Patterning of the developing limbs of *Drosophila* depends on localized expression of the secreted signaling proteins Hedgehog (Hh), Wingless (Wg) and Decapentaplegic (Dpp) (reviewed in Lawrence and Struhl, 1996; Neumann and Cohen, 1997b). Hh, Wg and Dpp proteins are thought to form concentration gradients that instruct cells about their prospective fate as a function of their position in the developing imaginal disc (Lecuit et al., 1996; Nellen et al., 1996; Zecca et al., 1996; Neumann and Cohen, 1997a; Strigini and Cohen, 1997). One of the interesting features of imaginal disc development is that patterning occurs during a phase of rapid growth. The imaginal disc primordia originate as small groups of about 25 cells in mid-embryogenesis (reviewed in Cohen, 1993). The nascent discs cease proliferation in the embryo and lie quiescent until the middle of the first larval instar when they begin to grow by rapid cell proliferation (Britton and Edgar, 1998). Patterning occurs during this proliferative phase in which the number of cells in the discs increases by approximately 1000-fold.

The rapid growth of the patterning field poses an interesting problem related to the formation of morphogen gradients. The localized sources of the secreted signaling proteins are thought to remain relatively constant during this rapid growth phase. For example, Hh is expressed continuously by posterior compartment (P) cells and induces Dpp expression in anterior (A) cells in second as well as third instar while the wing disc increases many fold in size (see Fig. 1). Similarly Dpp and Wg induce their target genes while the discs are growing during second and third instar (e.g. de Celis et al., 1996; Neumann and Cohen, 1996; Lecuit et al., 1997, 1998). Cells that are within range of these signals at early stages may be instructed to express particular target genes and adopt particular fates. As the disc grows it is likely that some of the progeny of those initially specified cells may be displaced out of range of the signal by growth.

This raises a question about the extent to which the response of cells to instruction by morphogens can change if they are exposed to different levels of ligand at different times in development. There is ample evidence that cells in the developing imaginal discs can be shifted from low-level responses to high-level responses by exposure to increased levels of morphogen (e.g. Basler and Struhl, 1994; Lecuit et al., 1996; Nellen et al., 1996; Zecca et al., 1996; Neumann and Cohen, 1997a). This is also true in *Xenopus* for cell fate changes induced by Activin in animal cap assays (Gurdon et al., 1994, 1995). Cells exposed to low levels of Activin express the target gene *Xbra*. Exposure to a higher dose of Activin can shift the response pattern of cells to induce expression of the high-level response genes Goosecoid and Eomesodermin. However, evidence from *Xenopus* also suggests that induction of the high-level response is not readily reversible. Reducing the dose of ligand does not allow cells to shift from a high-level response to a low-level response during the relatively short time period of the assay (hours). This has been termed the ‘ratchet effect’. In molecular terms, the ratchet effect might be explained by the relatively stable binding of Activin to its receptor. Slow dissociation of ligand from receptor prevents a shift down to...
a lower level of receptor occupancy when ligand is removed (Dyson and Gurdon, 1998).

Previous work has suggested that concentration-dependent responses to Wg and Dpp in patterning the proximal-distal (PD) axis of the leg are also not readily reversed by removal of ligand (Lecuit and Cohen, 1997). Dachshund (Dac) and Distal-less (Dll) are targets of Wg and Dpp. Their expression is induced when the leg disc consists of several hundred cells. As the disc grows, Dll and Dac are expressed in cells at a considerable distance from the source of the Wg and Dpp signals. Subsequent removal of the ability of cells to transduce the Wg or Dpp signals does not lead to loss of Dll or Dac expression. This suggests that their continued expression cannot be explained in terms of ligand stability and persistent signaling.

In this report, we make use of novel cell lineage-tracing methods to study the stability of different cell fate specification events during growth of the imaginal disc. We ask whether patterns of gene expression induced early in development in response to particular morphogen thresholds are reversible as the disc grows. We find that concentration-dependent responses to Hh and Dpp are reversible in the wing disc. We then ask whether specification of proximal and distal cell fates in the leg are reversible. We find that proximal cells can readily adopt more distal identity. However, distal cells are limited in their ability to adopt more proximal fates, even when given a growth advantage. These observations have important implications for the elaboration of pattern along the PD axis of the leg as the disc grows.

