Evolution of transcription factor function

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Functional assays in *Drosophila melanogaster* with orthologous transcription factors from other species suggest that changes in the protein-coding sequence may play a larger role in the evolution of transcription factor pathways than was previously believed. Interestingly, recent studies provide evidence that changes in transcription factor protein sequence can affect the regulation of only a subset of target genes, even in the same cells of a developing animal.

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Abbreviations
Bcd bicoid
CNS central nervous system
Ftz fushi tarazu
Zen zerknüllt

Introduction

Transcription factors regulate the spatio-temporal expression of thousands of genes, ensuring the proper development and functioning of the organism. Until recently, studies on the evolution of transcription factor pathways have focused largely on the apparent functional changes in *cis*-regulatory elements [1,2]. This focus is sensible if one accepts the common belief that the functional evolution of transcription factors would result in alterations in the expression of many genes and would therefore be likely to be disastrous for the organism. But with the knowledge that transcription factors have modular structures, sequence comparisons alone have permitted informed speculation on how the functions of transcription factors could be altered during evolution [3,4].

In the recent past, a few experimental studies, almost all on homeodomain proteins, have tested how sequence changes affect transcription factor functions in different animal lineages. The data suggest that changes in a transcription factor's coding sequence can alter the expression of a subset of downstream target genes without wholesale disruption of the entire downstream gene hierarchy. These changes can result in transcription factors acquiring new functions while retaining their overall role [5•,6•], or acquiring an entirely new role; in some cases this is correlated with the gain or loss of known cofactor interaction motifs [7•,8,9•,10•]. Here we focus on the evolution of new functions by orthologous transcription factors in different lineages.

Evolutionarily conserved roles of transcription factors — just how conserved are they?

Despite their variations in shape and complexity, most bilateral animals possess a core set of transcription factors that were inherited from a common ancestor > 500 million years ago and whose functions in controlling embryonic development have largely been conserved. For example, in both vertebrates and many invertebrates Hox transcription factors specify where different morphological features will develop on the head–tail axis of embryos, MEF-2 transcription factors specify skeletal muscle, Csx/Nkx2-5/Tinman transcription factors specify visceral mesoderm/heart, and Pax-6 transcription factors specify eye and anterior nervous system development [11]. The level of fine functional variation within orthologous factors that conserve broadly similar roles has been carefully studied in only a few instances.

The most convincing experiments evaluating the extent of functional conservation versus functional variation of distantly related transcription factors would come from studies in which orthologous coding sequences from, for example, a fly precisely replace endogenous coding sequences in a distantly related animal, for example a mouse. This would nearly guarantee that the distantly related orthologue would be expressed in the same patterns and levels as the endogenous gene. This experiment has not yet been accomplished in the precise manner described above, but this precision has been closely approached. For example, the *Drosophila engrailed* gene was introduced by homologous recombination into the locus of one of its mouse orthologues, *En1* [12]. In this instance, part of the endogenous *En1* gene remained at the recombined locus, and some *En1* regulatory sequences were not at the same positions relative to the promoter for *Drosophila engrailed* as they were before relative to the endogenous *En1* promoter. Nevertheless, *Drosophila engrailed* was expressed in mouse embryos in a pattern that was very similar to that of its *En1* orthologue. In mice homozygous for *Drosophila engrailed*, *En1* mutant phenotypes in the midbrain and cerebellum were largely rescued and most mice survived to adulthood, in contrast

to the early post-natal lethality observed in *En1* mutants. However, in the distal limbs, where En1 function is required for normal dorsal–ventral polarity, the *Drosophila engrailed* allele did not rescue the *En1* mutant phenotype even though it was expressed in the normal pattern and at approximately normal levels. There have been other similar rescue experiments; for example, the chicken *Hoxb1* gene partially rescues the function of its *Drosophila* orthologue, *labial* [13], and mouse *Pax3* partially rescues the function of a *Drosophila paired* mutation [14].

In such gene-swapping experiments, evidence for near-perfect rescue exists in only one case. Greer *et al.* [15] tested the mouse *Hoxa3* and *Hoxd3* paralogues, which began diverging after a duplication event 400–500 million years ago, for their ability to rescue each other's function. The coding regions of these two Hox paralogues were precisely substituted for each other using homologous recombination. Even in their normal chromosomal locations the two genes are expressed in nearly identical patterns, so the differences in the expression patterns of the swapped genes apparently amount to different transcript levels. The mice in which Hoxa3 protein was expressed in the amounts characteristic of Hoxd3 protein and vice versa had no apparent mutant phenotypes. However, in this case the two distantly related paralogues are likely to have been expressed in nearly identical patterns in the same animal for the past few hundred million years and are required in tandem for the proper development of many structures. This may have imposed stronger selection pressure on Hoxa3 to coevolve with Hoxd3 than is the case with paralogues expressed in different patterns, or with orthologues in different phylogenetic lineages. In sum, the current gene-replacement evidence for distant orthologues shows a great deal of functional conservation in some tissues, but in no cases are the functions identical, and in some cases an orthologous gene provides no detectable rescue.

