



**HAL**  
open science

# Hox dosage and morphological diversification during development and evolution

Samir Merabet, Julie Carnesecchi

► **To cite this version:**

Samir Merabet, Julie Carnesecchi. Hox dosage and morphological diversification during development and evolution. *Seminars in Cell and Developmental Biology*, 2024, 152-153, pp.70-75. 10.1016/j.semcdb.2022.11.009 . hal-04242153

**HAL Id: hal-04242153**

**<https://hal.science/hal-04242153>**

Submitted on 18 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Hox dosage and morphological diversification during development and evolution

Samir Merabet<sup>1,#</sup> and Julie Carnesecchi<sup>1</sup>

<sup>1</sup> IGFL, UMR5242, CNRS, ENS, University Lyon I, 32/34 Av. Tony Garnier, 69007 Lyon, France

# correspondance: samir.merabet@ens-lyon.fr

### Abstract

Hox genes encode for evolutionary conserved transcription factors that have long fascinated biologists since the observation of the first homeotic transformations in flies. Hox genes are developmental architects that instruct the formation of various and precise morphologies along the body axes in cnidarian and bilaterian species. In contrast to these highly specific developmental functions, Hox genes encode for proteins that display poorly selective DNA-binding properties *in vitro*. This “Hox paradox” has been partially solved with the discovery of the TALE-class cofactors, which interact with all Hox members and form versatile Hox/TALE protein complexes on DNA. Here, we describe the role of the Hox dosage as an additional molecular strategy contributing to further resolve the Hox paradox. We present several cases where the Hox dosage is involved in the formation of different morphologies in invertebrates and vertebrates, with a particular emphasis on flight appendages in insects. We also discuss how the Hox dosage could be interpreted in different types of target enhancers within the nuclear environment *in vivo*. Altogether our survey underlines the Hox dosage as a key mechanism for shaping Hox molecular function during development and evolution.

Key words: Hox, TALE, dose, transcription, morphogenesis

## Introduction

Hox genes are evolutionarily conserved regulators of morphological diversity in animals. They encode for homeodomain (HD)-containing transcription factors (TFs) which act by recognizing specific enhancers to regulate the expression of large numbers of downstream target genes [1,2]. Hox proteins have also recently been shown to control tissue-specific gene expression at the mRNA splicing regulatory level, enlarging their molecular repertoire for the fine tuning of gene expression *in vivo* [3,4].

A general paradox concerning the molecular mode of action of Hox proteins lies in the fact that their specific transcriptional programs *in vivo* contrast with their ability to recognize highly similar DNA-binding sites as monomers *in vitro*. The discovery of the generic TALE-class cofactors greatly contributed to better understand this *in vivo/in vitro* paradox. Two types of TALE cofactors interact with Hox proteins: the PBC and MEIS proteins. These proteins are highly conserved during evolution, with the presence of one (such as Extradenticle (Exd) or Homothorax (Hth) in *Drosophila*) or more (such as PBX1-4 or MEIS1-2 in human) representatives. PBC and MEIS cofactors interact on DNA with all Hox members and modulate both their DNA-binding properties and trans-regulatory activities [5,6]. In most cases, PBC/MEIS form trimeric complexes with the Hox protein, and the assembly of Hox/PBC/MEIS complexes has been described to rely on diverse Hox protein motifs in several instances [6]. This versatility is conserved from cnidarians to bilaterians and has been proposed to serve as a molecular scaffold for diversifying and specifying Hox patterning functions along longitudinal axes during animal evolution [7]. Beyond the TALE cofactors, many other types of TFs have been described to interact with Hox proteins [8]. Although it is expected that several of them could be Hox-specific, *in vivo* analyses showed that *Drosophila* TFs had a tendency to interact with two or more different Hox proteins [9]. The overall combination of interactions was however different for each *Drosophila* Hox protein, suggesting that Hox-specific transcriptional activity could result from the assembly of Hox-specific interactomes and not from individual Hox-specific cofactors [9].

Given their generic role as Hox cofactors, Hox-TALE-DNA interactions have been the subject of a number of studies. In particular, high throughput Selex-seq (Systematic Evolution of Ligands by Exponential Enrichment with massively parallel sequencing) and structural biochemistry showed that the TALE cofactors could help in revealing a “latent-specificity” in Hox DNA-binding properties [10]. This property was associated with the

recognition of specific DNA shapes and binding to divergent nucleotide sequences that were not necessarily of high affinity [10,11]. These non-consensus or so-called low affinity DNA-binding sites were also found to be distinctly enriched in genome-wide binding profiles of Hox proteins from different tissues [10]. In addition, their presence in several target enhancers (see below) reinforces their key contribution for Hox functional specificity *in vivo*. Importantly, the existence of low-affinity DNA-binding sites raises the question of the impact of the Hox expression level, or Hox dosage, in their selective recognition genome wide.