MATERIALS AND METHODS

**Drosophila strains**

*dppGal4* is described by Morimura et al. (1996), *tshGal4* and *DllGal4* by Calleja et al. (1996) and *gal4-459* by Thomas et al. (1995). We have mapped the insert to 32F2-4. The cytological location and expression pattern suggest that it is located at the *spalt* locus, so we refer to it as *SalGal4*. *UAS-Flp* is described in Campbell and Tomlinson (1998).

**Construction of UAS-Flp-EBD flies**

The estrogen-regulated Flip recombinase EBD fusion protein (Flp-EBD) is described in Nichols et al. (1997). Flp-EBD-251 was cloned into pUAST (Brand and Perrimon, 1993) and used to generate transgenic strains. Five different insertion lines were tested for background activity. An insertion on the X chromosome with the lowest background activity was used for the experiments reported here.

**Clone induction using UAS-Flp-EBD**

For induction of Flp-EBD activity, water-soluble β-estradiol (Sigma, E-4389) was added to the food at a final concentration of 0.3 mg/ml. To remove estrogen, larvae were taken out of their food, rinsed, hand selected according to stage and put into a fresh vial of untreated food. Care was taken to avoid overcrowding because the timing of disc development may vary if larvae develop more slowly due to overcrowding.

For lineage tracing, *UAS-Flp-EBD: act5C>stop>lacZ* flies were crossed to the respective Gal4 drivers. For induction of *Minute* clones in the Dll region larvae were derived from the cross *UAS-Flp-EBD: FRT82/+ × DllGal4/++; FRT82 armlacZ Dp(f+) M(3R)W123/+.*

**Antibodies**

Sources of antibodies used: anti-β-Gal (Cappel); mouse anti-Dll (Duncan et al., 1998); mouse anti-Tsh (Ng et al., 1996); mouse anti-Dac (Mardon et al., 1994); rabbit anti-Sal (Kuhnlein et al., 1994). Rat anti-Dll was kindly provided by Jun Wu.

**RESULTS**

**Reversibility of Hh- and Dpp-induced cell fates in the wing disc**

Hh induces *dpp* expression in anterior cells adjacent to the anteroposterior (AP) boundary of the wing disc (Basler and Struhl, 1994; Tabata and Kornberg, 1994). In mature third instar discs, a *dpp-lacZ* reporter gene is expressed in a narrow stripe of cells in the center of the disc, whereas in young third instar discs, the *dpp-lacZ* stripe occupies the central third of the disc (Fig. 1A,B). This comparison illustrates that the proportion of the disc occupied by Hh-responsive cells is relatively larger in small discs and decreases as the disc grows. Further, it suggests that Hh-responsive cells must be able to lose expression of Hh target genes as the cells are displaced out of range of the Hh signal by growth of the disc. To verify that this is indeed the case, we lineage-tagged cells born in the outer region of the disc. These discs staining with X-Gal at late third instar are broader than the region of cells that still express *dpp-lacZ*.

**Fig. 1. Lineage tracing of *dppGal4* and *SalGal4* in the wing.**

(A,B) X-Gal staining of *dpp-lacZ* discs. (A) Early third instar. The *dpp-lacZ* stripe occupies about one third of the width of the disc. (B) The *dpp-lacZ* stripe is relatively narrower in late third instar. The box in A shows the disc at same magnification as the one in B. (C) Scheme outlining lineage tracing with the use of *UAS-Flp*. The expression of Flp under Gal4 control leads to the activation of *lacZ* through an excision event. The expression of *lacZ* thereafter is independent of Gal4. (D) Lineage tracing in *UAS-Flp; dppGal4 UASGFP/act5C>stop>lacZ* shows that the region of cells that have once expressed *dppGal4* (marked by anti-β-Gal in red) is much broader than the region of cells that still express *dppGal4* (UASGFP, in green). A and P indicate anterior and posterior compartments. The arrow points to cells marked by *dppGal4* posterior to the *dppGal4* stripe in the notum. This might reflect a difference in the timing of establishment of the AP lineage restriction in notum and wing pouch. Alternatively, *dppGal4* might be expressed in some posterior cells in the notum at early times of development. (E) *SalGal4/UAS-Flp; act5C>stop>lacZ* disc. The region of cells that have expressed Sal at any point during development (labeled with anti-β-Gal, red) is broader than the region of cells expressing Sal (labeled with anti-Sal, green).
Lineage-tracing cells along PD axis of \textit{Drosophila} leg

