Int. J. Dev. Biol. 42: 445-451 (1998)

Hox genes, homeosis and the evolution of segment identity: no need for hopeless monsters

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ABSTRACT Significant changes have occurred in the developmental role of *Hox* genes, even within groups of arthropods that already have complex body plans and many different segment types. This is hard to reconcile with the 'selector gene' model for *Hox* gene function. Selector genes act as stable binary switches that direct lineages of cells to adopt alternative developmental fates. This model suggests that the regulation of selector genes can only evolve through mutations that alter the identity of whole developmental compartments –in the case of *Hox* genes, whole segments. Once segments have evolved distinct morphology and function, such mutations will result in dramatic homeotic transformations that are unlikely to be tolerated by natural selection. Thus we would expect the developmental role of these "master control genes" to become frozen as body plans become more complex. I argue for a revised model for the role and regulation of the *Hox* genes. This provides alternative mechanisms for evolutionary change, that may lead to incremental changes in segment morphology. The summation of such changes over long periods of time would result in differences in *Hox* gene function between taxa comparable to the effects of gross homeotic mutations, without the need to invoke the selective advantage of hopeful monsters.

KEY WORDS: *selector gene, gene regulation, enhancer modules, development, homeotic genes, ultrabithorax*

"It is not improbable that homoeosis of distant meromes may have given rise to permanent structural changes characteristic of whole groups of Arthropoda, supposing the abnormality once established to be favoured by natural selection."

(Lankester 1904, Structure and Classification of the Arthropoda, p. 536)

Introduction

Models of how genes act during development constrain our understanding of how they may change in evolution. García-Bellido's work provides a particularly clear example of this. From the 1970s onwards, Antonio led a school of developmental genetics that modeled development as a series of binary decisions, mediated by key controlling genes called selector genes (García-Bellido, 1975; Morata and Lawrence, 1977). The primary assumptions of this model are: i) that selector genes act as a series of binary switches to direct the developmental fate of groups of cells into alternative pathways; ii) that these decisions are lineally inherited and effectively irreversible during normal development; and iii) that a common combination of active selector genes specifies the identity of all cells in a developmental compartment of the fly.

This model provides a simple way to envisage the link between genes and morphology. It has been immensely fruitful. It has focused attention on the genetic subdivision and specification of the body -its internal representation, not its external form. It has emphasized the function of genes in normal development, not the bizarre phenotypes of mutant alleles. Many of the phenomena of *Drosophila* experimental genetics fit this paradigm well -not least the dramatic homeotic mutations that transform one region of the body into "the likeness of another" (Bateson, 1894).

For evolutionary biology though, this model of development is problematic. If selector genes work as stable binary switches, their role cannot change in small steps. They must be either "on" or "off". Any mutation that alters the regulation of a selector gene will be a mutation of major effect, a "hopeless monster" that is unlikely to be tolerated by natural selection. The developmental role of the selector gene will be constrained against evolutionary change, unless its activity specifies only minor developmental differences. This might be the case for *Hox* genes in an arthropod with many similar segments. In such an animal, switching one *Hox* gene on or off in a particular segment might make only a subtle change to its

Abbreviations used in this paper: kb, kibbase; bp, base pair; T2, Thorax segment 2; T3, Thorax segment 3; A1, Abdomen segment 1; A7, Abdomen segment 7.

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0214-6282/98/$25.00

Printed in Spain

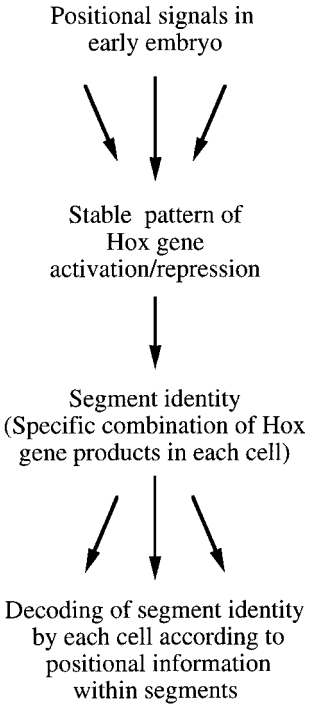

Fig. 1. Flow of information according to the 'Selector gene' model of *Hox* gene function. *The Hox genes respond only to axial information.*

