trast, contributions to sentinel duty are usually greater in males than in females, regardless of patterns of philopatry (*20–23*), perhaps because males gain information about the distribution of females by watching from raised positions.

Our results have three broader implications. First, they show that pronounced sex differences in behavioral development can occur in effectively monogamous species with little sexual dimorphism in body size. Differences similar to those we have described here may be expected to occur in other cooperative species. Second, our results emphasize that comparisons of cooperative behavior among helpers need to control for the effects of age, weight, sex, and nutritional status; attempts to investigate the influence of particular variables (such as kinship) on individual contributions to cooperative activities that do not control for these effects may generate misleading results. Lastly, our results suggest that differences between male and female helpers in their contributions to rearing young vary with the direct costs and benefits of cooperative activities to each sex generated by sex differences in philopatry. Previous analyses of cooperative behavior in meerkats have shown that individual differences in contributions to cooperative activities are unrelated to levels of kinship (*24*, *25*), and our results are consistent with the suggestion that mutualistic, direct benefits play an important role in the evolution and maintenance of cooperative breeding (*26*).

References and Notes

1. P. B. Stacey, W. D. Koenig, Eds., *Cooperative Breeding in Birds* (Cambridge Univ. Press, Cambridge, 1990).
2. A. Cockburn, *Annu. Rev. Ecol. System.* **29**, 141 (1998).
3. P. J. Greenwood, *Anim. Behav.* **28**, 1140 (1980).
4. Materials and methods are available as supporting material on *Science* Online.
5. T. H. Clutton-Brock *et al.*, *Science* **291**, 478 (2001).
6. T. H. Clutton-Brock *et al.*, *Proc. R. Soc. London Ser. B* **265**, 2291 (1998).
7. As group size increases, males reduce investment in babysitting to a greater extent than females (subadults, $\chi^2 = 10.08$, df = 1, $P = 0.002$; yearlings, $\chi^2 = 12.04$, df = 1, $P = 0.001$) as well as in pup feeding (subadults, $\chi^2 = 4.98$, df = 1, $P = 0.024$; adults, $\chi^2 = 3.35$, df = 1, $P = 0.067$). In contrast, in larger groups, females reduce their investment in digging (adults, $\chi^2 = 12.71$, df = 1, $P = 0.001$) and raised guarding (adults, $\chi^2 = 5.24$, df = 1, $P = 0.022$) to a greater extent than males. (For all other age categories, $P > 0.1$).
8. After the first year of life, helper contributions are not consistently related to body weight, and female helpers fed as pups do not contribute any more to cooperative activities than unfed controls ($P > 0.65$ for fed versus unfed ; $P > 0.3$ for interactions between fed and unfed, males and females).
9. J. A. Barnard, thesis, Cambridge University (2000).
10. A. S. Griffin, thesis, Edinburgh University (1999).
11. T. H. Clutton-Brock *et al.*, *J. Anim. Ecol.* **68**, 672 (1999).
12. T. H. Clutton-Brock *et al.*, *Science* **293**, 2446 (2001).
13. P. N. M. Brotherton *et al.*, *Behav. Ecol.* **12**, 590 (2001).
14. J. P. Rood, in *Advances in the Study of Mammalian Behavior*, J. F. Eisenberg, D. G. Kleiman, Eds. (American Society of Mammalogists, Lawrence, KS, 1983), pp. 454–488.
15. D. D. Owens, M. J. Owens, *Nature* **308**, 843 (1984).
16. J. Komdeur, *Proc. R. Soc. London Ser. B* **256**, 47 (1994).
17. J. R. Malcolm, K. Marten, *Behav. Ecol. Sociobiol.* **10**, 1 (1982).
18. E. A. Lacey, P. W. Sherman, in *The Ecology of the Naked Mole Rat*, P. W. Sherman, J. U. M. Jarvis, R. D. Alexander, Eds. (Princeton Univ. Press, Princeton, NJ, 1990), pp. 275–336.
19. M. J. O'Riain, N. C. Bennett, P. N. M. Brotherton, *Behav. Ecol. Sociobiol.* **48**, 471 (2000).
20. M. F. Clarke, *Behav. Ecol. Sociobiol.* **14**, 137 (1984).
21. K. J. McGowan, G. E. Woolfenden, *Anim. Behav.* **37**, 1000 (1989).
22. A. Zahavi, in (*1*), pp. 103–130.
23. S. D. Strahl, A. Schultz, in (*1*), pp. 131–155.
24. T. H. Clutton-Brock *et al.*, *Proc. R. Soc. London Ser. B* **267**, 301 (2000).
25. T. H. Clutton-Brock *et al.*, *Anim. Behav.* **61**, 705 (2001).
26. T. H. Clutton-Brock, *Science*, **296**, 69 (2002).

