Å, and c = 198.32 Å and contain one molecule per asymmetric unit.
21. The structure was refined to 2.2 Å resolution against data set NAT-Ox2 with the simulated annealing protocol in X-PLOR, followed by restrained isotropic B factor refinement and positional refinement. A bulk solvent correction allowed the inclusion of the low-resolution reflections. The current model (6293 atoms) consists of residues 1 to 380 and 394 to 780, 2 MGDs, one Mo(VI) ion with an oxo ligand, 279 water molecules, and Trp^{388} in two alternative conformations with occupancies of 0.5. The stereochemistry of the model is very good, with 87.7 percent of the residues in the most favored regions of the Ramachandram diagram and only two residues, Glu^{163} and His^{649}, in disallowed regions. However, both residues have well-defined electron density and the unusual main-chain torsion angles of His^{649} might be due to involvement in cofactor binding. The average coordinate error estimated from a Luzzati plot [V. Luzzati, *Acta Crystallogr.* **5**, 802 (1952)] is between 0.25 and 0.3 Å.
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Sending and Receiving the Hedgehog Signal: Control by the *Drosophila* Gli Protein Cubitus interruptus

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Drosophila limb development is organized by interactions between anterior and posterior compartment cells. Posterior cells continuously express and require *engrailed* (*en*) and secrete Hedgehog (Hh) protein. Anterior cells express the zinc-finger protein Cubitus interruptus (Ci). It is now shown that anterior cells lacking *ci* express *hh* and adopt posterior properties without expressing *en*. Increased levels of Ci can induce the expression of the Hh target gene *decapentaplegic* (*dpp*) in a Hh-independent manner. Thus, expression of Ci in anterior cells controls limb development (i) by restricting *hh* secretion to posterior cells and (ii) by conferring competence to respond to Hh by mediating the transduction of this signal.

Drosophila appendages develop from imaginal discs, each of which is composed of two adjacent but distinct cell populations, anterior (A) cells and posterior (P) cells (*1*). P cells continuously express *en* (*2–4*), which encodes a homeodomain protein (*3*, *5*) and which programs these cells to secrete the signaling molecule Hh (*6*). At the A/P boundary, but not in the rest of the P compartment, Hh induces in adjacent cells the expression of the organizing signal Dpp (*7*). Genetic analysis has shown that P cell identity is specified by *en* and the *en*-related gene *invected* (*inv*) (*8–11*), whereas A cell identity is the default fate for imaginal disc cells.

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The subdivision of the imaginal disc into A and P compartments can be visualized by the expression of the *cubitus interruptus* (*ci*) gene in all A compartment cells (*12*, *13*). Restriction of *ci* expression to A cells begins with the onset of *en* expression, which represses *ci* expression in the P compartment cells (*12*, *13*). Activation of *en* coincides with the establishment of specific affinities of A and P cells, which prevents intermixing between these cell populations. *ci* encodes a Zn-finger protein (Ci) belonging to the Gli family of transcription factors (*14*).

Anterior cell identity requires *ci* function. Loss-of-function mutations in *ci* are embryonic lethal (*15*). Thus, the effects of complete removal of *ci* function in imaginal disc cells have to be studied in genetic mosaics. *ci* is located on the fourth chromosome, and virtually no recombination is observed between fourth chromosome arms (*16*), making the generation of clones mutant for *ci* difficult. We have induced such clones by x-rays, albeit at very low frequency (*17*). Immunofluorescence studies of these *ci* null mutant clones have enabled us to make three observations: (i) *ci* clones form compact patches with smooth borders, as if cells within these clones minimize their contact with surrounding cells (Fig. 1, A to C and E to G). (ii) Most clones cause an up-regulation of Ci protein levels in surrounding cells (Fig. 1, A, C, E, and F). This accumulation

of Ci protein is similar to that of Ci along the A/P compartment boundary (*13*, *18*) (Fig. 1A). (iii) We found that *ci* mutant cells cause an autonomous, and often also nonautonomous, increase in proliferation that can result in outgrowths (Fig. 1, B and E).