phenotype. However, the same mutation would not be tolerated in an animal with segments that were very different from one another. In such an organism, the selector gene model predicts that the regulation of the *Hox* genes would be frozen by selective pressures. Incremental change in developmental processes would be relegated to genes that lie downstream in the developmental hierarchy. Perhaps for this reason, few developmental geneticists expected to find significant variation in the regulation of *"selector"* genes, or to consider how such variation may contribute to evolution.

The *Hox* genes of *Drosophila* provided much of the foundation for this selector gene paradigm, and yet from the start, they also provided data that challenged it. Many viable homeotic mutations are not complete transformations of one compartment into another. They are "incompletely penetrant" mutations effecting lesser changes in segment morphology and often affecting only a small part of a segment. Many of these mutations affect regulatory sequences, not protein function (Bender *et al.*, 1983). Thus regulation of the *Hox* genes is not absolutely constrained to conform to the selector gene model. In several cases *(Ultrabithorax, Ubx, Abdominal-B , Abd-B)*, the *Hox* genes show dosage effects (Lewis, 1978), suggesting that quantitative variation in the levels of *Hox* gene products can affect segment morphology in subtle ways.

Recent studies suggest that these effects are not just artefacts of laboratory mutations, but rather that they parallel the complex role that regulation of the *Hox* genes actually plays in the control of morphogenesis. The *Hox* genes do not just function as binary switches between developmental states of whole compartments (Castelli-Gair and Akam, 1995; Castelli-Gair 1998). Allelic variation at Hox loci does exist in natural populations (Gibson and Hogness, 1996) though the morphological consequences of this variation await study. Patterns of *Hox* gene expression have changed during the diversification of the arthropods, not only in animals with very similar segments, but also in such groups as the insects and crustaceans, which have complex body plans and diverse segment morphologies (Kelsh *et al.*, 1994; Warren *et al.*, 1994; Averof and Patel, 1997; Rogers *et al.*, 1997).

Models for *Hox* gene function are moving beyond a rigid binary hierarchical view of gene action in development, towards a model that recognizes the complexity of regulatory information that can be integrated by single promoters. These promoters are "microprocessors". Their organization holds the key to our understanding of morphological evolution, and makes it much easier to envisage how the function of the *Hox* genes can evolve.

The selector gene model for *Hox* gene function

The pioneering genetic analysis of Ed Lewis (1978,1981) led to a model for *Hox* gene function that fully conformed to the selector gene paradigm (Fig. 1). The model envisaged that maternal information provides positional signals along the axis of the early embryo. Regulatory elements of the *Hox* genes respond to these signals with on/off decisions in each segment (or, as now understood, parasegment), so that a unique set of *Hox* genes will be expressed in all cells of each metamere. This set of Hox proteins was seen as a "Hox code", giving each cell a "segment identity". The complexity of developmental patterning within segments lay entirely downstream of the *Hox* genes, in the decoding of segment identity by each cell according to its Hox code.

An important component of this model was a memory mechanism that caused the 'on' or 'off' state of the *Hox* genes early in development to be stably propagated. This allowed positional information in the blastoderm to specify stable *Hox* codes that could direct the differentiation of cells much later in development, for example during adult metamorphosis.

Lewis' genetic analysis identified unique genetic elements for each segment controlled by the Bithorax complex, but it was already clear that single genes were important for the normal development of more than one segment. For example the Ubx gene specifies not only the development of the third thoracic segment, but also the specific characteristics of the first abdominal segment. This was resolved by a combinatorial coding model (Struhl, 1982). This model preserves the idea that *Hox* genes are binary logical switches, but asserts that different *Hox* genes acting together can specify novel segment identities -presumably by specifying in combination a developmental pathway that is different from that specified by either of the two gene products acting alone.