Depending on the phenotype(s) being scored, there is another complication in the interpretation of orthologue replacement experiments or experiments where a phylogenetically distant orthologue mimics a gain-of-function phenotype produced by an endogenous gene. If only one or two endogenous downstream genes need to be regulated to achieve a specific phenotype, inducing that phenotype with an orthologous factor does not provide strong support for extensive functional conservation. In at least two cases, there is evidence for such a scenario. It was shown that brief ectopic expression of mouse Hoxb6 protein in a developing fly will partially transform the antennae into legs, which is quite similar to the phenotype seen after brief ectopic expression of the fly orthologue Antennapedia [16]. However, loss of function in the antenna primordia for any of three genes, *Distal-less*, *homothorax*, or *spineless-aristapedia*, results in a similar phenotype, and it has been shown that the *homothorax* gene is indeed transcriptionally repressed in the antenna primordia by ectopic Antennapedia [17], as is *Distal-less* (C Gross, W McGinnis, unpublished data). Therefore it seems likely that the ability of the Hoxb6 orthologue to mimic ectopic Antennapedia function resides in its ability to repress transcription of one or more of these three genes. *Distal-less*, *homothorax*, and *spineless-aristapedia* all encode transcription factors, and their loss might result in a stable alteration of downstream gene cascades. In this context, it is relevant that recent studies have shown, at least in some cell types, that a Hox gene need regulate only one target gene to accomplish its normal morphogenetic function in those cells during *Drosophila* development [18•,19•].

There is at least one more case where the conservation of orthologous transcription factor function may have been overestimated. When expressed in the imaginal disc primordia of adult *Drosophila* structures, mouse Pax6 protein can induce the development of ectopic *Drosophila* eyes in a similar way to its *Drosophila* orthologue Eyeless [20]. Later studies have shown that *Drosophila* Eyeless activates the expression of the other 'eye' transcription factor genes *sine oculis*, *eyes absent*, and *dachshund*, which in certain combinations can induce ectopic eye development themselves [21,22]. So the ability of Pax6 to induce ectopic eyes may not indicate extensive conservation of the ability to regulate entire batteries of downstream genes, as in this assay it would need to activate only two downstream eye-promoting genes to mimic the function of its orthologue Eyeless. Therefore, the apparent similarity of distantly related transcription factor functions in some ectopic expression assays (as well as in some gene-swap assays) may not always indicate amazing functional conservation, as complex phenotypes such as the development of a leg or an eye may require very few endogenous downstream genes to be activated or repressed.

Modest divergence in transcription factor functions in different lineages

We know from the fossil record that proto-hexapods with similar morphologies to modern silverfish appeared ~400 million years ago. Molecular evidence indicates that these early insect-like creatures branched from a crustacean lineage [23]. Two recent studies suggest that mutations in the Hox transcription factor sequence contribute to the difference in limb number between multi-limbed crustaceans and hexapod insects. In *Drosophila*, the Hox proteins Ultrabithorax and Abdominal-A are required to repress limb development in the abdomen, whereas in the crustacean *Artemia* the orthologues of these proteins apparently do not repress limbs [24]. One study found that the loss of serines and threonines from the C-terminal region of Ultrabithorax proteins during the transition from an *Artemia*-like ancestor to insects could explain how Ultrabithorax evolved a limb-repression function, and suggested that the loss of serine/

threonine phosphorylation sites — which are not found in any insect Ultrabithorax orthologues, but which are present in many multi-limbed arthropod Ultrabithorax orthologues — contributed to the macroevolutionary change in limb number between these two arthropod lineages [5•]. Interestingly, this study suggested that a modulatory domain for an existing repression function rather than the repression function itself was evolving. The *Artemia* Ultrabithorax protein — although unable to repress the limb promoting gene *Distal-less* in *Drosophila* embryos — still retained a transcription-repressive function on another target gene, *Antennapedia*, which is also normally expressed in the epidermal limb primordia.

A complementary study involved swaps of protein domains between an onychophoran (a proto-arthropod) version of Ultrabithorax and its *Drosophila* orthologue followed by tests of limb repression in *Drosophila* embryos [6•]. The authors found evidence that a C-terminal region from *Drosophila* Ultrabithorax, which contained a glutamine/alanine-rich motif present in all insect Ultrabithorax proteins, provides a transcriptional repression function that is missing from its onychophoran ancestor. Some non-insect arthropods conserve a portion of the glutamine/alanine rich motif, but in none of these is it as extensive as the motif found in insect Ultrabithorax orthologues. This study suggested that a new repression domain evolved in the insects that was not present in ancestral versions of Ultrabithorax. Therefore it seems that the evolution of amino acids involved in transcriptional-repression functions — by mutations that abolished a repression-modulatory domain and in the same region apparently generated an additional repression domain — may explain one step in the evolution of the hexapod body plan from arthropod ancestors with limbs on more body segments. (See also Update.)

Another recently reported case of apparent transcription factor evolution was in the forkhead class protein FOXP2. Mutations in humans that reduce the dose of FOXP2 are correlated with defects in verbal articulation. Enard *et al.* [25] analyzed the sequence of FOXP2 orthologues from gorillas, chimpanzees, orangutans, rhesus monkeys, and mice, and found that at some point after the divergence of the gorilla and human lineages a two-amino-acid sequence variation in FOXP2 was fixed and maintained only in the human lineage. The authors speculate that this event in the evolutionary branch leading to humans may have contributed to their acquisition of speech and language compared to other primates [25]. This speculation seems highly unlikely given the minimal evidence, but it is not completely impossible.

