Ubx, Drosophila and Butterfly Wings

Introduction to Ubx

Models of Developmental Gene Regulation

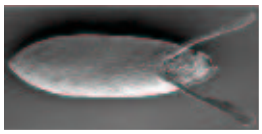
Intrinsic & Extrinsic

Embryonic Segmentation

Ubx & the Wing Disc

Evolution of Wings

Ubx & the Butterfly Wing



The development of a fertilized egg into a complex adult is one of the most challenging mysteries of biology. The mature form can be regarded as a three dimensional array of cells, each expressing a set of genes that specifies its type. The nature and activities of these cells produces the phenotype of the individual.

Introduction to Ubx

Homeosis: The transformation of one body part into another has long been used as a insight into developmental processes and their genetic control. William Bateson, who originated the term, described many monstrosities produced by homeosis. He regarded them as evidence for discontinuities in evolution.

The bithorax complex of Drosophila was originally identified through mutations that transformed thoracic identity.

The first of these mutations, bx (bithorax), was discovered by Bridges in 1923.

Ubx was first identified as a dominant allele of bx (bx^D), then as a gene in its own right.

E.B. Lewis described the genetics of the complex bx region in the American Zoologist (1963, 3: 33). It was only with the complete DNA sequence and its molecular expression that the nature of these mutations could be understood.

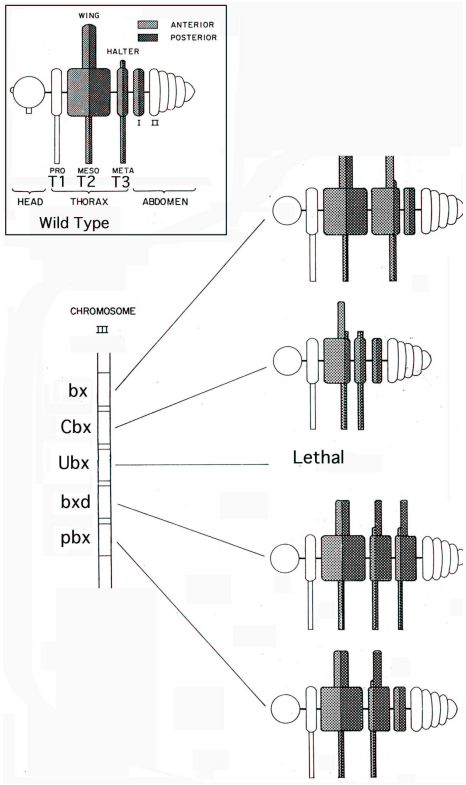
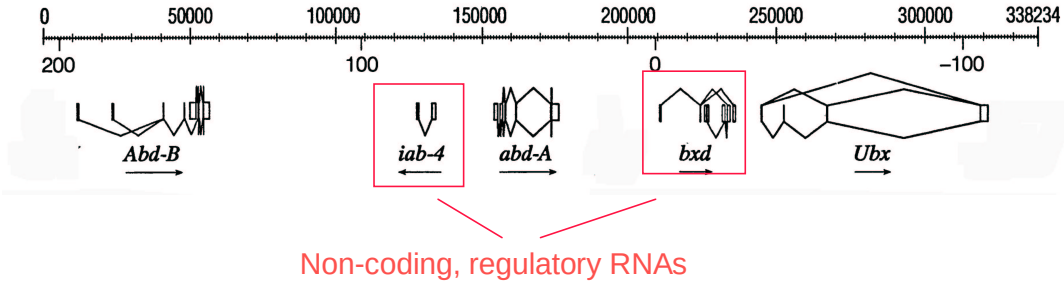


Diagram of homeotic transformation and genes according to Lewis.

The Molecular Nature of Ubx

The complete DNA sequence of the bx region revealed three protein-coding genes. Some of the mutations that were thought by Lewis to define genes were actually in regulatory elements.

BX-C Region (Martin et al. PNAS 92: 8398, 1995.



Non-coding, regulatory RNAs

Regulation of Ubx expression is complex. There are at least two regions that affect Ubx expression, the ~40 kb upstream region (which contains the bxd RNA unit) and the second intron of Ubx.

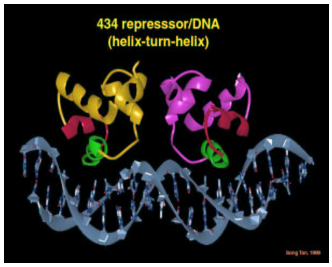
Some “pseudoalleles” described by Lewis are mutations in bxd. There are early (during embryogenesis) bxd transcripts and late bxd transcripts. The mechanism of bxd regulation is unknown, but is connected with the phenomenon of “transvection” whereby a product from one homolog can influence the regulation of the trans-Ubx copy when in close chromosomal proximity.

Other pseudoalleles, such as Cbx, map to the second intron of Ubx.

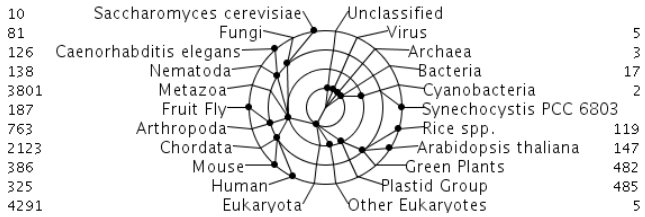
[see Mattick & Gagen MBE 18:1611, 2001]

The homeobox domain and Hox genes.