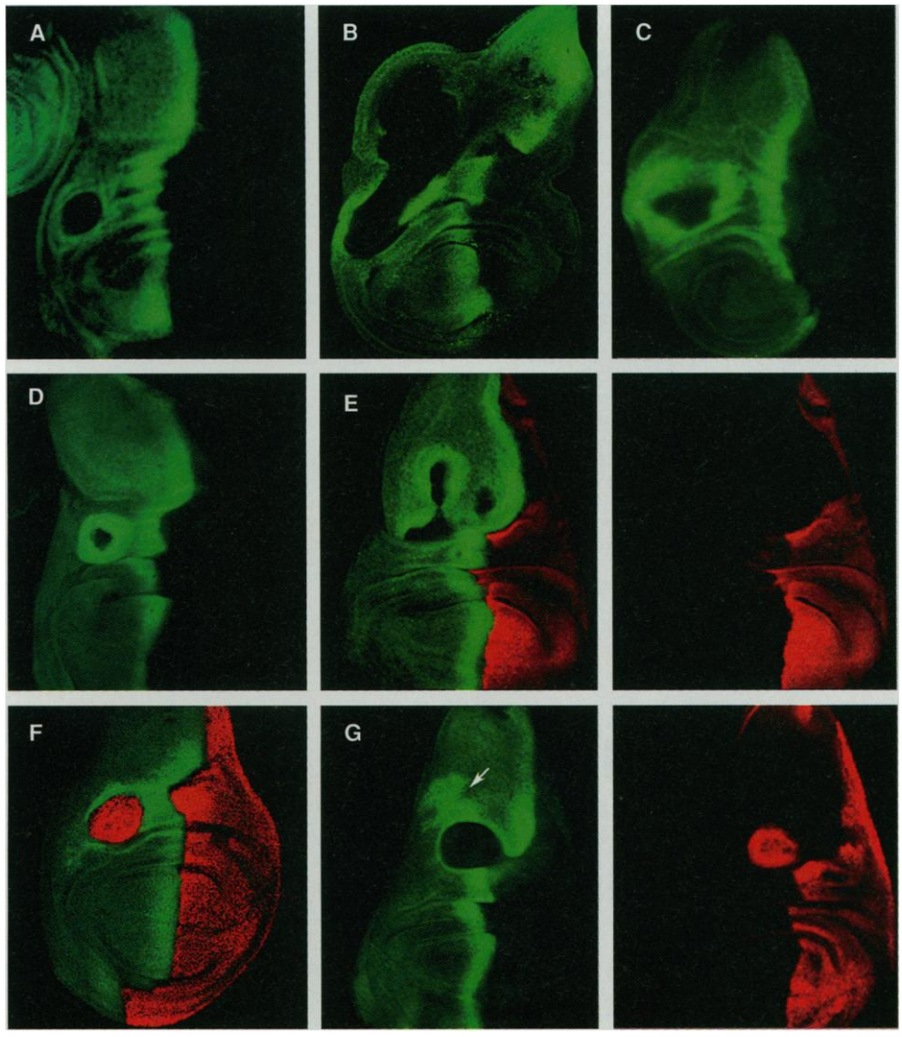

Fig. 1. Clones of cells mutant for *ci* express *hh* and cause pattern reorganizations. All panels show late third instar larval wing imaginal discs. Ci expression is always in green, anterior is to the left, and dorsal is up. (**A** through **C**) Wing discs carrying clones of ci^{null} mutant cells marked by the lack of Ci staining. In the wild type, Ci protein is distributed uniformly at low levels in all A cells and at higher levels in a band, three to five cells wide, adjacent to the A/P compartment border. The presence of a patch of ci^{-} cells, in an otherwise ci^{+} tissue, can reprogram A cells to accumulate Ci protein around the ci^{-} cells [(A through C) and (E through G)] and to induce excess proliferation (B and E). A total of 52 *ci* clones were analyzed for this study. (**D**) A wing disc carrying a *Tubα1>en* clone in the A compartment that was marked by the presence of *hh-lacZ* expression (*10*). *Tubα1>en* clones are circular, autonomously repress *ci* expression, and nonautonomously up-regulate the Ci protein levels in a ring around the clone. This behavior is similar to the effects of removal of *ci* function [compare *ci* clones in (A) and (C) with the *Tubα1>en* clone in (D)]. (**E**) An *en-lacZ* wing disc carrying a ci^{-} clone double-stained with anti-Ci (green, left panel) and anti–β-galactosidase (red, left and right panels) to visualize *en* expression. None of the clones showed *en* expression, as assessed either by the enhancer trap line *ryxho25* (*4*, *19*) (six clones) or by the En monoclonal antibody 4D9 (*43*) (three clones). (**F** and **G**) Autonomous activation of *hh* expression in *ci* mutant cells. We used two independently generated $ci^{protein\text{-}null}$ alleles, $ci^{D+revA9\text{-}101A}$ (*17*) (F) and ci^{DR50} (*13*) (G). Because both revertants have been induced on the same ci^{D} chromosome, we cannot exclude the presence of additional lesions on these chromosomes. The up-regulation of Ci protein levels (in green) around the *ci* clones is likely to be a consequence of the *hh* expression within the clones (red staining in F and G, right panel). (G) In this disc, the *ci* clone (marked by the absence of Ci staining) is the result of a reciprocal recombination event. The twin spot clone (determined by the higher Ci protein level) has a typical irregular shape (arrow), in contrast to the smooth, round shape of all *ci* clones. All 15 clones that were analyzed by double staining showed *hh-lacZ* expression; 12 clones were induced with the $ci^{D+rev9A\text{-}101A}$ allele, and 3 with ci^{R50}.