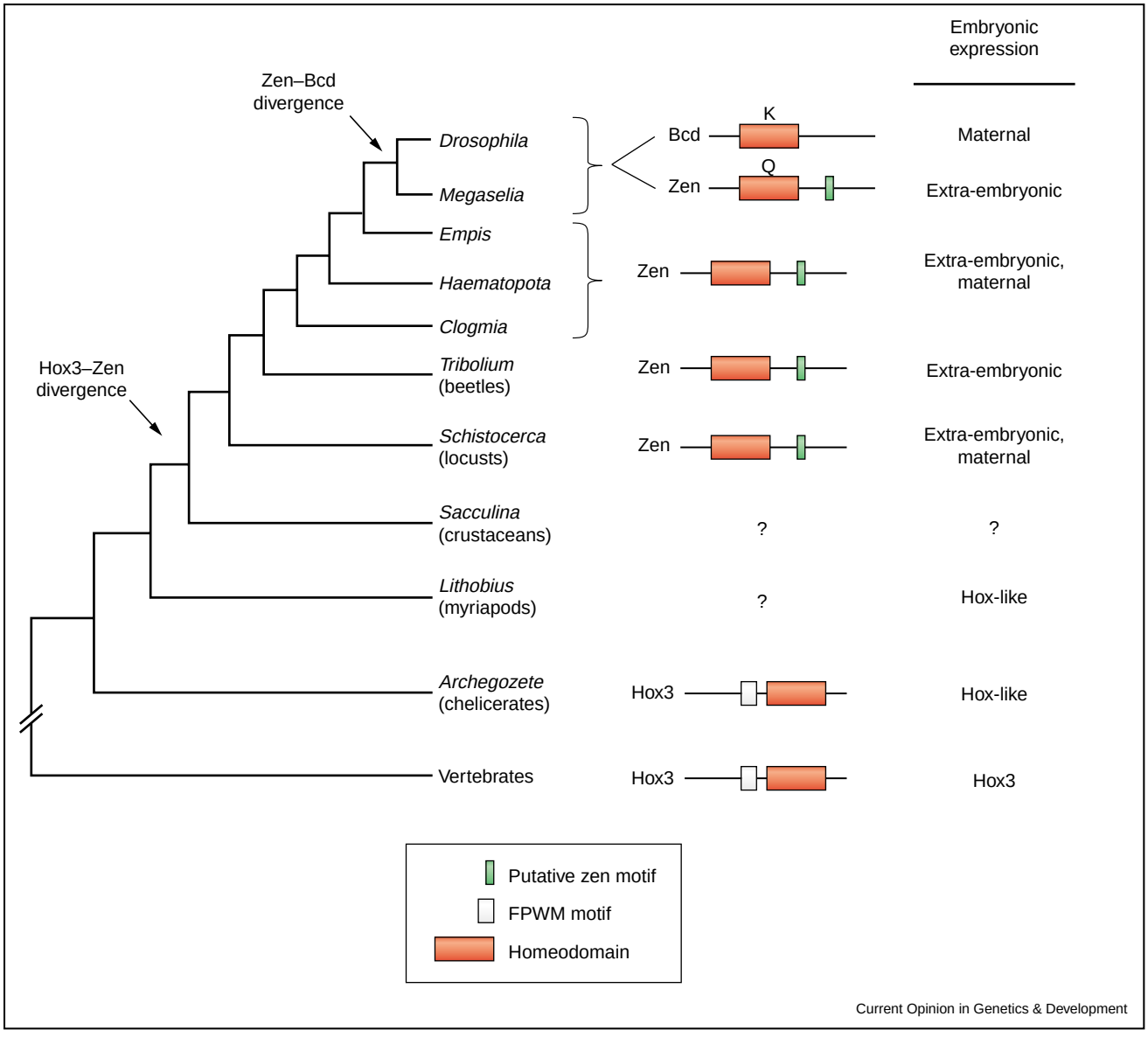
Complete divergence of transcription factor function

Studies of Zerknüllt (Zen) and Bicoid (Bcd) orthologues in different insect lineages indicate that they are homeodomain proteins that have undergone rapid evolution. In fact, the evolution of the *bcd* gene has been so rapid in cyclorraphan Dipterans that its derivation from Hox genes was unrecognized until recently. In *Drosophila*, *bcd* is maternally expressed and functions as an anterior determinant, whereas *zen* is expressed in extra-embryonic tissues and functions in dorsal–ventral patterning. Although the *bcd* and *zen* genes of *Drosophila* have diverged greatly in function, analysis of their counterparts in non-cyclorraphan Dipterans indicates that they are paralogues [8] and that the complete divergence of *bcd* from *zen* in expression and function occurred within the Dipteran lineage [7•,26•,27]. The *zen* gene, in turn, has been found to be a derivative of a Hox3 orthologue. Phylogenetic analysis of the homeodomain sequences of Zen from *Tribolium* and *Schistocerca* groups them with Hox3 genes, but they also share motifs outside the homeodomain with *Drosophila* Zen [28]. Evidence for *zen*'s ancestral homeotic function comes from expression studies of the *Hox3/zen* orthologue in three chelicerates and a myriapod [29–31,32•]. These studies show a Hox-like expression pattern for *Hox3/zen* along the anterior-posterior axis, suggesting that it has a role in segment identity. Like the non-Dipteran insect orthologues, chelicerate *Hox3/zen* genes group more closely, in phylogenetic analyses, to chordate Hox3 even though chelicerates are more closely related to *Drosophila* than to chordates [29–31]. Whether the *Hox3/zen* orthologues from these other arthropods also have extra-embryonic expression is not known. Functional assays to test for potential homeotic functions of the basal arthropod *Hox3/zen* orthologues are still needed, but it seems likely that *Hox3/zen* had a Hox-like function in the early arthropods that is still retained in some lineages but that is lost in the insect lineage (Figure 1). Taken together, these findings provide indirect evidence that *cis*-regulatory changes were involved in *Hox3/zen* functional evolution.

