

# Implications of the spatial and temporal regulation of *Hox* genes on development and evolution

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**ABSTRACT** Nearly 20 years have passed since Ed Lewis revealed the importance of *Hox* genes in the specification of different segments in the anterior-posterior axis of the fly. Pioneering studies by several authors, among others García-Bellido and his student Ginés Morata, helped to elaborate a theory of segmental specification that was strengthened with the arrival of molecular techniques to the field of Developmental Biology. The conservation of *Hox* genes in metazoans at the level of sequence, function and complex organization has resulted in the export of this *Drosophila* theory as a paradigm to interpret the development of axial specification in organisms less amenable to experimental study. There are two main ways to interpret how *Hox* genes work in *Drosophila*. One considering *Hox* genes as "segment identity" factors giving global properties to the segments in which they are active. Another considering *Hox* genes as encoding spatially restricted transcription factors required for a number decisions taken at the cellular level. Here I use published and unpublished experimental data to illustrate that early activation of the *Hox* genes does not establish a gene code that leads to "segment identity". I will stress the point that *Hox* expression patterns develop with the embryo, that there are many genes involved in this modulation, and that the changing pattern of expression is important to achieve the final shape of the animal. I will show that, by interpreting *Hox* gene function in this way, some apparently paradoxical results in the *Hox* field can be answered. Finally, I discuss the implications of dynamic *Hox* gene expression on the evolution of segment morphology.

**KEY WORDS:** *gene regulation, hox development, evolution*

## Introduction

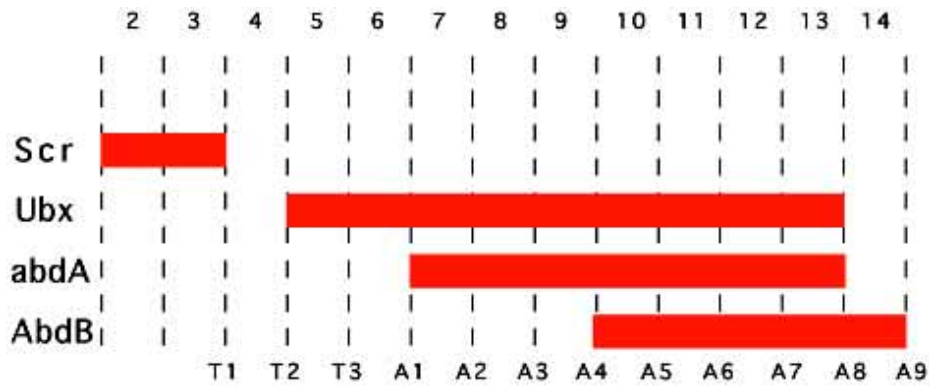
A wild type fly has a well defined head, thorax and abdomen. Genetic analysis showed that flies lacking the three *Hox* genes of the bithorax complex: *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*), are only composed of a head and an extended thorax as all the abdominal segments resemble thoracic segments (Lewis, 1978; Sánchez-Herrero *et al.*, 1985). Lewis' work led García-Bellido to propose that *Hox* genes regulate body morphology acting as selectors of alternative developmental pathways (García-Bellido, 1975). What they would do is to regulate other genes under their command so that in a certain segment a particular *Hox* gene would modulate downstream genes involved in making the abdomen, while in a more anterior segment another *Hox* gene will modulate genes that generate a thorax. These genetic propositions implied that, in *Drosophila*, the *Hox* genes encode regulatory proteins, and that they are expressed in different patterns along the anterior-posterior axis. Both proposals were confirmed by molecular biology studies: the *Hox* genes encode

transcription factors active in different segments where they control the activation of target genes required for the development of the structures typical of that segment.

An important experiment that influenced our current views on segment specification was the observation that, in the epidermis, *Hox* gene function is cell autonomous. Morata and García-Bellido showed that if a small group of cells stop expressing the *Ubx* gene in T3 they would form T2 structures (Morata and García-Bellido, 1976). Comparing the number of cells of the wing and the haltere at different stages in development, they found that the differences between these homologous thoracic appendages are established very early in development. However, they also showed that *Ubx* function is required continuously to maintain cell affinities and patterns of cell division during development. Consistent with these observations, molecular studies proved that the *Hox* genes are expressed from early embryogenesis until the end of development (reviewed in Akam, 1987).