The interface between wild-type and *ci* mutant cells resembles an A/P compartment boundary (Fig 1, A and C). Moreover, *ci* mutant clones behave similarly to anterior clones that ectopically express *en* (*Tubα1>en*) (*10*). Anterior expression of *en* under the control of the *Tubα1* promoter causes autonomous repression of *ci* transcription (Fig. 1D). As with ci^{-} clones (Fig. 1, A to C), cells surrounding the *en*-expressing clones accumulate higher levels of Ci protein (Fig. 1D). However, the described behavior of *ci* mutant clones is a consequence not of *en* expression within these clones (Fig. 1E) but of the lack of *ci* activity, suggesting that the phenotype caused by ectopic *en* expression in A cells might be a consequence of the ability of En to repress *ci* transcription.

Ci prevents *hh* expression in anterior cells. We monitored *hh* expression in wing discs carrying *ci* mutant clones (*19*) and found that loss of *ci* activity always caused autonomous activation of *hh* expression (Fig. 1, F and G). Thus, *ci* activity is required in A cells to prevent them from expressing *hh*. Ectopic expression of *ci* by means of the Gal4 system (*20*) does not consistently reduce *hh-lacZ* expression in P cells (*21*), indicating that although Ci is required to repress *hh* transcription, it is not sufficient to do so in P cells (*22*). The clonal analysis indicates that an essential function of Ci is to prevent *hh* expression, thereby defining the anterior border of the *hh* expression domain.

Ci transduces the Hh signal by activating the target genes *dpp* and *ptc*. The Hh signaling pathway induces in adjacent, non–*en*-expressing cells the expression of *dpp* (*7*). The activities of the segment polarity gene product Patched (Ptc) (*11*, *23*, *24*) and the cyclic AMP–dependent protein kinase A (Pka-C1) (*24*, *25*) block inappropriate *dpp* expression in the absence of Hh signaling. Loss of *ptc* (*26*) or *pka-C1* function in imaginal disc cells causes an autonomous accumulation of Ci protein (Fig. 2B) (*27*). Because the transcriptional activity of the *ci* gene is not changed in these clones (Fig. 2A), it can be inferred that Ci levels must normally be down-regulated posttranscriptionally by Ptc and Pka and that this negative regulation is alleviated in those cells that receive the Hh signal (*18*, *27*). This conclusion explains the increased levels of Ci protein in cells surrounding *ci* clones and at the A/P boundary (Fig. 1, A to C, E, and F). The control of Ci protein levels by the Hh signaling pathway could be of crucial importance if Ci functions in a concentration-dependent manner. In wild-type wing discs, the high levels of Ci protein along the A/P boundary spatially correlate with the domain of *dpp*

and high *ptc* expression. To test whether high levels of Ci are sufficient to activate *dpp* expression independently of Hh, we ectopically expressed increased levels of Ci (Fig. 2, C and D) and found that *dpp* transcription was induced in A cells and even in *en*-expressing P cells (Fig. 2, C and D) (*28*). Consistent with a role for *ci* in inducing *dpp* transcription, we observed that a 50% reduction in *ci* exacerbates the phenotype of hypomorphic dpp^{disc} alleles (*29*). So, as has been inferred from genetic analysis in the embryo (*18*, *30*), *ci* functions downstream of the Hh signaling pathway to activate transcription of the Hh target genes. From our ectopic expression experiment, we conclude that Ci plays a direct role in inducing *dpp* expression. Notably, Ci is predicted to be a Zn-finger transcription factor and is the first identified component of the Hh-signaling cascade that is able to activate *dpp* transcription in both A and P cells. Ectopic expression of Ci is also able to activate *ptc* transcription in P cells (*31*).