Surprisingly, the role of the Hox dosage on Hox function remains a poorly investigated issue. Here, we present studies which exemplify the importance of the Hox dosage for controlling specific morphologies during development. In particular, we describe the role of the Hox dosage in the context of flight appendage formation during development and evolution in insects. We also discuss and speculate on the transcriptional readout of Hox dosage when considering the nuclear and chromatin environment *in vivo*.

## **1- Hox dosage in animal development**

One of the first studies describing a dose-dependent effect of Hox proteins was the analysis of Hox gene mutants from the group 4 in mice [12]. More particularly, the authors compared the skeletal phenotype of single, versus double or triple mutant combinations for *Hoxa4*, *Hoxb4* and *Hoxd4*. They observed a clear dosage-dependent increase in the double and triple mutants, explaining the functional redundancy between the paralogs [12]. Previous analysis of other Hox mutant mice did not reveal this aspect of redundancy and dosage-dependent phenotype [13,14]. Given that *Hoxa4*, *b4* and *d4* encode for Hox proteins with highly similar HDs, it was suggested that this dosage dependency could reflect similar transcriptional activities on common downstream target genes [12].

Another pioneer study described dose-dependent effects of the Hox mode of action for controlling the number and size of digits in vertebrates. This effect implied the posterior genes *Hoxd11*, *Hoxd12*, *Hoxd13* and *Hoxa13* as major determinants of digit morphogenesis [15]. Importantly, progressive decrease of dosage with those Hox genes induced an increased severity in digit size and number defects in the mouse, highlighting a common Hox-dose dependent mechanism for controlling the size and number of digits. A dosage-dependent phenotype with the same four Hox genes was also described for external genitalia formation [16]. Based on developmental and phylogenetic arguments, the authors

proposed an attractive model for the dose-dependent involvement of posterior Hox genes of the clusters A and D in diversifying more largely the length and number of digits during vertebrate evolution [15].

Interestingly, Hox dosage is known to be instrumental for different aspects of leg morphogenesis in insects. One example comes from the role of the Hox gene *Ultrabithorax* (*Ubx*) in repressing the formation of non-sensory microtrichiae, or trichomes, on the posterior femur of the second and third legs in different *Drosophila* species [17]. The pattern of trichomes distribution was not identical between *Drosophila* species that display different profiles of *Ubx*. In addition, this pattern was shown to be dose-dependent and to require high levels of *Ubx* expression for efficient repression [17]. Interestingly, the same Hox gene *Ubx* has been described to modulate leg length depending on its expression level in the water strider *Limnoporus dissortis* [18]. This species is characterized by longer legs on the second thoracic segment (T2) than on the third thoracic segment (T3). This morphological difference was shown to depend on a low *Ubx* expression level in the T2 leg, which promotes growth, while a high *Ubx* expression level in the T3 leg has an opposite repressive role on growth. Decreasing *Ubx* expression levels shortened or lengthened the T2-leg or T3-leg, respectively. These observations underline that different tissues respond distinctly to Hox dose variations depending on their respective endogenous Hox expression level.

## **2- Hox dosage and flight appendage morphogenesis in insects during development and evolution**

Flying insects cover thousands of different species which have been classified in several major orders [19]. The ancestral state in insects consisted of two pairs of similar wings on their second (T2 forewing, FW) and third (T3 hindwing, HW) thoracic segments [19,20]. Most existing lineages, such as the Odonata, Hymenoptera or Hemiptera, retain the ancestral state. Still, flight appendages diverged over the course of evolution, not only between species, but also between the T2 and T3 flight appendages of the same species. These modifications applied to the shape, size, and/or color pattern. For example, the FW and HW can be of different shapes and color patterns in butterflies (Lepidoptera order), while it is mostly the size that distinguishes the two pairs of wings in bees (Hymenoptera order). In more extreme cases, the wing evolved into a different flight organ, as exemplified with the

FW transformed into a thick protective envelop called elytron in the Coleoptera, or the HW transformed into a small dumbbell-shaped organ called haltere in the Diptera (Figure 1).