The next stage was to find how the *Hox* genes are activated in different segments. Trans-regulation of *Hox* genes was found to be

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**Fig. 1. Classical *Hox* code model scheme.** Horizontal bars represent the region where each *Hox* gene is expressed and required. This model summarizes protein expression and functional data obtained from analysis in the embryo and in the adult. Stress is placed on the parasegmental domains of *Hox* expression. Numbers represent parasegmental units and vertical lines the parasegmental borders (that is, the boundary between engrailed and wingless expression). The position of thoracic (T) and abdominal (A) segments is represented under the diagram. Modified from Lawrence and Morata, 1994.

controlled by two classes of genes. One class, required only transiently to activate *hox* expression, is integrated by the genes involved in subdividing the embryo into segments (Nüsslein-Volhard *et al.*, 1984; Ingham and Martínez-Arias, 1986; White and Lehmann, 1986; Harding and Levine, 1988; Irish *et al.*, 1989; Jack and McGinnis, 1990; Casares and Sánchez-Herrero, 1995). The other class, required for the maintenance of *Hox* gene expression, is integrated by genes of the *Polycomb* and *Trithorax* groups which are dispensable for the early activation (Struhl and Akam, 1985; Wedeen *et al.*, 1986; Breen and Harte, 1993).

These results put together have given rise to the *Hox* code theory (reviewed in Lawrence and Morata, 1994): The early activation of a *Hox* gene by the segmentation genes in a metamer provides the cells expressing it with a "segmental identity". This identity is maintained by the *Polycomb* and *trithorax* group genes by keeping the early state of *Hox* expression during development. A cell therefore has the same identity all along development and accordingly gives rise to the structures specific of that segment. As each segment expresses different combinations of *Hox* genes, each has a different code resulting in the anterior posterior diversification of the segments of the animal.

The *Hox* code theory initially developed in *Drosophila* is now used as a paradigm to explain how *Hox* genes work in other organisms. It is therefore important to highlight the points that the *Hox* code hypothesis cannot explain but are never discussed in reviews about *Drosophila* development. While in earlier work *Hox* genes were seen as affecting the development of particular structures (Ventral pits, Keilin organs... Lewis, 1978) or the cellular properties of cells (cell affinities, mitotic rates... García-Bellido, 1975; Morata and García-Bellido, 1976), the focus has later shifted to metameric units studied as a whole. We do not talk about cell properties or particular structures and organs but of "segment identity". While this view is easier to use when talking in abstract terms it does not always work when applied to the development of particular organs. In what follows I will discuss why we should study *Hox* genes not as regulators of segment identity but as spatially restricted transcription factors involved in the control of cell behaviors and the specification of segment structures.

### Relativity in the development of segment identity: the function of *HOX* transcription factors changes during development

Figure 1 represents the common summary about *Hox* gene expression in *Drosophila* presented in reviews (Akam, 1987;

Lawrence and Morata, 1994; Carroll, 1995). *Ubx* is required for the development of T3 and A1, *abd-A* for A2-A7 and *Abd-B* for A5-A9. This summary compiles data that include experimental results obtained in the embryo and in the larva and its weakness is, paradoxically, its simplicity and elegance. The model in Figure 1 shows a static view of *Hox* expression in which *Hox* genes are continuously expressed within the same parasegmental boundaries. This situation is not real, as all *Hox* genes have a temporal and spatial expression that does not fit with that idealization.

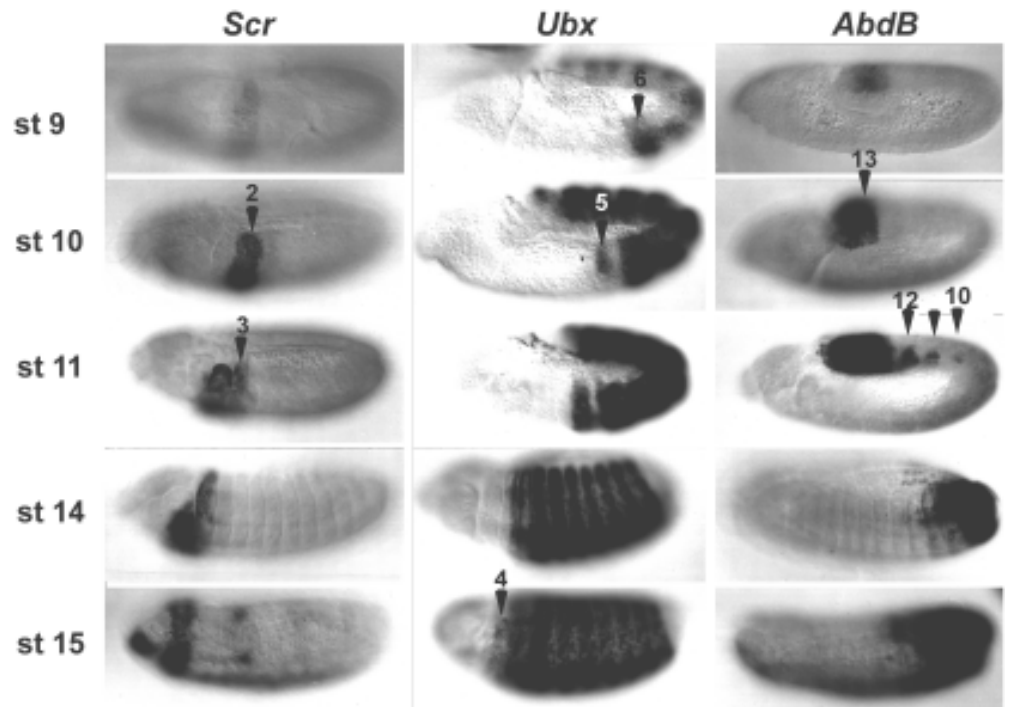
To illustrate some exceptions I will concentrate on the expression of the *Scr*, *Ubx* and *Abd-B* genes (Fig. 2). First, in all cases the *Hox* genes are expressed in fewer segments at early than at late stages of development. Second, at a single time point, a segment is a mosaic of cells expressing and not expressing a given *Hox* gene. Third, although the expression of *Hox* genes is frequently parasegmental, there are many cases in which this is not true (Fig. 3 and Martínez-Arias *et al.*, 1987; DeLorenzi and Bienz, 1990; Irvine *et al.*, 1991).