Combinatorial action of transcription factors is well documented as a general model (e.g., Johnson, 1995), but there is little evidence that two *Hox* genes acting together in a single cell (e.g., *Antennapedia* and *Ubx*) can elicit a qualitatively different response from one or the other of these genes acting alone. Most available evidence suggests that one gene product will compete or dominate in its effects over the other (Gonzalez-Reyes *et al.*, 1990; Lamka *et al.*, 1992).

There are other mechanisms that might allow a single *Hox* gene to specify more than one segment identity, while preserving an effectively digital coding of segment identity. Differential splicing of *Hox* gene products is well documented, and could in principle result in the expression of unique protein variants in each segment. However, where it has been examined, the evidence is all against such a role for the different splicing variants (Mann and Hogness, 1990; Subramanian *et al.*, 1994). Alternatively segments might be

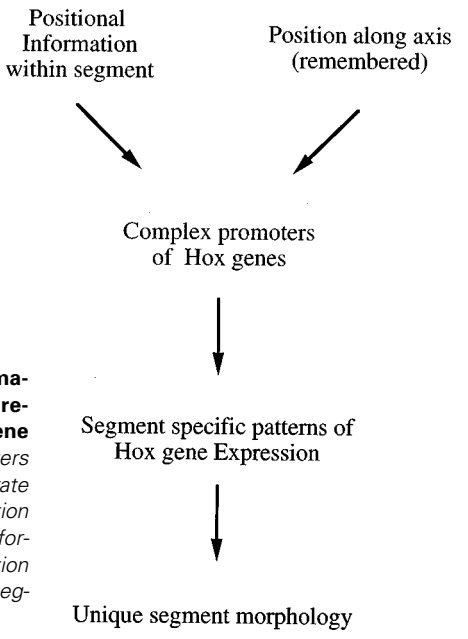

Fig. 2. Flow of information according to a revised model of *Hox* gene function. *The promoters of the Hox genes integrate information about position along the axis, with information about the position of each cell within the segment.*

characterized by different levels of Hox proteins which could exert qualitatively different developmental effects -effectively a multi-threshold model for the interpretation of Hox codes. There is certainly evidence to support the idea that levels of Hox proteins are important for their developmental effects (Smolik-Utlaut, 1990). However, our work (Castelli-Gair *et al.*, 1990,1994; Castelli-Gair and Akam, 1995) and that of others (Mann, 1994) suggest that a more radical revision of the model is required, which does away with the idea of a digitally encoded segment identity, at least at the level of *Hox* gene expression.

A revised model for *Hox* gene function

Two key assumptions distinguish a revised "post-selector gene" model for *Hox* gene function from the orthodox "selector gene" version. The first is that details of the spatial and temporal pattern of *Hox* gene expression within segments matter for the normal development of segment morphology. The second is that the *Hox* genes themselves integrate two types of information in each cell - the position of that cell along the axis (remembered from the early embryo) and the local spatial and temporal signals that impinge on the cell throughout development (Fig. 2).

I discuss these points below. To do so, I must first highlight some features of the regulatory architecture of the *Hox* genes.

The regulatory architecture of the *Hox* genes

The sizes of the *Drosophila Hox* genes range from 30-130 kb (Lindsley and Zimm, 1992). In the case of *Ubx*, 1.4 kb of coding sequence is embedded in a 100 kb regulatory region. We do not know how much of this DNA is significant for function, or the details of its organization.

In many respects, the regulatory architecture of the *Hox* genes appears to be similar to that of other eukaryote promoters that have been analyzed in more detail (Pankratz *et al.*, 1990; Stanojevic *et al.*, 1991; Kirchhamer and Davidson, 1996). The promoters are modular. Individual enhancer modules span a few hundred bases of DNA, and contain multiple binding sites for each of a small set of transcription factors (typically numbering 4-6). A single module will drive expression of the gene in one cell type at one stage of development. Expression in other tissues, or even in the same tissue at other stages of development, will be mediated by other, independent modules. Repressors that shut down expression when bound to one module need have no effect on the activity of other modules. Thus modules act additively.

