Expression of wingless in the Drosophila embryo: a conserved cis-acting element lacking conserved Ci-binding sites is required for patched-mediated repression

Derek Lessing* and Roel Nusse†

Howard Hughes Medical Institute, Department of Developmental Biology, Beckman Center, Stanford University, Medical Center, Stanford, CA 94305, USA

*Present address: MCDB Department, PO Box 208103, Yale University, New Haven, CT 06520-8103, USA

†Author for correspondence (e-mail: rrusse@cmgm.stanford.edu)

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SUMMARY

Patterning of the Drosophila embryo depends on the accurate expression of wingless (wg), which encodes a secreted signal required for segmentation and many other processes. Early expression of wg is regulated by the nuclear proteins of the gap and pair-rule gene classes but, after gastrulation, wg transcription is also dependent on cell-cell communication. Signaling to the Wg-producing cells is mediated by the secreted protein, Hedgehog (Hh), and by Cubitus interruptus (Ci), a transcriptional effector of the Hh signal transduction pathway. The transmembrane protein Patched (Ptc) acts as a negative regulator of wg expression; ptc embryos have ectopic wg expression. According to the current models, Ptc is a receptor for Hh. The default activity of Ptc is to inhibit Ci function; when Ptc binds Hh, this inhibition is released and Ci can control wg transcription.

We have investigated cis-acting sequences that regulate wg during the time that wg expression depends on Hh signaling. We show that approximately 4.5 kb immediately upstream of the wg transcription unit can direct expression of the reporter gene lacZ in domains similar to the normal wg pattern in the embryonic ectoderm. Expression of this reporter construct expands in ptc mutants and responds to hh activity. Within this 4.5 kb, a 150 bp element, highly conserved between D. melanogaster and Drosophila virilis, is required to spatially restrict wg transcription. Activity of this element depends on ptc, but it contains no consensus Ci-binding sites. The discovery of an element that is likely to bind a transcriptional repressor was unexpected, since the prevailing model suggests that wg expression is principally controlled by Hh signaling acting through the Ci activator. We show that wg regulatory DNA can drive lacZ in a proper wg-like pattern without any conserved Ci-binding sites and suggest that Ci can not be the sole endpoint of the Hh pathway.

Key words: hedgehog, Drosophila, wnt, wingless, patched, Cubitus interruptus, segmentation

INTRODUCTION

A critical period in anteroposterior patterning in the Drosophila embryo takes place after gastrulation. At this time, the borders of each segment primordium, or parasegment, are defined by the expression domains of two genes, wingless (wg) and hedgehog (hh) (Martinez Arias, 1993). However, at this stage, the parasegment boundaries are not yet stable. Two observations demonstrate the necessity of properly regulated wg and hh gene activities for the segmentation process. First, loss-of-function mutations in either wg or hh disrupt segmentation. A wild-type embryo has an alternating pattern of denticles and naked cuticle on its ventral surface; a belt of denticles and the adjacent naked cuticle form a segment. In both wg and hh mutants, naked cuticle is lost and denticles are continuous (Nüsslein-Volhard and Wieschaus, 1980). Second, ectopic expression of either gene has a drastic effect on segmentation. If wg is expressed throughout the embryo, a completely naked cuticle results (Noordermeer et al., 1992); ubiquitous Hh causes a rearrangement in each segment of the cuticle such that the posterior portion of each denticle array is replaced by a mirror image of the anterior portion (Ingham, 1993; Tabata and Kornberg, 1994).

wg and hh are expressed in non-overlapping domains but their expression is nevertheless mutually dependent. wg transcription fails in a hh embryo, implying that a signal passes from the hh-expressing cell to the neighboring anterior cell to maintain wg expression. Since hh transcription is lost in a wg mutant, a parallel signaling event takes place from the wg cell to the adjacent posterior cell. Both genes encode secreted proteins which are, in fact, the signals forming this regulatory circuit (DiNardo et al., 1994).

A better understanding of Wg activity will involve not only discovering how the Wg ligand is interpreted in responding
cells, but also how wg expression is restricted in each parasegment. In the embryo, wg is expressed in an approximately one-cell-wide stripe located at the posterior edge of each parasegment (Baker, 1988). Many members of the segment polarity class of genes, to which wg and hh belong, encode proteins that transduce the Hh signal and are required to maintain proper wg expression. For example, the patched (ptc) product is a transmembrane protein that is a putative receptor for Hh (Marigo et al., 1996; Stone et al., 1996). In ptc embryos, wg stripes expand anteriorly; therefore ptc acts to repress wg expression (Ingham et al., 1991; Martinez Arias et al., 1988).