For a long time, the formation of FW in insects has been considered as a Hox-independent process, while HW formation and diversification was shown to result from the activity of *Ubx* in the third thoracic segment [20]. These distinct Hox contributions on the T2 or T3 segment were deduced from pioneering work showing the absence of any obvious expression and function of the Hox gene *Antennapedia* (*Antp*) in the FW primordia of the beetle *Tribolium castaneum* [21] and the fruit fly *Drosophila melanogaster* [22]. In addition, the HW was transformed into an elytron upon the loss of *Ubx* in the T3 segment in the beetle, and the same type of haltere-to-wing transformation was observed when affecting *Ubx* expression in the T3 segment in the fruit fly [21] [22]. *Ubx* was also shown to be expressed in the wing primordia in two different ant species [23], the silkworm *Bombyx mori* [24], the butterflies *Bicyclus anynana* [25,26] and *Junonia coenia* [25] and the honeybee *Apis mellifera* [24], raising the question of how a highly conserved Hox protein could have diversified its activity to trigger the formation of various flight appendages during insect evolution.

The advent of new genetic tools and more sensitive antibodies demonstrated that *Ubx* was in fact not the only Hox gene involved in flight appendage formation in insects. More particularly, *Antp* was shown to be required for proper elytron and HW formation in *Tribolium* [27], and to be expressed and required for the formation of the FW and HW in *Bombyx* [27]. In addition, analysis in *Drosophila* revealed the contribution of *Antp* for proper FW formation [28]. Interestingly, *Antp* is dynamically expressed at a low level in the region of the wing primordium that gives rise to the distal wing in the *Drosophila* adult [28].

Surprisingly, *Antp* can replace *Ubx* and rescue haltere formation in a *Ubx* mutant background when expressed at high *Ubx*-like doses [28]. Conversely, decreasing the *Ubx* dose in the haltere into an *Antp*-like dose led to haltere-to-wing transformation [28]. These results underlined that the dose, instead of the nature of the Hox protein, is decisive for making a wing or a haltere in *Drosophila*.

Observations in other insect species further illustrated a striking correlation between the expression level of both *Antp* and *Ubx*, and the morphological similarities or differences between the FW and HW in four wings insect species. For example, the damselfly *Ishnura elegans*, which has almost identical FW and HW, displays similar levels of *Antp* and *Ubx* in

the FW and HW primordia [28]. In contrast, higher levels of Antp and Ubx were measured in the HW primordia of *Apis*, which gives rise to a smaller wing than the FW in the adult [28]. A differential Hox expression level was also observed between the FW and HW primordia in *Bombyx*, which has FW and HW of different shapes [28].

The role of the Hox dose has also been linked to the nutritional status in the rice planthopper insect *Nilaparvata lugens* (*Nl*), which expresses *Ubx* in both the FW and HW primordia [29,30]. Importantly, the level of *Ubx* expression is impacted by the quality of the diet: high quality induced its expression, which led to the short wing form, while low-quality ripe plants had the opposite effects with a reduction of its expression and the formation of long-wing form.

Altogether, these observations suggest that the Hox dosage (here considering Antp and Ubx) could be widely used for shaping flight appendages in insects. In particular, there is a recurrent observation that higher doses could trigger size reduction. Extreme cases are the flies, with the transformation of HW into small balancing organs. Whether the transformation of FW into elytra in the beetle could also result from a particular Hox expression level remains to be determined. Moreover, Hox dosage could explain the inverted pattern of the haltere and wing in the free-living male of the endoparasitic Strepsiptera insects when compared to the flies [31]. Instead of deploying ectopic expression of *Ubx*, as previously suggested [32], the modulation of the Hox dosage, with high Antp and low Ubx, could be sufficient to induce the formation of halteres or wings in the T2 and T3 segment, respectively (Figure 1). This phenotype could be artificially reproduced in *Drosophila*, demonstrating that Hox dose variation in specific places of the body is compatible with the overall development of the animal.

Classically, the functional outcome of Hox gene activity was considered as resulting from specific spatial and temporal expression profiles, thus underestimating the key role of protein dosage. Now, the importance of protein dosage has been underestimated. Altogether, the presented studies showed that the Hox dosage is a molecular strategy promoting a diversity of morphological variations, from subtle wing size and/or shape modification to the formation of a completely new organ. In this context, we propose a speculative model based on Antp and Ubx expression levels to explain flight appendage diversification in insects (Figure 1).

### 3- Molecular aspects of the Hox dosage at the transcriptional level

The *Drosophila* haltere disc can be divided into three main regions along the proximal-distal axis (Figure 2). These regions give rise to different structures in the adult haltere: (i) the capitellum, which originates from the most distal region called the pouch, (ii) the pedicellus and scabellum, which respectively originate from the distal and proximal hinge region, and (iii) the notum, which originates from the most proximal region of the haltere disc (Figure 2). Interestingly, these different regions express different levels of Ubx, with a high-to-low gradient from the distal to the most proximal part of the disc (Figure 2). Thus, there is a striking correlation between the fate of the different regions of the haltere disc and the dose of Ubx.