The early activation of *Ubx* has been examined most thoroughly. At least seven distinct modules drive *Ubx* expression in blastoderm and early germband stages. All are active in the same broad region of the embryo, but with slightly different parasegmental specificities (Müller and Bienz, 1991; Pirrotta *et al.*, 1995). Qian *et al.* (1991,1993) have characterized one of these elements in detail. A 500bp core element drives expression of the Ubx gene in a pattern that resembles its earliest activation, in parasegments 6, 8, 10 and 12 of blastoderm and extended germ band stage embryos. This module has binding sites for the Engrailed, Hunchback, Fushitarazu, Twist and Tailless proteins, a set of spatially regulated transcription factors that together account for most aspects of the observed expression pattern. The core element is embedded in a region of several kilobases of DNA that can modify and extend its activity, though whether this flanking region contains independent enhancer modules remains unclear; it appears not to function in isolation. Expression of *Ubx* in the imaginal discs depends on entirely separate and independent enhancers (Pirrotta *et al.*, 1995).

Other mechanisms are superimposed on this modular architecture. One is a memory mechanism, that has the property of modifying the activity of an enhancer module throughout the whole of development, according to information present in the blastoderm stage embryo (García-Bellido and Capdevila, 1978; Paro, 1995). I discuss this mechanism in detail below, because how it works constrains the evolutionary flexibility of the *Hox* genes. Another is a global repression system that allows one *Hox* encoded protein to downregulate all transcription from the promoter of another *Hox* gene. This mechanism ensures that only one *Hox* protein is expressed at high levels in most cell types. It does not otherwise concern us here.

Peifer *et al.* (1987) proposed the first detailed model for the memory mechanism. They suggested that enhancer modules active in the same segment were clustered together into chromatin domains that operated as a unit -i.e., they were made accessible for subsequent activation ("open for business") or they were shut down irreversibly (silenced), by mechanisms initiated in the blastoderm. The existence of such chromatin domains is supported by several observations. Reporter genes inserted at random into the Bithorax complex frequently acquire segmental patterns of activity, even though they are not targeted to specific enhancers (McCall *et al.*, 1994). Specific stretches of DNA behave as boundary elements that limit the spread of active or inactive domains (Gyurkvics *et al.*, 1990; Hagstrom *et al.*, 1996). Chiang *et al.* (1995) suggest that each such domain has a single principal binding site for Polycomb protein, a key component of the machinery that is believed to mediate the memory mechanism.

Studies of regulatory fragments in transgenic constructs provide a rather different view, suggesting that there are more sites through

which Polycomb proteins can act, and that silencing operates directly on single modules rather than on whole chromatin domains (Simon *et al*., 1993; Chan *et al*., 1994; Christen and Bienz, 1994; Bienz and Müller, 1995). The truth may lie in a combination of these views - that whether or not an enhancer module is "open for business" in a particular segment depends on the local binding of "memory" proteins, but the binding of these is only loosely integrated along the chromosome: Local changes in DNA sequence might alter the sensitivity of an enhancer module to repression, or release it altogether from sensitivity to this mechanism. Such a mechanism seems necessary to account for the expression of Hox proteins in particular cell types of segments that otherwise do not express the gene - for example, the expression of U*bx* in just a few specific neural cells of parasegment 4 (White and Wilcox, 1985).

The importance of "within segment" *Hox* gene regulation

In the *Drosophila* blastoderm, *Hox* genes are typically transcribed in parasegmental stripes. However, once the segment polarity and dorso/ventral patterning genes have erected a scaffold of pattern within each segment, patterns of *Hox* gene transcription rapidly become more complex (White and Wilcox, 1985; Carroll *et al*., 1986; Mahaffey and Kaufman, 1987; Mahaffey *et al*., 1989; Diederich *et al*., 1991; Engström *et al*., 1992; Castelli-Gair and Akam, 1995). In a few cases, we know that this complexity matters. One case that has been analysed in detail is the role of *Ubx* in the embryo. The temporal and spatial pattern of *Ubx* expression can account for many details of the differential development of T2, T3 and A1 in the larva. (See Castelli-Gair, 1998, in this issue for further details).