Such exceptions complicate the understanding of segment specification. For example, what is the segment identity of a cell that has had two different *Hox* codes during development? What happens to cells of the same segment which express different *Hox* codes? These can be viewed as minor exceptions when looking at the global *Hox* expression, but should not be ignored as they can result in dramatic morphological changes when considering the development of a particular organ (see below).

As the protein patterns are changing with the developmental stage of the embryo, unless we define experimentally when *HOX* protein function is required, we are not able to decide what aspects of these expression patterns are significant for development. Because during embryogenesis the embryo is forming a collection of different cells (muscle, epidermis, nervous system) the requirement of *HOX* function has to be answered independently for each different cell type. As doing this complicates matters we should first find out if such complex approach is necessary.

We can test if the conventional model of *Hox* gene function presented in Figure 1 can predict the outcome of a simple experiment: If we prevent the seventh abdominal segment (A7) from expressing *Abd-B*, would this segment transform to a more anterior segment? The answer is yes and no. In Figure 4 we see the result of preventing *Abd-B* expression in A7 in the embryo and in the adult. Such a mutation strongly affects the A7 segment in the adult while many A7 structures in the embryo remain normal (PNS, CNS, oenocytes) (Heuer and Kaufman, 1992). The usual response to this observation has been to suggest that the homeotic change in

**Fig 2. Patterns of expression of Scr, Ubx and Abd-B protein during early embryogenesis.** Each column shows the expression of a different Hox gene at progressively later stages of development. The expression is very dynamic and comes up in different segments at different stages. Scr is first expressed in PS2, later it expands to PS3. At stage 9 Ubx expression is confined to the abdomen (PS6-13), at stage 10 it expands to the thorax up to PS5. At stage 15 onwards Ubx is expressed in PS4, a region where no Ubx function has been found. Abd-B expression at early stages is confined to the last abdominal segments (PS 13-14). At later stages Abd-B is expressed also in PS10-12. In many (para)segments there are cells expressing and not expressing a particular Hox gene. This mosaic expression is particularly clear for Scr in PS3; for Ubx in PS5 and for Abd-B in PS10-12.



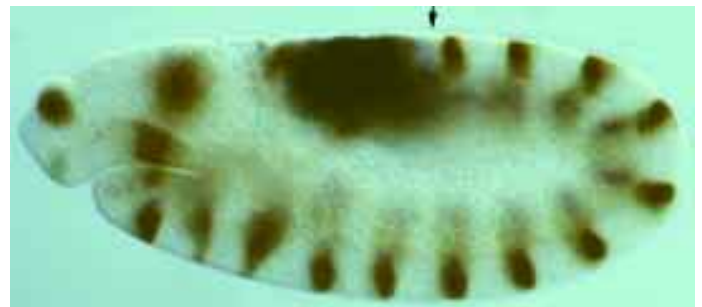
these cells is too subtle to be detected. A simpler answer is that we do not see changes because there are none. In the wild type fly *Abd-B* is only required in A7 for the formation of some structures. These include the larval denticle belts and the cells that will form the adult epidermis. The normal development of A7 structures like the PNS or the oenocytes does not require *Abd-B*; in fact unrestricted expression of ABD-B protein disrupts the normal development of A7 (Lamka *et al.*, 1992; Castelli-Gair *et al.*, 1994).