Cubitus interruptus (Ci), another segment polarity gene, is also required for wg expression (Forbes et al., 1993). Ci is a member of the Gli family of zinc finger-containing proteins; Ci binds to Gli consensus sites upstream of the wg promoter and can activate transcription in Drosophila Schneider cells (Von Ohlen et al., 1997) or in yeast (Alexandre et al., 1996). Based on genetic evidence, Ci is part of the Hh signaling pathway; the gain-of-function allele Cf (Von Ohlen et al., 1997) or Ci driven by the hairy promoter (Alexandre et al., 1996) can substitute for hh function. Moreover, processing of Ci protein is modified by Hh signaling (Aza-Blanc et al., 1997). In the simplest model of the Hh pathway, Ptc activity results in downregulation of Ci in most cells of the parasegment. This inhibition of Ci is relieved when Hh binds to Ptc, resulting in Ci activation of wg in a single-cell-width domain at the parasegmental border. Ci is the only known transcription factor regulated by Hh.

We have taken two approaches to identify cis-acting regulatory sequences of the wg gene. First, we cloned wg from another Drosophila species, D. virilis. These two species are separated by 60 million years (Beverley and Wilson, 1984), sufficient time for significant divergence of non-functional DNA upstream of the wg transcription unit. Embryonic development is largely identical for both species; wg is expressed in the same pattern in D. virilis as it is in D. melanogaster, so conserved regulatory sequences are likely to have the same function.

Second, we carried out reporter construct assays in embryos. We found that 4.5 kb upstream of wg is sufficient for generating wg-like stripes in the embryonic germ band. Deletion of a small conserved element resulted in expansion of these reporter stripes. Further, this element can confer spatial repression that was lost in a ptc mutant background. A conserved cis-acting regulatory element is therefore responsive to ptc activity and the Hh pathway. The element most likely binds a transcriptional repressor, since its absence results in ectopic reporter gene expression; however, as we note in the discussion, there are no likely segment polarity proteins which are good candidates for mediating the function of this conserved element.

MATERIALS AND METHODS

Molecular biology

Primer extensions were performed as described in Nusse et al. (1990) and ribonuclease protection experiments were based on protocols in Sambrook et al. (1989). D. virilis wg was isolated from a genomic library obtained from M. Scott, constructed in EMBL3A. Two overlapping but distinct phage clones were isolated with a D. melanogaster probe derived from sequences straddling the start site of transcription for wg. Nested deletions (Erase-a-Base kit, Promega) of overlapping subclones of D. melanogaster and D. virilis DNA were sequenced with the Sequenase II kit (USB).

Reporter constructs were derived from a 5.3 kb EcoRV genomic fragment of D. melanogaster wg DNA. The sequence of this fragment is available in Genbank, accession number U84292; subsequent numbering in parentheses refers to the sequence in this databank entry. The sequence of the D. virilis wg DNA is available under AF046865. For WLZ4.5L, PCR was used to amplify all of the upstream DNA present in the EcoRV fragment, the proximal promoter for wg, and all of the 5’ untranslated region, just short of the initiating codon of the main open reading frame (1-5226). WLZAG was made by fusing a PCR product of wg DNA downstream of element G (1410-5226) to an EcoRV-ClaI fragment (1-1170), whose 3’ end is just upstream of element G. Appropriate primers were designed to make the deletion WLZ2.5L (2005-5226) and to specifically amplify element G (1190-1410), which was then added to WLZ2.5L to form WLZGc2.5L. All constructs are based on pW-ATG-lac1 (Kuhn et al., 1988), a P-element vector with a promotorless lacZ gene.

In situ hybridizations and antibody stains

lacZ and wg transcripts were hybridized to digoxigenin-coupled RNA probes, visualized with anti-digoxigenin antibody (Boehringer Mannheim). The lacZ probe was synthesized from plasmid pH8lac, which was constructed by inserting the Xbal-EcoRI lacZ fragment of pW-ATG-lac1 into Bluescript (Stratagene). pH8lac was linearized with XhoI and the probe was made with T7 RNA polymerase. The wg probe was made with T3 RNA polymerase from Xbal-linearized pCV template, a plasmid containing the complete wg cDNA.