27. We thank H. and J. Kotze and the Northern Cape Conservation Authority for permission to work on land at Vanzylsrus and the National Parks and their staff for permission to work in Gemsbok Park. The study would not have been possible without the support of members of the Mammal Research Institute at the University of Pretoria (including J. Skinner, J. du Toit, and M. Haupt) as well as the help of over 40 assistants, students, postdocs, and visitors, who contributed to data collection. In particular, we are grateful to P. Brotherton, E. Cameron, D. Gaynor, N. Green, R. Kansky, M. Manser, and J. O'Riain. In planning and carrying through the work, we have benefited from the guidance of S. Creel, D. Macdonald, and M. Manser. Funded by grants from the Natural Environmental Research Council and the Biotechnology and Biological Sciences Research Council.

Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5579/253/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S4

1 March 2002; accepted 7 June 2002

Leg Patterning Driven by Proximal-Distal Interactions and EGFR Signaling

M. I. Galindo, S. A. Bishop, S. Greig, J. P. Couso*

wingless **and** ***decapentaplegic*** **signaling establishes the proximal-distal axis of** ***Drosophila*** **legs by activating the expression of genes such as** ***Distalless*** **and** ***dachshund*** **in broad proximal-distal domains during early leg development. However, here we show that** ***wingless*** **and** ***decapentaplegic*** **are not required throughout all of proximal-distal development. The tarsus, which has been proposed to be an ancestral structure, is instead defined by the activity of** ***Distalless***, ***dachshund***, **and a distal gradient of epidermal growth factor receptor (EGFR)–Ras signaling. Our results uncover a mechanism for appendage patterning directed by genes expressed in proximal-distal domains and possibly conserved in other arthropods and vertebrates.**

Animal appendages develop along a proximal-to-distal (PD) axis, from proximal body wall to distal tip. This axis is not inherited from the embryo and is established anew in each developing appendage. In *Drosophila* legs, the combination of a dorsal signal provided by the *BMP4* homolog *decapentaplegic* (*dpp*) with a ventral signal provided by the *Wnt* homolog *wingless* (*wg*) establishes the PD axis (*1*), in addition to organizing the dorsal-ventral appendage pattern (*2*, *3*). Signaling from *wg* and *dpp* activates the expression of the genes *Distalless* (*Dll*) and *dachshund* (*dac*) early in leg development (*4*, *5*). *Dll* encodes a homeodomain protein expressed and required medially in the femur and tibia. However, the leg comprises 10 segments along the PD axis, whose specification involves further genes (*6*). Here we describe how, after 84 hours of development, PD patterning becomes *wg*- and *dpp*-independent. Instead, a mechanism mediated by genes expressed in PD domains, such as *Dll*, *dac*, and the epidermal growth factor receptor (EGFR) ligand *vein*, activates the expression of further genes and generates distal leg fates such as the tarsus. Previous data in *Drosophila* and on homologous genes in other arthropods and vertebrates suggest that this PD patterning mechanism might be conserved and ancestral.

We studied the timing of *wg* requirements for PD development with a temperature-sensitive mutant (*7*). Removal of *wg* function before 72 hours after egg laying (hours AEL) produces truncated legs lacking the distal parts and showing ventral patterning defects (Fig. 1, A and B). However, shifts of animals from permissive to restrictive temperature at

School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, UK.