This observation explains the morphology of the A7 segment in the wild type embryo. The PNS in the abdomen is identical from A2 to A7 (Ghysen *et al.*, 1986) despite the fact that A2-A4 do not express *Abd-B* and A5-A8 do (Figs. 1 and 2). This does not mean that *Abd-B* is dispensable for PNS development as *Abd-B* mutants affect the PNS of A8 (Fig. 4). Why do A7 and A8 respond in such a different way to *Abd-B* expression? One possible answer could be that there is a difference in the temporal expression of *Abd-B* between A7 and A8 (Fig. 2). Expression is detectable previous to 6 h in A8 and it is only later seen in A7 and more anterior segments in a restricted spatial expression.

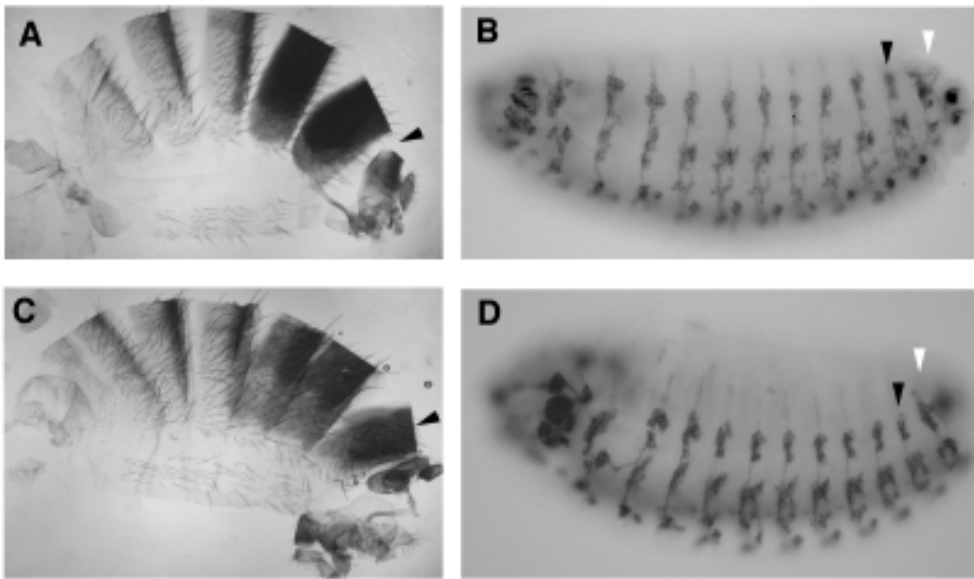
To test if *Abd-B* expression at different stages of development has different effects on development we can control when we express *Abd-B* using a heat shock promoter. The results of expressing ectopically *Abd-B* for one hour at different stages of development are shown in Figure 5. Unrestricted expression of ABD-B at 3-5 h of development results in the appearance in anterior segments of the posterior spiracles normally restricted to A8. Unrestricted expression at 5-7 h does not result in the appearance of ectopic posterior spiracles but affects sensory organ development. In the abdomen of wild type flies basiciconal sensilla are only present in segments A8 and A9, while in embryos heat shocked from 5-7 h they also appear from A1-A7. Unrestricted expression at 8 h results in none of the previous transformations but still affects the development of denticle belts.

These experiments should not be over-interpreted as the transient *Abd-B* expression they generate is an artificial situation never present in normal embryos. However, these results highlight the fact that cells respond differently to *Hox* expression at different stages of development. Therefore we cannot talk about a "Hox code" because in a particular group of cells the outcome of *Hox* expression changes with time.

In the above example the PNS morphology can be affected by *Hox* function at a stage when *Abd-B* is not expressed in A7. At a later stage when ABD-B is expressed in A7 it will not be capable of affecting PNS morphology, but will be able to affect the elements that give rise to larval denticle belts and imaginal abdominal cuticle.



**Fig. 3. An example of non parasegmental HOX expression.** A *wingless-lacZ* embryo at stage 11 double stained for  $\beta$ -gal (brown) and *Abd-B* (black). *Wingless* is expressed in the most posterior cells of each parasegment. At this stage *Abd-B* expression does not reach the *wingless* stripe (arrow) revealing that *Abd-B* is not expressed in a parasegmental domain everywhere in the segment. Note that the expression of *Abd-B* in other cells is more anterior, reaching the parasegment boundary.