However, there is also evidence suggesting that protein sequence changes played a role in the transition of *Hox3* to *zen*, and from *zen* to *bcd*. The homeodomain of the Hox3 orthologue from the spider *Cupiennius salei* is located towards the C terminus of the protein, a position more similar to that observed in the chordate Hox3 (and other Hox) proteins than to Zen proteins, where the DNA-binding domain is located towards the N-terminus [30]. Additionally, the basal insect Hox3/Zen protein acquired motifs outside the homeodomain that are similar to motifs in the *Drosophila* Zen protein and lost the YPWM motif that is conserved only in Hox proteins and their close relatives [28]. So it appears that there has been a dramatic change in expression pattern that is correlated with an apparent loss of Hox-like protein function, as evidenced by the loss and gain of protein motifs (Figure 1).

The divergence between Zen and Bcd protein coding sequences was also apparently accompanied by a divergence of *cis*-regulatory sequences during the period when

Figure 1



Evolution of Hox3/Zen/Bcd protein motifs and function. The embryonic expression patterns and protein diagrams denoting sequence motifs in Hox3/Zen orthologues are depicted on a phylogenetic tree. At some point before or during the early insect divergence, Hox3 lost its Hox-like expression and acquired extra-embryonic and maternal expression. During this period, the FPWM motif was lost from the Hox3 precursor, the homeodomain acquired a more N-terminal position and Zen-like motifs were acquired. The arrow marking the Hox3–Zen divergence indicates the most recent possible divergence of Zen from Hox3 on the basis of known data, but this could have occurred before this time and after divergence of the clade which include myriapods, crustaceans, and insects. Then, in the insect lineage, maternal expression of *zen* was lost and the *bcd* gene was acquired through duplication and divergence of *zen* in the Drosophilids. During the evolution of the Bcd protein, amino acid number 50 of the homeodomain mutated to a lysine (K). The residue is glutamine (Q) in all of the Hox3 and Zen proteins. On the tree, the phylogenetic relationship of the animals is simplified and the length of the lines is not indicative of relative divergence times.

cyclorraphan Dipterans evolved, although in this and the other cases described in this review it is unknown whether the novel expression pattern or the protein sequence changes evolved first. Rapid changes in the Bcd protein sequence, including a change within the homeodomain that led to the acquisition of RNA-binding ability [33], resulted in a Bcd that was highly divergent from Zen, and in Bcd eventually acquiring an anterior embryonic polarity function. Interestingly, Bcd is a clear example of a protein acquiring a DNA-binding specificity that differs strikingly from its Hox ancestors as a result of a substitution of lysine for glutamine at residue 50 of the homeodomain. This change does not result in a novel homeodomain binding specificity, as it converts the

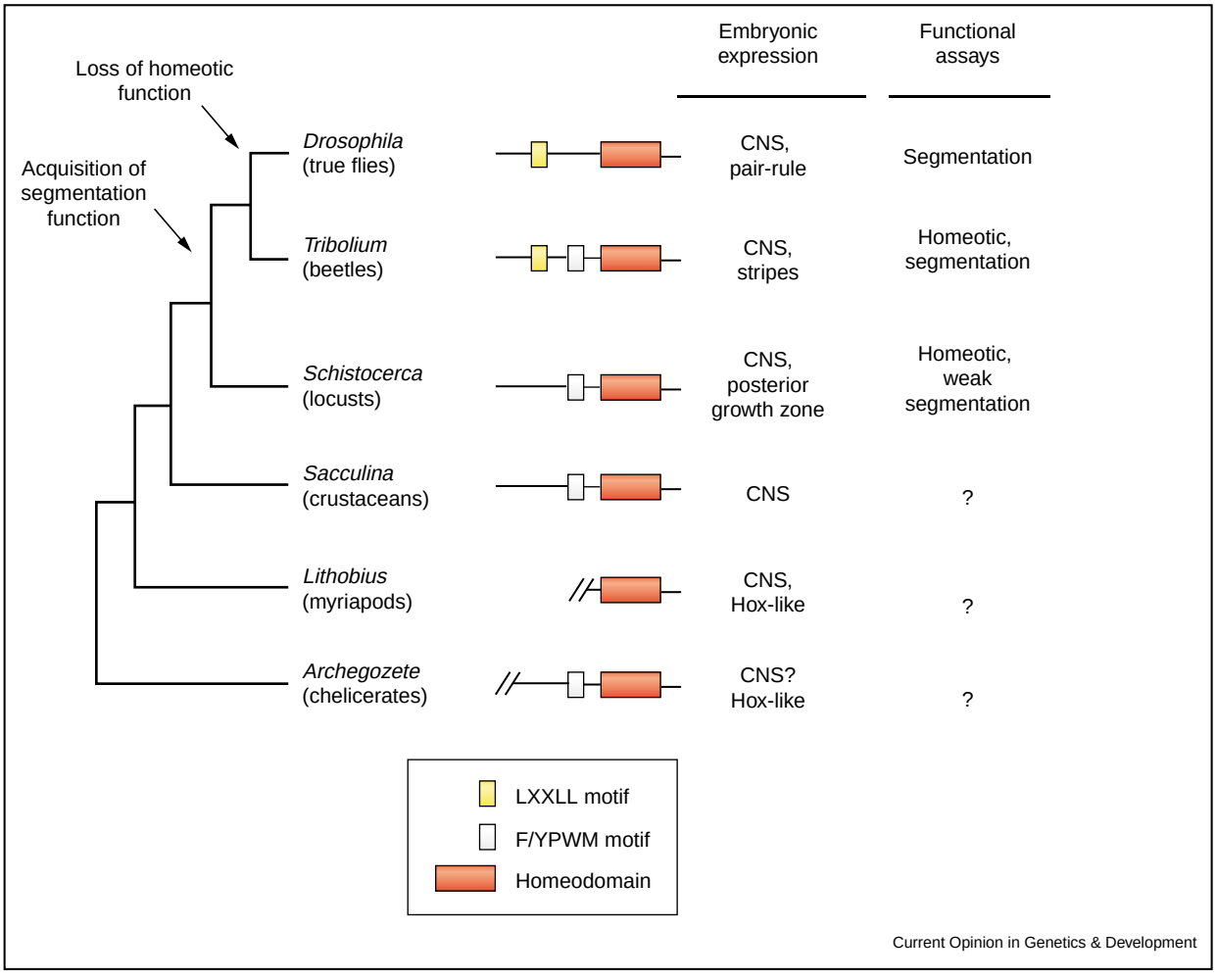
binding specificity of Bcd to that of Orthodenticle/Otx family members, which are ancient pre-existing anterior transcription factors in the homeodomain class [34]. This conversion of DNA-binding specificity would have altered the expression of all Bcd targets. It is possible that this dramatic change may not have been strongly selected against if Bcd was only transiently present in early embryos, as it is in present-day *Drosophila* embryos, and if it could bind new targets but initially had little regulatory effect on them. Then, as other domains in Bcd evolved, the protein acquired the ability to regulate downstream enhancers that could bind lysine-50 homeodomains.