In addition to a specific pattern of Ubx doses, the haltere disc is characterized by a specific expression pattern for the Exd and Hth cofactors [33]. In particular, Exd and Hth cofactors are present in regions with high/medium or low Ubx doses (see below). Notably, the activity of an autoregulatory module of *Ubx* was shown to be specifically controlled by low levels of Ubx with Exd and Hth [33], and this regulation occurred through low-affinity Ubx-Exd binding sites (see also below). These results demonstrated that the regulation of a specific target cis-regulatory module associates with a particular Ubx dosage.

Previous work identified the bulk transcriptome and genome-wide binding occupancy of Ubx in the whole haltere disc, however impairing the making of a direct correlation with specific Ubx doses genome-wide [34–36]. A recent study tackled this issue by considering more specifically regions expressing or not the Exd and Hth cofactors [37]. The analysis was also performed by doing a time-sensitive knockdown of Ubx in the distal domain and the results were systematically compared to the wing disc, which does not express Ubx. This work revealed that haltere-specific chromatin accessibility was different depending on the presence or not of the Exd/Hth cofactors: transcriptional repression and reduction of chromatin accessibility was associated with Hox monomer binding while the presence of Exd/Hth correlated with transcriptional activation and increase of chromatin accessibility [37]. These different effects were also observed in regions expressing different doses of Ubx (Figure 2). Notably, regions expressing high or low level of Ubx with Exd/Hth were associated with atypical/low-affinity or consensus/high-affinity DNA-binding sites, respectively [37] (as defined from SELEX-seq assays [10]). Based on these observations, we propose a speculative model associated with the Ubx dose: high Ubx dose combined without (in the pouch) or with

(in the distal hinge) the Exd/Hth cofactors allows the recognition of low-affinity binding sites. In contrast, low Ubx dose with Exd/Hth (in the proximal hinge and notum) would more likely recognize high affinity DNA-binding sites (Figure 2). Thus, the Ubx dose could be tightly linked to the DNA-binding site recognition mode and chromatin accessibility in the haltere disc.

Whether this molecular mode of action applies to other Hox proteins, in the context of non-TALE cofactors, and/or to Ubx in other tissues, remains to be determined. It is interesting to notice that low-affinity binding sites for Ubx have been described in several enhancers of the target gene *shavenbaby (svb)* [11]. This gene is repressed by Ubx in the abdominal segments of the *Drosophila* embryo and this repression was found to rely on different redundant enhancers which contained several low-affinity DNA-binding sites for Ubx/TALE complexes. Changing these DNA-binding sites into consensus/high-affinity DNA-binding sites led to ectopic activation of *svb* by different Hox proteins in the *Drosophila* embryo, showing that low-affinity DNA-binding sites are pivotal for conferring Hox specificity *in vivo* [11]. In addition, multiple low-affinity DNA-binding sites are important for stabilizing enhancer activation against Ubx dose variation or environmental stress, demonstrating a role in transcriptional robustness [11]. Interestingly, the different enhancers of *svb* are regrouped into specific microenvironments in the nucleus, allowing the formation of hubs that are enriched for Ubx and Hth [38]. These observations highlighted that Hox dosage could be locally controlled at specific loci in the nucleus, with the formation of local spots of concentration ensuring robust and active transcription with low-affinity enhancers. This mechanism probably involves the local recruitment of other TFs and chromatin modifiers. It illustrates more generally the importance of the nuclear architecture for controlling TFs and chromatin distribution. Along the same line, the nuclear architecture was recently described to increase in heterogeneity as embryonic development progresses, a phenomenon called “nuclear morphogenesis” which was interpreted as reflecting an increase in the complexity of gene regulation during development [39]. Considering the abovementioned dose-dependent role of Ubx on chromatin accessibility in the haltere disc, we suggest that Hox dosage could also impact on enhancer localization for proper regulation with other TFs in specific domains of the nucleus. This hypothesis could be tested by modifying artificially the level of Ubx in the proximal (with increasing doses) or distal (with decreasing doses) region

of the haltere disc and assess whether these modifications could mimic the nuclear pattern of hubs normally observed in the distal or proximal region, respectively.