BX-C genes code for a protein domain (~60 aa) called a “homeodomain” (HD). It is capable of forming a DNA-binding helix-turn-helix (HTH) motif. The DNA sequence (~180 nts) that codes for this protein doimain is called the “homeobox” (HB). The homeobox was first discovered in Drosophila segmentation genes, but quickly found to have homologs in many other organisms. The homeobox is conserved across metazoa and is frequently found in genes regulating body plan.



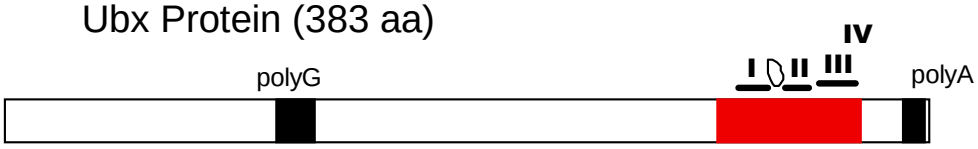
An HTH dimer in a bacterial repressor molecule



Antennapedia-like Homeodomain Tree

http://www.ebi.ac.uk/interpro/IEntry?ac=IPR001356#

Ubx Protein (383 aa)



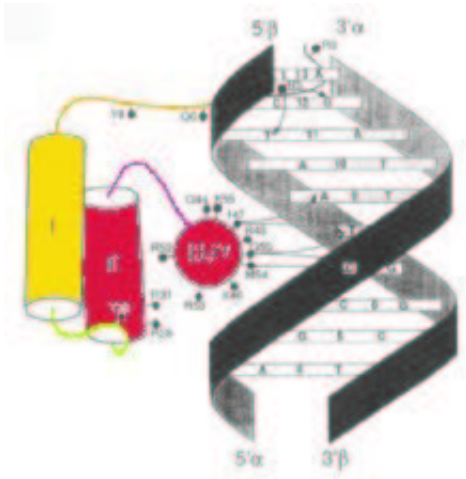
59 aa HD in **RED**. The four alpha helices are indicated by bars. Note: There are splice variants of the Ubx protein.

HMUX_DROME: P02834

See also
http://www.ebi.ac.uk/interpro/DisplayIproEntry?ac=IPR001827

The Homeodomain

The ~60 aa homeodomain from Gehring et al. *Annu. Rev. Biochem. 1994. 63: 487-526*.



The HD has an N-terminal arm plus alpha helix (yellow). The HTH motif is formed by helix II + [II,IV] (red).

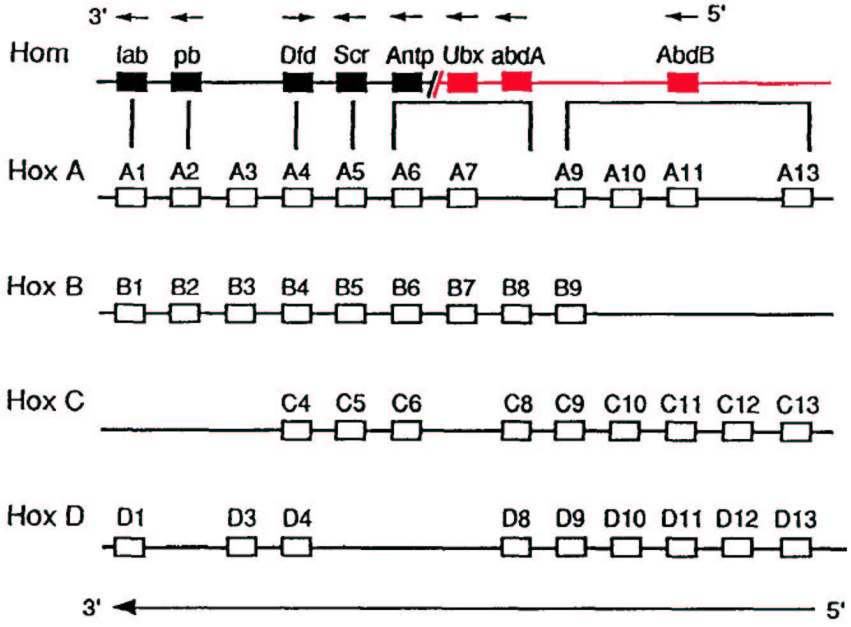
The contacts between the HD and the DNA target are at least partly determined by the amino acid sequence. Thus, the specific DNA target of a homeodomain protein is influenced by the HD amino acid sequence. However, the target sequence (~8-10 bp) is too small to explain the target specificity of homoeodomain proteins.

The HD can bind as a monomer or dimer with another HTH protein.

Many HD proteins have additional protein domains. An example is the EH box in the *engrailed* family.

Hox and HOM genes

Although many Drosophila genes have a HB, only two major clusters of homeotic genes are recognized, BX-C and ANT-C (antennapedia). Together these have been named “HOM” gene clusters (in invertebrates). Genes in vertebrates that are homologs are called “hox” genes. The gene family has evolved by extensive gene duplication, both of gene type within a cluster and by cluster duplication (perhaps followed by gene elimination, see figure).



Hox genes in Drosophila and mammals, from Gehring et al. *Annu. Rev. Biochem. 1994. 63: 487-526*. BX-C is in red and ANT-C in black. They are not linked, whereas each hox cluster is linked.

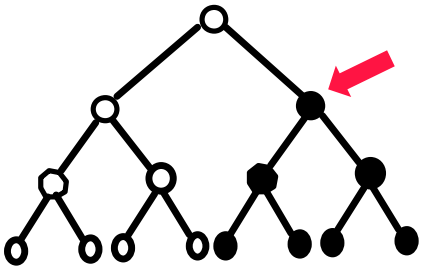
The Regulation of Developmental Genes

Mutations in homeotic genes transform tissue identity. They play and important role in determining cell fate and identity. A few general principles for specifying cell identity during development will be described before looking at Ubx in particular.

