRNAs). However, there is growing evidence that the sORFs present in some ncRNAs are actually translated into small peptides, the abundance of which is probably greatly underestimated (*5*–*7*). Whereas sORF-encoded peptides may represent an overlooked repertoire of bioactive molecules (*8*), their functions and the mechanisms by which they operate are largely unknown.

We and others recently identified an evolutionarily conserved sORF gene, referred to as *polished rice* (*pri*) or *tarsal-less* (*tal*) in *Drosophila,* and *mille-pattes* (*mlpt*) in *Tribolium* (*9*–*11*). *pri* mRNA is a polycistronic transcript that encodes four similar peptides, 11 to 32 amino acids in length, that play a redundant role in *Drosophila* embryogenesis (*9*, *10*). Embryos that lack *pri* display prominent defects, including the absence of trichomes and aberrant tracheal architecture (*9*, *10*). Reduced *pri* activity in imaginal development results in abnormal leg morphogenesis (*10*, *12*). Similarly, *mlpt* knockdown in *Tribolium* leads to appendage defects and the transformation of segmental identity (*11*).

To gain insight into the molecular function of Pri peptides, we focused on their role in trichome formation during *Drosophila* embryogenesis.

Epidermis differentiation results in a pattern of smooth cells and cells that form apical extensions, called trichomes (ventral denticles and dorsal hairs) (Fig. 1A) (*13*). Modifications of the trichome pattern that have been examined in insects (resulting from laboratory-induced mutations or evolutionary diversification) are so far all attributable to changes in expression of *shavenbaby* (*svb*) (*14*–*16*). Indeed, *svb* encodes a transcription factor that directly regulates the expression of target effectors, which are collectively responsible for trichome formation (*17*, *18*). Although the absence of *pri* results in trichome loss, the expression of *svb* is not altered in *pri* mutants (*9*). Reciprocally, *pri* is expressed normally in *svb* mutants (*9*), showing that *svb* and *pri* act in parallel in trichome formation (fig. S1). Expression of Svb target genes, such as *miniature* and *shavenoid* (*17*), is lost in *pri* mutants, whereas the expression of other epidermal genes is unaffected (Fig. 1B and S2). The activity of isolated Svb-responsive enhancers was also strongly reduced in *pri* mutants (fig. S3). Therefore, *pri* is specifically required for the transcription of Svb downstream targets in trichome cells.

How can Pri peptides regulate the expression of Svb target genes without affecting *svb* expression? The *svb* locus encodes three overlapping protein isoforms: Svb and the germline-specific proteins OvoA and OvoB (Fig. 2A) (*19*, *20*). Ovo/Svb proteins all share the same DNA-recognition and transcriptional-activation domains but differ in their N-termini (Fig. 2A). The shortest isoform, OvoB, is a transcriptional activator and induces trichomes when artificially expressed in the epidermis (Fig. 2F) (*19*). OvoA contains an extended N-terminal region, which switches its function toward active transcriptional repression and thus dominantly inhibits trichome formation (Fig. 2C) (*19*). Svb contains a further N-terminal extension, compared to OvoA, and promotes the formation of ectopic trichomes like OvoB (Fig. 2D) (*20*). To evaluate the specificity of Pri/Svb interaction, we examined the influence of *pri* on the different Ovo/Svb isoforms with respect to trichome formation. In wild-type embryos, seven rows of ventral cells per segment express *svb* and form trichomes (Fig. 2B) (*13*, *15*). Upon its ectopic expression in smooth cells, Svb [or Svb:green fluorescent protein (GFP)] induced supernumerary trichomes in control embryos (Fig. 2D) but not in *pri* mutants (Fig. 2E). In contrast, OvoB (or OvoB:GFP) was insensitive to *pri*, with ectopic trichomes forming in both control and *pri* mutant embryos (Fig. 2, F