Hh-responsive region using \textit{dppGal4} to direct expression of FLP recombinase. In larvae carrying \textit{dppGal4}, \textit{UAS-Flp} and \textit{act5c>stop>lacZ}, FLP recombinase is expressed in cells expressing \textit{dppGal4} and mediates excision of the flip-out ‘stop’ cassette from the inactive reporter construct to generate an active \textit{act5c>lacZ} transgene (Struhl and Basler, 1993, illustrated in Fig. 1C). After excision of the cassette, reporter gene expression is regulated by the actin promoter and is clonally inherited in all the progeny of \textit{dppGal4}-expressing cells in which the recombination event took place. Cells expressing \textit{lacZ} (red) fill most of the anterior compartment of the wing pouch, hinge and the notum (Fig. 1D). By comparison, the \textit{dppGal4} domain is much narrower (visualized using \textit{UASGFP}, green). This indicates that cells born in the \textit{dppGal4} domain contribute to most of the A compartment of the wing and that they change their pattern of gene expression as they are displaced out of range of Hh.

Dpp signaling induces Spalt expression in the wing pouch. Clones of cells unable to transduce the Dpp signal lose Spalt expression, suggesting that expression of Spalt depends on continuous input of the Dpp signal (Lecuit et al., 1996; Nellen et al., 1996). Spalt is first induced in early third instar discs (de Celis et al., 1996). To ask whether cells that initiate Spalt expression at this stage revert to a more lateral identity as the disc grows in the course of normal development, we lineage-tagged cells born in the \textit{dppGal4} domain contribute to most of the A compartment of the wing and that they change their pattern of gene expression as they are displaced out of range of Hh.

Fig. 2. Tsh, Dac and Dll expression in the course of leg development. Different stages of leg discs expressing Tsh (blue), Dll (green) and Dac (red). (A,C,E) Horizontal sections and (B,D,F) cross sections of the same discs. The nature of the disc as an epithelial sack of tissue can easily be seen in these sections. (A,B) Very early third instar disc. Dac is starting to be expressed in a few cells at the border between Dll and Tsh expression (A, arrow). Dac expression starts at one side of the disc, Tsh and Dll expression patterns are still complementary on the other side. Dac expression is turned on in cells that still express Dll (arrows in A,B) and in cells expressing Tsh (arrowheads in A,B). (Box in B) Higher magnification of the overlapping Dac/Dll and Dac/Tsh expression is shown above, Tsh and Dll staining is shown below. Some cells at the Dll/Tsh boundary express low levels of both Dll and Tsh (box in A). (C,D) Horizontal and cross section through an early-mid third instar disc. The Dac domain has appeared between the Dll and Tsh domains. (C) Dll and Dac only overlap in a very narrow stripe of cells (appears yellow, arrows). (D) A proximal ring of Dll expression starts to show up at this stage (arrows). (EG) Late third instar disc shown at 1/2 the magnification of A-D. (E) Dll and Dac expression overlap in a broad domain (arrow), and (F) the epithelium of the disc is now highly folded. The proximal ring of Dll expression is indicated with arrows, it appears yellow where Dac and Dll overlap and white where all three proteins overlap. (G) Dll expression from the same disc. The femur shows low levels of Dll expression (arrows), as compared to no Dll expression in the Tsh-expressing domain (arrowhead). (H) Optical section through a partially everted early pupal disc. The top layer of the epithelial sac that can still be seen in D (peripodial membrane) is broken through by the evert ing leg.