How do cells become specialized into different types?

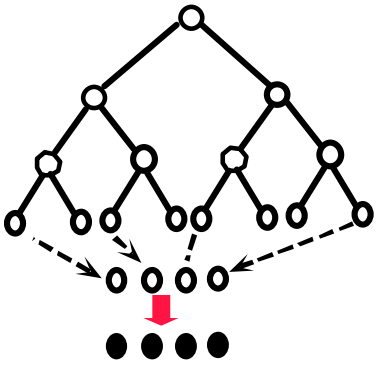
Inheritance (listen to your mother). A cell autonomous mode => intrinsic.

Requires memory of gene state following mitosis. Different cell types are mosaics of different clones.



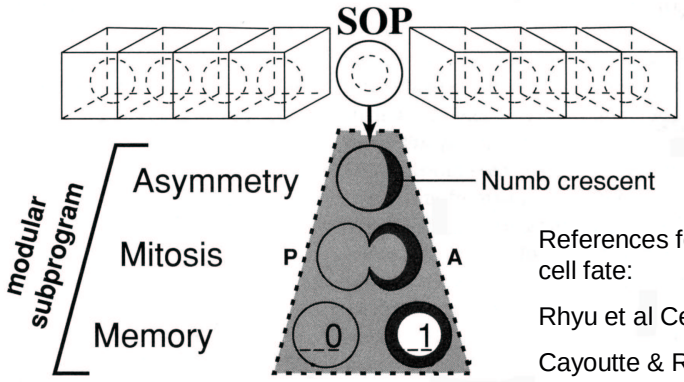
Environment (listen to your neighbors). A cell non-autonomous mode => extrinsic.

A group of cells in a common environment responds to a diffusible substance (morphogen).



Determination of a Drosophila bristle - a MODEL for inheritance. *[Adapted and stolen from Held, Fig. 2.1.]*

Sensory **O**rgan **P**recursor cell

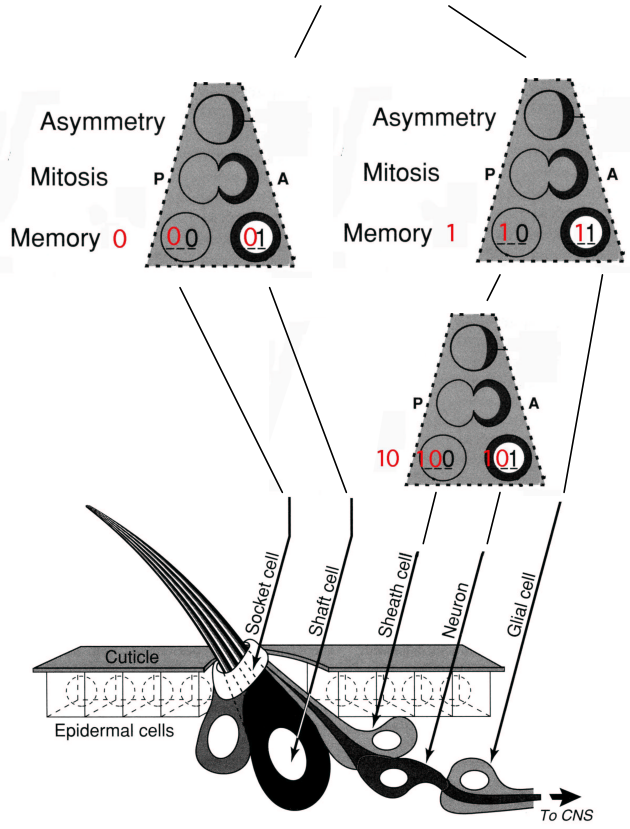


Membrane-tethered

References for Numb & asymmetrical cell fate:

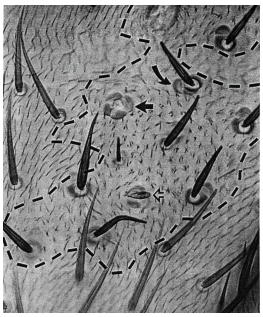
Rhyu et al Cell 76:477, 1994

Cayoutte & Raff Nat. Neurosci. 5: 1265, 2002



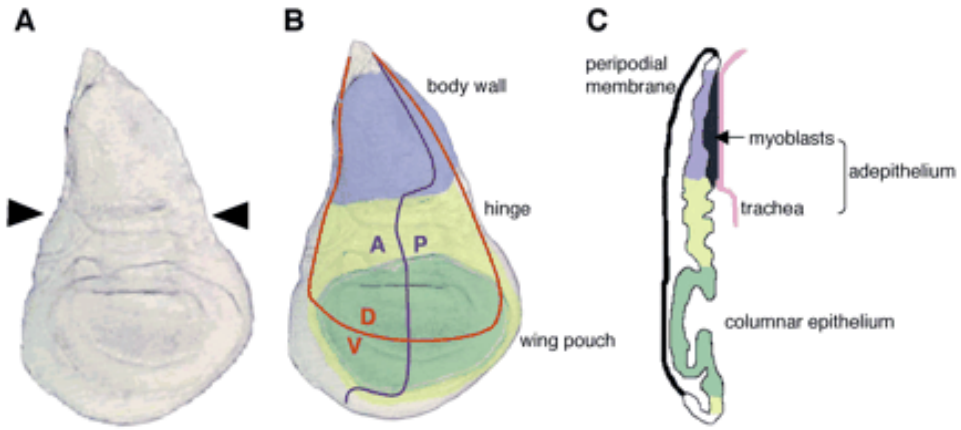
Reset program & remember mother

Asymmetrical distribution of Numb acts as a signal for alternate pathways (perhaps by inhibiting Notch). Defective numb clone (dotted) and bristle abnormalities from Rhyu ey al.