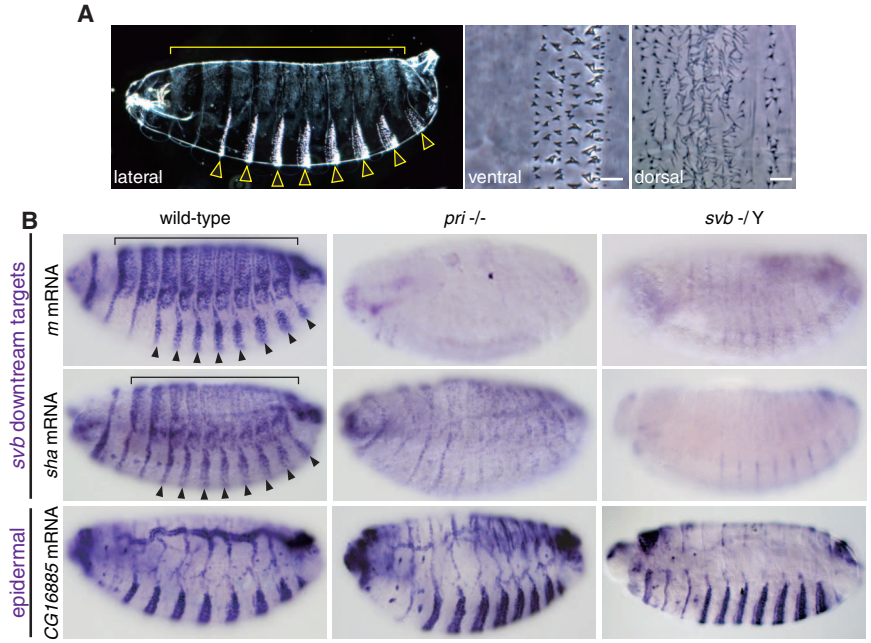

Fig. 1. *pri* is required for the expression of Svb target genes. (**A**) Embryonic cuticle specimens, showing the two types of trichomes, ventral denticles (arrowheads and middle close-up) and dorsal hairs (bracket and right close-up). (**B**) Two Svb downstream targets, *miniature* (*m*) and *shavenoid* (*sha*) are expressed in trichome cells at stages 15 and 16 in wild type, but not in *pri* and *svb* mutants. As a control of *pri* specificity for Svb function, the epidermal expression of *CG16885* (independent of *svb*) was not affected in *pri* mutants. Anterior is to the left and dorsal is to the top, except for the close-ups in (A). Scale bar, 10 μm.