Anti-Slp, made in rat, was used at 1:300 dilution. Anti-β-galactosidase made in rabbit (Cappel) was used at 1:500. Antibodies were preabsorbed before use against wild-type embryos.

Antibody stains and in situ hybridizations were performed as described (Grossniklaus et al., 1992; Lehmann and Tautz, 1994). For double stains, we first incubated embryos with the antibody and developed this signal, then followed this with in situ hybridization.

Other methods

DNA injections of embryos and recovery of transgenic lines was essentially as described (Spradling, 1986). For each construct, at least four separate lines were generated, each line representing an independent insertion of the P-element construct into the genome.

Contig assembly and subsequent sequence analysis was done with the GCG suite of programs (Genetics Computing Group, Madison, WI). Reporter stripe widths were quantitated from scanned
photographs of embryos with NIH Image, a program in the public domain which was developed at the US National Institutes of Health (http://rsb.info.nih.gov/nih-image). WLZ4.5L and wg stripes were measured from images of two wild-type and two ptc- embryos each. Four wild-type and three ptc- embryos were examined for WLZGc2.5L stripe quantification. Stripe widths were normalized for the length of each embryo.

RESULTS

wg transcription start sites

To map the wg promoter, we first determined the start site of transcription by two different approaches: primer extension and ribonuclease protection (data not shown). Total cellular RNA was analyzed from Drosophila embryos or, as a negative control, from Schneider cells, which do not express wg (R. N., unpublished data). Both types of experiments indicated a transcription start site 13 bp upstream of the cDNA reported in Rijsewijk et al. (1987), in a context that is an exact match for the consensus arthropod RNA polymerase II initiator (Cherbas and Cherbas, 1993). Both the primer extension and ribonuclease protection reveal a second transcription start 100 bp downstream of the first; this is not similar to the consensus initiator. Sequence surrounding the first, but not the second, transcription start site is conserved with D. virilis (see Fig. 1 and below). Neither transcription start has an associated TA TA box.

wg from Drosophila virilis

D. melanogaster and D. virilis diverged approximately 60 million years ago (Beverley and Wilson, 1984). The embryonic development of these two species is very similar, as indicated by conserved genes required for segmentation and the conserved regulation of these genes. For example, the segment polarity gene engrailed (en) is regulated in part by tracts of 20-100 bp that are 90% identical compared to D. virilis (Kassis et al., 1989). Similarly, conserved regulatory DNA from D. virilis hairy will direct lacZ in a hairy-like pattern in D. melanogaster embryos (Langeland and Carroll, 1993).

We cloned wg from D. virilis to begin an analysis of the regulation of wg in D. melanogaster. A probe derived from D. virilis wg DNA was used for in situ hybridization to D. virilis embryos, showing an expression pattern indistinguishable from that of D. melanogaster wg (not shown). We sequenced the first exon of D. virilis wg and approximately 5 kb of upstream DNA. A comparison with the corresponding region from D. melanogaster is shown in Fig. 1.

Putative binding sites for homeodomain, winged-helix domain and Tcf transcription factors, some of which are known regulators of wg, are noted in Fig. 1. Before its dependence on the Hh pathway, wg is regulated in the cellular blastoderm by fushi tarazu and even-skipped, both of which encode homeodomain proteins (Ingham et al., 1988). During germ-band extension, wg requires Slp activity, provided by a pair of
Homologous proteins, each of which contain a winged-helix DNA-binding domain (Grossniklaus et al., 1992). Finally, Wg regulates its own expression by a Hh-independent mechanism (Hooper, 1994; Yoffe et al., 1995); this autoregulation may be carried out in the nucleus by Arm and DTcf-1, which together form a Wg-dependent transcriptional regulator (Brunner et al., 1997; Riese et al., 1997; van de Wetering et al., 1997).

Also of interest are binding sites not found in the conserved sequences in Fig. 1. Protein Kinase A regulates wg in the embryo (Ohlmeyer and Kalderon, 1997). Although it has not yet been demonstrated in Drosophila, PKA regulates the transcription factor CREB, which binds the cAMP response element (or CRE; Sassone-Corsi, 1995). No CREs were found in the wg regulatory DNA shown in Fig. 1, even among unconserved sequences. Ci is part of the Hh signaling pathway and can activate wg transcription directly in vitro (Von Ohlen et al., 1997). The sequence alignment shows no conserved Ci-binding sites (TGTTGTTGTC; Kinzler and Vogelstein, 1990). However, six possible sites are in a region for which we do not have the corresponding DNA from D. virilis (i.e., D. melanogaster nucleotides 1-1166, according to the sequence we have deposited in Genbank, accession number U84292).