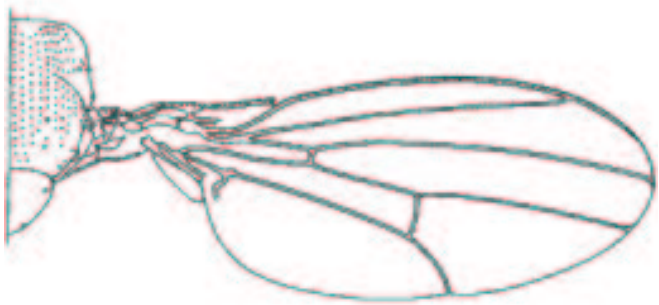


Expression of Dpp at the A/P boundary in 3-rd instar wing discs - a MODEL for extrinsic regulation.

The wing disc starts as a group of ~20 cells in the embryo and becomes ~50,000 cells in the 3rd instar disc. The Dorsal/Ventral & Anterior/Posterior compartments develop early.

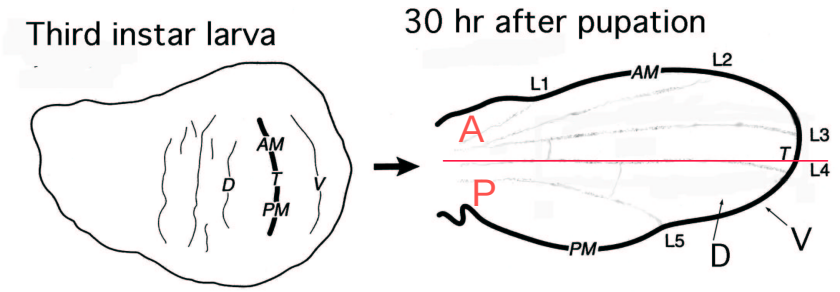


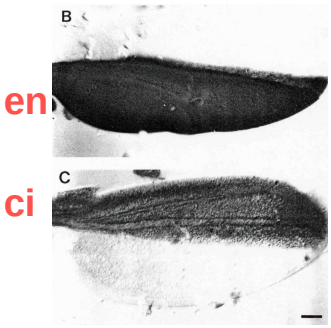
From: Butler et al. Development 130, 659-670, 2003



Expression of Dpp at the A/P boundary in 3-rd instar wing discs - a MODEL for extrinsic regulation.

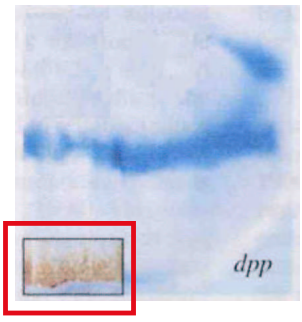
The Dorsal/Ventral & Anterior/Posterior compartments develop early. They are marked by expression of apterous/wg=wingless & *en*grailed/ci=cubitus-interruptus (respectively).





Regions of ci and en expression in the pupal wing (Blair Dev. 115: 21, 1992).

The A/P boundary is just about where the wing vein L4 will later appear. A band of dpp expression appears at this boundary in the 3rd instar disc.



Biehs et al

Dev. 125: 4245, 1998

The insert shows that the protein (brown) extends beyond the cellular-RNA expression (blue).

The third instar Drosophila wing disc produces a band of Dpp expression at the A/P boundry that is important in the specification of cell fate, including the formation of veins.

"ACTORS" at the D/V & A/B boundaries.

From Flybase (http://flybase.bio.indiana.edu) and Swiss-Prot (http://us.expasy.org)

D/V

apertous => **AP**, a homeodomain-containing protein (469 aa). Has two LIM Zn-binding domains. Nuclear localization.

wingless => **WG**, a secreted protein (468 aa). Binds to transmembrane receptors on neighboring cells to regulate gene expression.

A/P

ci => **CI**, a Zn-finger transcription factor (1397 aa).

dpp (decapentaplegic) => **DPP**, a secreted glycoprotein (588 aa), binds to TGFb (transforming growth factor-beta) receptors.

engrailed => **EN**, a homeodomain-containing transcription factor (552 aa).