Bifunctional role of Ci in regulating *dpp* expression. In reciprocal experiments, we investigated *ptc* and *dpp* expression in wing discs containing *ci* mutant clones (*32*). We found that expression of *ptc-lacZ* in cells surrounding *ci* mutant clones (Fig. 3A) mimics its expression along the A/P compartment boundary—consistent with a positive regulation of *ptc* by Ci (see Fig. 4 legend). In contrast, we found that regulation of *dpp* transcription by Ci is more complex. Although cells surrounding *ci* clones occasionally expressed *dpp* (Fig. 3B), cells lacking *ci* consistently express *dpp* (Fig. 3B). This expression of *dpp* in *ci* mutant cells implies that *dpp* expression is normally repressed by the low levels of Ci in the wild-type A cells. Because *ci* mutant cells express *hh* (Fig. 1, F and G) and do not express *en* (Fig. 1E), Hh signaling might bypass Ci function to induce *dpp* expression. However, we think these data indicate that the Zn-finger protein Ci acts both as a repressor and as an activator of *dpp* transcription in a concentration-dependent manner (Fig. 4). Other Gli-like proteins, such as YY1 (Yin Yang 1) in humans and Krüppel in *Drosophila*, can act as repressors as well as activators (*33*, *34*), depending on their concentration (*33*, *35*). Furthermore, the activity of YY1 is negatively modulated by the E1A-associated adapter protein p300 (*36*), a homolog of CBP (CREB binding protein) (*37*). Haploinsufficiency for the cofactor CBP gives rise to the Rubinstein-Taybi syndrome characterized by abnormal development of extremities (for example, broad thumbs and overgrown toes) (*38*). A CBP homolog in *Drosophila* might play an analogous role in Ci-mediated *dpp* transcription. Constitutive phosphorylation by Pka might be required for this CBP homolog and Ci to repress *dpp* transcription. Conversely, reduction of Pka or CBP activity or an increase of Ci levels (by experimental overexpression or by Hh signaling) might overcome this regulation and result in *dpp* activation (Fig. 4).

Ci mutant phenotypes. The bifunctional roles of *ci* in controlling gene expression are reflected in the phenotypes of viable *ci* alleles. Wings from homozygous ci^{W} mutants have, at low penetrance, a mirror-symmetric outgrowth composed of anterior wing material (Fig. 5A) (*39*, *40*), resembling duplications caused by ectopic *dpp* expression (*10*). In ci^{W} mutant wing discs, *dpp*, but not *hh*, is ectopically expressed in patches of A cells associated with the anterior outgrowths (Fig. 5B). One could explain these defects by inferring that the lower levels of Ci protein in ci^{W} mutants (*13*) are too low to adequately repress *dpp* transcription in A