Evolution of transcription factor function linked to cofactor interactions

At present, the best evidence for a direct relationship between protein sequence changes and changes in cofactor interactions can be found in the evolution of the pair-rule gene, *fushi tarazu* (*ftz*). Like *zen* and *bcd*, *ftz* maps in the Hox cluster in insects, encodes a homeodomain protein, and is thought to have duplicated from a Hox gene and undergone a complete divergence in function [35].

Functional assays of Ftz protein orthologues in *Drosophila* [9•,10•] and expression studies of *ftz* genes in different arthropods [32•,35,36] indicate that *ftz* genes in different lineages have one, two or three different functions. CNS or CNS-like expression is found for *ftz* orthologues in a myriapod [32•], a crustacean [36], and several insects [37,38], suggesting that one ancient function of *ftz* might be in CNS development. Functional evidence for this conservation lies in the ability of *Schistocerca* Ftz to rescue *Drosophila ftz* CNS function via activation of *even-skipped* in neuronal precursors [10•]. It is not yet known though

Figure 2



Evolution of Ftz protein function in arthropods. Expression studies of *ftz* in various arthropods suggest that Hox-like and CNS functions are ancestral functions of Ftz protein that are still retained in extant arthropods. Functional assays in *Drosophila* embryos show a correlation between the acquisition of segmentation function and the loss of homeotic function with the acquisition of an LXXLL motif and the loss of a YPWM motif, respectively, in the insect lineage.

which sequences are required for CNS function and whether these are conserved in the arthropods.

Löhr *et al.* [9•] tested the homeotic and segmentation abilities of Ftz proteins from *Drosophila*, *Tribolium* and *Schistocerca*. Their findings suggest that *Tribolium* Ftz and *Schistocerca* Ftz possess some Hox-like functions whereas *Drosophila* Ftz does not. Among the insects, with the exception of Abdominal-B orthologues, homeotic function correlates with the presence of a YPWM motif. The YPWM motif is part of an interaction domain for the Hox cofactor Extradenticle/Pbx and is conserved in Hox proteins [39]. Ftz protein sequences from crustaceans [36] and chelicerates [35] reveal the presence of a slightly altered YPWM motif, FPWM (Figure 2), whereas sequence data from centipede Ftz, which exhibits Hox-like expression, is inconclusive with regard to the presence of a YPWM domain. Whether these Ftz proteins from non-insect arthropods also have Hox-like functions is unknown, although it seems very likely.

Assays for segmentation function in *Drosophila* embryos indicate that *Tribolium* Ftz possesses similar functions to *Drosophila* Ftz, but that *Schistocerca* Ftz shows little or no segmentation function. Again, Löhr *et al.* [9•] found that segmentation ability is correlated with a specific protein sequence, an LXXLL motif. The LXXLL motif has been implicated as a domain that enables cofactors to facilitate interactions with nuclear hormone receptors [40], and Ftz protein has been shown to interact with the nuclear hormone receptor Ftz–F1 through an LXXLL motif in order to carry out its segmentation function in flies [41,42,43•,44•]. Löhr *et al.* [9•] further propose that competition of cofactors resulted in an exchange of homeotic function for segmentation function via the loss of the YPWM motif, allowing *Drosophila* Ftz to act solely in segmentation during early embryogenesis.

The evolution of the Ftz protein in arthropods appears to be an example of a transcription factor that has altered the set of target genes it regulates from genes involved in segmental identity to genes involved in segmentation. Although not as well studied, this transition has also occurred in the evolution of *even-skipped/Evx* orthologues. In many vertebrates, *Evx* genes map adjacent to the most posterior genes in Hox complexes, are expressed in the posterior termini of developing embryos, and are essentially Hox-cluster genes required for posterior identities. Orthologues of *even-skipped* are also expressed at the posterior termini of some arthropod embryos [45•]. Thus, it seems likely that, like *ftz*, the *even-skipped*-like Hox genes diverged and acquired a pair-rule segmentation function at some point during insect evolution, although they retain an ancestral Hox function in many other taxa [46]. To what extent the batteries of downstream segmentation and segment-identity genes overlap is not yet known, but recent results suggest that some Hox proteins within the canonical complexes have roles in maintaining segment boundaries [18•,47], in addition to their well-known segment-identity roles.