*To whom correspondence should be addressed. E-mail: J.P.Couso@biols.susx.ac.uk

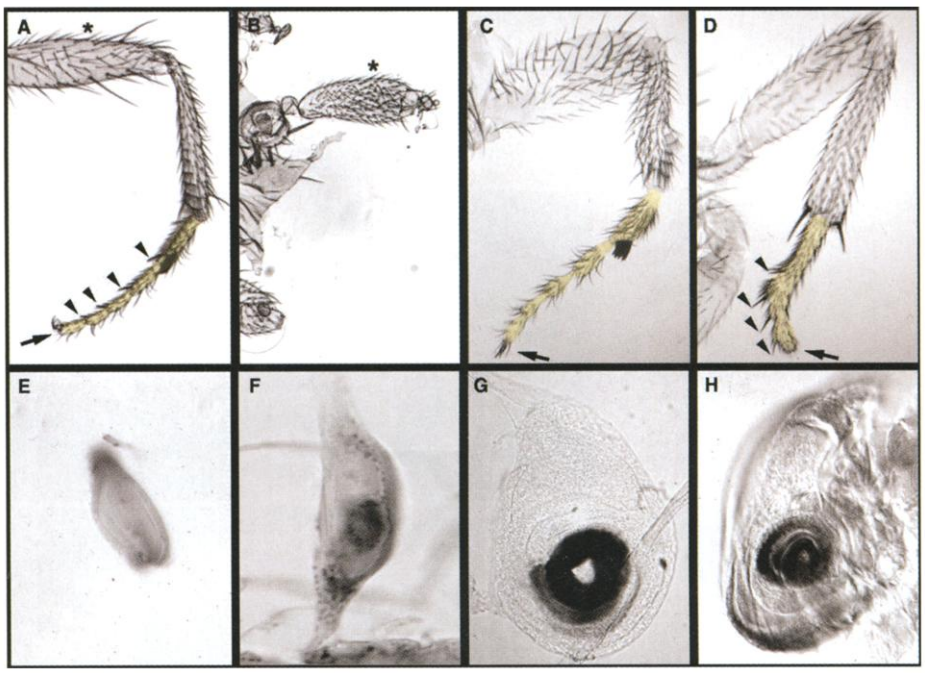


Fig. 1. *wg* and *dpp* signaling are not required for PD development and *rn* and *bab* activation after 84 hours AEL. (**A**) Wild-type leg showing femur (asterisk), five tarsal segments (yellow) with ventral apical bristles (arrowheads) and pretarsal claws at distal tip (arrow). (**B**) Leg from which *wg* protein was removed before 72 hours AEL, showing a truncated PD axis lacking tibia, tarsal segments, and claws (asterisk labels femur). (**C**) Leg after removal of *wg* from 84 hours AEL, showing a normal PD axis despite loss of ventral structures such as apical bristles. Claws are present (arrow) but ventral pretarsal components are missing. (**D**) Leg deprived of *Mad* function from 84 hours AEL. PD organization is intact, despite defects mirroring those of C). The pretarsus lacks claws (arrow) but retains ventral elements, and apical bristles remain while dorsal structures on the opposite side are missing. (**E**) 80 hours AEL leg disc showing no *bab* expression. (**F**) *bab* expression appears in the center of the disc as the central tarsal fold forms from 90 to 96 hours AEL and is quickly restricted to a ring. (**G**) *bab* expression at 120 hours AEL. (**H**) *bab* expression is still present after removal of *wg* function from 84 hours AEL, although the disc is ventrally defective.

approximately 84 hours AEL produces legs with ventral patterning defects but with an otherwise intact PD organization (Fig. 1C).

To assess the temporal requirements for *dpp* signaling, we identified a temperature-sensitive allele of *Mothers against decapentaplegic* (*Mad*), the *dpp* signal transducer (*7*). Removal of *Mad* function before 72 hours AEL also produces truncations of the PD axis, whereas removal from 84 hours AEL until the end of development renders a normal PD organization despite dorsal patterning defects (Fig. 1D). Clones of null *Mad* mutant cells (*7*) show the same results: clones induced before 72 hours AEL show elimination of entire tarsal segments, whereas after 84 hours AEL, only dorsal pattern features are affected. These results show that the input from *dpp* and *wg* signals into PD development ends by 84 hours AEL.

