Disruption of promoter-enhancer communication leads to flies with no thorax

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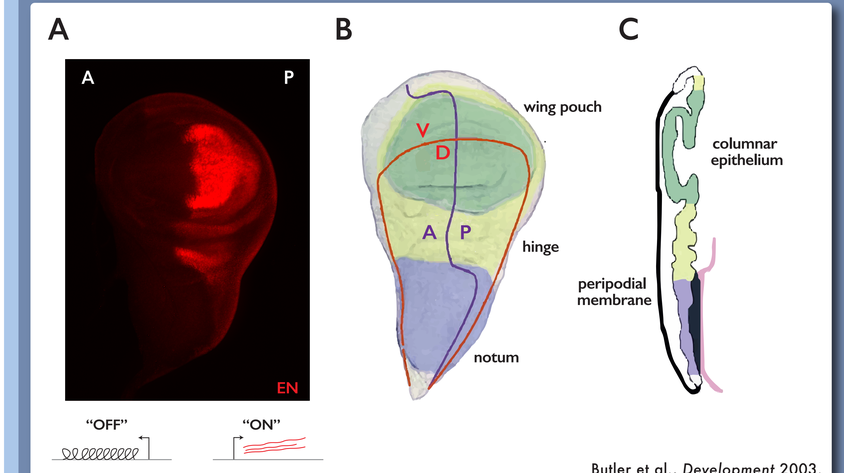
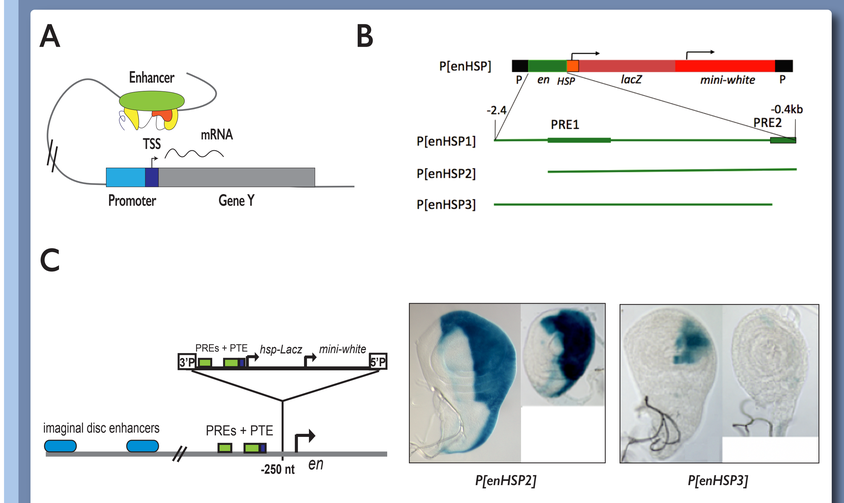
In Drosophila, the *invected* (inv) and *engrailed* (en) genes exist within a co-regulated complex and are expressed throughout early development. Although the inv/en promoters are separated by 54 kb, their expression is regulated by the same enhancers distributed across a 70 kb region, suggesting that enhancers can activate multiple promoters over long distances. Previous studies have identified a 2 kb regulatory fragment upstream of the en promotor, which may serve as a promoter tethering element by facilitating interactions between the en promoter and distant enhancers. We have generated a transgenic line containing the 2 kb regulatory fragment fused to a β-galactosidase reporter gene inserted near the en promoter. When coupled with a wild-type chromosome, transgenic organisms expressed β-galactosidase and En only in the posterior compartment of the wing imaginal discs, consistent with appropriate enhancer communication. However, in the absence of a wild-type chromosome, transgenic organisms exhibited impaired imaginal disc development in addition to a de-repression of β-galactosidase in the anterior compartment of the wing imaginal discs, suggesting the endogenous enhancers have been hijacked by the regulatory fragment within the transgene. **Taken together, our data suggest that a specific regulatory fragment may serve as a promoter tethering element and is required to facilitate interactions between the en promoter and imaginal disc enhancers at discrete developmental stages.**



Butler et al., *Development* 2003.

Figure 1. The Drosophila wing is derived from the wing imaginal disc. (A) The expression of the segment polarity gene *engrailed* establishes the anterior/posterior axis in developing imaginal discs. (B) Fate map of the third instar wing disc showing the anterior-posterior (AP) and dorsal-ventral (DV) compartment boundaries. In adult flies, the wing pouch (green) gives rise to the wing blade, the hinge (yellow) forms the link to the notum (blue) or thorax. (C) The imaginal disc is composed of multiple cell layers: the peripodial membrane, and the columnar epithelium that gives rise to the adult epidermis.



Kwon et al., *Development* 2009.