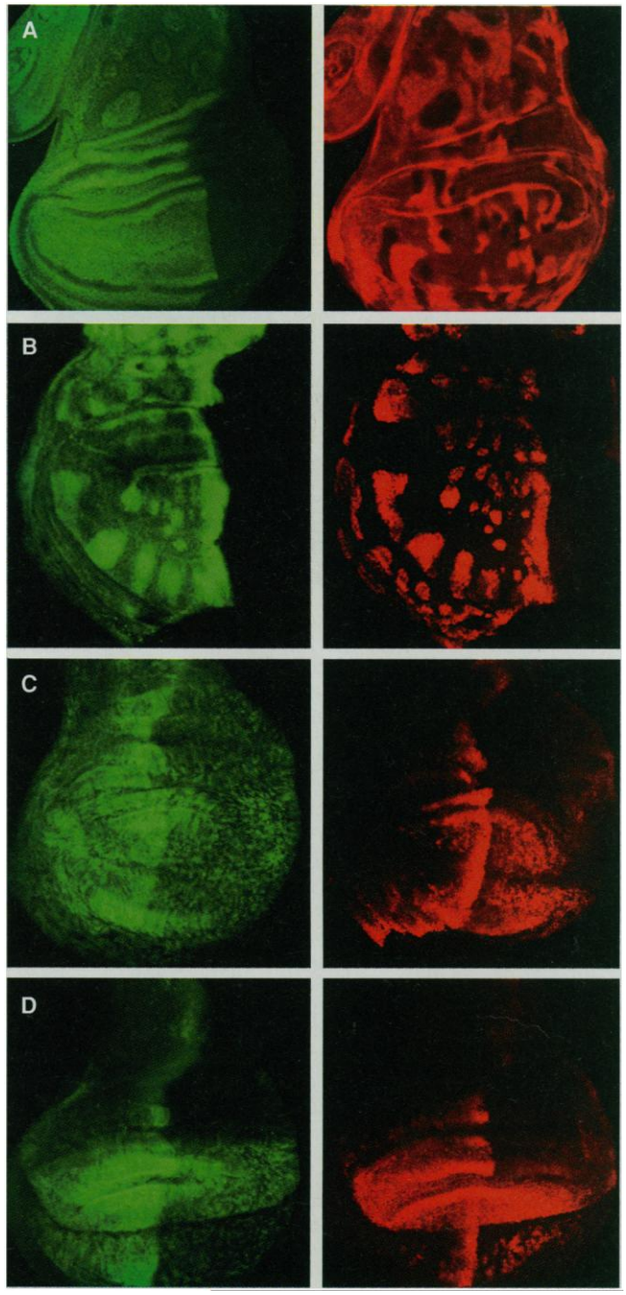

Fig. 2. *ptc*-mediated control of Ci protein levels prevents inappropriate activation of *dpp* transcription. All panels show third instar wing discs. Anterior is to the left, and dorsal is up. (**A** and **B**) Two wing discs carrying multiple ptc^{S2} clones. The clones are marked by the lack of the *CD2* marker gene expression [red in (A), twin spots with two copies of the *hs-CD2* transgene are also visible] or the presence of *dpp-lacZ* expression [red in (B)]. The transcription of *ci* was monitored by means of an enhancer trap line in *ci* [green in (A)] and the distribution of the Ci protein by staining with anti-Ci [green in (B)]. In contrast to *ci-lacZ* expression, Ci protein levels are up-regulated in *ptc* mutant clones, indicating that Ci levels in A cells that are not exposed to the Hh signal are posttranscriptionally down-regulated during normal development. (**C** and **D**) Expression of high Ci levels by means of the Gal4/UAS system can activate *dpp* transcription in A and P cells. (C) Overexpression of *ci* driven by the Gal4 line C765. This Gal4 enhancer trap line is expressed by most wing imaginal cells at the late third instar larval stage. The ectopic expression of *ci* (green staining) causes excess proliferation, likely because of the activation of *dpp* expression (red) in A and P cells. In wild-type discs, *dpp* expression is confined to a stripe of cells running along the A/P compartment boundary (visible in Figs. 2C, 3A, and 4B). (D) The Gal4 line MS1096 is expressed at high levels by dorsal wing cells and at low levels in ventral wing cells. *dpp* expression is activated (red) in those cells that express high but not low levels of Ci (green). The genotypes of the wing discs are *FRT42* ptc^{S2} *hs-CD2;* ci^{PlacZ}/+ (A), dpp^{P10638} *FRT42* ptc^{S2} (B), dpp^{P10638}/+; *Gal4[C765]/ UAS-ci* (C), and *Gal4[MS1096]/+;* dpp^{P10638}/+; *UAS-ci*/+ (D).

cells. Conversely, adult wings heterozygous for another *ci* allele, the ci^{Cell}, have been described as narrower and as lacking wing material between veins L3 and L4, the region adjacent to the A/P compartment boundary (*39*). This defect is typical for a "reduced *dpp* function" phenotype. The ci^{Cell} allele encodes a COOH-terminally truncated protein that inappropriately accumulates to high, uniform levels in all A cells (*13*). Presumably, the Ci^{Cell} protein acts in a dominant-negative fashion by competing with wild-type Ci in the activation of *dpp* transcription along the compartment boundary.

Multiple roles of the Gli-related protein Ci in controlling limb development. Taken together, our results indicate that *ci* controls multiple fundamental properties of A compartment cells. A cells that have lost *ci* activity minimize contact with surrounding wild-type A cells, suggesting that A cell identity is not a default fate that imaginal cells adopt in the absence of *en*. The analogy to the behavior of *en/inv* mutant clones in the P compartment raises the possibility that loss of *ci* function in the A compartment generates cells with "P-type affinity." In this instance, Ci could be regarded as the primary determinant of compartment affiliation, and the A/P compartment boundary would be defined by the apposition of *ci*-expressing and non–*ci*-expressing cells. In this scenario, the primary function of *en* in the compartmentalization process of imaginal discs would be to selectively prevent *ci*