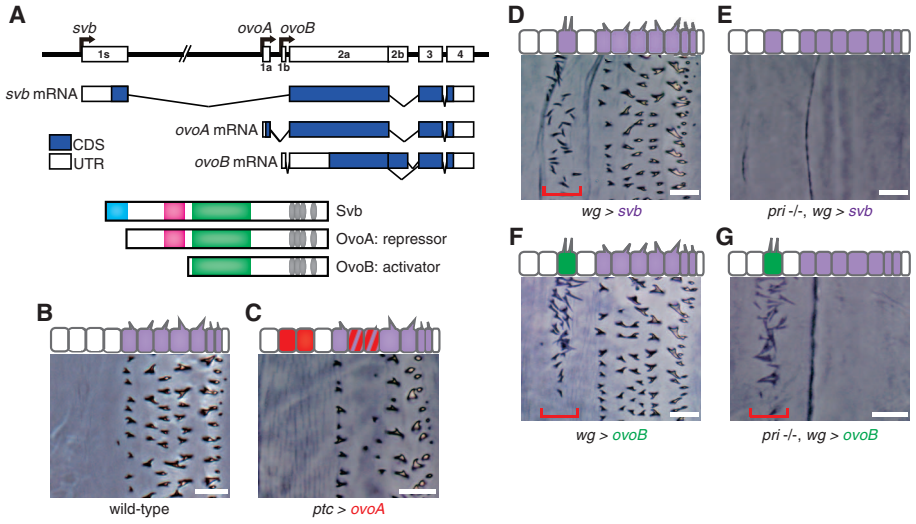

Fig. 2. The ability of Svb to induce trichomes depends on *pri*. (**A**) Scheme of the *ovo*/*svb* locus and protein isoforms. Coding (CDS) and untranslated regions (UTR) of mRNAs are represented by blue and white boxes, respectively. The Svb-specific protein region is in turquoise; the repression, activation, and DNA-binding domains are in red, green, and gray, respectively. (**B** to **G**) Micrographs of ventral trichomes (A4 segment) and illustrations of epidermal cells expressing *svb* (purple), *ovoA* (red), and *ovoB* (green) in embryos of different backgrounds. *pri* mRNA is widely expressed in epidermis and reinforced in trichome cells (fig. S1F). (B) Wild-type embryo. (C) Embryo expressing *UAS-ovoA:GFP* under the control of *ptc-GAL4*. (D) and (E) Embryos expressing *UAS-svb:GFP* under the control of *wg-Gal4* in the presence (D) or absence (E) of *pri*. (F) and (G) Embryos expressing *UAS-ovoB:GFP* under the control of *wg-Gal4* in the presence (F) or absence (G) of *pri*. Red brackets indicate ectopic trichomes. Scale bar, 10 μm.

[1]Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology (NIBB), National Institutes of Natural Sciences, 5-1 Myodaiji-Higashiyama, Okazaki 444-8787, Japan. [2]Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan. [3]Université de Toulouse, Université Paul Sabatier, Centre de Biologie du Développement, Bâtiment 4R3, 118 route de Narbonne, F-31062 Toulouse, France. [4]CNRS, UMR 5547, Centre de Biologie du Développement (CBD), F-31062 Toulouse, France. [5]Department of Basic Biology, School of Life Science, Graduate University for Advanced Studies (SOKENDAI), 38 Myodaiji-Nishigonaka, Okazaki 444-8585, Japan. [6]Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan.

*These authors contributed equally to this work.
†Present address: RIKEN Center for Developmental Biology, 2-2-3 Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan.
‡To whom correspondence should be addressed. E-mail: payre@cict.fr (F.P.); kageyama@nibb.ac.jp (Y.K.)

and G). In the latter case, we observed only OvoB-induced ectopic trichomes and no Svb-dependent endogenous trichomes (Fig. 2G). These results show that whereas *pri* has no effect on the shorter OvoB isoform, *pri* peptides specifically control the ability of Svb to induce trichomes.

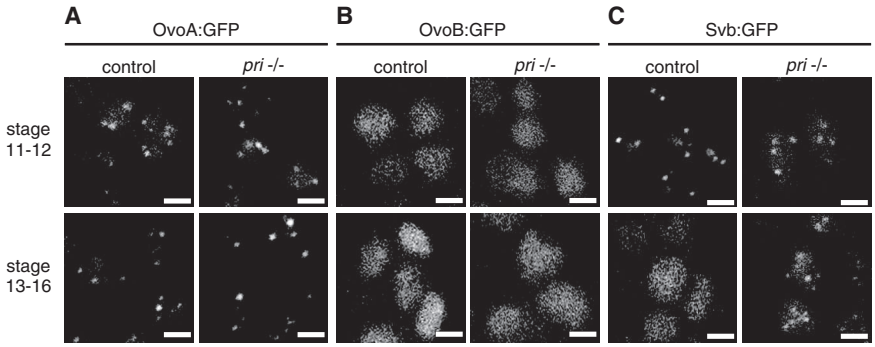

Fig. 3. *pri* regulates the subnuclear localization of Svb in living embryos. Distribution of (**A**) OvoA:GFP, (**B**) OvoB:GFP, and (**C**) Svb:GFP driven by *wg-GAL4* in control (*pri* +/–) or *pri* mutant embryos at stages 11 and 12 and stages 13 to 16. In all cases, the GFP signal was restricted to nuclei (fig. S4). Although the distribution of OvoA (focal) and OvoB (diffuse) was insensitive to *pri*, Svb switched from foci to diffuse in a *pri*-dependent manner. Scale bar, 10 μm.