Self-maintenance regulatory mechanisms maintain *Dll* and *dac* expression beyond this point (*5*, *8*). However, new genes with PD domains of expression and function become active. *rotund* (*rn*) encodes a zinc-finger nuclear protein expressed in a transient ring during 84 to 96 hours AEL (*9*) (fig. S1, A and B). Tarsal segment loss in *rn* mutants shows that this period is crucial for tarsal development, and a *rn-lacZ* reporter with persistent *lacZ* expression reveals that the ring of *rn*-expressing cells gives rise to most of the tarsus (fig. S1C). The *bric-a-brac* (*bab*) (*10*) gene encodes a nuclear protein with a BTB/POZ domain required for appropriate tarsal differentiation, whose expression starts in the presumptive tarsus shortly after *rn*, from 90 hours AEL until the end of development (Fig. 1, E to G). At 120 hours AEL, after

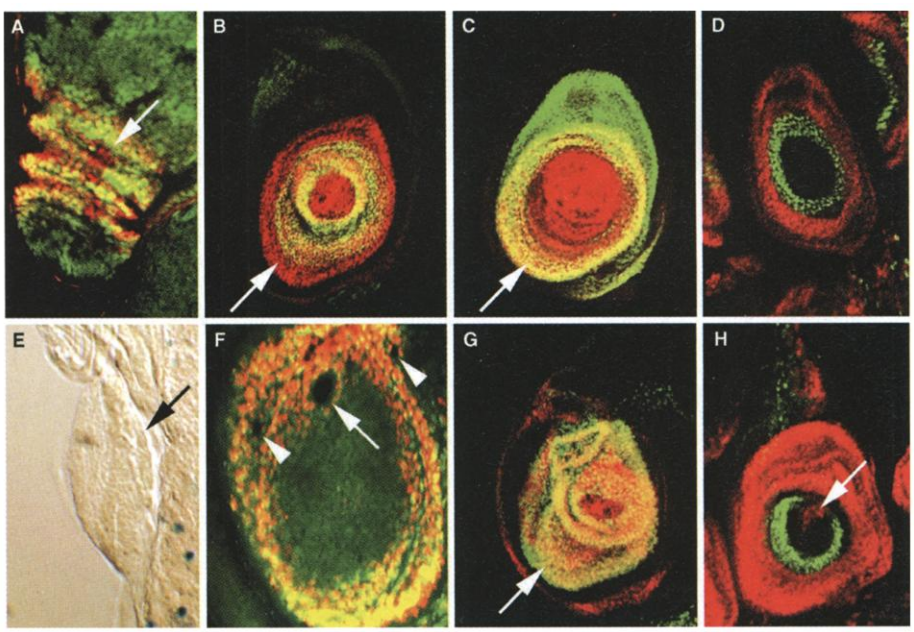


Fig. 2. *Dll* and *dac* define tarsal development and gene expression. (**A**) *bab* expression (red label, yellow in overlap) is unaffected in clones of *Mad*$^-$ cells (absence of green, arrow). (**B**) The ring of *bab* expression is included within the *Dll* domain (red) and appears yellow, but central and medial (arrow) *Dll*-expressing cells do not express *bab*. (**C**) *Dll* (red) and *dac* (green) expression overlap in the medial cells (yellow, arrow) of a 120 hours AEL leg disc. (**D**) *rn* (green) and *dac* (red) domains abut at 96 hours AEL. (**E**) *rn* expression is not activated in *Dll*$^-$ leg discs (arrow; compare with Fig. 1F). (**F**) *Dll*$^-$ clones (loss of green) generated at 80 hours AEL. *bab* expression (yellow) is absent autonomously in *Dll*$^-$ cells (arrow), even in single isolated ones (arrowheads). (**G**) In *dac*$^-$ mutants, *bab* expression is de-repressed and coincides medially with *Dll* [arrow; staining as in (B)]. (**H**) *bab* expression (green) is eliminated (arrow) by ectopic expression of *dac* (red).

extensive cell proliferation, *bab* expression occupies a wider area, similar to *rn-lacZ*, corresponding to presumptive tarsal segments one to four (Fig. 1G). Because the onset of *rn* and *bab* expression occurs after 84 hours AEL, *wg* and *dpp* signaling should neither be activating *rn* and *bab* nor be directly required for their expression. As expected, removal of *wg* or *dpp* signaling in temperature-sensitive mutants or in clones of *Mad*$^-$ cells from 84 hours AEL does not affect *rn* or *bab* expression (Figs. 1H and 2A and fig. S1D).