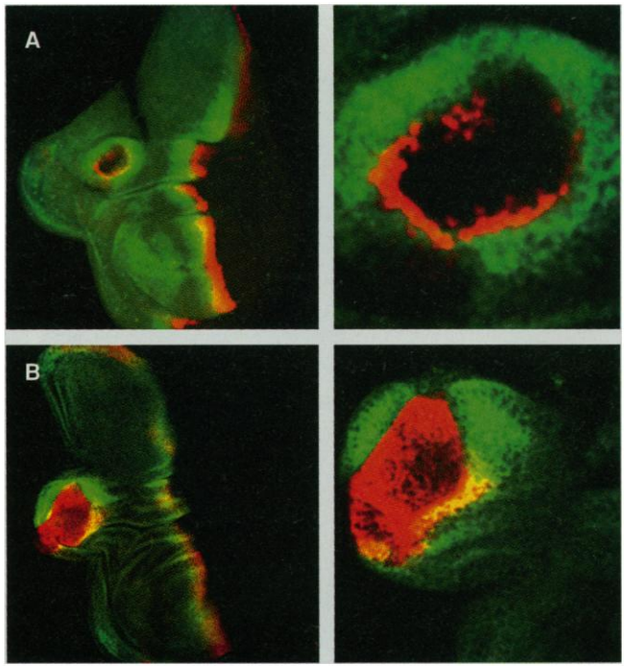

Fig. 3. Regulation of *ptc* and *dpp* by Ci. In all panels Ci staining is in green. (**A**) The high levels of Ci protein around the ci^{-} clones induce *ptc-lacZ* expression (red) at a level comparable with the wild-type *ptc* expression along the A/P border. Ten clones were recovered in a *ptc-lacZ* background. (**B**) In addition to some nonautonomous expression around the clones (see yellow stain in the right panel), *dpp-lacZ* expression (red) is observed within the *ci* mutant clones. The increase in proliferation that results in outgrowths is likely due to the activation of *dpp* in *ci* mutant cells. Thirteen clones were analyzed for *dpp* expression by two different reporter lines (*32*), and in all cases *dpp* was expressed by the ci^{-} cells in the A compartment.

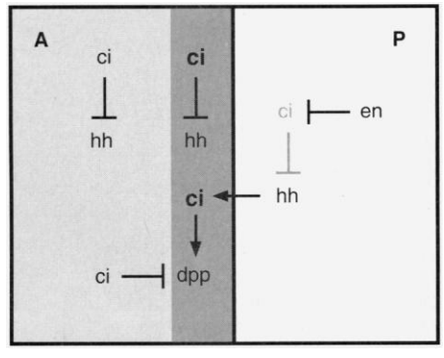

Fig. 4. Model for the multiple functions of *ci* in preventing the production of, but conferring competence to, the Hh signal. The ability of En to repress *ci* transcription subdivides the wing imaginal disc into two cell populations: anterior, *ci*-expressing cells (A, shaded) and posterior, non–*ci*-expressing cells (P, unshaded). Ci performs two functions during limb patterning. First, constitutive *ci* activity represses *hh* in all A cells, thereby generating a non–*hh*-expressing (A) and a *hh*-expressing population of cells (P). Second, Ci is also a central component of the Hh signal transduction pathway. The selective expression of *ci* in A cells is therefore also responsible for creating a Hh-responsive (A) and a non–Hh-responsive population of cells (P). This dual function of Ci links the control of Hh signal production with the control of Hh signal reception. *Regulation of* dpp *and* ptc *expression by different levels of Ci.* Pka and Ptc activity prevent the stabilization (or posttranscriptional modification) of Ci protein in all A cells that are not exposed to Hh signal. Therefore, Ci protein is present at low levels in these cells (indicated by ci). Hh signal transduction allows stabilization (or modification) of the Ci protein by antagonizing the posttranscriptional regulation imposed by Pka and Ptc. The Ci protein levels are high (indicated by ci in bold type) in cells near the A/P boundary that are exposed to the Hh signal (dark shaded). We propose that at higher concentration Ci activates *dpp* and *ptc* transcription (for example, by forming homodimers or heterodimers with other factors). In contrast, at low concentration, Ci protein is likely unable to form homodimers and functions alone, or in combination with cofactors (for example, a CBP homolog), as a repressor of *dpp* expression. Our findings raise the possibility that Pka and Ptc activity repress *dpp* and *ptc* transcription by modulating Ci levels (or activity). Lack of *pka* or *ptc* function results in increased Ci protein levels and activation of *dpp* and *ptc* transcription independently of the Hh signal. Ectopic expression of *ci* shows that *ptc* expression is positively regulated by *ci* function (*31*), indicating that repression of *ptc* by *en* is likely to be indirect. In contrast, *dpp* expression is regulated at different levels; our results suggest that *dpp* is actively repressed or induced by low or high Ci levels, respectively.