**Fig. 4. The lack of Abd-B function has different effects in larval and adult structures.** The abdomen of an adult wild type male is formed by six segments. **(A)** The seventh abdominal segment of the larva does not give rise to adult abdominal structures in the male due to repression by Abd-B (the arrowhead points to the position between the A6 segment and the genitalia). In adult Abd-B mutant males an A7 segment forms **(C)**. By contrast Abd-B does not seem to influence many A7 structures in the embryo. In the wild type embryo **(B)**, the PNS of the A7 segment (black arrowhead) resembles that of abdominal segments where Abd-B is not expressed and not that of A8 where Abd-B is expressed (white arrowhead points to the spiracle neurones of A8). The lack of requirement

for Abd-B in the PNS of A7 is illustrated by the absence of defects in the A7 PNS of an Abd-B mutant embryo **(D)**. In the Abd-B mutant embryo the PNS of A8 is affected as shown by the absence of A8 spiracle neurones.

Other *Hox* genes also have different developmental effects when expressed at different times. *Ubx* has three different effects on leg development. In A1 *Ubx* prevents leg formation; in T3 *Ubx* is required continuously for the normal leg morphology while in T2 *Ubx* has to be active before 6 h of development and then turned off (Morata and Kerridge, 1981).

The regulation of the *Distalless (Dll)* gene, a gene required for leg development that is a direct target of *Ubx*, illustrates at the molecular level how dynamic *Hox* expression influences the morphology of the animal (Castelli-Gair and Akam, 1995). UBX represses *Dll* expression in A1 but not in T3 despite the fact that UBX is expressed in both segments in the relevant cells. The reason for this difference resides in the temporal expression of *Ubx* in T3 and A1 and in the regulation of *Dll* itself. The expression of *Dll* in the leg primordia is controlled by two enhancers (Vachon *et al.*, 1992). One enhancer is sufficient for the early activation of *Dll* in both the thoracic and abdominal segments, but the binding of UBX protein to this enhancer blocks *Dll* expression in the abdominal segments (Vachon *et al.*, 1992). Blocking of the early *Dll* enhancer does not occur in T3 as no *Ubx* is expressed in T3 at this stage of development. The early enhancer is not sufficient to maintain *Dll* expression in the leg primordia for which a second enhancer is required. The second enhancer is not repressed by UBX and depends on *Dll* autoregulation (Castelli-Gair and Akam, 1995). The use of the two enhancers and the temporal expression of *Ubx* in T3 and A1 explains the different effects of UBX protein on the regulation of *Dll*. If *Dll* gets activated in the leg primordia before UBX is expressed, the DLL protein autoregulatory loop will maintain *Dll* expression independently of UBX presence at later stages (the situation found in T3). On the other hand if UBX gets activated earlier, the initial expression of *Dll* will be blocked and the feedback loop will not occur, preventing the formation of a leg (the situation found in A1). In this way differences in timing of expression of about one hour can have drastic consequences on the morphology of the organism.

Not only leg development depends on the correct temporal modulation of *Ubx* expression. In a single PNS organ, the dorsal Kölbchen, ABD-A or UBX expression has different temporal effects (Castelli-Gair *et al.*, 1994). Early expression affects the dorsal-ventral migration of the sensory organ but not the specification of the organ as abdominal or thoracic; while later expression will select the abdominal differentiation of the sensory organ. This case is different from that described for the leg, in the sense that the location and differentiation of the sense organ seem to be independent decisions, while the presence or absence of a leg seems to involve a program that is turned on and maintained: a developmental subroutine. However, in both cases minor changes in *Hox* expression can have important effects on the final morphology of a segment, as this will be the result of a complex outcome that involves the cell's competence to take certain decisions.

Recent results studying the regulation of the mouse *Hoxd-11* gene prove that tight temporal regulation of *Hox* gene expression is also required for the normal segment morphology in vertebrates. The deletion of a conserved enhancer results in the local delay of *Hox* expression. Although those cells do express normal levels of *Hox* genes at later stages, this delay in *Hox* expression results in the transformation of sacral vertebrae into lumbar morphology (Zákány *et al.*, 1997).

The results summarized above do not imply that every minor detail of the *Hox* expression pattern has an influence on segment morphology. Ectopic induction of *Hox* genes does not have an effect at all stages of development. Also, in the wild type fly some *Hox* genes are expressed in regions where they do not affect the segment morphology. For example, *Ubx* is expressed in T1 at late stages of development without influencing its development. The most extreme case of this is that of the *proboscipedia* gene. While *proboscipedia* is expressed in the head of both embryo and larva, the expression in the embryo is not required for the development of the larval head, while larval expression is required for the adult head.

In summary, *Hox* genes are not controlling segment identity, but cellular behavior that will result in a certain segment morphology. This is what *Hox* genes do in unsegmented organisms like *C. elegans* (Salsler and Kenyon, 1996) and is probably what they did in the common ancestor of all metazoans. Segment identity is a subjective concept that originates from the observation that in a particular species, a number of cell characteristics are always associated in a given segment.