A different patterning mechanism must generate the domains of expression of *rn* and *bab* and must lead to tarsal growth and dif-

ferentiation. The presumptive tarsus is included within the *Dll*-expression domain (Fig. 2B), and *Dll* function is required for *bab* and *rn* activation and tarsal development at 84 hours AEL, as shown by *Dll* mutants and *Dll*$^-$ clones [Fig. 2, E and F; (*7*)]. However, neither the *Dll*-expressing cells in the center of the disc (the presumptive distal tip), nor the most peripheral *Dll*-expressing cells, express *rn* or *bab* (Fig. 2B). These peripheral cells appear to be medial cells expressing both *Dll* and *dac* (compare B and C of Fig. 2), and double-staining reveals that indeed the early rings of *rn* and *bab* expression are abutted by *dac* (Fig. 2D). These observations suggest that *rn* and *bab* expression, promoted by *Dll*, is repressed in medial *Dll*-expressing cells by *dac* and in the central cells by another factor. In clones of *dac*$^-$ cells induced at 80 hours AEL, we observe ectopic outgrowths and ectopic expression of *bab* in medial cells near the endogenous *bab* domain [fig. S1F (*7*)]. Staining of entire *dac*$^-$ mutant discs reveals this effect clearly: *bab* expression now extends to all medial *Dll*-expressing cells (Fig. 2G). This result agrees with ectopic expression of *dac* in the tarsal region, which represses *bab* and eliminates tarsal structures (Fig. 2H and fig. S1G).

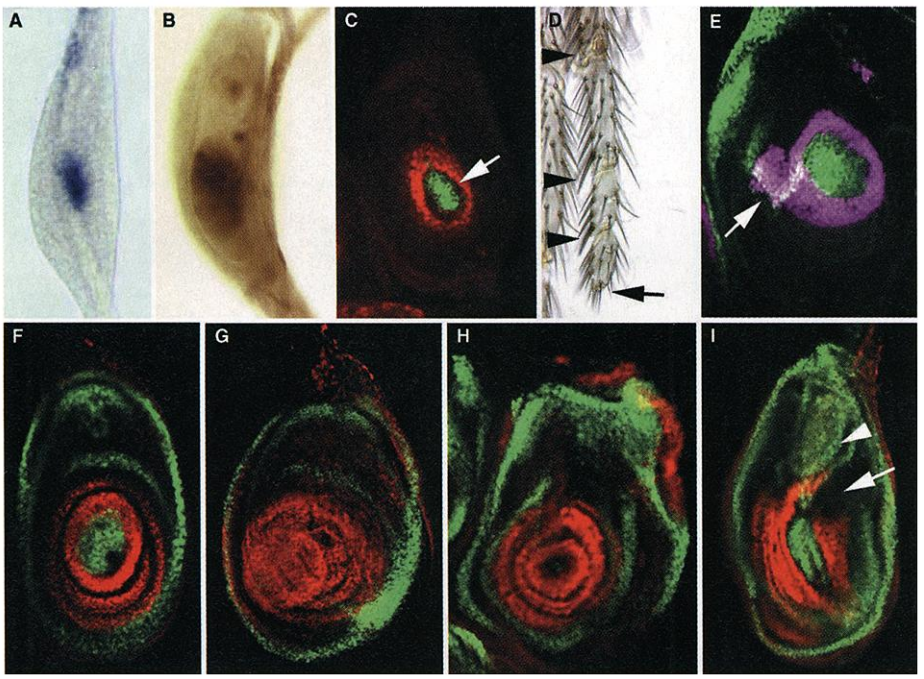
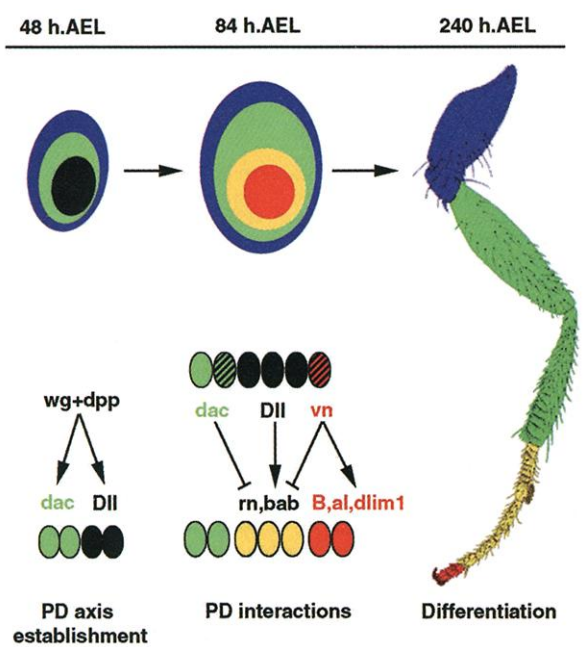