Fig. 3. Expression of \textit{tshGal4} and \textit{DllGal4} in young leg discs. (A,B) Mid-second instar discs labeled to visualize Dll protein (red) and \textit{tshGal4}-directed β-Gal expression (green). At this stage, the Dll domain is only 3-4 cells in diameter. The arrow in B indicates a cell expressing Dll and β-Gal. (C) Late second or early third instar disc labeled to visualize Dll protein (red) and \textit{DllGal4}-directed β-Gal expression (green). The rest of the disc is unlabelled.
particular threshold response to the Hh or Dpp morphogens. Rather, cells in the wing disc appear to be able to revert to lower threshold responses when morphogen levels decrease.

**Reversibility of proximal-distal cell fates induced by Wg and Dpp**

Wg and Dpp signaling define domains ofDll, Dac, Tsh and Hth expression along the PD axis of the leg (Lecuit and Cohen, 1997; Abu-Shaar and Mann, 1998; Wu and Cohen, 1999). These genes are expressed in concentric rings in the disc. After eversion, the central Dll domain will give rise to the distal leg, including tarsus and distal tibia (Diaz-Benjumea et al., 1994; Lecuit and Cohen, 1997). The Dac domain corresponds to the presumptive femur and proximal tibia segments of the leg (Mardon et al., 1994; Lecuit and Cohen, 1997). The proximal Hth and Tsh domain will form proximal leg elements and body wall (Abu-Shaar and Mann, 1998; Wu and Cohen, 1999).

Clonal analysis has shown that removing the ability of cells to transduce the Wg and Dpp signals does not lead to loss of Dll expression (Lecuit and Cohen, 1997). This raises a question about the extent to which distal cells can revert to more proximal identities as the leg disc grows. In the second instar, the disc is subdivided into two domains: a central Dll-expressing region and a surrounding region expressing Hth and Tsh (Abu-Shaar and Mann, 1998; Wu and Cohen, 1999). As the Dac domain arises de novo in a disc that formerly had only two cell populations, cells that express Dac must be derived from proximal cells that previously expressed Tsh and Hth, from distal cells that previously expressed Dll, or from both populations.

To begin address this question, we examined the expression domains of Dac, Dll and Tsh in second and early third instar imaginal discs. By early third instar Dac expression begins to separate the ring-like Hth/Tsh domain from the central Dll domain (Fig. 2A,B). Cells can be found that express both Dac and Dll as well as cells that express both Tsh and Dac (Fig. 2A,B). At this stage, we can also find cells that express low levels of Dll and Tsh where the two expression domains meet (box Fig. 2A). These observations suggest that there may be a transitional zone between Hth/Tsh-expressing proximal cells and Dll-expressing distal cells, and that this is the region where Dac expression initiates. By early-mid third instar the Dac domain forms a band that overlaps slightly the central Dll domain and the Tsh domain where the outer ring of Dll expression is initiated (Fig. 2C,D, see also Wu and Cohen, 1999). As the disc matures, the Dac and Dll domains overlap considerably in the tibia and basitarsus (yellow, Fig. 2E-H). The outer ring of Dll overlaps both Dac and Tsh (and Hth) in the trochanter.

To ask to what extent cells born in the Tsh-expressing or Dll-expressing domains contribute to the Dac-expressing femur and tibia segments in the course of normal development, we made use of tshGal4 and DllGal4 to lineage tag cells. We first asked if the expression patterns of the GAL4 drivers reflect those of the endogenous proteins in second and third instar discs. tshGal4, UASlacZ larvae were labelled with anti-β-Gal and anti-Dll to compare Gal4-driven β-Gal expression to Dll. In second instar, β-Gal expression is largely complementary to Dll expression (Fig. 3A,B). As noted above for the endogenous proteins, we observe occasional cells expressing both markers (arrow, Fig. 3B). DllGal4-driven β-Gal expression is comparable to that of Dll in a slightly older disc (Fig. 3C). The GAL4 expression patterns are comparable to endogenous patterns at later stages as well (not shown).