### Modulation of *Hox* expression

The above results show that the spatial and temporal regulation of *Hox* gene expression has to be finely tuned during normal development. In *Drosophila*, it is thought that we have already got a good understanding of how *Hox* gene expression is controlled. In the blastoderm, the genes that are involved in subdividing the embryo into segments also activate *Hox* expression in restricted anterior-posterior domains. This is achieved by the combination of activator genes (*fushi-tarazu*, *evenskipped*, *tailless*, etc) in conjunction with repressors (*hunchback*) that establish the domains of *Hox* expression. Both activators and repressors are only transiently expressed. As, in general, *Hox* genes do not autoregulate, the patterns that segmentation genes establish have to be maintained later in development by other genes. These are the genes of the *Polycomb* and *trithorax* groups.

The above elements suggest an early crystallized pattern of *Hox* expression that is propagated during development, but there must be other genes responsible for the subtle *Hox* gene modulation. Which are these genes? It has been proposed that the segmentation genes of the segment polarity class are involved in the local modulation of *Hox* patterns (Martínez-Arias and White, 1988). In contrast to other segmentation genes, segment polarity genes are not expressed transiently, but they are maintained during development. The best documented case is the down regulation of the expression of *Ubx* in the posterior compartment by the segment polarity gene *engrailed* (*en*). As we have seen this down regulation is important for the formation of the legs, due to the modulation of the levels of UBX protein (Mann, 1994) or timing of *Ubx* expression (Castelli-Gair and Akam, 1995). In *en* mutants the expression of *Ubx* in A1 increases (Martínez-Arias and White, 1988; Mann, 1994). Conversely ectopic expression of *en* using a heat shock promoter results in the down regulation of *Ubx* in all segments as a result of which the leg marker *Dll* is expressed in all the abdominal segments (Mann, 1994).

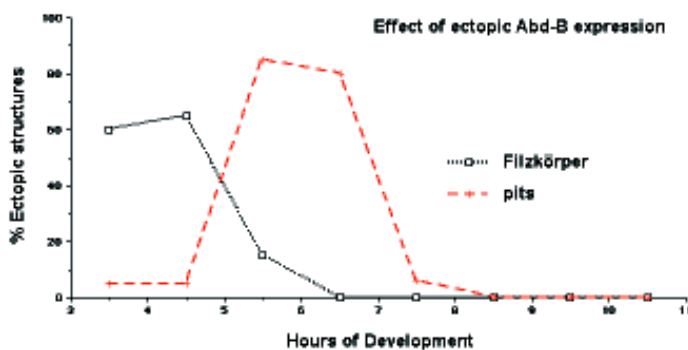
We know very few genes modulating *Hox* expression. The complex modulation of *Hox* expression suggests that they must form a large and diverse class of genes responsible for the temporal as well as dorsal-ventral and even cell specific regulation. The reason for the scarce knowledge of *Hox* modulators probably resides in the subtlety of their effects on development. Good candidates to be *Hox* modulators are the zinc-finger proteins encoded by the *spalt* (*sal*) and *sal-related* (*sal-r*) genes (Kühnlein *et al.*, 1994; de Celis *et al.*, 1996). In *sal* mutants (or both) *Ubx* becomes expressed in more anterior segments (Fig. 6). *sal* has been classified as a Pc group gene (Casanova, 1989). Several reasons suggest that *sal* is not a Pc group gene but forms part of this less well characterized class of *Hox* modulators. First, genes of the Pc-G are required to repress all *Hox* genes in regions where they were not initially activated; in contrast, *sal* mutants

only affect *Ubx* expression while *Scr*, *abd-A*, and *Abd-B* expression is not affected (data not shown). Casanova suggested that *Scr* is expressed ectopically in double mutants for *sal* and the bithorax complex (Casanova, 1989) but ectopic *Scr* expression can be detected in bithorax complex mutants alone (Pelaz *et al.*, 1993). Second, in *sal* mutations the spatial expression of *Ubx* in PS3 and PS4 mimics the spatial expression of *Ubx* in PS5, suggesting that *sal* interacts with the subset of enhancers responsible for that pattern. Third, the result of this anterior *Ubx* expression is not a complete transformation of one segment into another. While the PNS and the denticle belt patterns in the thoracic cuticle in *sal* mutants are normal the formation of the anterior spiracle is repressed (Fig. 6) resulting in the formation of a segment with mosaic characteristics not to be found in the wild type fly.