Salser and Kenyon (1996) reach a similar conclusion from a study of *Hox* gene function in *C. elegans*. The *mab-5* gene switches on, off, on and off again to regulate proliferation, differentiation and morphogenesis in a single cell lineage. In these two cases it is clear that the differential regulation of a *Hox* gene within a single segment or lineage is critical for the development of normal morphology.

Are the *Hox* genes special?

By accepting a role for the regulation of *Hox* genes within compartments, we demote them from their privileged status as stable binary switches. The revised model allows the activity of the *Hox* genes to be modulated in a complex way throughout development, by local signals, hormone receptors or any of the other stimuli that commonly mediate gene regulation. In this regard, it makes the *Hox* genes like any other genes. It predicts that small changes, particularly in the structure of their promoter modules, will change the phenotype of segments.

The important and unusual characteristic of the *Hox* genes is that their response to this "real time" information can be segment specific, even though the information impinging on each cell may be the same in each segment. This is because the enhancers themselves are differentially available in different segments, and because, in some cases, a single gene is regulated by a different set of enhancers in each segment. *Ubx* for example is regulated by the "abx" enhancers in parasegment 5, which integrate patterning information in one way, but by the "bxd" enhancers in parasegment 6, which specify a different within segment pattern (Peifer *et al*., 1987; Simon *et al*., 1990). One reflection of this is that the *engrailed* gene appears to activate Ubx expression in parasegment 5, but repress it in parasegment 6.

According to this model, the number of different segment identities is limited not by the number of *Hox* genes or protein products, or their combinations, but by the extent to which enhancer modules can be differentially regulated in different segments. In *Drosophila*, the memory functions of the Bithorax Complex can discriminate between serially homologous cells in adjacent segments, allowing cells to respond differentially to a conserved set of positional signals in each segment. These differences in *Hox* gene expression may not appear until late embryogenesis, even though the information that specified the differences was registered by the *Hox* genes in the blastoderm.

The effect of mutation on *Hox* gene function

With this model, we can distinguish two ways that regulatory mutations in the *Hox* genes might alter segment morphology. Some mutations may alter the sensitivity of individual enhancers to "within segment" information. This will change the detailed morphology of one or more segments, by modifying a particular developmental process. However, the change would not necessarily be recognized as a "homeotic mutation". My colleague David Stern has recently found evidence for such variation affecting the *Ubx* gene of *Drosophila* (D. Stern, in preparation). Certain fine details of leg morphology depend directly on the pattern of *Ubx* activity during pupal stages. Naturally occurring differences in leg morphology depend, at least in part, on allelic differences at the *Ubx* locus.

Mutations of this type have contributed to the divergence of the Diptera and the Lepidoptera. *Ubx* and *abdominal-A* expression are locally repressed in the abdominal leg primordia of butterflies but not of *Drosophila*. This allows formation of the abdominal prolegs in the caterpillar (Warren *et al.,* 1994). Note though, that this repression affects only the enhancer that drives expression in abdominal segments A3-A6. In A1 and A2, one or both of these genes must be driven by an enhancer that remains active in the leg primordia, blocking development of the prolegs in these segments.

Other mutations may affect the action of the memory mechanism. In this case, the differential expression of *Hox* genes between segments will be altered. In the extreme case, if a whole set of enhancer modules respond inappropriately to positional signals, normal morphology will be expressed in the wrong location, and a recognizable homeotic mutation will result. The *Contrabithorax* mutation Cbx^{1} is an example of such a change, where a large piece of DNA carrying imaginal disc enhancers has been transposed, within the Bithorax complex and in the process become active in the wrong segment (Bender *et al*., 1983). Much of the wing develops as a haltere.