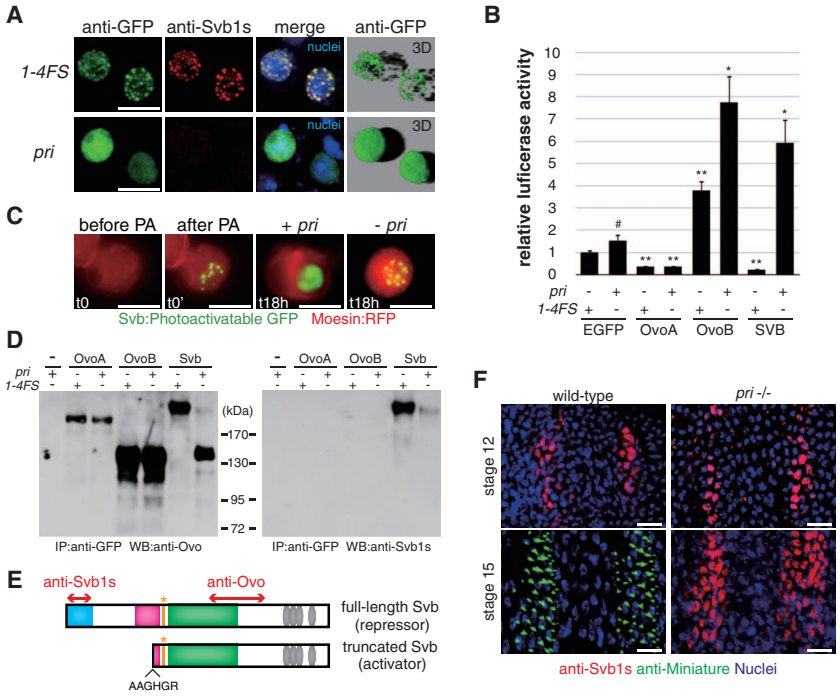

Fig. 4. Pri converts the Svb protein from a transcriptional repressor to an activator by N-terminal truncation. (**A**) Subnuclear localization of Svb:GFP in S2 cells when *pri* is co-expressed. *1-4FS*, a full-length *pri* mRNA with frame-shift mutations in ORF1-4 (*9*), was used as control. Cells were stained with antibody to GFP (green) and anti-Svb1s (red). Nuclei are in blue. The rightmost panel is a three-dimensional representation of Svb nuclear distribution. (**B**) Transcriptional activity of OvoA, OvoB, and Svb in S2 cells. Luciferase activity was used as a reporter for the transcriptional activity of the Enh-*m* enhancer. Error bars represent SE, and significance against GFP/*1-4FS*–transfected cells was evaluated with *t* tests (*$P < 0.05$, **$P < 0.01$, #$P > 0.05$). (**C**) Distribution of Svb:PA-GFP (green) in S2 cells, before photoactivation (t0), after photoactivation (t0′), and after the induction of *pri* expression (t18h). Without *pri* induction, Svb:PA-GFP was retained in foci (–*pri,* t18h). Red is Moesin:red fluorescent protein (RFP) used as a transfection control. (**D**) Western blots analysis of S2 cells expressing OvoA:GFP, OvoB:GFP, and Svb:GFP. Protein extracts were immunoprecipitated with antibody to GFP and probed with antibody to Ovo or anti-Svb1s. (**E**) Schematic representation of predicted form of truncated Svb. The N terminus of truncated Svb matches the sequence AAGHGR, which is located 56 amino acids upstream of the OvoB-initiating methionine (asterisk). Red arrows indicate the regions used to generate antibodies. (**F**) Ventral views of wild-type and *pri* mutant embryos stained with anti-Svb1s (red), and antibody to Miniature (green) that underlies nascent trichomes. Nuclei are in blue. Scale bar, 10 μm.