Preliminary lineage-tagging experiments showed that embryonic expression of the GAL4 drivers poses a technical problem. Tsh is expressed throughout the embryonic ectoderm of the trunk prior to specification of the imaginal disc primordia (Fasano et al., 1991). Consequently, tshGal4 UAS-Flp lineage tags the ectodermal cell population from which the imaginal disc primordia originate in the embryo. All disc cells from tshGal4 UAS-Flp act5c>stop>lacZ larvae express β-Gal (not shown). Likewise, Dll is expressed in the embryonic ectoderm as the disc primordia are being recruited (Cohen et al., 1993). The early pattern of Dll expression includes more cells than ultimately constitute the imaginal discs, and DllGal4 UAS-Flp also lineage tags virtually all of the cells in the disc (not shown; see also Campbell and Tomlinson, 1998).

To minimize the problem of GAL4-induced FLP activity in the embryo, we made use of a modified version of the FLP recombinase, FLP-EBD, in which the FLP recombinase is linked to the hormone-binding domain of the estrogen receptor (Nichols et al., 1997). The level of recombinase activity of FLP-EBD is regulated by estrogen. In the absence of added estrogen, tshGal4 UAS-Flp-EBD act5c>stop>lacZ shows a moderate background level of recombinase activity. The level of background activity was found to be lower at 18°C, where Gal4 activity is reduced. In larvae raised at 18°C without added estrogen, we observe a few clones per disc on average (Fig. 4A,B). 55% of discs showed one or more clones in the proximal part of the leg, but no distal clones (e.g. Fig. 4A, n=53 leg discs). 45% showed one or more clones in the distal part of the leg (e.g. Fig. 4B). When larvae were fed with estrogen at 25°C in third instar, many small β-Gal-expressing clones were recovered in the proximal ring where Tsh is expressed (Fig. 4C, arrows). Some larger clones were observed in random positions, presumably reflecting estrogen-independent background activity (Fig. 4C, arrowhead). In contrast, when tshGal4 UAS-Flp-EBD act5c>stop>lacZ larvae were fed estrogen beginning in second instar (starting at the equivalent of 48-60 hours of development at 25°C), many β-Gal-expressing clones were found in all regions of the discs (Fig. 4D, 100% of discs, n=33). The number of β-Gal-expressing clones per disc was much higher than in untreated controls. The clones were located in femur, tibia and tarsal segments, as well as in the trochanter and coxa (where Tsh is expressed). The frequency of clones located in the distal region suggests that the progeny of cells that express tshGal4 normally contribute to the formation of distal leg segments.

In the absence of added estrogen, DllGal4 UAS-Flp-EBD act5c>stop>lacZ discs show a low background level of recombinase activity, resulting in less than one β-Gal-expressing clone per disc on average (at 18°C, not shown). Larvae fed with estrogen and kept at 25°C from first or second instar onward show many clones in the femur, tibia and tarsal segments, but no clones in the coxa and more proximal regions of the disc (Fig. 4E). By comparison, feeding larvae with estrogen for 15 hours in mid third instar leads to many small clones in the Dll expression domain and an occasional clone in the femur region (arrowhead, Fig. 4F). This control suggests that the pattern of FLP-EBD activity accurately reflects the expression of Dll protein at the time of estrogen treatment (as
in the untreated controls, we observe occasional background clones in random positions). The observation of marked clones in the femur and tibia when Flp activity is induced early suggests that some cells born in the Dll domain lose (or reduce) Dll expression and contribute to formation of the Dac domain.

**Lineage tracing the progeny of cells expressing Dll in second instar**

The experiments presented thus far show that proximal cells can lose Tsh expression to give rise to any part of the disc in the course of normal development. In contrast, Dll-expressing cells do not normally give rise to coxa. In these experiments, larvae were fed estrogen continuously starting at first or second instar, and therefore the fate of cells that express Dll or Tsh at any point thereafter was traced. Our results indicate that cells of the femur region, which express little or no Dll or Tsh at the end of development, have expressed both Tsh and Dll at some point in second or third instar. To more accurately define the time of Dll expression in the cells that later give rise to the femur, DllGal4 UAS-Flp-EBD act5c>stop>lacZ larvae were fed with estrogen in the second instar and transferred to estrogen-free food. In this experiment, we lineage-tagged cells that expressed Dll during second instar.