In addition, nuclear hubs could also serve as reservoirs of TFs. These reservoirs could be used to buffer strong Hox dose variations (up or down) under stress condition, allowing the maintenance of specific Hox transcriptional programs. Finally, nuclear hubs could also serve as a point source for the diffusion of TFs towards distant promoters. In this scenario, it will be interesting to analyze whether local nuclear hubs are enriched in enzymes like acetylases, which have been proposed to promote the diffusion of TFs from distant enhancers to the target promoter [40].

## **Conclusion**

The Hox paradox has long been investigated by studying the DNA-binding and protein-protein interaction properties of Hox proteins with their generic TALE cofactors without considering the role of the dose [41]. In this review, we presented several lines of evidence which clearly demonstrate the importance of the Hox dosage, both at the macroscopic/phenotypic and molecular level. We proposed speculative modes of action linking the Hox dosage and the TALE partnership with the recognition of different types of DNA-binding sites and distinct impacts on chromatin accessibility. We also emphasized how the Hox dosage could be micro-shaped by the nuclear architecture to form local hubs with specific enhancers.

In the future, the role of the Hox dose will certainly be investigated in a variety of model systems. For example, it will be interesting to know whether Hox dosage variation and local nuclear hubs could be a general principle associated with Hox function across the animal phyla (from Cnidaria to Bilateria). Along the same line, the Hox dosage could also potentially impact on their numerous molecular activities other than transcriptional regulation, such as genome repair [42,43], DNA replication [44,45], RNA splicing [4] or translation [46]. An important challenge will be to understand the impact of the Hox dose at both large-scale and single molecule resolution levels. The advent of MERFISH technology coupled to single cell RNA-seq [47] could provide an unprecedented resolution of the impact of the Hox dose on hundreds of target genes in the same cell. In addition, approaches like single particle tracking PhotoActivated Light Microscopy (sptPALM, [48]) could offer a precise measurement of both the enrichment and the dynamics of individual Hox or Hox-

TALE interactions *in vivo*. Deciphering the Hox dosage at these resolution scales will strongly contribute to our understanding of the mechanistic of Hox function and certainly reveal novel and unexpected molecular facets of Hox proteins.

### Figure legends

**Figure 1. Hox dosage and the diversification of flight appendages in insects: correlations and speculations.** A simplified evolutionary tree of insects is shown with a representative species for each order. Pictures are not at scale and were obtained from WordPress (<https://wordpress.org/>). Cartoons schematize the forewing (FW) and hindwing (HW) morphology, and the Hox dose includes both Antp and Ubx expression levels in the FW and HW primordium of each corresponding species. This Hox level is not known in the male Strepsiptera (S) representative *Xenos vesparum* or in the Coleoptera (C) representative *Tribolium castenum*.

Other branch orders and representative species are: Odonata (O; damselfly *Ischnura elegans*); Hemiptera (He; planthopper *Nilaparvata lugens*); Hymenoptera (Hy; honeybee *Apis mellifera*); Lepidoptera (L; silk moth *Bombyx mori*); Diptera (D; fruit fly *Drosophila melanogaster*).

**Figure 2. Ubx dosage and the specification of different structures in the *Drosophila* haltere.**

**A.** SEM acquisition of adult wild type haltere with its four main regions (from distal to proximal): the capitellum (1), the pedicellus (2), the scabellum (3) and the notum (4). **B.** Haltere imaginal disc stained for Ubx (gray). The Ubx expression level is high in the pouch (1), which does not express the Exd and Hth cofactors, and which gives rise to the capitellum in the adult haltere. The Ubx expression level is also high in the distal hinge region (2), although to a less extent than in the pouch. This region expresses Exd and Hth and gives rise to the pedicellus in the adult haltere. The Ubx expression level is low in the proximal hinge (3) and notum (4), which give rise to the scabellum and notum in the adult haltere. These regions express Exd and Hth. **C.** Quantification of the Ubx expression level in the different regions of the haltere imaginal disc. The quantification is represented as a relative percentage of the level measured in the pouch in each individual disc (100%). One-way Anova test shows the statistical significance between the different regions (\*\*\*\* pvalue $\leq$ 0,0001). **D.** Speculative model on the Ubx dosage and the recognition of different types of DNA-binding sites in the

genome. High levels allow the recognition of low affinity binding sites, without (region 1) or with (region 2) the Exd and Hth cofactors. Low levels of Ubx with Exd and Hth restrict the recognition mode to highly-affinity/consensus DNA-binding sites (regions 3-4). Compared to high affinity binding sites, low affinity binding sites are less enriched in high throughput *in vitro* DNA-binding experiments and display divergent nucleotide sequences [10]. See also the main text.