We next examined whether Pri peptides affect the synthesis or trafficking of Ovo/Svb proteins. Using transgenic C-terminal GFP-fusions (proven functional as described above), we observed that *pri* does not influence the production of Ovo/Svb proteins or their import to the nucleus (Fig. 3 and fig. S4). However, we noticed different patterns of their intranuclear distribution. Regardless of *pri* activity, throughout embryogenesis OvoA accumulated in discrete foci (Fig. 3A), and OvoB was distributed diffusely in the nucleoplasm (Fig. 3B). During stages 11 and 12, before *pri* is expressed in the epidermis (fig. S1), Svb formed intranuclear foci, like OvoA (Fig. 3C). At the onset of *pri* epidermal expression (stage 13 onwards) (fig. S1), the nuclear distribution of Svb became diffuse (Fig. 3C). Therefore, Svb distribution changes from a pattern similar to the OvoA repressor to that of the OvoB activator, and the timing of this conversion correlates with the expression of *pri*. This redistribution of Svb was abolished in *pri* mutants, in which Svb remained in nuclear foci throughout embryogenesis (Fig. 3C). Thus, *pri* participates in the conversion of nuclear distribution of Svb from punctate to diffuse.

The nonpunctuated, diffuse nuclear distribution of Svb in epidermal cells correlates with its ability to induce trichomes, suggesting that Svb redistribution coincides with active transcription of its targets. We explored this hypothesis using assays in *Drosophila* Schneider cells (S2 cells), which are of embryonic origin. Similarly to observations in embryos, the nuclear pattern of Svb was converted from punctate to diffuse in a *pri*-dependent manner in S2 cells (Fig. 4A). We quantified the transcriptional activity of Svb/Ovo using the Enh-*m* enhancer, which is directly activated by Svb in vivo (*17*) (fig. S3). OvoB strongly stimulated the transcription driven by Enh-*m,* and OvoA repressed transcription, both with or without *pri* (Fig. 4B). In contrast, Svb behaved like OvoA in the absence of *pri*, but similar to OvoB, activated Enh-*m* in the presence of Pri peptides (Fig. 4B). Inactivation of the Svb-binding site of Enh-*m* (*17*) suppressed this activation (fig. S5), indicating that *pri* is required for the direct activation of Enh-*m* by Svb. These results demonstrate that Pri peptides switch the transcriptional activity of Svb from that of a repressor accumulated in nuclear foci to a nucleoplasmic activator.

To explore the mechanisms by which Pri peptides trigger this switch in Svb intranuclear distribution, we examined whether Pri requires de novo synthesis of the Svb protein. Using a photoactivatable-GFP (PA-GFP), we observed that photoactivated Svb:PA-GFP switched from foci to diffuse distribution after the induction of *pri* expression (Fig. 4C). Therefore, the same Svb molecules are relocated within the nucleus, suggesting that the action of Pri peptides relies on posttranslational modifications of Svb. Accordingly, Western blot analysis showed that whereas the size of OvoA and OvoB proteins (including that of their minor species) were not affected by *pri*, Svb exhibited a differential electrophoretic mobility in a *pri*-dependent manner (Fig.

4D). In the absence of *pri*, Svb appeared slightly larger than OvoA, as deduced from the cDNA sequences (Fig. 2A). Upon *pri* expression, the Svb protein displayed a faster mobility, corresponding to a truncation of approximately 50 kD, without apparent modification in the size of *svb* mRNA (fig. S6). An antibody raised against the N-terminal Svb-specific region (anti-Svb1s) recognized only the larger Svb protein but not the truncated product formed upon *pri* expression. This truncated Svb protein was detected by antibodies to Ovo and GFP (Fig. 4, A and D, and fig. S7A), showing that it lacks the N-terminal region but retains an intact C terminus. To further characterize Svb truncation, we purified the truncated Svb protein and microsequenced its N-terminal end (fig. S8A). The N terminus of truncated Svb matches the sequence AAGHGR, which is located 56 amino acids upstream of the OvoB-initiating methionine and within a protein region that shows strong evolutionary conservation in insects (Fig. 4E and fig. S8B). The corresponding DNA sequence displays synonymous nucleotide substitutions across species and lacks canonical or alternative initiation codons (fig. S8B), further supporting the view that Svb truncation results from a posttranslational cleavage. Hence, the *pri*-induced truncated form of Svb contains the DNA-binding and activation domains but not the repression domain, explaining why it acts as a transcriptional activator.