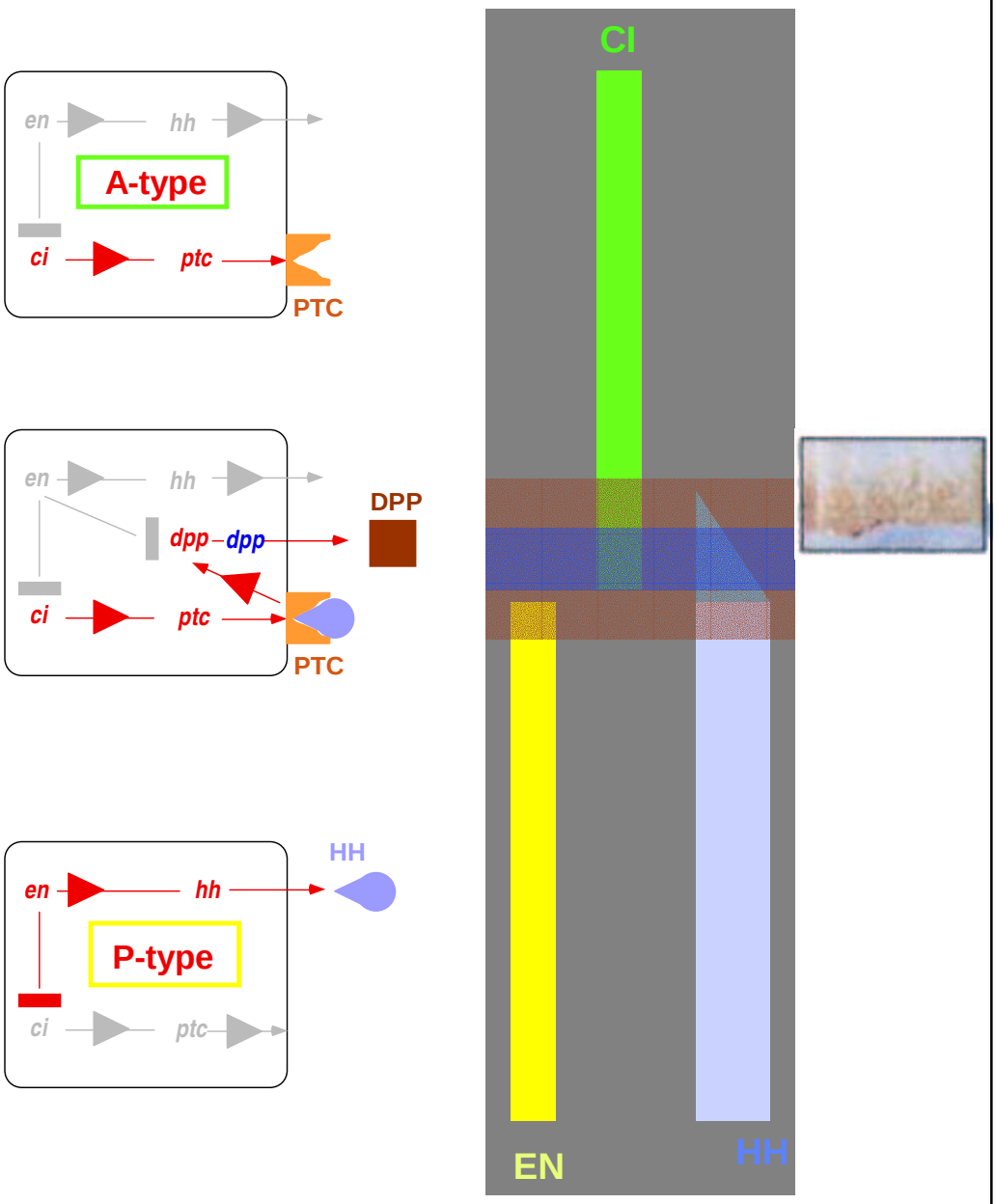
As Well -

hh (*hedgehog*) => **HH**, a secreted protein (471 aa), target of *engrailed* regulation. Contains the "sonic hedgehog domain"

ptc (*patched*) => **PTC**, a transmembrane receptor protein (1286 aa) with cytoplasmic and extracellular domains. Interacts with sonic hedgehog domain.

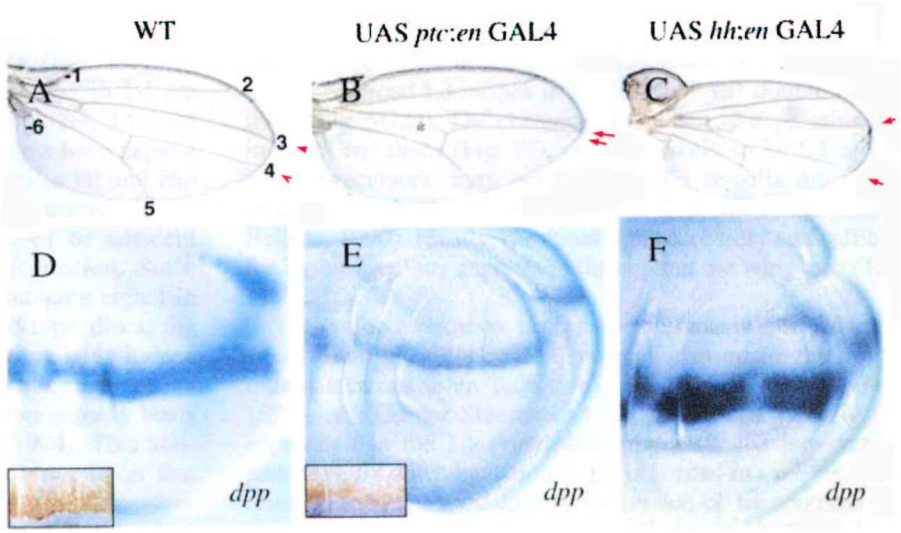
Secreted Proteins, Receptors & Transcription

A MODEL for the DPP stripe *[Adapted and stolen from Held, Fig. 6.4 & see also Blair, 1992.]*



What does DPP do?

One role for DPP protein (data from Biens et al. Dev. 125: 4245, 1998) may be to regulate the formation of veins L4 and L3. Secreted DPP spreads out from the A/P boundary, forming an additional border (in A). Biens et al studied the effect of HH & DPP on vein formation in 3rd instar discs. When the width of the dpp expression stripe was decreased by expression of a ptc transgene, the distance between L3 & L4 was decreased, and when the dpp stripe was increased by expression of an hh transgene, the L3 - L4 distance increased.