Conclusions

Cis-regulatory sequence mutations have been thought to be pre-eminent in the evolution of transcription factor pathways. This is in part because *cis*-regulatory evolution was the only variation that was widely assayed until recently and in part because the conservation of transcription factor functions, although real, has been overemphasized. The advent of detailed assays for the role of homeodomain protein sequence variations, accompanied by comparison of orthologue expression patterns in different taxa, has shown that both protein expression pattern changes and protein sequence mutations in these proteins have contributed to their functional evolution in developmental pathways. It will be fascinating to determine whether changes in expression pattern have typically occurred before protein functional divergence or vice versa.

Update

Evolutionary variation in Hox repression evolution may have also occurred within the crustacean lineage. Studying the development of the crustacean *Daphnia*, Shiga et al. [48•] found that the diversification of anterior appendage morphology and Distal-less expression patterns was associated with evolutionary variation in the expression pattern of the *Daphnia* Antennapedia Hox protein. When the *Daphnia* Antennapedia protein was tested in *Drosophila* embryos, it possessed a much stronger limb-suppressing activity than its *Drosophila* cognate.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Tautz D: **Evolution of transcriptional regulation**. *Curr Opin Genet Dev* 2000, **10**:575-579.

2. Carroll SB: **Endless forms: the evolution of gene regulation and morphological diversity**. *Cell* 2000, **101**:577-580.

3. Frigerio G, Burri M, Bopp D, Baumgartner S, Noll M: **Structure of the segmentation gene *paired* and the Drosophila PRD gene set as part of a gene network**. *Cell* 1986, **47**:735-746.

4. Morgenstern B, Atchley WR: **Evolution of bHLH transcription factors: modular evolution by domain shuffling?** *Mol Biol Evol* 1999, **16**:1654-1663.

5. • Ronshaugen M, McGinnis N, McGinnis W: **Hox protein mutation and macroevolution of the insect body plan**. *Nature* 2002, **415**:914-917.

We carried out domain swaps followed by mutagenesis to locate the residues that prevent a crustacean Ultrabithorax protein from repressing limbs and allow *Drosophila* Ultrabithorax to repress limbs in *Drosophila* embryos. Coupled with comparative analysis of Ultrabiothorax protein sequence from a variety of insects and other arthropods, the results indicated that serine and threonine residues in consensus phosphorylation sites were mutated during the evolution of insects from crustaceans. This eventually resulted in a version of Ultrabithorax protein that repressed limbs in the abdomen of proto-insects, contributing to the formation of the hexapod body plan.

6. Galant R, Carroll SB: **Evolution of a transcriptional repression**
• **domain in an insect Hox protein**. *Nature* 2002, **415**:910-913.
The authors mapped the domain differences that distinguish the functions of an onychophoran version of Ultrabithorax protein, which does not repress limbs in *Drosophila* embryos, from *Drosophila* Ultrabithorax, which does repress limbs. A C-terminal fragment of *Drosophila* Ultrabithorax, which contains a Gln/Ala-rich motif, was found to have a repressive function in cell culture assays, and was also found to confer a limb-repressive function when appended to the C-terminus of onychophoran Ultrabithorax. Although the deletion of this domain from *Drosophila* Ultrabithorax appears to have a minor influence on its limb-repression abilities, the study suggests that a repression domain evolved in insect Ultrabithorax that contributes to the suppression of limbs in hexapods.

7. Brown S, Fellers J, Shippy T, Denell R, Stauber M, Schmidt-Ott U:
• **A strategy for mapping *bicoid* on the phylogenetic tree**. *Curr Biol* 2001, **11**:R43-R44.
A search was conducted for a *bcd* gene in the *Tribolium* Hox cluster at the conserved position between *zen* and *Dfd/Hox4*. The authors propose that *bcd* was acquired after the radiation of holometabolous insects and is characteristic of more derived insects. They also propose that *bcd* hijacked the function of an existing anterior determinant.

8. Stauber M, Jackle H, Schmidt-Ott U: **The anterior determinant bicoid of *Drosophila* is a derived Hox class 3 gene**. *Proc Natl Acad Sci USA* 1999, **96**:3786-3789.

9. Löhr U, Yussa M, Pick L: ***Drosophila fushi tarazu*: a gene on the**
• **border of homeotic function**. *Curr Biol* 2001, **11**:1403-1412.
This paper provides an extensive analysis of the function of *Drosophila*, *Tribolium* and *Schistocerca ftz* utilizing ectopic expression assays in *Drosophila*. Along with [5•] and [6•], it also provides experimental evidence for a link between protein sequence changes and changes in transcription factor function.

10. Alonso CR, Maxton-Kuechenmeister J, Akam M: **Evolution of Ftz**
• **protein function in insects**. *Curr Biol* 2001, **11**:1473-1478.
The authors performed functional tests of Ftz orthologues of *Drosophila* and *Schistocerca* using overexpression assays in *Drosophila* embryos, assaying the activation or repression of target genes involved in CNS and segmentation functions. They concluded that *Schistocerca* Ftz closely mimicked its *Drosophila* orthologue in the regulation of CNS target genes but not in the regulation of segmentation target genes.