### Acknowledgements

We thank Abderrahman Khila and René Rezsöházy for helpful comments on the manuscript. Work in the laboratory of S.M. is supported by the Centre National de la recherche Scientifique (CNRS), Ecole Normale Supérieure (ENS) de Lyon, Région Auvergne-Rhône-Alpes, Institut National du Cancer (InCA) and European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement (RNA-NetHOX, 101024467) for JC.

### References

1. Lohmann, I.; McGinnis, W. Hox Genes : It ' s All a Matter of Context. **2002**, *12*, 514–516.
2. Hueber, S.D.; Lohmann, I. Shaping segments: Hox gene function in the genomic age. *BioEssays* 2008, *30*, 965–979.
3. Carnesecchi, J.; Sigismondo, G.; Domsch, K.; Baader, C.E.P.; Rafiee, M.R.; Krijgsveld, J.; Lohmann, I. Multi-level and lineage-specific interactomes of the Hox transcription factor Ubx contribute to its functional specificity. *Nat. Commun.* **2020**, *11*, doi:10.1038/s41467-020-15223-x.
4. Carnesecchi J, Boumpas P, van Nierop Y Sanchez P, Domsch K, Pinto HD, Borges Pinto P, L.I. The Hox transcription factor Ultrabithorax binds RNA and regulates co-transcriptional splicing through an interplay with RNA polymerase II. *Nucleic Acids Res.* **2022**, *50*, 763–783, doi:10.1093/nar/gkab1250.
5. Mann, R.S.; Lelli, K.M.; Joshi, R. *Chapter 3 Hox Specificity. Unique Roles for Cofactors and Collaborators*; 2009; Vol. 88; ISBN 9780123745293.
6. Merabet S., M.R. To Be Specific or Not: The Critical Relationship Between Hox And TALE Proteins. *Trends Genet.* **2016**, *Jun;32*, 334–47.

7. Hudry, B.; Thomas-Chollier, M.; Volovik, Y.; Duffraisse, M.; Dard, A.; Frank, D.; Technau, U.; Merabet, S. Molecular insights into the origin of the Hox-TALE patterning system. *Elife* **2014**, *3*, e01939, doi:10.7554/eLife.01939.
8. Rezsöházy, R.; Saurin, A.J.; Maurel-Zaffran, C.; Graba, Y. Cellular and molecular insights into Hox protein action. *Development* **2015**, *142*, 1212–1227, doi:10.1242/dev.109785.
9. Baeëza, M.; Viala, S.; Heim, M.; Dard, A.; Hudry, B.; Duffraisse, M.; Rogulja-Ortmann, A.; Brun, C.; Merabet, S. Inhibitory activities of short linear motifs underlie hox interactome specificity in vivo. *Elife* **2015**, *2015*, doi:10.7554/eLife.06034.001.
10. Slattery, M.; Riley, T.; Liu, P.; Abe, N.; Gomez-alcala, P.; Dror, I.; Zhou, T.; Rohs, R.; Honig, B.; Bussemaker, H.J.; et al. Cofactor Binding Evokes Latent Differences in DNA Binding Specificity between Hox Proteins. *Cell* **2011**, *147*, 1270–1282, doi:10.1016/j.cell.2011.10.053.
11. Crocker, J.; Abe, N.; Rinaldi, L.; McGregor, A.P.; Frankel, N.; Wang, S.; Alsaawadi, A.; Valenti, P.; Plaza, S.; Payre, F.; et al. Low Affinity Binding Site Clusters Confer Hox Specificity and Regulatory Robustness. *Cell* **2015**, 191–203, doi:10.1016/j.cell.2014.11.041.
12. Gerald S.B. Horan, Ramiro Ramirez-Solis, Mark S. Featherstone, Debra J. Wolgemuth, Allan Bradley, and R.R.B. Compound mutants for the paralogous *hoxa-4*, *hoxb-4*, and *hoxd-4* genes show more complete homeotic transformations and a dose-dependent increase in the number of vertebrae transformed. *Genes Dev.* **1995**, *9*, 1667–1677.
13. Condie, B.G. and M.R.C. Mice homozygous for a targeted disruption of *Hoxd-3* (*Hox-4.1*) exhibit anterior transformations of the first and second cervical vertebrae, the atlas and axis. *Development* **1993**, *119*, 579–595.
14. Rancourt, D.E., T. Tsuzuki, and M.R.C. Genetic interaction between *hoxb-5* and *hoxb-6* is revealed by nonallelic noncomplementation. *Genes Dev.* **1995**, *9*, 108–122.
15. Zákány, J.; Fromental-Ramain, C.; Warot, X.; Duboule, D. Regulation of number and size of digits by posterior Hox genes: A dose-dependent mechanism with potential evolutionary implications. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 13695–13700, doi:10.1073/pnas.94.25.13695.
16. van der Hoeven, F., Zakany, J. and Duboule, D. Gene transpositions in the *HoxD* complex reveal a hierarchy of regulatory controls. *Cell* **1996**, *85*, 1025–1035.