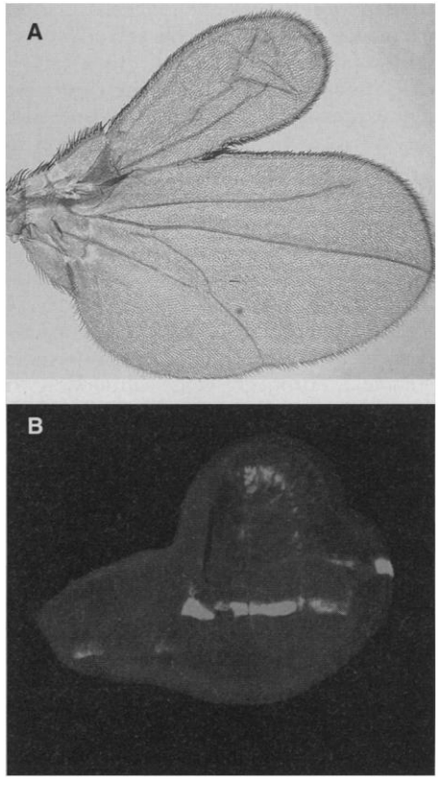

Fig. 5. Duplication of anterior wing pattern in the viable ci^{W} mutant and the association of ectopic *dpp* expression with such outgrowths. In both panels, anterior is up. (**A**) An adult wing of a female fly homozygous for the ci^{W} mutation. The reduced Ci levels that occur in this mutant (*13*) are likely to be responsible for the anterior pattern duplication. In addition, this mutation causes misexpression of *ci* in the posterior compartment (*13*). The interruption of the vein L4 is due to the ectopic expression of *ci* in P cells. (**B**) A third instar larval wing disc homozygous for the ci^{W} mutation stained with anti–β-galactosidase to visualize *dpp-lacZ* expression. Note the ectopic expression of *dpp* associated with the anterior outgrowth. We did not detect ectopic *hh* expression in such wings.

transcription in one population of disc cells, thereby generating a *ci* "on"/*ci* "off" interface of cells.

Our results indicate that *ci* expression is responsible for preventing the expression of *hh* in one population of imaginal disc cells (the A cells) at the same time that it renders these cells competent to respond to Hh and mediates the transduction of this signal (Fig. 4). The dual function of *ci*—to prevent production of, but to confer competence to, the Hh signal—might represent an evolutionarily conserved aspect of patterning by Hh family members. It is interesting to note possible parallels in the function of Ci and its vertebrate homologs Gli. First, like Ci, Gli proteins appear to be expressed in patterns complementary to Sonic Hh (*41*). Second, like reduction of *ci* activity, haploinsufficiency for the Gli3 gene appears to be responsible for the genetic disorders *Greig cephalopolysyndactyly syndrome* (GCPS) in humans and *Extra-toes* (*Xt*) in mice (*42*). Mutations in *extra-toes* cause anterior digit duplications similar to the phenotype of homozygous ci^{W} flies (Fig. 5A). These observations are consistent with the view that Gli protein function in Hh signaling is not restricted to *Drosophila*.