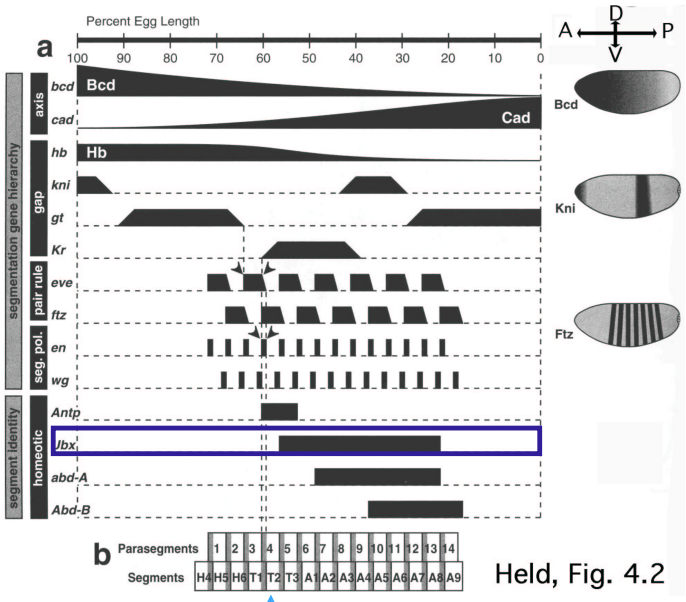


An en promoter drives the production of GAL4 which activates the pts or hh transgene which is under control of UAS.

Embryonic Segmentation in Drosophila

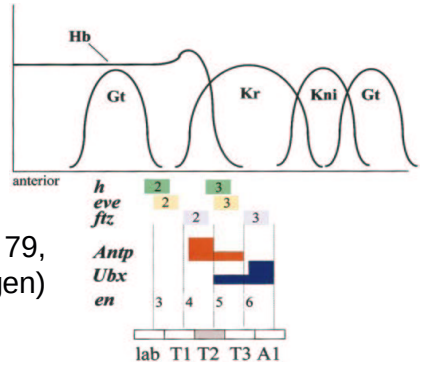
Ubx has a role both in early, embryonic development and later, during the larval stage within imaginal discs where it takes part in the morphogenesis of imago structures.

Ubx expression is restricted to T3 and abdominal segments that are more posterior.



Held, Fig. 4.2

Hb (hunchback) plays an important role in localizing Ubx. Hb is expressed maternally as a gradient, then zygotically first as a gradient (above) , then as a stripe in PS4 (box), repressing the formation of Ubx in T2.



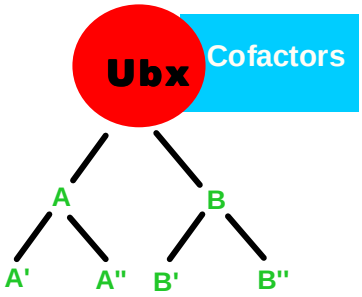
Summary from Wu et al. Dev. Biol. 237: 79, 2001 for regulatory protein (morphogen) gradients and targets.

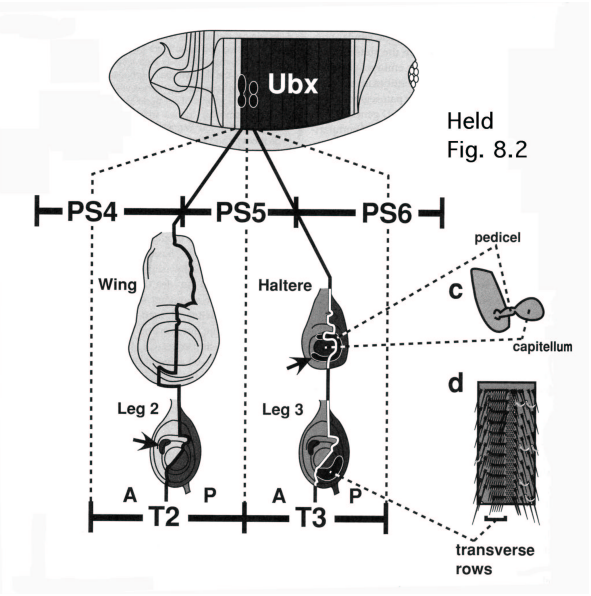
What does Ubx Do?

The larval stage is an individual in its own right having its own segment diversity. The embryonic pattern of Ubx expression is only partly concerned with establishing imaginal discs, it is also required for larval development. For example, Ubx with the homeododomain protein EXD (*extradenticle*) regulate downstream genes in Drosophila mesoderm.

Because Ubx mutations transform the fate of adult cells in T3 (haltere) to T2 (wing), interest has focused on the 3rd instar imaginal discs.

Master Gene Model of Ubx Regulation





Held Fig. 8.2

Ubx expression (red) in 3rd instar imaginal discs (Weatherbee et al Genes & Dev. 12: 1474, 1998).

Ubx Controls Genes at Many Levels - A "Micromanager"

Gene active in wing disc

Dpp

spalt related

(vein development)

blistered

(dorsal-ventral adhesion)

Wingless

(D/V boundary)

Wg

ac-sc

(bristles)

Gene repressed in haltere disc

Ubx ⊣ ***spalt related***

Ubx ⊣ ***blistered***

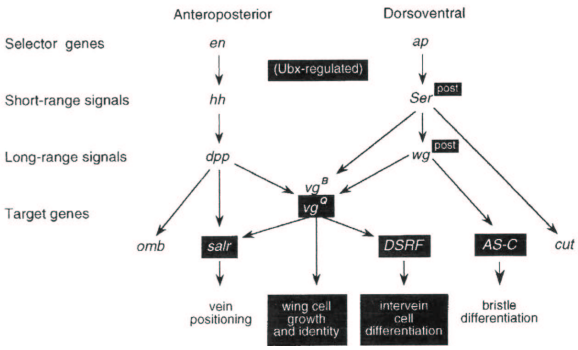
Ubx ⊣ ***Wingless***

Repressed in posterior compartment (smaller size), activity changed in anterior compartment.