Consistent with this idea, we observed a *pri*-dependent truncation of the endogenous Svb protein during embryogenesis. In wild-type embryos, anti-Svb1s detected a transient nuclear signal in trichome cells, at stages 11 and 12, that disappeared at later stages (Fig. 4F and fig. S7B). The loss of the Svb N-terminal region coincided with the onset of *pri* expression in the epidermis (fig. S1). Indeed, *pri* is required for Svb truncation in vivo—as revealed by the persistence of anti-Svb1s signal in *pri* mutants—throughout embryogenesis (Fig. 4F). We conclude that Pri peptides convert Svb from a transcriptional repressor to an activator via the truncation of its N-terminal region.

This study demonstrates that 11– to 32–amino acid peptides encoded by sORFs orchestrate epidermal differentiation through the control of Svb transcriptional activity. At stages 11 and 12, *svb* is already expressed and restricted to presumptive trichome cells, in which the full-length Svb repressor probably prevents the premature expression of cellular effectors. At stages 13 and 14, the expression of *pri* in epidermal cells then triggers N-terminal truncation of the Svb protein, probably through a proteolytic release of the repressor domain, causing a rapid conversion of Svb function toward activation. Thus, although *svb* expression defines the spatial pattern of trichomes, the action of Pri peptides defines the temporality of trichome formation.

Besides the mechanisms of epidermal differentiation, our studies suggest broader functions for Pri peptides. Although *pri* is also required for tracheal morphogenesis (*9*), we observed normal trachea in *svb* mutant embryos (fig. S9), indicating that Pri peptides probably regulate additional developmental factors. Recent large-scale analyses indicate that thousands of unexplored transcripts are also probably encoding polypeptides of less than 100 amino acids in mice and humans (*1*, *21*, *7*). Future functional analyses should elucidate how small peptides encoded by transcripts improperly termed ncRNAs contribute to various biological processes including development and differentiation.

References and Notes

1. P. Carninci *et al.*, RIKEN Genome Exploration Research Group and Genome Science Group (Genome Network Project Core Group), *Science* **309**, 1559 (2005).
2. S. Inagaki *et al.*, *Genes Cells* **10**, 1163 (2005).
3. T. Ota *et al.*, *Nat. Genet.* **36**, 40 (2004).
4. J. L. Tupy *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 5495 (2005).
5. K. Hanada, X. Zhang, J. O. Borevitz, W. H. Li, S. H. Shiu, *Genome Res.* **17**, 632 (2007).
6. J. P. Kastenmayer *et al.*, *Genome Res.* **16**, 365 (2006).
7. M. C. Frith *et al.*, *PLoS Genet.* **2**, e52 (2006).
8. Y. Hashimoto, T. Kondo, Y. Kageyama, *Dev. Growth Differ.* **50** (suppl. 1), S269 (2008).
9. T. Kondo *et al.*, *Nat. Cell Biol.* **9**, 660 (2007).
10. M. I. Galindo, J. I. Pueyo, S. Fouix, S. A. Bishop, J. P. Couso, *PLoS Biol.* **5**, e106 (2007).
11. J. Savard, H. Marques-Souza, M. Aranda, D. Tautz, *Cell* **126**, 559 (2006).
12. J. I. Pueyo, J. P. Couso, *Dev. Biol.* **324**, 192 (2008).
13. F. Payre, *Int. J. Dev. Biol.* **48**, 207 (2004).
14. A. P. McGregor *et al.*, *Nature* **448**, 587 (2007).
15. F. Payre, A. Vincent, S. Carreno, *Nature* **400**, 271 (1999).
16. E. Sucena, I. Delon, I. Jones, F. Payre, D. L. Stern, *Nature* **424**, 935 (2003).
17. H. Chanut-Delalande, I. Fernandes, F. Roch, F. Payre, S. Plaza, *PLoS Biol.* **4**, e290 (2006).
18. I. Fernandes *et al.*, *Dev. Cell* **18**, 64 (2010).
19. J. Andrews *et al.*, *Development* **127**, 881 (2000).
20. I. Delon, H. Chanut-Delalande, F. Payre, *Mech. Dev.* **120**, 747 (2003).
21. P. P. Amaral, M. E. Dinger, T. R. Mercer, J. S. Mattick, *Science* **319**, 1787 (2008).