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17. Clones of *ci* mutant cells were induced as follows: Embryos and first instar larvae (12 to 54 hours after egg laying) were exposed to an x-ray source (Phillips, 150 kV and 14 mA at 24-cm focal distance) at a final dose of 13 gray. In the absence of suitable cell markers for the fourth chromosome, we used a rat Ci monoclonal antibody (anti-Ci) to mark *ci* cells in the anterior compartment by the lack of Ci staining. The two *ci* amorphic alleles used, $ci^{D+rev9A\text{-}101A}$ and ci^{DR50}, do not produce detectable levels of Ci protein [J. Locke and K. D. Tartof, *Mol. Gen. Genet.* **243**, 234 (1994)]. *ci* clones in the experiment shown in Fig. 1, A and B, were induced in a $ci^{D+rev9A\text{-}101A}/M^{57g}$ background, and the *ci* clone shown in Fig. 1C was induced in a $ci^{DR50}/\pm$ background. The large size of the clone shown in Fig. 1B can be attributed to the M^{+} advantage in the clone (*19*). The frequency of the *ci* clones was very low, one wing clone for 50 to 100 larvae dissected.
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19. For the experiments in which *en* or *hh* expression was monitored, *ci* clones were generated in the progeny of females $ci^{D+rev9A\text{-}101A}/M^{57g}$ crossed to males carrying either the lethal enhancer trap line *ryxho 25* (*4*) for *en* or the viable P30 line [C. Ma, Y. Zhou, P. A. Beachy, K. Moses, *Cell* **75**, 927 (1993)] for *hh* expression. The frequency of these clones was, therefore, reduced at least by half. A fraction of *ci* clones marked by the ectopic expression of *hh-lacZ* was stained with a mouse monoclonal antibody to En/Inv, confirming our results obtained with the $en^{ryxho\ 25}$ line. In the experiment shown in Fig. 1, F and G, two different *ci* amorphic alleles, generated independently, were used (*17*). We obtained the same results with both alleles, demonstrating that the effects of *ci* clones are not influenced by genetic backgrounds. Surprisingly, twin spots are rarely found (detected as increased Ci staining) in these experiments, indicating that the majority of *ci* mutant cells result from the loss of the ci^{+} chromosome. The cells in these *ci* clones are haplo fourth. In Fig. 1G, we show an example of a true recombination event between the fourth chromosome arms. The irregular shape of the twin spot indicates that all aspects of *ci* clones can be attributed to the lack of *ci* activity.
20. The full-length *ci* cDNA was inserted into the vector pUAST [A. H. Brand and N. Perrimon, *Development* **118**, 401 (1993)]. Three independent UAS-Ci strains were analyzed.
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22. The inability of ectopically expressed Ci to repress *hh* in *en*-expressing cells may be due to the existence of a Ci cofactor (X) that, like *ci*, is present only in A cells as a result of direct *en* repression. In P cells that lack *en* activity, both the expression of *ci* and that of the hypothetical factor X will be derepressed, resulting in *hh* repression.
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26. *ptc* mutant clones were generated by means of the FLP FRT technique [K. G. Golic, *Science* **252**, 958 (1991)]. The *ptc* allele used was ptc^{S2}. *ptc* mutant clones were detected by two methods: (i) by the loss of the CD2 marker within the *ptc* mutant tissue and (ii) by the ectopic expression of *dpp-lacZ* (*11, 23–25*). Transcription of *ci* was monitored by means of an enhancer trap line in the *ci* locus.
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28. *En* expression is not reduced in P cells that ectopically express Ci (*21*). Therefore, high levels of Ci can override the partial repression of *dpp* expression by En [M. Sanicola, J. J. Sekelsky, S. Elson, W. M. Gelbart, *Genetics* **139**, 745 (1995)].
29. The *dpp* alleles used were dpp^{s8} and dpp^{d12} and the *ci* allele was the $Df(4)M^{62f}$ that uncovers the *ci* locus. The dpp^{s8}/dpp^{d12} flies reach adulthood and display a typical *dpp* wing phenotype (*9*). Females $dpp^{s8}/+$; $Df(4)M^{62f}/+$ were crossed to males dpp^{d12}/CyO. Flies that have a 50% reduction of *ci* (recognized as *Minute* flies) are synthetically lethal in combination with this *dpp* mutant combination. To exclude an effect of the *M* mutation associated with the $Df(4)M^{62f}$ chromosome, we crossed females $dpp^{s8}/+$; M^{57g}, $ci^{+}/+$ to males dpp^{d12}/CyO as control, and dpp^{s8}/dpp^{d12}; M^{57g}, $ci^{+}/+$ flies were found in the progeny.
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31. *Ptc* expression is readily induced in all P cells, even by relatively low ectopic Ci expression levels (*40*), suggesting that, unlike *dpp* (*28*), *ptc* is not negatively regulated by En. Therefore, *ptc* expression is normally restricted to A cells because *ci* is not expressed in P cells. We observe higher levels of *ptc* expression in P cells than in A cells presumably because Ptc protein in P cells is inactive in repressing *ptc* expression because P cells are continuously exposed to the Hedgehog signal.
32. *ptc* expression was monitored by means of an enhancer trap line in *ptc*. Our *ptc-lacZ* line did not allow one to score the low level of *ptc* transcription present in A cells that are not exposed to the Hh signal. We could not, therefore, determine whether the low level of *ptc* expression is lost in *ci* mutant clones. *dpp* expression was monitored by means of either the *dpp* reporter construct BS3.0 [R. K. Blackman *et al.*, *Development* **111**, 657 (1991)] or an enhancer trap line dpp^{P10638}.
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40. The ci^{W} allele has another, dominant effect on *posterior* wing pattern (gap in vein L4). ci^{W} discs show a low level of *ci* misexpressed in the P compartment. This results in the transcriptional activation of *ptc* in P cells, indicating that *ci* positively regulates *ptc* transcription and that restriction of *ptc* expression to A cells by *en* is mediated indirectly by preventing *ci* expression in P cells.
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