17. Stern DL. A role of Ultrabithorax in morphological differences between *Drosophila* species. *Nature* **1998**, Dec3;396, 463–6.
18. Refki, P.N.; Armisen, D.; Crumière, A.J.J.; Viala, S.; Khila, A. Emergence of tissue sensitivity to Hox protein levels underlies the evolution of an adaptive morphological trait. *Dev. Biol.* **2014**, *392*, 441–453, doi:10.1016/j.ydbio.2014.05.021.
19. Misof, B.; Liu, S.; Meusemann, K.; Peters, R.S.; Donath, A.; Mayer, C.; Frandsen, P.B.; Ware, J.; Flouri, T.; Beutel, R.G.; et al. Phylogenomics resolves the timing and pattern of insect evolution. *Science (80-. )*. **2014**, *346*, 763–767, doi:10.1126/science.1257570.
20. Tomoyasu, Y. Ultrabithorax and the evolution of insect forewing/hindwing differentiation. *Curr. Opin. Insect Sci.* 2017, *19*, 8–15.
21. Tomoyasu, Y.; Wheeler, S.R.; Denell, R.E. Ultrabithorax is required for membranous wing identity in the beetle *Tribolium castaneum*. *Nature* **2005**, *433*, 643–647, doi:10.1038/nature03272.
22. Carroll, S.B.; Weatherbee, S.D.; Langeland, J.A. Homeotic genes and the regulation and evolution of insect wing number. *Nature* **1995**, *375*, 58–61, doi:10.1038/375058a0.
23. Abouheif E, W.G. Evolution of the gene network underlying wing polyphenism in ants. *Science (80-. )*. **2002**, *297*, 249–52.
24. Prasad N, Tarikere S, Khanale D, Habib F, S.L. A comparative genomic analysis of targets of Hox protein Ultrabithorax amongst distant insect species. *Sci. Rep.* **2016**, Jun 14, 27885.
25. Tong, X.; Hrycaj, S.; Podlaha, O.; Popadic, A.; Monteiro, A. Over-expression of Ultrabithorax alters embryonic body plan and wing patterns in the butterfly *Bicyclus anynana*. *Dev. Biol.* **2014**, *394*, 357–366, doi:10.1016/j.ydbio.2014.08.020.
26. Saenko, S. V.; Marialva, M.S.P.; Beldade, P. Involvement of the conserved Hox gene *Antennapedia* in the development and evolution of a novel trait. *Evodevo* **2011**, *2*, doi:10.1186/2041-9139-2-9.
27. Fang C, Xin Y, Sun T, Monteiro A, Ye Z, Dai F, Lu C, T.X. The Hox gene *Antennapedia* is essential for wing development in insects. *Development* **2022**, *149*, doi:10.1242/dev.199841.
28. Paul R, Giraud G, Domsch K, Duffraisse M, Marmigère F, Khan S, Vanderperre S, Lohmann I, Stoks R, Shashidhara LS, M.S. Hox dosage contributes to flight appendage