The converse situation is more common in experimental genetics; a set of segment specific enhancers become inactive or separated from their promoter, and their contribution to the final pattern is lost. This appears to be the mechanism of action of most of the *bithorax* mutant alleles. Some of these are deletions of hundreds of base pairs. Others are insertions of transposable elements that place new "boundary elements" into the Ubx gene, isolating the promoter from the activity of most of the parasegment 5 enhancers (Bender *et al.,* 1983; Gerasimova and Corces, 1996).

If the "open for business" model of chromatin domains is accurate, then mutations affecting the memory mechanism would be expected to act on entire (para)segmental units. However, if specific enhancer modules are independently opened or closed, the system is potentially much more flexible. The segmental limits of expression of each *Hox* gene could then be modulated independently in particular tissues, and at different phases of development. For example, segment specific regulation in sensory bristle cells could be independent of earlier phases of expression in the developing epidermis. A model that incorporates the flexibility to vary segmental boundaries of expression in particular tissues seems to accord much better with the observed patterns of *Hox* gene expression than the more rigid, strictly lineal memory mechanism originally proposed.

Just how flexible this system might be depends in large measure on the complexity of the regulatory input to the *Hox* genes. We have no good estimate of this. At least 10 discrete DNA fragments containing Ubx enhancers have been described to date (Müller and Bienz, 1991; Simon *et al.*, 1993; Pirrotta *et al.*, 1995), though many have been defined only as fragments several kilobases long. However, the regulatory elements that drive Ubx expression during adult development have been assayed almost exclusively in late larval imaginal discs. No attempt has been made to map elements active during later stages of adult patterning, after pupariation, when much of the fine detail of morphology is established (e.g., the pattern of wing veins and mechanosensory elements). In no case have the details of cell by cell regulation in the nervous system or other complex tissue been investigated. My own prejudice is to think that the elements defined so far represent only the tip of the iceberg; that there may be as many as a hundred modules that function in distinct spatio-temporal domains. These would contain literally thousands of protein binding sites, and perhaps as much as 10 kilobases of sequence where single base changes could affect the patterns of Ubx expression, in most cases in very minor ways. At present, the best way to identify such functional sequence is probably to compare the extent of sequence conservation between different species (Kreitman and Ludwig, 1996). Little such information is available for *Ubx* (Wilde and Akam, 1987), but low resolution data from heteroduplex mapping is available for the *engrailed* gene, which shows comparably complex spatial and temporal regulation (Kassis, 1990). Of the 70 kb *engrailed* regulatory region, 33 dispersed blocks totalling about 20 kb are sufficiently conserved to hybridize between distantly related *Drosophila* species (Kassis *et al.*, 1985).

A gradualist scenario for the evolution of segment

The *Hox* genes might justifiably be considered master control genes (Gehring, 1996) for segment identity. For most segments of the insect trunk, they provide the only conduit for channeling axial information from the early embryo to cells at the later stages of development. When their function is eliminated, homeotic transformations result, generating less complex body plans which, in some cases, mimic inferred ancestral states -so called atavic mutations (García-Bellido, 1977). It is tempting to shift this process into reverse, and to assume that segment diversification has been achieved by a series of overt homeotic mutations generating novel complexity. (Lankaster, 1904; Goldschmidt, 1940).

I do not reject the possibility that overt homeotic mutations have contributed to morphological evolution. However, I think it unlikely that complex patterns of segment specialization have evolved this way. With the revised model for *Hox* gene function, it is not difficult to propose an alternative scenario. Assume that an animal already has an array of *Hox* genes differentially expressed along the A/P axis of the body, and that these control substantial differences between segments. This is the condition in which the traditional selector gene hypothesis predicts that the developmental role of the *Hox* genes will be "frozen" by selection against hopeless monsters. However, consider a mutation that allows one enhancer module to become active several segments more anteriorly. Such a mutation is known in flies -*Hab*, a single base change in a Kruppel protein binding site (Shimell *et al.*, 1994). This particular mutation affects an enhancer that is active in very early development, and leads to a semi-lethal mutation. However, if this enhancer affected expression only in the mechanosensory bristles of the adult epidermis, then few aspects of segment morphology would be affected. Segments would not transform into the likeness of something else; only the segmental distribution of bristles would be changed. Formally, though, this *Hox* gene will have become part of the "*Hox* code" for several new segments. Subsequently, if that enhancer becomes responsive to hormonal or other local signals appearing earlier in development, additional aspects of the segment phenotype may come under its control.