Fig. 3. EGFR-Ras signaling prevents *bab* expression and promotes distal leg development. (**A**) *vn* transcript and (**B**) distribution of activated MAPK in the center of 80 hours AEL leg discs. These patterns remain until 96 hours AEL. (**C**) *bab* (red) and *vn* (green) expression at 96 hours AEL. *vn* is expressed in the center of the disc where *bab* is absent. Note the gap between the two domains (arrow), revealing the range of *vn* action. (**D**) Legs deprived of EGFR from 80 hours AEL have four tarsi, ending in a joint instead of pretarsus (labels as in Fig. 1A). (**E**) Ectopic EGFR activation generates ectopic expression (arrow) of *al* (green) and *Bar* (purple; white overlap). (**F** to **I**) *bab* (red) and *dlim1* (green) expression in 120 hours AEL discs from wild type (F), *DllGal4 UAS-argos* (G), dominant-negative EGFR (H), and ectopically activated EGFR (I). *dlim1* expression is lost from the leg tip, which is invaded by *bab* expression in (G) and (H), whereas in (I) *bab* is lost (arrow) and *dlim1* is activated ectopically (arrowhead).

The *vein* (*vn*) gene is expressed at the very center of the leg disc shortly after 72 hours AEL (Fig. 3A). The onset of *vn* expression requires both *wg* and *dpp* signaling and *Dll*, but by 84 hours AEL, when *rn* and *bab* are activated, *vn* is no longer dependent on *wg* and *dpp* (fig. S1H). *vn* encodes a neuregulin-like secreted protein that is the only ligand of EGFR (*11*) active here. We detect activation of the EGFR-Ras signal transducer mitogen-activated protein kinase (MAPK) (*12*) in the center of the leg disc from 80 hours AEL to 96 hours AEL, in the region devoid of *rn* and *bab* expression (Fig. 3B). Secretion of the *vn* protein could lead to the EGFR-mediated repression of *rn* and *bab* several cell diameters away from the center (Fig. 3, A to C; fig. S1E). Blocking *vn* signaling with ectopic expression of the secreted EGFR antagonist *argos* throughout the *Dll* domain produces mutant phenotypes only near the *vn*-expressing cells, and these phenotypes are similar to those of *vn*, MAPK, and *Ras* mutant conditions: the pretarsus is missing at the tip of the leg, and tarsus five is missing or abnormal (fig. S1, I and J). *bab* expression invades the center of the disc, which loses the expression of the distal tip marker *dlim1* (*13*) (Fig. 3, F and G). Using both a temperature-sensitive allele of EGFR and a dominant-negative version of the EGFR protein, we deprived leg discs of EGFR function during 80 to 96 hours AEL (*7*) and observed similar results: loss of distalmost structures and *bab* expression covering the disc center (Fig. 3, D and H), which has also lost the expression of the pretarsal markers *dlim1* and *aristaless* (*al*) (*1*), as well as the tarsus five marker *Bar* (*14*) (fig. S1K). Reciprocally, in legs expressing ectopically an activated version of EGFR, tarsal structures and the expression of *bab* are lost, whereas ectopic expression of *dlim1*, *al*, and *Bar* is observed (Fig. 3, E and I). Hence, a central gradient of EGFR-

Fig. 4. Proximal-distal leg development in *Drosophila*. Before 72 hours AEL, the combination of *wg* and *dpp* signaling establishes the expression of *Dll* (black) and *dac* (green) in the developing disc. After 84 hours AEL, PD development becomes *wg*- and *dpp*-independent and is driven by PD interactions. *rn* and *bab* expression (yellow) is inserted in the *Dll*-expressing cells in between *vn* at the disc center (red), and the medial ring of *dac* expression (green). *dac* promotes femur and tibia development, whereas *vn* promotes distal development through the activation of *al*, *dlim1*, and *Bar*. From 96 to 120 hours AEL, the leg pattern is completed by definition of further PD regions and intercalation of joints between segments. During pupal metamorphosis the disc everts and the fly leg differentiates.