A full-length reporter construct

As a starting point for functional studies of wg regulatory regions, we fused nearly the entire region of D. melanogaster wg shown in Fig. 1 to the reporter lacZ. The endogenous wg proximal promoter and 5′ untranslated region were included to form construct WLZ4.5L, which was introduced into the germ line to make transgenic animals. Each WLZ4.5L line expresses the transgene initially at the cellular blastoderm in a pattern much like wg itself. wg transcription is initiated in anterior stripes before posterior ones, and odd-numbered stripes appear before even-numbered ones; reporter stripes roughly follow this pattern, except that the even-numbered stripes are delayed compared to those of wg (Fig. 2A,D). lacZ stripes are about one cell wide and are immediately anterior to the cells that express engrailed (en) and hh, as shown by a double labeling of reporter transcripts and En protein (not shown). There are also differences compared to endogenous wg expression: reporter expression is lacking in some spots in the head and is excessive in the prospective hindgut. Moreover, during late germ-band extension at stage 11, expression of the reporter construct fades from the epidermis (compare Fig. 2C and F; we note loss of lacZ transcription at stage 11 in all other reporter constructs described below). Endogenous wg at this time has split into separate dorsal and ventral domains in the epidermis of each parasegment.

While the expression of the full-length reporter construct is

**Fig. 4.** Deletion constructs WLZ2.5L (A), WLZDG (B) and WLZGc2.5L (C). (A,B) In situ hybridizations to lacZ transcripts; (C) antibody stains of β-galactosidase. Wild-type embryos are shown for all three constructs; note that the narrow stripes driven by WLZGc2.5L in normal embryos expand in a ptc− background.

**Fig. 5.** Relative widths of stripes driven by wg reporter constructs. (A) Double stain for lacZ transcripts (blue) and Slp protein (orange). Slp is visible just past the anterior edges of some lacZ stripes (yellow arrowheads). (B) Quantification of the change in stripe width due to the ptc mutation. ‘wg’, mean stripe widths of endogenous wg; ‘WLZ4.5L’ and ‘WLZGc2.5L’, mean lacZ stripe widths. The number of stripes measured for each category is in white; bars indicate s.e.m. See Fig. 3 for stains of wg and WLZ4.5L in wild-type and ptc− mutant embryos.
not maintained past stage 11 in the epidermis, it nevertheless behaves like wg in response to the Hh signal. Previous work has shown that wg expands in an anterior direction in ptc− embryos (Ingham et al., 1991; Martinez Arias et al., 1988; Fig. 3A and B). In a ptc− background, the reporter stripes expand just as wg stripes do (Fig. 3C and D). Ectopic grooves at stage 11 are a morphological landmark of ptc− embryos; these grooves are at the anterior end of both the expanded wg and lacZ stripes. When hh driven by the heat shock promoter is uniformly expressed throughout the embryo, the result is the same as for a ptc mutant: wg expands, as does the full-length reporter stripe (Fig. 3F). The HS-hh experiment was done with a slightly different construct (Fig. 3G) which behaves the same as WLZ4.5L.

Deletions of regulatory DNA cause expanded stripes
Construct WLZ2.5L is an approximately 2 kb deletion from the full-length starting point. In wild-type embryos, this transgene expresses lacZ in wide stripes (Fig. 4A, compare to Fig. 2E). We then removed from WLZ4.5L only the distal-most conserved element, box G, to get construct WLZΔG. Just as for the larger deletion, lacZ is expressed in wide stripes (Fig. 4B). Since deletion of box G causes ectopic lacZ expression, the simplest explanation for how the element functions is that it binds a transcriptional repressor.

To determine the positioning and width of each stripe within the parasegment, we examined embryos stained for lacZ transcripts in conjunction with antibody labeling for En and Sloppy-paired (Slp) as markers. Each wg stripe abuts the anterior edge of the En domain in the adjacent parasegment; similarly the wide WLZΔG stripes do not overlap with En (not shown). wg expression coincides with the posterior end of the Slp domain. In a ptc− embryo, wg expands anteriorly to exactly fill the entire Slp stripe (Cadigan et al., 1994b). The WLZΔG stripes also expand anteriorly, although the anterior edges are somewhat ragged and do not exactly match the anterior edge of the Slp domain (Fig. 5A). We conclude that deletion of element G has an effect similar to the ptc mutation on wg expression, causing an anterior expansion of expression.