Figure 2. (A) Sequences within the core promoter communicate with distant enhancers over many kilobases and facilitate accurate gene expression throughout development. (B) Constructs with fragments of en regulatory sequence and the heat shock promoter (HSP) drive transcription from the *lacZ* transgene. The locations of Polycomb response elements (PREs) are shown as green boxes. (C) PRE2 behaves as a promoter tethering element (PTE), recruiting imaginal disc enhancers to the *lacZ* gene and driving stronger β-gal expression from *P[enHSP2]* than *P[enHSP3]*.

How does P[enHSP2] disrupt *engrailed* expression during imaginal disc development?

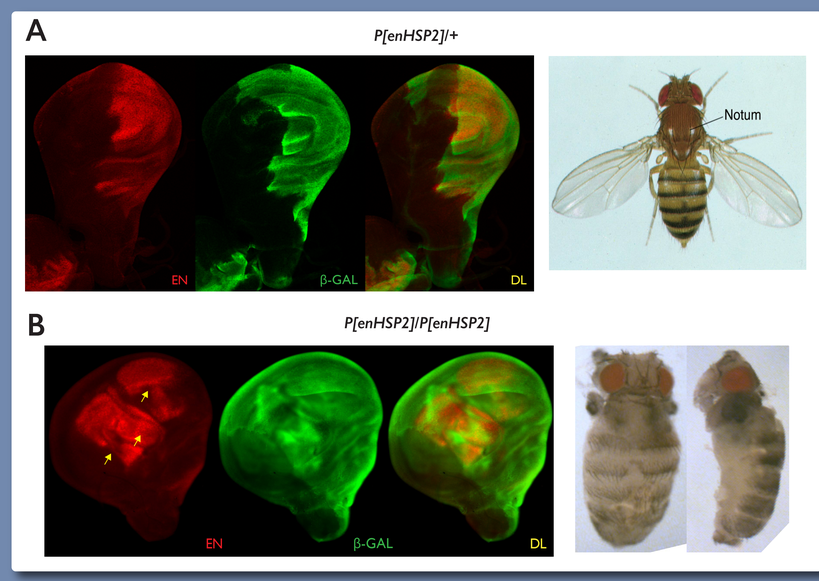


Figure 3. *P[enHSP2]* homozygotes exhibit impaired notum development and lack thoraxes. Imaginal wing discs double-labeled (DL) for En (red) and β-gal (green). (A) En and β-gal are expressed only in the posterior tissues of the wing discs of larvae with one copy of the *P[enHSP2]* transgene, resulting in the normal development of adult structures. (B) Two copies of the *P[enHSP2]* transgene disrupt En and β-gal expression, causing duplications of the wing pouch (yellow arrow) and a loss of the adult thorax.

P[enHSP2] causes a recessive cis-acting loss of *engrailed* expression

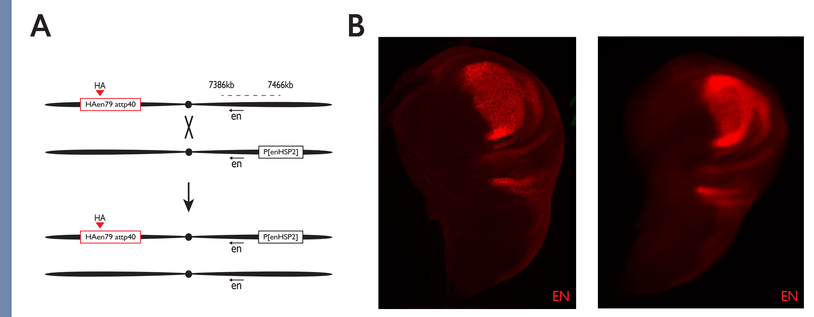
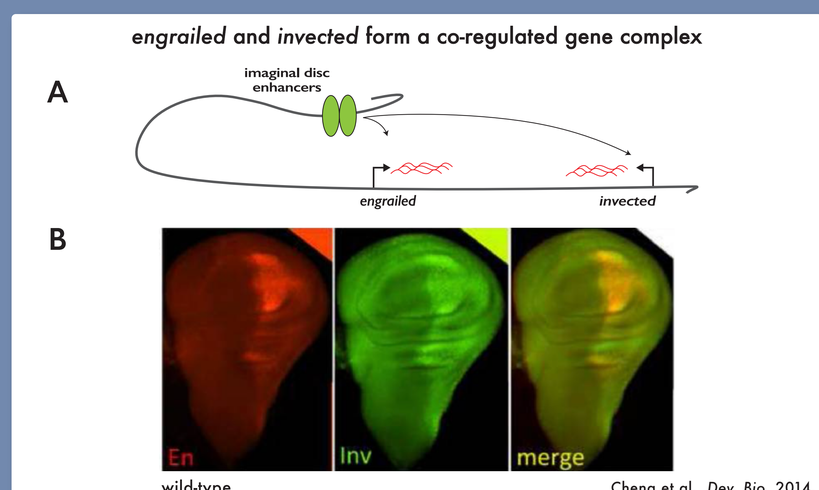


Figure 4. The *HAen79* transgene contains the necessary sequences to rescue *en/inv* double mutants. We hypothesize the *P[enHSP2]* homozygous mutant phenotype is recessive and should be restored in flies with *HAen79*. (A) A schematic of the recombination event used to generate the *HAen79 P[enHSP2]* chromosome. (B) Wing discs from third instar larvae that were homozygous and heterozygous for *HAen79 P[enHSP2]* exhibited similar En (red) expression patterns.