Ras signaling promotes simultaneously and independently [fig. S1L (*7*)] three effects: activation of *al* and *dlim1* at the very center, activation of *Bar* in nearby cells, and repression of *bab* from the whole area. Regarding tarsal segments 1 to 4, *vn* acts as the repressory element working in parallel to *dac* to restrict the activation of *rn* and *bab* to the middle of the *Dll* domain. Tarsal segments 1 to 4 are thus defined in those *Dll*-expressing cells not simultaneously exposed to either the *dac* protein or EGFR-Ras signaling triggered by *vn* (Fig. 4 and fig. S1E).

Thus, interactions between genes and signals expressed in PD domains constitute a patterning mechanism for the development of new PD fates. We suspect that further PD interactions exist in *Drosophila* legs, for example, during trochanter development proximal to the *dac* domain (*7*).

The *Drosophila* tarsus develops in the absence of homeotic genes, suggesting that it is an ancestral ground-state limb structure (*15*). Because tarsal development is driven by PD interactions, altogether these results might suggest that these interactions are an ancestral process, possibly to be found in other animals. In primitive insect limbs, a transition in *dpp* expression (*16*, *17*) signals a *wg*- and *dpp*-independent patterning phase, whereas the conserved expression of *Dll*, *dac*, and *al* suggests a conservation of their functional roles (*18*). In vertebrate limbs, expression and requirements of *Dll*, *dac*, and *al* homologs are similar to those of insects (*19*–*21*), and experimental embryology has shown that insect and vertebrate limbs react to PD axis alterations in the same way (*22*).

Further work is required to clarify these issues and to obtain a complete understanding of PD patterning in animal appendages. Our results show the importance of an element whose role had long been suspected (*1*, *22*), that is, the existence of PD interactions that complete the initial PD organization generated by dorsal-ventral and anterior-posterior patterning cascades.

References and Notes

1. G. Campbell, T. Weaver, A. Tomlinson, *Cell* **74**, 1113 (1993).
2. J. P. Couso, M. Bate, A. Martinez-Arias, *Science* **259**, 484 (1993).
3. J. Jiang, G. Struhl, *Cell* **86**, 401 (1996).
4. F. Diaz-Benjumea, B. Cohen, S. Cohen, *Nature* **372**, 175 (1994).
5. T. Lecuit, S. Cohen, *Nature* **388**, 139 (1997).
6. J. P. Couso, S. A. Bishop, *Int. J. Dev. Biol.* **42**, 345 (1998).
7. Materials and methods and other supplementary material are available on *Science* Online.
8. J. Castelli-Gair, M. Akam, *Development* **121**, 2973 (1995).
9. S. E. St Pierre, M. I. Galindo, J. P. Couso, S. Thor, *Development* **129**, 1273 (2002).
10. D. Godt, J. Couderc, S. Cramton, F. Laski, *Development* **119**, 799 (1993).
11. B. Schnepp, G. Grumbling, T. Donaldson, A. Simcox, *Genes Dev.* **10**, 2302 (1996).
12. R. Schweitzer, B. Shilo, *Trends Genet.* **13**, 191 (1997).
13. J. I. Pueyo, M. I. Galindo, S. A. Bishop, J. P. Couso, *Development* **127**, 5391 (2000).
14. T. Kojima, M. Sato, K. Saigo, *Development* **127**, 769 (2000).
15. F. Casares, R. S. Mann, *Science* **293**, 1477 (2001).
16. E. Jockusch, C. Nulsen, S. Newfeld, L. Nagy, *Development* **127**, 1617 (2000).
17. N. Niwa *et al.*, *Development* **127**, 4373 (2000).
18. A. Abzhanov, T. C. Kaufman, *Dev. Biol.* **227**, 673 (2000).
19. R. Robledo, L. Rajan, X. Li, T. Lufkin, *Genes Dev.* **16**, 1089 (2002).
20. R. J. Davis, W. Shen, T. A. Heanue, G. Mardon, *Dev. Genes Evol.* **209**, 526 (1999).
21. S. Qu *et al.*, *Development* **125**, 2711 (1998).
22. S. V. Bryant, V. French, P. J. Bryant, *Science* **212**, 993 (1981).