DllGal4 UAS-Flp-EBD act5c>stop>lacZ larvae were kept at 18°C for 2-4 days and then fed with estrogen at 25°C for 12-15 hours. Second instar larvae were hand selected and raised at 18°C on fresh food (without estrogen) until late third instar (5-9 days at 18°C). Marked clones (green) were generally found in the Dll domain (red, Fig. 5). The distribution of clones marked in second instar was compared to the mature Dll-expression domain by analysis of serial optical sections and optical cross-sections. Of 23 clones examined in 12 discs, 18 were located in the distal part of the Dll domain, corresponding to the presumptive tarsus. Five clones included cells at the proximal edge of the Dll domain, corresponding to the presumptive distal tibia. A disc containing both types of clone is shown in Fig. 5A. The clone indicated with an arrow extends close to the proximal edge of the Dll domain. The clone indicated by an arrowhead, though large, remains in the tarsal cap of the disc. One clone (of 23) crossed from the Dll-expressing domain into the more proximal tibia. As there is a basal section of the epithelium, arrow). In a basal section (Fig. 6A),...
the boundary between Minute+ and Minute mutant tissue runs along the edge of the Dll expression domain for a considerable distance (arrows), but can cross the Dll ring and extend into more proximal territory (asterisk). In Fig. 6C, the arrow indicates the border of the clone within the Dll ring and the arrowhead shows a Minute+ tissue that crossed the ring on the opposite side of the disc. Both types of clones were observed in most of the 11 discs examined. We conclude that cells born in the Dll domain can adopt proximal fate. The observation that these clones often follow the Dll ring suggests that proximalization does not occur readily, possibly because Dac or Dll expression are not readily lost. This contrasts with the observation that Tsh-expressing cells easily change their pattern of gene expression and adopt more distal fate. Cells that cannot lose Hth (and presumably Tsh) expression cannot cross out of the Dll ring (Wu and Cohen, 1999).

DISCUSSION

In this report, we have examined the ability of cells to change their developmental fate in response to changes in the level of morphogen signaling. We find that cells readily reverse their responses to Hh and Dpp in the wing disc. The reversibility of the response to endogenous Dpp differs from our previous finding that cells expressing the activated form of the Dpp receptor Tkv* under control of dppGal4 allowed expression of Dpp-target genes in anterior cells that had been displaced out of range of the Hh signal (Lecuit et al., 1996). In light of the present results, we infer that the maintenance of Spalt expression in those experiments resulted from perdurance of the activated receptor and that this does not reflect the normal state of the Dpp gradient. The reversibility of responses to Dpp is consistent with several reports showing that cells mutant for components of the Dpp signal-transduction pathway lose target gene expression and appear to adopt more lateral fates (Nellen et al., 1996, Lecuit et al., 1996, Singer et al., 1997). The relative lability of cellular responses to morphogen signaling in the imaginal discs contrasts with the apparently non-reversible ‘ratchet effect’ described in Xenopus. These systems differ in two important respects: patterning in Xenopus takes place in a few hours, without growth, whereas disc patterning takes place over 3 days in a rapidly growing tissue.

In the developing leg disc, we find that some responses to Wg and Dpp are readily reversible, while others are not. Lineage tracing of cells born in the TshGAL4 domain suggests that cells readily lose Tsh (and Hth) expression and instead express Dll and Dac. In young discs, we observed a small proportion of cells expressing low levels of both Dll and Tsh at the edge between these domains. It is possible that these were cells in transition between the domains. Our results suggest that cells born in the presumptive body wall readily contribute to formation of more distal leg regions. Under normal circumstances cells born in the Dll-expressing distal domain of the leg do not contribute to the body wall. However, we have seen that they are not prohibited from doing so when given a strong growth advantage.