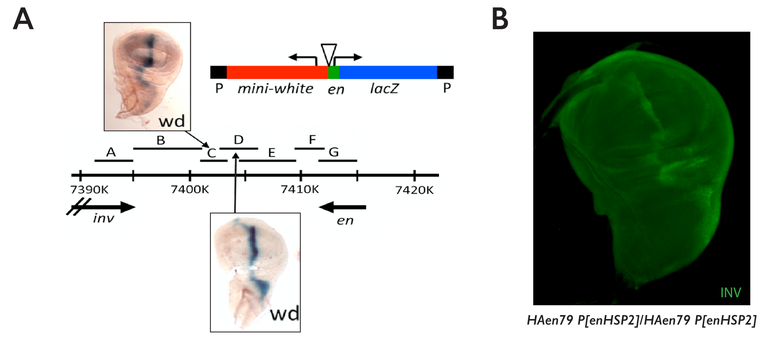
engrailed and *invected* form a co-regulated gene complex



Cheng et al., *Dev. Bio.* 2014.

Figure 5. *engrailed* and *invected* form a co-regulated gene complex which shares enhancers spread across a 62kb regulatory region. (A) Imaginal disc enhancers interact with *en* regulatory DNA to drive *inv/en* expression. (B) Inv (green) and En (red) are co-expressed in the posterior tissues of third-instar larvae.

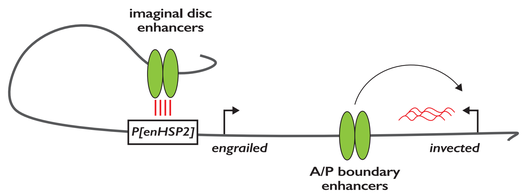
Anterior/posterior boundary enhancers stimulate invected expression in P[enHSP2] homozygotes



Cheng et al., *Dev. Bio.* 2014.

Figure 6. *P[enHSP2]* prevents the imaginal disc enhancers from driving the co-expression of Engrailed and Invected. (A) A schematic of the *lacZ* construct used to determine the expression patterns of specific regulatory fragments within the *engrailed/invected* domain. (B) Imaginal discs from *HAen79 P[enHSP2]* homozygotes express Inv (green) in patterns consistent with the regulatory fragments C and D.

P[enHSP2] prevents the imaginal disc enhancers from accessing the engrailed promoter.



Conclusions

1. Impaired notum development results in loss of the adult thorax in P[enHSP2] homozygous mutants
2. Developmental defects associated with P[enHSP2] are recessive
3. Imaginal disc enhancers are tethered to the HSP by cis-acting regulatory sequences, resulting in a loss of en/inv expression

Future Directions

PhiC31-mediated genome engineering enables rapid cassette exchange at the *engrailed* locus.

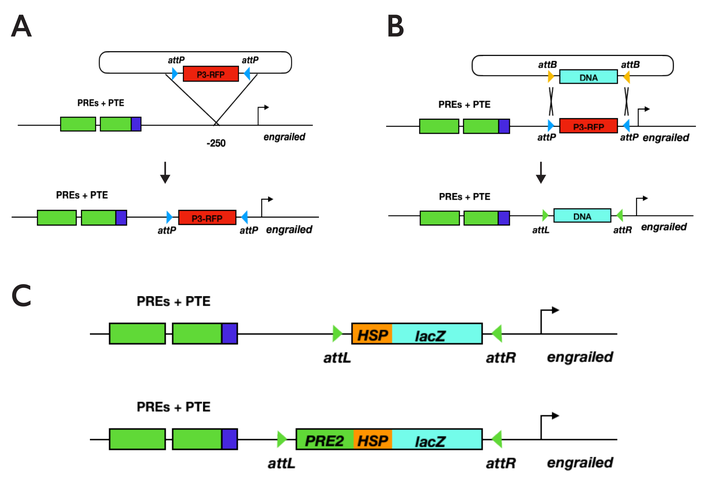


Figure 7. Genetic engineering at the engrailed locus using minimal 34bp attP sites. (A) The attP P3-RFP vector will be inserted at -250 from the TSS in a wild-type background. (B) Cis-acting regulatory elements will be exchanged with the P3-RFP using an attB donor vector. (C) Proposed future experiments include a control without *engrailed* regulatory sequence and a construct with PRE2.

Acknowledgements & Funding

Rainbow Transgenic Flies, Inc

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Healthy pregnancies. Healthy children. Healthy and optimal lives.

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