Conserved box G is a ptc-responsive element
To examine whether box G is sufficient for narrowing the stripes, we added the element back to construct WLZ2.5L. With two copies of box G (WLZGc2.5L), all wild-type transgenic lines have narrow stripes, similar to the full-length reporter (Fig. 4C). A single copy of box G added back to WLZ2.5L has a similar effect. Three out of four lines carrying this construct also have narrow stripes (not shown), but the fourth has very weakly expressed stripes that are somewhat wider than normal. It seems therefore that a construct with a single copy of box G is more susceptible to influences from its insertion site.

We then crossed WLZGc2.5L into a ptc− background. If box G mediates the activity ptc, then the narrow stripes of WLZGc2.5L should expand in a ptc− embryo, asdoes wg itself and WLZ4.5L. As shown in Fig. 4C, this is indeed the case. We quantified the effect of ptc by measuring stripe widths from digital images of embryos (Fig. 5B; see Methods). WLZGc2.5L stripes are on average 1.7 times wider in ptc− mutants compared to wild type; although this is not as impressive as the 2.2-fold difference seen for wg, the response is much like the full-length construct WLZ4.5L.

DISCUSSION
What is the role of Ci in regulating wg?
Loss of Ci function results in the loss of wg transcription (Forbes et al., 1993). Recently Ci was shown to exist in two different forms; besides the full-length protein, a carboxy-terminal truncation, Ci75, is nuclear and inhibits the expression of Hh target genes in imaginal disks. The proteolysis of the Ci precursor protein that yields Ci75 is inhibited by Hh signaling (Aza-Blanc et al., 1997). Since Ci apparently has two roles carried out by different protein products, it is instructive to look at embryos lacking all Ci activity due to a homozygous deficiency removing the Ci locus; wg expression is lost in such embryos (Von Ohlen et al., 1997). Clearly the principal role of Ci can not be to directly repress wg transcription. Possibly, Ci has an indirect effect on wg, perhaps by downregulating expression of another inhibitor of wg expression.

In contrast, full-length Ci acts as a transcriptional activator in vitro (Von Ohlen et al., 1997). Of the six binding sites in wg regulatory DNA denoted in Fig. 1, the distal-most four can bind Ci in a sequence-specific manner. In a transient cotransfection system, a 1 kb wg fragment containing these four Ci sites activated transcription from a heterologous promoter, an effect that was lost when the Ci sites were mutated. The mutated sites also bound Ci much less efficiently. Thus, these four Ci-binding sites within upstream wg DNA are sufficient for mediating transcriptional activation in vitro. However, within the context of the entire upstream region shown in Fig. 1, including the endogenous wg proximal promoter and element G, Ci-dependent transcriptional activation is much less dramatic (Von Ohlen et al., 1997).

Our data show that these distal Ci-binding sites are not required in vivo for directing reporter expression. For example, WLZ2.5L embryos have stripes, albeit abnormally wide ones, although this construct lacks the distal 2 kb present in the full-length construct. WLZGc2.5L directs narrow, wild-type-width stripes, which are regulated by ptc. Neither WLZ2.5L nor
WLZGc2.5L contains any of the sites for which Von Ohlen et al. (1997) found an in vitro role in mediating Ci function. How can this apparent contradiction be resolved? We have tried to examine the role of Ci in regulating wg reporter constructs, but we could not distinguish any differences in lacZ expression in a collection of embryos derived from heterozygous Ci parents. lacZ expression had completely faded from the embryos at about the same time that wg transcription fails in Ci mutants, i.e., by the completion of germ-band extension. This negative result suggests two possible explanations.