- morphology in *Drosophila*. *Nat. Commun.* **12**, 2892.
29. Liu, F.; Li, X.; Zhao, M.; Guo, M.; Han, K.; Dong, X.; Zhao, J.; Cai, W.; Zhang, Q.; Hua, H. Ultrabithorax is a key regulator for the dimorphism of wings, a main cause for the outbreak of planthoppers in rice. *Natl. Sci. Rev.* **2020**, doi:10.1093/nsr/nwaa061.
  30. Fu, S.J.; Zhang, J.L.; Chen, S.J.; Chen, H.H.; Liu, Y.L.; Xu, H.J. Functional analysis of Ultrabithorax in the wing-dimorphic planthopper *Nilaparvata lugens* (Stål, 1854) (Hemiptera: Delphacidae). *Gene* **2020**, *737*, doi:10.1016/j.gene.2020.144446.
  31. Footitt, R.G.; H. Adler, P.; Kathirithamby, J. Biodiversity of Strepsiptera. In *Insect Biodiversity*; 2018; pp. 673–704.
  32. Whiting MF, W.W. Insect homeotic transformation. *Nature* **1994**, *368*, 696.
  33. Delker, R.K.; Ranade, V.; Loker, R.; Voutev, R.; Mann, R.S. Low affinity binding sites in an activating CRM mediate negative autoregulation of the *Drosophila* Hox gene Ultrabithorax. *PLoS Genet.* **2019**, *15*, doi:10.1371/journal.pgen.1008444.
  34. Slattery, M.; Ma, L.; Négre, N.; White, K.P.; Mann, R.S. Genome-wide tissue-specific occupancy of the hox protein ultrabithorax and hox cofactor homothorax in *Drosophila*. *PLoS One* **2011**, *6*, doi:10.1371/journal.pone.0014686.
  35. Choo, S.W.; White, R.; Russell, S. Genome-wide analysis of the binding of the Hox protein Ultrabithorax and the Hox cofactor Homothorax in *Drosophila*. *PLoS One* **2011**, *6*, e14778, doi:10.1371/journal.pone.0014778.
  36. Agrawal, P.; Habib, F.; Yelagandula, R.; Shashidhara, L.S. Genome-level identification of targets of Hox protein Ultrabithorax in *Drosophila*: novel mechanisms for target selection. *Sci. Rep.* **2011**, *1*, 205, doi:10.1038/srep00205.
  37. Ryan Loker, Jordyn E Sanner, R.S.M. Cell-type-specific Hox regulatory strategies orchestrate tissue identity. *Curr. Biol.* **2021**, *31*, 4246–4255, doi:10.1016/j.cub.2021.07.030.
  38. Tsai, A.; Muthusamy, A.K.; Alves, M.R.; Lavis, L.D.; Singer, R.H.; Stern, D.L.; Crocker, J. Nuclear microenvironments modulate transcription from low-affinity enhancers. *Elife* **2017**, *6*, doi:10.7554/elife.28975.
  39. Tsai A, Muthusamy AK, Alves MR, Lavis LD, Singer RH, Stern DL, C.J. Nuclear microenvironments modulate transcription from low-affinity enhancers. *Elife* **2022**, *6*, doi:10.7554/eLife.28975.
  40. Karr JP, Ferrie JJ, Tjian R, D.X. The transcription factor activity gradient (TAG) model:

- contemplating a contact-independent mechanism for enhancer-promoter communication. *Genes Dev.* **2022**, *36*, 7–16, doi:10.1101/gad.349160.121.
41. Merabet, S.; Mann, R.S. To Be Specific or Not: The Critical Relationship Between Hox And TALE Proteins. *Trends Genet.* **2016**, *xx*, 1–14, doi:10.1016/j.tig.2016.03.004.
  42. Rubin E, Wu X, Zhu T, Cheung JC, Chen H, Lorincz A, Pandita RK, Sharma GG, Ha HC, Gasson J, Hanakahi LA, Pandita TK, Sukumar S. Rubin E, Wu X, Zhu T, Cheung JC, Chen H, Lorincz A, Pandita RK, Sharma GG, Ha HC, Gasson J, Hanakahi LA, Pandita TK, S.S. A role for the HOXB7 homeodomain protein in DNA repair. *Cancer Res.* **2007**, *67*, 1527–35.
  43. BC., F. Architects meets Repairers: The interplay between homeobox genes and DNA repair. *DNA Repair (Amst).* **2019**, *Jan*, 34–48.
  44. Comelli L, Marchetti L, Arosio D, Riva S, Abdurashidova G, Beltram F, F.A. The homeotic protein HOXC13 is a member of human DNA replication complexes. *Cell Cycle* **2009**, *8*, 454–459.
  45. Salsi V, Ferrari S, Ferraresi R, Cossarizza A, Grande A, Z. V. HOXD13 binds DNA replication origins to promote origin licensing and is inhibited by geminin. *Mol. Cell. Biol.* **2009**, *29*, 5775–5788.
  46. Topisirovic, I.; Kentsis, A.; Perez, J.M.; Guzman, M.L.; Jordan, C.T.; Borden, K.L.B. Eukaryotic translation initiation factor 4E activity is modulated by HOXA9 at multiple levels. *Mol. Cell. Biol.* **2005**, *25*, 1100–12, doi:10.1128/MCB.25.3.1100-1112.2005.
  47. Gao D, Ning J, Liu G, Sun S, D.X. SpatialMap: Spatial Mapping of Unmeasured Gene Expression Profiles in Spatial Transcriptomic Data Using Generalized Linear Spatial Models. *Front Genet* **2022**, *26*, 893522.
  48. Manley S, Gillette JM, L.-S.J. Single-particle tracking photoactivated localization microscopy for mapping single-molecule dynamics. *Methods Enzym.* **2010**, *475*, 109–20.