22. We thank the Kyoto Drosophila Genetic Resource Center (DGRC); Bloomington *Drosophila* Stock Center for fly strains; the Indiana DGRC; G. Patterson, J. Lippincott-Schwartz, and C. Hill for plasmids; S. Takada and T. Okubo (NIBB) for technical advice; Y. Latapie and B. Ronsin (CBD) and J. D'Alayer (Institut Pasteur) for excellent technical assistance; and members of the Kobayashi laboratory for helpful comments and discussion. This work was supported by a Research Fellowship for Young Scientists from the Japan Society for the Promotion of Science; the JST PRESTO program; the Ministry of Education, Culture, Sports, Science and Technology KAKENHI (20370091); Agence Nationale de la Recherche (Programme Blanc "Netoshape"); Fondation pour la Recherche Médicale (Equipe 2005); and Association pour la Recherche sur le Cancer (1111).

Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5989/336/DC1
Materials and Methods
Figs. S1 to S9
References

10 February 2010; accepted 20 May 2010
10.1126/science.1188158

Hedgehog Signaling Regulates Segment Formation in the Annelid *Platynereis*

Nicolas Dray,[1]*† Kristin Tessmar-Raible,[2,3]* Martine Le Gouar,[1]* Laura Vibert,[1] Foteini Christodoulou,[3] Katharina Schipany,[2] Aurélien Guillou,[4] Juliane Zantke,[2] Heidi Snyman,[3] Julien Béhague,[1,4] Michel Vervoort,[1,4] Detlev Arendt,[3] Guillaume Balavoine[1,4]‡

Annelids and arthropods share a similar segmented organization of the body whose evolutionary origin remains unclear. The Hedgehog signaling pathway, prominent in arthropod embryonic segment patterning, has not been shown to have a similar function outside arthropods. We show that the ligand Hedgehog, the receptor Patched, and the transcription factor Gli are all expressed in striped patterns before the morphological appearance of segments in the annelid *Platynereis dumerilii*. Treatments with small molecules antagonistic to Hedgehog signaling disrupt segment formation. *Platynereis* Hedgehog is not necessary to establish early segment patterns but is required to maintain them. The molecular similarity of segment patterning functions of the Hedgehog pathway in an annelid and in arthropods supports a common origin of segmentation in protostomes.

In the fruit fly, the axial patterning of each individual segment is controlled and maintained by two signaling pathways operating across the segment: Wnt/β-catenin and Hedgehog (*1*, *2*). The function of the Hedgehog (Hh) pathway in segment formation is conserved in other holometabolous insects (*3*) and probably also in noninsect arthropods (*4*, *5*). By contrast, mesodermal somites in vertebrates are patterned by nonhomologous segment polarity genes (*6*). Here, we investigated the role of the Hh pathway during segment formation in an annelid representative of the third great branch of Bilaterians, Spiralians (fig. S1). The nereidid *Platynereis dumerilii* presents two phases of segment formation: larval metamorphosis and juvenile posterior growth (fig. S2). Using degenerate polymerase chain reaction (PCR), we cloned four genes of the Hh pathway in *Platynereis* coding, respectively, for orthologs of the ligand Hedgehog (*Pdu-hh*), its receptor Patched *(Pdu-ptc)*, the transmembrane activator Smoothened (*Pdu-smo*),