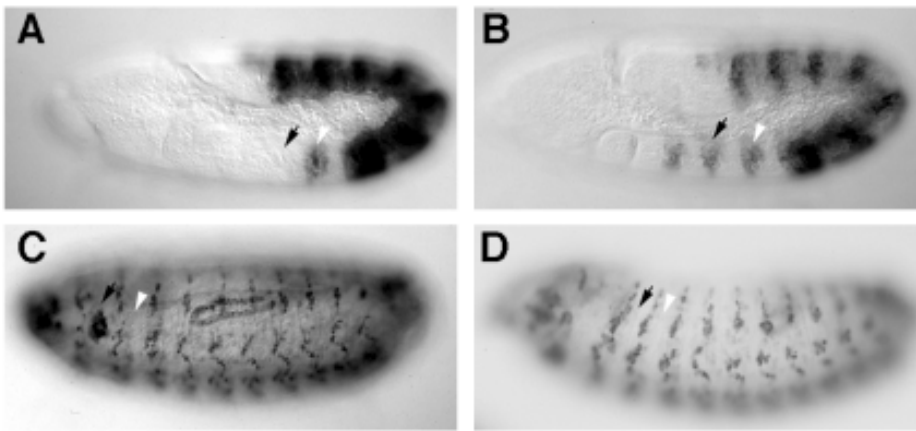
The existence of a large number of modulator genes with small effects on the *Hox* regulatory regions provides a plasticity to the evolution of different segment morphologies that is unlikely to have been missed in evolution. Minor changes in the *cis* regulatory elements of the target genes to which these modulator proteins bind could result in the modification of segment morphology without creating animals of improbable viability.

### *Hox* gene regulation and the evolution of body shape

From early studies it was realized that the *Hox* genes could be paramount in the evolution of body shapes. Ed Lewis proposed that the evolution of the insects body plan from a precursor Arthropod had depended on the appearance of new *Hox* genes by duplication of ancestral ones. These new genes would be expressed in more posterior segments and their evolution would result in the appearance of new segmental structures. Initially, with only one *Hox* gene all the trunk segments would look alike. After duplication, the gene would acquire new functions leading to the appearance of a distinct thorax and abdomen, further duplication and divergence events leading to the transformation of four winged insects into two winged flies. Mutations in *Hox* genes would reverse evolutionary processes resulting in atavistic trans-



**Fig 5. The expression of *Abd-B* has different effects at different stages of development.** Graph indicating the defects induced by ectopic *Abd-B* expression at different stages. Ectopic expression between 3 and 5 h induces the formation of spiracular structures (filzkörper). Filzkörper are not formed when the expression is induced between 5 and 7 h. However at this time PNS structures (ventral pits) are affected. Ectopic expression at eight hours induces neither pits nor spiracles, however, the shape of the denticle belts is affected producing the occasional transformation to the typical A8 denticle belt shape.



**Fig. 6. Specific alterations of the spatial and temporal *Hox* expression result in organ specific homeotic transformations.** *UBX* protein expression at stage 10 in wild type (A) and *sal* mutant embryos (B). The *sal* mutant embryo shows in PS3 and PS4 the expression normally present in PS5. (C,D) PNS morphology of the resulting embryos as revealed by staining with anti-Cut antibody at stage 15. The anterior spiracle can be detected in the wild type (C black arrow), but is missing in the *sal* mutant embryo (D). In all panels the black arrow points to the posterior compartment of the first thoracic segment in the region where the anterior spiracle develops. The white arrowhead points to the homologous position in the second thoracic segment. The pres-

ence of a spiracle in the second thoracic segment is prevented by the expression of *UBX* (white arrowhead in A). Note that *Ubx* also controls the organization of the PNS cells in the abdomen. The organization of these cells is not affected in the *sal* mutant, most likely as a result of the restricted spatial and temporal expression of *UBX*.

formations (García-Bellido, 1977). That is, these mutations in *Hox* genes would take the organism "back" in evolution.

The finding that *Hox* genes are present in vertebrates and in non segmented animals (McGinnis *et al.*, 1984; Costa *et al.*, 1988) seemed inconsistent with the view that Arthropod body shape and *Hox* genes evolved together: if the ancestors of insects already had a complete set of *Hox* genes, their invention could not be responsible for the evolution of segment diversity in arthropods.

Despite of this, *Hox* genes have a great potential to change body shapes as shown by the effect of mutations and it is unlikely that they have not participated directly in some aspects of evolution. *Hox* genes are unlikely to have participated in the sudden invention of a new type of segment but, as we have seen, by modulating its patterns of expression in time or space they can generate changes in either a specific cell type or a structure.