11. Veraksa A, Del Campo M, McGinnis W: **Developmental patterning genes and their conserved functions: from model organisms to humans**. *Mol Genet Metab* 2000, **69**:85-100.

12. Hanks MC, Loomis CA, Harris E, Tong C-X, Anson-Cartwright L, Auerbach A, Joyner A: ***Drosophila engrailed* can substitute for mouse *Engrailed 1* function in mid-hindbrain, but not limb development**. *Development* 1998, **125**:4521-4530.

13. Lutz B, Lu H-C, Eichele G, Miller D, Kaufman TC: **Rescue of *Drosophila labial* null mutant by the chicken ortholog *Hoxb-1* demonstrates that the function of Hox genes is phylogenetically conserved**. *Genes Dev* 1996, **10**:176-184.

14. Xue L, Noll M: **The functional conservation of proteins in evolutionary alleles and the dominant role of enhancers in evolution**. *EMBO J* 1996, **15**:3722-3731.

15. Greer JM, Puetz J, Thomas KR, Capecchi MR: **Maintenance of functional equivalence during paralogous Hox gene evolution**. *Nature* 2000, **403**:661-665.

16. Malicki J, Schughart K, McGinnis W: **Mouse Hox 2.2 specifies thoracic segmental identity in *Drosophila* embryos and larvae**. *Cell* 1990, **63**:961-967.

17. Yao L-C, Liaw G-J, Pai C-Y, Sun YH: **A common mechanism for antenna-to-leg transformation in *Drosophila*: suppression of *homothorax* transcription by four HOM-C genes**. *Dev Biol* 1999, **211**:268-276.

18. Lohmann I, McGinnis N, Bodmer M, McGinnis W: **The *Drosophila***
• **Hox gene Deformed sculpts head morphology via direct regulation of the apoptosis activator reaper**. *Cell* 2002, **110**:457-466.
The Hox protein Deformed directly activates the apoptosis-promoting gene *reaper* to maintain a segment boundary in the head of *Drosophila* embryos. Surprisingly, expression of *reaper* alone at the boundary in a *Deformed* mutant embryo is capable of rescuing the segmentation defect.

19. Brodu V, Elstob PR, Gould AP: ***abdominal A* specifies one cell**
• **type in *Drosophila* by regulating one principal target gene**. *Development* 2002, **129**:2957-2963.
In a *Drosophila* chordotonal organ precursor cell, the Hox protein Abdominal-A activates the expression of the *rhomboid* gene, which promotes the secretion of an active form of Spitz, a ligand of the epidermal growth factor receptor. The Spitz signal is required for the differentiation of adjacent cells called oenocytes. Surprisingly, expression of *rhomboid* alone in chordotonal precursor cells of *abdominal-A* mutant embryos is capable of rescuing the oenocyte defect.

20. Halder G, Callaerts P, Gehring WJ: **Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila***. *Science* 1995, **267**:1788-1792.

21. Chen R, Amoui M, Zhang Z, Mardon G: **Dachshund and eyes absent proteins form a complex and function synergistically to induce ectopic eye development in *Drosophila***. *Cell* 1997, **91**:893-903.

22. Pignoni F, Hu B, Zavitz KH, Xiao J, Garrity PA, Zipursky SL: **The eye-specification proteins So and Eya form a complex and regulate multiple steps in *Drosophila* eye development**. *Cell* 1997, **91**:881-891.

23. Friedrich M, Tautz D: **Ribosomal DNA phylogeny of the major extant arthropod classes and the evolution of myriapods**. *Nature* 1995, **376**:165-167.

24. Averof M, Akam M: **Hox genes and the diversification of insect and crustacean body plans**. *Nature* 1995, **376**:420-423.

25. Enard W, Przeworski M, Fisher SE, Lai CSL, Wiebe V, Kitano T, Monaco AP, Paabo S: **Molecular evolution of FOXP2, a gene involved in speech and language**. *Nature* 2002, **418**:869-872.

26. Stauber M, Prell A, Schmidt-Ott U: **A single *Hox3* gene with**
• **composite *bicoid* and *zerknüllt* expression characteristics in non-Cyclorrhaphan flies**. *Proc Natl Acad Sci USA* 2002, **99**:274-279.
This paper covers the characterization of the maternal and zygotic expression patterns of *Hox3/zen* orthologues from three basal flies. The authors propose a model whereby a *Hox3/zen/bcd* ancestor in insects underwent duplication and one gene evolved a zygotic function in extra-embryonic tissue (*zen*) whereas the other evolved a maternal function as an anterior determinant (*bcd*).

27. Brown SJ, Fellers JP, Shippy TD, Richardson EA, Maxwell M, Stuart JJ, Denell RE: **Sequence of the *Tribolium castaneum* homeotic complex: the region corresponding to the *Drosophila melanogaster* antennapedia complex**. *Genetics* 2002, **160**:1067-1074.

28. Falciani F, Hausdorf B, Schroder R, Akam M, Tautz D, Denell R, Brown S: **Class 3 Hox genes in insects and the origin of *zen***. *Proc Natl Acad Sci USA* 1996, **93**:8479-8484.

29. Telford MJ, Thomas RH: **Of mites and zen: expression studies in a chelicerate arthropod confirm *zen* is a divergent Hox gene**. *Dev Genes Evol* 1998, **208**:591-594.

30. Damen WG, Tautz D: **A Hox class 3 orthologue from the spider *Cupiennius salei* is expressed in a Hox-gene-like fashion**. *Dev Genes Evol* 1998, **208**:586-590.