I envisage that some such process occurred during the evolution of the *Abd-B* gene in insects. In all insects *Abd-B* appears to play a role in the specification of the most posterior abdominal segments. In a beetle and a locust its role appears to be limited to segments posterior to A7, at least in the embryonic epidermis (Beeman *et al.*, 1993; Kelsh *et al.*, 1994). In flies it is expressed progressively more anteriorly in later stages of embryogenesis (DeLorenzi and Bienz, 1990), and is needed for the modification of pigmentation and other features of the final adult pattern (Sánchez-Herrero *et al.*, 1985).

An analogous process is suggested by the observations of Averof and Patel (1997), who surveyed the expression of Ubx/abd-A class Hox proteins in a range of crustaceans. They infer that the common ancestor of the Crustacea expressed these Hox proteins from the first thoracic segment backwards, but that in several descendant groups, the genes encoding these proteins have been shut off in the most anterior thoracic segments. This change correlates with the development of these segments as maxillipeds, which have a morphology quite distinct from that of the more posterior thoracic segments. Maxillipeds have arisen repeatedly in Crustacea that already had distinct gnathal and thoracic segments. This change, and the accompanying shift in *Hox* gene expression, could have been the result of a single "homeotic" mutation, but the available data already suggest an alternative model. Averof and Patel note that the patterns of expression change with time in some species. Levels of protein in the anterior thoracic segments are progressively down regulated during development, compared with their more posterior neighbors. Such temporal changes can be expected to have incremental effects on morphology, and may provide conditions where selection could lead to the complete loss of expression from one or more segments. The end result is a difference between species that mimics the effects of an overt homeotic mutation, but the mechanism that generated this change need not have involved mutations of large effect.

Macroevolutionary implications

The last common ancestor of all arthropods probably had a set of *Hox* genes not dissimilar from those present in a fly (Averof and Akam, 1993; Grenier *et al*., 1997), and of comparable complexity to those in a basal chordate (García-Fernandez and Holland, 1994). The *Hox* proteins themselves have probably changed rather little in the last 500 million years, for insect and vertebrate proteins are to some extent interchangeable (Manak and Scott, 1994) -though the assays that test this have been rather crude.

We must look to the complexity of *Hox* gene regulation for the origins of the "high tech" arthropod body plans that characterize insects and many Crustacea; especially to the proliferation of independent enhancer modules that allow a single gene to show different patterns of expression in segments at different positions along the body axis. In *Drosophila*, enhancer modules can discriminate the position of each segment, allowing unique segment morphologies. This seems unlikely to be the case for arthropods with large numbers of very similar segments. Here we may expect to see enhancer modules showing graded patterns of activity over blocks of many segments.

At present we have no idea how enhancer modules arise and diversify. Is it by the duplication and divergence of existing modules, in a process akin to structural gene duplication; by the insertion of whole new fragments into the proximity of the existing genes; or *de novo*, by the stochastic appearance of sequence elements that have some enhancer activity? In flies and vertebrates it seems that different routes have been taken - in the flies, by multiplying the complexity of regulatory elements *in cis* to single copies of each *Hox* gene; in vertebrates by allowing the diversification of duplicate copies of the whole cluster. In both cases, the end result is to allow a more varied and subtle response to positional information, both between and within segments.

Acknowledgments

Many colleagues have helped to shape the ideas expressed here. I acknowledge particularly the influence of my former colleagues Michalis Averof and James Castelli-Gair, and of David Stern, who allows me to cite unpublished work, and who commented in detail on the manuscript. Work from this laboratory has been supported by the Wellcome Trust.

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