The progeny of Dll-expressing cells in second instar are mostly fated to give rise to the tarsus and do not contribute to femur. In contrast, we observed that femur, tibia and tarsal segments derive from cells that have expressed Dll in early third instar. The difference between these stages suggests that

Fig. 4. Lineage tracing the progeny of Dll- and Tsh-expressing cells from second instar. (A,B) X-Gal staining to an everted pupal disc from a UAS-Flip;EBD; tshGAL4; act5c>stop>lacZ larvae kept at 18°C without estrogen treatment. Background clones appear in (A) the proximal region of the disc only, or (B) include tarsal segments. (C) Anti-Dll (red) and anti-β-Gal (green) staining to a disc of a larvae of the same genotype that was fed estrogen in third instar, just before fixation. The majority of clones (small) is seen in the proximal region (arrows). The disc also shows a larger background clone in the Dll-expressing region (arrowhead). (D) X-Gal staining to a UAS-Flip;EBD; tshGAL4; act5c>stop>lacZ disc of a larvae that was fed estrogen from second instar onwards. All discs in this experiment show clones in the tarsal segments. (E) X-Gal staining of a UAS-Flip;EBD; DllGAL4; act5c>stop>lacZ disc from a larvae that was fed with estrogen beginning in first instar. Clones of lacZ expression can be found in femur (fe), tibia (ti) and tarsus (ta). The coxa region (co) is free of clones. The marked cells at the edge of the disc (arrow) are in the outer ring of Dll expression, which runs below the coxa where the disc epithelium folds back on itself (see Fig. 2). (F) Anti-Dll (red) and anti-β-Gal (green) staining to a disc of a larvae of the same genotype that was fed estrogen in late third instar. Clones are found in the Dll domain. One clone is seen in the femur (arrowhead).
new cells must be induced to turn on Dll in order to provide the population of cells that contribute to the femur. These cells must derive from the Tsh domain in second instar and acquire Dll and Dac expression (illustrated in Fig. 7). At later stages, Dll is expressed at very low levels in the femur, where it may be repressed by Dac. Downregulation of Dll expression in the femur is unlikely to be a direct response to a lowering of Wg and Dpp signaling, because clones of cells unable to respond to these signals do not show abnormal Dll or Dac expression (Lecuit and Cohen, 1997). This contrasts with the situation in the wing where removal of Dpp signaling leads to loss of Spalt expression (Nellen et al., 1996, Lecuit et al., 1996). The low level of Dll expression in the femur is in part due to Dac activity, as dac mutant clones show elevated levels of Dll (Abu-Shaar and Mann, 1998). The transient induction of Dll in the precursors of the femur is consistent with genetic analyses
showing that formation of all leg segments except coxa depends on Dll activity in early development, whereas the low level of Dll expressed later is apparently not required for normal femur development (Cohen and Jürgens, 1989; Gorinkiel et al., 1997; Campbell and Tomlinson, 1998).

When the distal part of a leg imaginal disc, or of an amphiobian or a cockroach leg is removed, distal structures will regenerate from the cut edge. If the distal part of the leg disc is cultured alone, distal structures will regenerate from the cut edge, leading to a duplication of the fragment. The fact that distal structures regenerate but proximal structures do not has not been termed distal transformation (French et al., 1976; Schubiger and Schubiger, 1978; Bryant et al., 1981). These experiments show that cells have a general tendency to distalize, whereas their capability to proximalize is restricted.

Our experiments show that distal transformation happens during normal development. Some proximal cells switch their pattern of gene expression as the disc grows and acquire distal fate. Distal cells do not normally switch to proximal fate, but can do so if forced during early development. The regeneration studies suggest that the ability to shift from distal to proximal fate may be lost as development proceeds.

We thank Ann-Mari Voie and Anna Cyrklaf for technical assistance and Birgit Kerber and David Hipfner for suggesting modifications to the manuscript and the members of the laboratory for discussion. K. W. was supported by an EMBO-LTF.

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