31. Abzhanov A, Popadic A, Kaufman TC: **Chelicerate *Hox* genes and the homology of arthropod segments**. *Evol Dev* 1999, **1**:77-89.

32. Hughes CL, Kaufman TC: **Exploring the myriapod body plan:**
• **expression patterns of the ten Hox genes in a centipede**. *Development* 2002, **129**:1225-1238.
Characterization of the expression patterns of all the genes from the Hox cluster of the centipede *Lithobius atkinsoni*. The paper also includes a fairly comprehensive comparison of all arthropod Hox gene expression patterns and discusses the implications for *ftz* and *Hox3/zen* evolution.

33. Niessing D, Driever W, Sprenger F, Taubert H, Jaeckle H, Rivera-Pomar R: **Homeodomain position 54 specifies transcriptional versus translational control by Bicoid**. *Mol Cell* 2000, **5**:395-401.

34. Laughon A: **DNA-binding specificity of homeodomains**. *Biochemistry* 1991, **30**:11357-11367.

35. Telford MJ: **Evidence for the derivation of the *Drosophila fushi tarazu* gene from a Hox gene orthologous to lophotrochozoan Lox5**. *Curr Biol* 2000, **10**:349-352.

36. Mouchel-Vielh E, Blin M, Rigolot C, Deutsch JS: **Expression of a homologue of the *fushi tarazu (ftz)* gene in a cirripede crustacean**. *Evol Dev* 2002, **4**:76-85.

37. Brown SJ, Hilgenfeld RB, Denell RE: **The beetle *Tribolium castaneum* has a fushi tarazu homolog expressed in stripes during segmentation**. *Proc Natl Acad Sci USA* 1994, **91**:12922-12926.

38. Dawes R, Dawson I, Falciani F, Tear G, Akam M: **Dax, a locust Hox gene related to *fushi-tarazu* but showing no pair-rule expression**. *Development* 1994, **120**:1561-1572.

39. Mann RS, Chan SK: **Extra specificity from extradenticle: the partnership between HOX and PBX/EXD homeodomain proteins**. *Trends Genet* 1996, **12**:259-262.

40. Heery D, Kalkhoven E, Hoare S, Parker M: **A signature motif in transcriptional co-activators mediates binding to nuclear receptors**. *Nature* 1997, **387**:733-736.

41. Yu Y, Li W, Su K, Yussa M, Han W, Perrimon N, Pick L: **The nuclear hormone receptor Ftz–F1 is a cofactor for the *Drosophila* homeodomain protein Ftz**. *Nature* 1997, **385**:552-555.

42. Guichet A, Copeland JWR, Erdelyi M, Hlousek D, Zavorszky P, Ho J, Brown S, Percival-Smith A, Krause HM, Ephrussi A: **The nuclear receptor homologue Ftz–F1 and the homeodomain protein Ftz are mutually dependent cofactors**. *Nature* 1997, **385**:548-552.

43. • Schwartz CJE, Sampson HM, Hlousek D, Percival-Smith A, Copeland JWR, Simmonds AJ, Krause HM: **FTZ-factor1 and Fushi tarazu interact via conserved nuclear receptor and coactivator motifs**. *EMBO J* 2001, **20**:510-519.
The Ftz–F1 and Ftz proteins interact via an LXXLL motif and homeodomain residues in the Ftz protein. The LXXLL motif was originally defined as a sequence required for interactions between hormone receptors and their cofactors.

44. • Yussa M, Lohr U, Su K, Pick L: **The nuclear receptor Ftz–F1 and homeodomain protein Ftz interact through evolutionarily conserved protein domains**. *Mech Dev* 2001, **107**:39-53.
As in [43•], these authors defined the Ftz LXXLL motif and the Ftz homeodomain as the principal interaction surfaces with its cofactor Ftz–F1, an orphan hormone receptor. This paper also shows that a mouse homologue of Ftz–F1, called SF-1, can rescue the segmentation defects of *Drosophila Ftz–F1* mutants.

45. • Hughes CL, Kaufman TC: **Exploring myriapod segmentation: the expression patterns of *even-skipped*, *engrailed*, and *wingless* in a centipede**. *Dev Biol* 2002, **247**:47-61.
In centipede embryos, the *even-skipped* orthologue is expressed in patterns that suggest the gene has segment boundary functions as well as posterior segment identity functions.

46. Ferrier DEK, Minguillon C, Cebrian C, Garcia-Fernandez J: **Amphioxus *Evx* genes: implications for the evolution of the midbrain-hindbrain boundary and the chordate tailbud**. *Dev Biol* 2001, **237**:270-281.

47. Gavalas A, Davenne M, Lumsden A, Chambon P, Rijli FM: **Role of *Hoxa-2* in axon pathfinding and rostral hindbrain patterning**. *Development* 1997, **124**:3693-3702.

48. • Shiga Y, Yasumoto R, Yamagata H, Hayashi S: **Evolving role of Antennapedia protein in arthropod limb patterning**. *Development* 2002, **129**:3555-3561.
Shiga *et al.* found that the altered morphology of the first thoracic limb appendage in the crustacean *Daphnia* correlates with altered patterns of *Distal-less* and *Antennapedia* in the limb primordium. In *Drosophila* embryos, *Daphnia* Antennapedia protein, unlike its *Drosophila* homologue, has a repressive effect on *Distal-less* transcription, and the protein region conferring this activity was mapped to the N-terminal region of the protein. Their results suggest that *Daphnia* Antennapedia diversifies anterior leg morphology by repressing Distal-less, and that this repressive function evolved after the crustaceans and insects split.