First, Ci may be required for expression of wg reporters, but possible differences in Ci mutants are not detectable, because expression of the constructs in wild-type embryos fades during germ-band extension (Fig. 2F). If Ci indeed directly regulates reporter expression, then Ci must bind wg regulatory DNA through redundant sites besides the ones marked in Fig. 1. Such hypothetical sites must be located in the proximal 2.5 kb upstream of wg, i.e., they must be present in the WLZ2.5L and WLZGc2.5L constructs. There are no conserved Ci consensus binding sites in this region, nor are there conserved sequences with 1 bp or 2 bp mismatches from this consensus. Conceivably Ci could bind to a quite different site, perhaps by forming a heterodimer with some other transcriptional regulator; however, we note that deletion of three consensus Ci-binding sites adversely affects a ptc reporter construct in vivo (Alexandre et al., 1996; as is the case for wg, ptc transcription is regulated by Hh signaling). Relatives of Ci in mammals (Kinzler and Vogelstein, 1990) and nematodes (Zarkower and Hodgkin, 1993) bind to this same consensus site; interestingly, these two groups arrived at the same binding site sequence independently and did not uncover alternative sites that bound Ci with the same affinity.

A second explanation for our data is that Ci may have a minimal role in regulating our reporter constructs. The 4.5 kb upstream of wg that we analyzed is not sufficient to completely duplicate the pattern of wg expression in the embryo; besides the lack of expression at stage 11 and after, lacZ is not expressed in the head as wg is. This is in accordance with the effects of a wg rescue construct (Sampedro et al., 1993) in which approximately 4 kb of wg upstream DNA was fused to sequences encoding a temperature-sensitive Wg protein. At the permissive temperature, this transgene only partially rescues the cuticle of a wg embryo, which fails to hatch. Thus this tract of wg regulatory DNA is not sufficient for normal embryonic development. Further, construct WLZ4.5L is not as fully dependent on ptc as endogenous wg is (Fig. 5B shows that these lacZ stripes do not expand to the same extent as wg itself in ptc mutants). Other activators of wg, such as Slp, might sustain expression of the reporter constructs studied here without complete regulation by Ci. Therefore a portion of wg regulatory DNA in isolation may not depend on Ci while still responding to ptc and hh.

Significance of element G and the nature of proteins that bind it

The 150 bp G box, 91% identical with its counterpart from D. virilis, mediates repression of wg in a ptc-dependent manner. Fig. 4C shows that the G box is sufficient for conferring wild-type width to reporter stripes, which in turn expand in a ptc mutant background, thus behaving like wg itself. Deletion of element G results in wide stripes in a wild-type embryo (Fig. 4B), suggesting that this is a binding site for a transcriptional repressor active in cells anterior to the wild-type wg domain. A repressor that binds element G could possibly act in parallel to ptc and hh; in such a case, the repressor’s activity would be overcome by a Hh-regulated activator, i.e. Ci. We favor the simpler explanation that a repressor is another endpoint of Hh signaling.

Among segment polarity gene products that are transcriptional regulators – En, Slp, Gooseberry (Gsb) and Ci – none are likely to be repressors directly controlling wg via element G. The En homeodomain protein does repress transcription (Jaynes and O’Farrell, 1991; Schwartz et al., 1995), but its (positive) effect on wg is indirect, since En is present in different cells than those where wg is transcribed. The proteins Slp and Gsb, on the other hand, are located in the same cells that express wg. Loss of slp function results in the loss of wg expression at the same time as in hh mutants, but this is not the phenotype expected for a repressor of wg. The slp locus is transcribed as two tandem mRNAs that encode very similar, largely redundant proteins (Cadigan et al., 1994a). There is no evidence that Slp proteins directly regulate wg, but they each have a winged-helix DNA-binding domain and a transcriptional activation domain, and winged-helix-binding sites are present in wg reporter constructs (Fig. 1). gsb function is required somewhat later in development, after wg transcription fades laterally in the germ band; gsb is only required for the ventral domains of each wg stripe (Hidalgo, 1991). We observe that our reporter construct expression is lost during this time and suspect that other cis-acting sequences mediate this later expression of wg by gsb.

Finally, Ci exists in a form that negatively regulates Hh target genes in the imaginal disk. However, this putative repressor form of Ci binds to the same site as full-length Ci (Aza-Blanc et al., 1997) and we have shown that these sites are dispensable for generating ptc-regulated stripes in the germ band. At the present time, the relationship of activator and repressor forms of Ci in the embryo is unclear. A trans-acting regulator binding element G is likely to be a novel protein repressing transcription, at least from the perspective of the Hh signaling. Since the G box is responsive to ptc, the regulator(s) that bind to it are likely to be regulated by the Hh pathway (Fig. 6). We propose therefore that Ci is not the sole output of the Hh pathway in the regulation of wg transcription.

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