⊣ ***ac-sc***

(*sc* expressed in pedicelluar part, positively regulated by Ubx).

Ubx acts independently on target wing genes at different levels, implying the "accumulation of a complex network of Ubx-regulated interactions". Weatherbee et al Genes & Dev. 12: 1474, 1998.



Evolution of Wings

Homeotic genes repress wing development. Wing formation appears to be a ground state. For example (Carroll et al. Nature 375: 58, 1995):

WT: wing (T2) and haltere (T3) markers (*vestigial* & *snail*) are active in Drosophila **embryonic primordia** (~20 cells) before the imaginal discs appear.

Ubx^- + $abdA^-$ mutants *vestigial* & *snail* are active from T2 through A7.

BX-C proteins (Ubx, abdA & AbdB) are expressed in abdominal segments of an apterygote (T3 - T7).

BX-C genes predate the evolution of Arthropoda and so must have had some function other than repressing wings.

Apterygota

Archaeognatha

Zygentoma (=Thysanura)

Pterygota

Wings on T2/3, no limb rudiments on Abdomen

How Drosophila Got Its Wings

Carroll et al (Nature 375: 58, 1995) presented a hypothesis about the evolution of wing number.

Wings evolved on all thoracic and abdominal segments (b = palaeodictyopteran nymph).

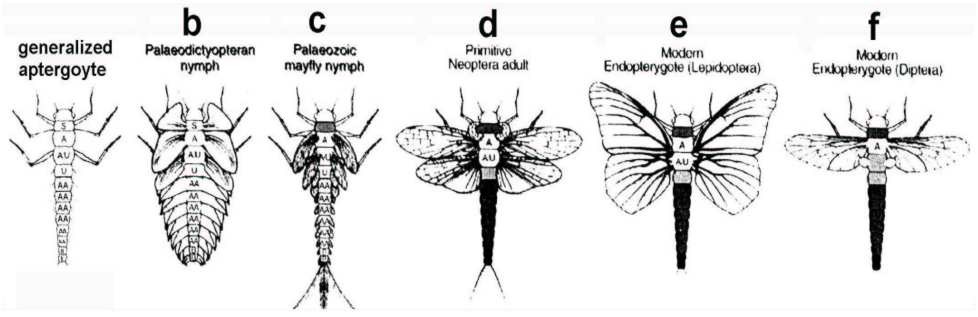
Later, different homeotic genes (S, U, AA, B: BX-C = U+AA+B) that originally (as well as presently) had other functions evolve to repress the wing program in different segments.

S (Sex combs reduced) was co-opted into repressing wings in T1 (shaded band in c = Paleozoic nymph).

Lepidoptera have repression of the wing program in A2-A7 by abd-A (AA) & Abd-B (B). Repression in A1 is postulated to have occurred through UbX.

Ubx represses wing in T3 of Dipterans to form the haltere.

Although A (Antennapedia) is expressed in Drosophila T2 & T3, it is not required for wing formation and Carroll et al do not suggest it has any role in the evolution of wing number.



How the Butterfly Got Its Spots?

(Well, just a little that relates to Ubx)

The buckeye butterfly, Precis coenia (Hübner) has two large multicolored eyespots on the dorsal hindwing and one large eyespot on dorsal forewing. The ventral wings are patterned differently. As well, there are summer and winter ventral patterns. “Nijhout (1980) demonstrated by grafting experiments of pupal wing epidermis that the wing eyespots are determined from a central area of the future eyespot called the focus.”

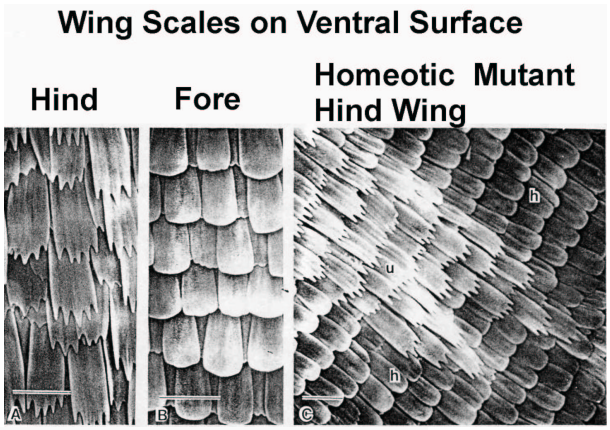
[http://www.uni-ulm.de/biologie1/Koch/Haustiere/Precis/precise.html]

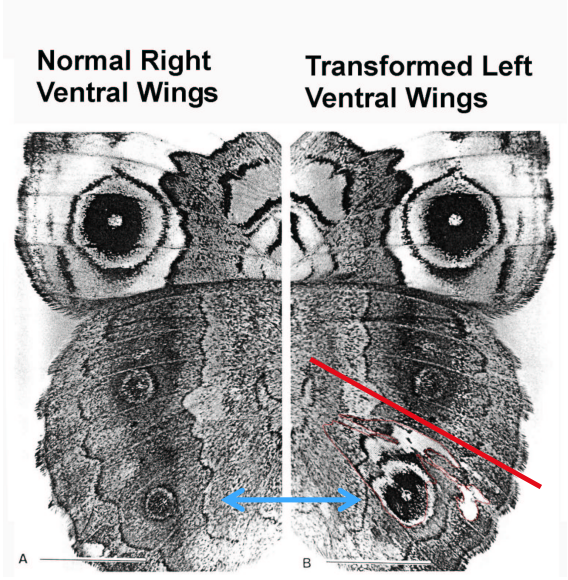
Dorsal wings, the left spot was damaged during pupal development.