Modulation of *Hox* expression can be achieved by minor changes in an enhancer. The *cis* regulatory elements in *Drosophila* have enhancers with major effects in body shape which are in general those involved in gene maintenance. There is also evidence of a different class of enhancer that is acting in a redundant or collaborative way. For example, a construct containing 8.8 Kb of *Ubx cis* regulatory sequences (the BRE element) contains enhancers capable of driving expression in A1 (PS6) in the embryo; a mutation (the *bx34<sup>e-priv</sup>* allele) that removes these sequences develops a normal A1 segment showing that other enhancers can compensate for the absence of the BRE element (Peifer and Bender, 1986; Qian *et al.*, 1991). Experiments studying the adult structures of A1 also suggest the presence of redundant *Ubx cis* regulatory elements. There are two *Ubx cis* regulatory regions that when removed independently do not affect the development of A1, however, when removed simultaneously the A1 segment is completely missing (Castelli-Gair *et al.*, 1992). Many other examples of enhancers acting collaboratively have been described. It is at this level that subtle evolution could act.

There are several cases in which changes in *Hox* spatial expression have been correlated with the evolution of segment morphology. A very nice case has been shown in Crustaceans (Averof and Patel, 1997). Genes homologous to *Drosophila's Ubx* and *abd-A* are expressed in the thorax in a region that normally forms swimming appendages. However, in some species one or

more thoracic appendages are transformed into feeding appendages (maxillipeds). The number of maxillipeds varies among species. Studying thirteen different species of Crustaceans Averof and Patel show that *UBX* /*ABD-A* protein expression correlates with thoracic segments bearing swimming appendages, while thoracic segments that do not express these proteins are transformed into maxillipeds.

Another case has been shown in Lepidoptera (Warren *et al.*, 1994). Butterfly caterpillars have abdominal prolegs. Prolegs differ from thoracic legs in morphology but both express the homolog of *Dll*. As we have previously seen *Dll* is repressed in the abdomen of *Drosophila* by *UBX* and *ABD-A*. Why is *Dll* expressed in the abdomen of butterflies? Warren and collaborators show that *Dll* activation in the abdomen of the butterfly is delayed with respect to the thorax. At later stages however, *ABD-A* protein is down regulated in the abdomen allowing activation of *Dll*.

These two examples suggest that changes of segment patterns during evolution could have been due to the modulation of *Hox* expression. However, a note of caution should be said in the interpretation of expression patterns in the absence of experimental data. Not all expression cases will turn out to be so clearly cut. For example, most insects have wings in T2 and T3. In flies the T3 pair of wings has evolved into specialized balancing structures, the halteres. Because when flies do not express *Ubx* in T3 the halteres are transformed into wings, one could predict that butterflies do not express the homolog of *Ubx* in T3. This is not the case, butterflies do express *Ubx* in the T3 wings (Warren *et al.*, 1994), a fact that is not surprising if we think on the possible existence of developmental subroutines as that described for *Dll* in the leg. It is under this light that we can interpret this result. *Ubx* in butterflies would not prevent the development of wings in T3 if *Ubx* is expressed at a moment in which wing development has been irreversibly triggered.

The application of molecular techniques to the study of comparative development is going to provide many interesting data about how developmental mechanisms evolved. Unfortunately, the interpretation of these data will depend very much on the views of the researcher. To take full advantage of these advances we should try to use model organisms where hypothesis can be tested or to develop experimental approaches in organisms where such

approaches are not available. This will be the only way to get a clear understanding of the evolution of developmental mechanisms.

## Materials and Methods

The *Abd-B<sup>M5</sup>* and *Abd-B<sup>D14</sup>* double heterozygous flies were used for Figure 4c and *Abd-B<sup>M1</sup>* null allele for Figure 4d (Casanova *et al.*, 1986; Zavortink and Sakonju, 1989). Two *sal* mutant alleles were used *sal<sup>lB57</sup>* and *Df(2L)5* a deletion removing both *sal* and *sal-r* (Nüsslein-Volhard *et al.*, 1984; de Celis *et al.*, 1996). The following antibodies were used: a polyclonal anti-CUT (Blochinger *et al.*, 1990), and the monoclonals FP3.38 anti-UBX (White and Wilcox, 1984), 6H4 anti-SCR (Glicksman and Brower, 1988) and 1A2E9 anti-ABD-B (Celniker *et al.*, 1989).

For heat shock experiments eggs from *HS-ABD-Bm F4* flies (Lamka *et al.*, 1992) were collected every hour and aged for different periods at 25°C. A single 1 h heat shock was induced by immersion in a 37°C water bath. Heat shocked eggs were transferred to agar plates and allowed to develop prior to cuticle preparation. Cuticle preparations and antibody stainings were done as described in Castelli-Gair and Akam (1995).

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