23. We thank A. Simcox and G. Campbell for unpublished results and discussions on EGFR signaling, C. Sainsot for technical assistance, and I. Pueyo, F. Casares, and J. Bacon for manuscript comments. This work was supported by the Wellcome and Leverhulme Trusts.

Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5579/256/DC1
Materials and Methods
Supporting Text
Fig. S1

28 March 2002; accepted 13 June 2002

Mediation of Poly(ADP-Ribose) Polymerase-1–Dependent Cell Death by Apoptosis-Inducing Factor

Seong-Woon Yu,[1]* Hongmin Wang,[1]* Marc F. Poitras,[1] Carmen Coombs,[1] William J. Bowers,[5] Howard J. Federoff,[5] Guy G. Poirier,[6] Ted M. Dawson,[1,2,4] Valina L. Dawson[1,2,3,4]†

Poly(ADP-ribose) polymerase-1 (PARP-1) protects the genome by functioning in the DNA damage surveillance network. PARP-1 is also a mediator of cell death after ischemia-reperfusion injury, glutamate excitotoxicity, and various inflammatory processes. We show that PARP-1 activation is required for translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus and that AIF is necessary for PARP-1–dependent cell death. *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, H_2O_2, and *N*-methyl-D-aspartate induce AIF translocation and cell death, which is prevented by PARP inhibitors or genetic knockout of PARP-1, but is caspase independent. Microinjection of an antibody to AIF protects against PARP-1–dependent cytotoxicity. These data support a model in which PARP-1 activation signals AIF release from mitochondria, resulting in a caspase-independent pathway of programmed cell death.

PARP-1 is a nuclear enzyme that responds to DNA damage and facilitates DNA repair (*1*–*3*). Once activated, PARP-1 transfers 50 to 200 molecules of ADP-ribose to a variety of nuclear proteins, including histones and PARP-1 itself (*1*–*4*). PARP-1 activation mediates cell death in ischemia-reperfusion injury after cerebral ischemia (*5*, *6*) and myocardial infarction (*7*), inflammatory injury, reactive oxygen species-induced injury (*8*), glutamate excitotoxocity (*9*, *10*), and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injury (*11*, *12*). The molecular mechanisms of PARP-1–induced cell death are not known, although one possibility is that PARP-1 signals to downstream cell death effectors.

To examine the mechanism by which PARP-1 activation kills cells, immortalized mouse embryonic fibroblasts from wild-type and PARP-1–knockout (KO) mice (*13*) were exposed to *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), a DNA-alkylating agent that potently activates PARP-1 and elicits PARP-1–dependent cytotoxicity. Exposure to 0.5 mM MNNG for 15 min induces nearly complete cell death in wild-type fibroblasts within 12 to 24 hours, whereas PARP-1–KO fibroblasts are resistant to the toxic effects of MNNG (Fig. 1A). PARP-1 activation consumes nicotinamide adenine dinucleotide (NAD^+) through the adenosine 5′-diphosphate (ADP)-ribosylation of nuclear associated proteins (*8*), resulting in an 80% reduction of NAD^+ levels within 15 min in wild-type fibroblasts (Fig. 1B). By 30 min, NAD^+ levels are no longer detectable. In contrast, NAD^+ levels in PARP-1–KO fibroblasts are maintained to greater than 60% of control values for up to 24 hours (Fig. 1B). The broad-spectrum caspase inhibitors, boc-as-

[1]Departments of Neurology, [2]Neuroscience, and [3]Physiology; and [4]the Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA. [5]Department of Neurology, Center for Aging and Developmental Biology, University of Rochester, NY 14642, USA. [6]Health and Environment Unit, Laval University Medical Research Center, CHUQ, Ste-Foy, Quebec G1V 4G2, Canada.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: vdawson@jhmi.edu