Ventral wings (summer left, winter right)

Hindsight

Nijhout & Rountree isolated a homeotic mutants of *P. coenia* in which (randomly occurring) patches of cells normally found on the ventral forewing are now found on the ventral hindwing (h in panel c of figure) [the dorsal surface is not transformed].





Hind -> Fore transformation in *P. coenia* (Nijhout & Rountree).

Transformed patches are posterior to the line (A/P axis?).

Note that eyespot focus seems to be retained (blue).

Weatherbee et al (Curr. Biol. 9: 109, 1999) studied Ubx expression in this mutant and found the the Ubx protein was absent from transformed wing imaginal disc patches.

No picture available

Ubx is normally expressed in the hindwing (but not the forewing) [P. coenia] imaginal disc, suggesting that expression of Ubx regulates patterns of the hindwing type and the the default is pattern of the forewing type. This could be by repression of genes concerned with forewing patterns as well as by induction of genes specifically concerned with hindwing pattern elements.

Weatherbee et al show that when the Ubx-deficient (transformed) region includes the hindwing eye spot focus, larger forewing-like spots are generated which include scales that are wild type in morphology. This suggests that a Ubx-deficient focus produces a morphogen that diffuses away from genetically modified cells producing a forewing-like eyespot.

No picture available

The *Distalless* gene (*Dll*) is a marker for the developing eyespot focus (see Brakefield & French BioEsssays 21: 391, 1999). Both fore- and hind-wings foci initially have similar numbers of *Dll* expressing cells. Later, *Dll*-expression expands to more cells in the fore- than in the hind-wing.

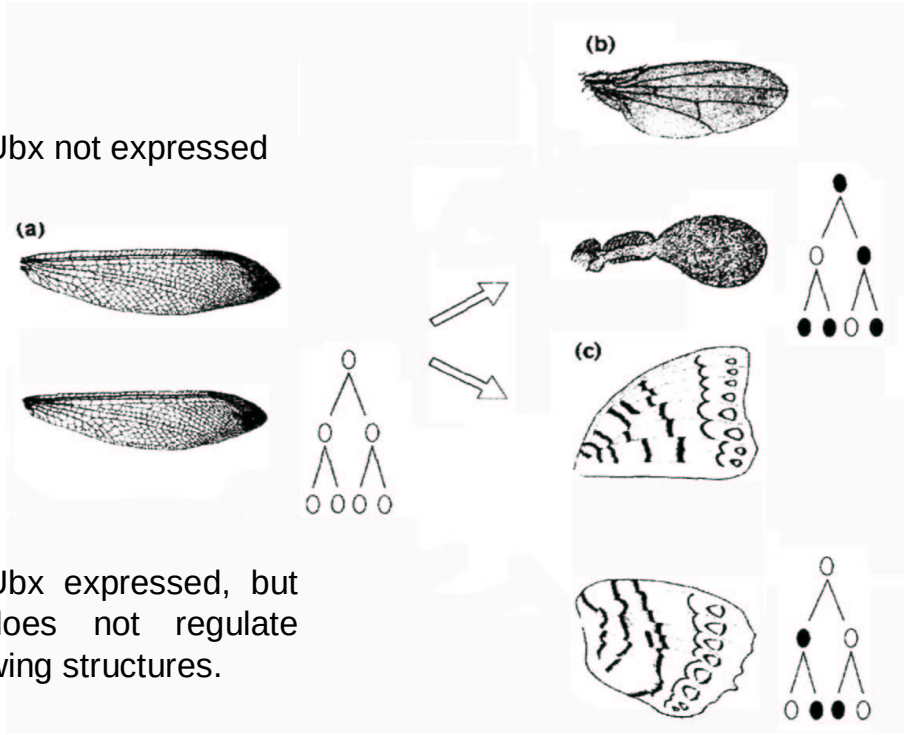
Weatherbee et al found that when a hindwing Ubx-deficient patch included the focus, *Dll*-expression increased compared to wild type, suggesting that Ubx normally depresses the production of focal morphogen. Outside the Ubx-deficient patch where Ubx is normally expressed, *Dll* is expressed at (normal) low levels, suggesting that Ubx controls genes (such as *Dll*) that are downstream of the focal signal.

Recalling the evolutionary model of Carroll et al, the 4 wing ancestor of Lepidoptera & Drosophila expressed Ubx in T3 (hindwing), but it had nothing to do with wing production. Dipterans use Ubx to repress wing in T3 and regulate expression of haltere genes. Lepidopterans use Ubx in T3 to regulate differences in hindwing vs forewing pattern elements.

Summing Up

It is clear that the regulation of many T3 genes that are downstream of Ubx have diverged between the Lepidopteran *vs* Dipteran lineages. Wing genes have become repressed in Dipterans, while pattern genes have become repressed and modified in Lepidopterans.

Ubx not expressed



Ubx expressed, but does not regulate wing structures.

Modified and stolen model from Weatherbee et al (Curr. Biol. 9: 109, 199). Left: Fore- and hind-wings of ancestor express identical wing genes. Ubx is expressed in T3 (hindwing), but does not regulate wing genes. Right: Ubx expression in T3 is adapted to regulate fore- *vs* hind-wing differences. Dark circles in the hindwing gene hierarchies resented [non-overlapping]genes that come under Ubx control.