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Haltere development in *D. melanogaster:* implications for the evolution of appendage size, shape and function

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ABSTRACT Differential specification of dorsal flight appendages, wing and haltere, in *Drosophila* provides an excellent model system to address a number of important questions in developmental biology at the levels of molecules, pathways, tissues, organs, organisms and evolution. Here we discuss the mechanism by which the Hox protein Ubx recognizes and regulates its downstream targets, implications of the same in growth control at cellular and organ level and finally the evolution of haltere from ancestral hindwings in other holometabolous insects.

KEY WORDS: *Ultrabithorax, Drosophila, wing, haltere, evolution, Bombyx, Apis, Tribolium, ChIP-seq, RNA-seq*

Introduction

Post-fertilization, early developmental events in *Drosophila melanogaster* involves establishment of morphogen gradients of maternal effector genes. These genes are transcribed in nurse cells and deposited in the eggs through a highly regulated transport mechanism. The protein products of these genes diffuse freely in the zygote, thereby, establishing a concentration gradient of positional information. These proteins are transcription factors and they in turn activate another set of transcription factors (the gap genes) in a concentration-dependent manner. By this process, information generated by the gradients of individual proteins are converted to discrete information modules represented by the expression and function of different sets of proteins. Sequential activation of different sets of genes divides the embryo into distinct developmental units (segments/compartments/germ layers etc). At the end of this cascade of gene regulatory events are the Hox proteins, which are expressed along the antero-posterior axis in a segment-specific pattern and regulate development of various tissues and organs (Busson, 1993).

There is a direct correlation to the evolution of Hox genes to evolution of diversity along the antero-posterior axis in the animal kingdom (reviewed in Hughes and Kaufman, 2002, Pearson *et al.*, 2005). In *Drosophila*, there are 8 Hox genes expressed along the anterior-posterior axis. The flight appendages in *Drosophila*, the wing and haltere, are present on the second (T2) and third thoracic (T3) segment, respectively. While the wing is considered to be a Hox-free state, the halteres are specified by the Hox protein Ultrabithorax (Ubx) by the suppression of wing development pathways in the T3 segment (Carroll *et al.*, 1995), (reviewed in Tomoyasu, 2017). Loss of *Ubx* function in developing haltere discs induces haltere-to-wing transformations (Lewis, 1978), whereas ectopic expression of Ubx in developing wing discs leads to wing-to-haltere transformations (Cabrera *et al.*, 1985, Castelli-Gair *et al.*, 1990, White and Akam, 1985, White and Wilcox, 1985). The differential development of wings and halteres constitutes a good genetic system to study cell fate determination at different levels such as growth, cell shape, size and its biochemical and physiological properties. They also represent the evolutionary trend that has established the differences between serial homologues such as fore and hind wings in insects, wings and legs in birds and fore and hind limbs in mammals.

For the past two decades, we have been studying the events downstream of Ubx in the context of haltere specification in *Drosophila*. Much of our work revolves around two major questions related to Ubx function. 1) As Ubx specifies haltere fate and represses wing fate, what are the growth and patterning events that are regulated by Ubx to repress wing development and specify haltere development? and 2) Being a member of Hox transcription factors, all of which have very similar DNA-binding homeodomain, what is the mechanism by which Ubx recognizes and regulates its targets genes?

At the experimental level, we have employed, (as the technology progressed), techniques such as classical genetics, EP (Enhancer-Promoter) screen and RNAi (RNA Interference) screen to study developmental reprogramming by Ubx and enhancer-trap, microarray/RNA-seq, ChIP-chip and ChIP-sequencing to identify

Abbreviations used in this paper: AP, anterio-posterior; DV, dorso-ventral; N, notch; Ubx, Ultrabithorax.

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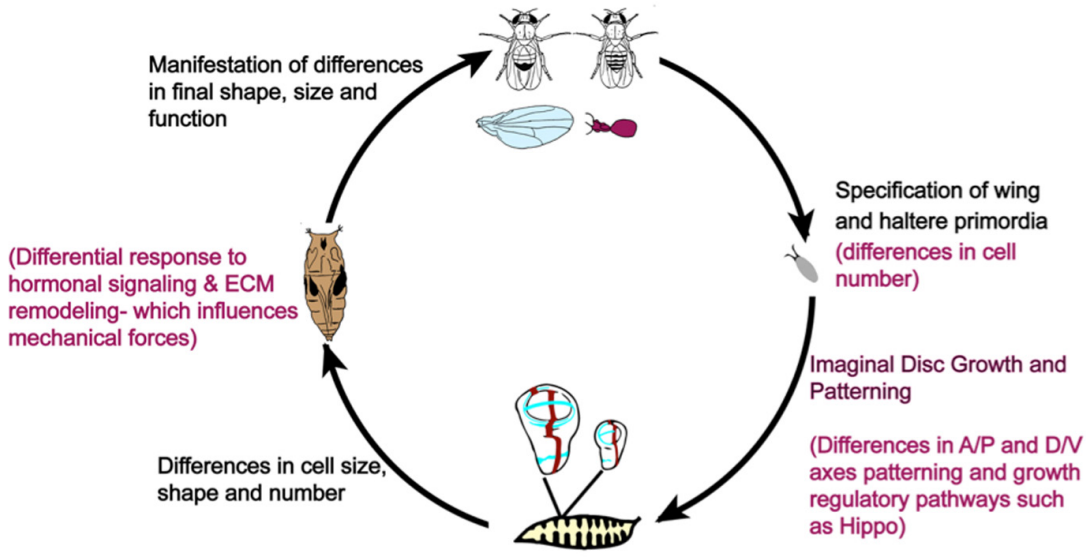

Fig. 1. Life cycle of *Drosophila* indicating key steps in the development of wing and halteres. *Events/traits regulated by Ubx in developing haltere are shown in magenta. Ubx regulates haltere development throughout the life cycle. It regulates cell number of the primordium in the embryo. While it doesn't affect the proliferation rate or cell size or shape in larval discs, it does regulate patterning events (gene expression patterns) that results in differential cell number, size and shape later during pupal development. Ubx also regulates certain events in the pupa, such as clearance of ECM, hormonal signalling etc to bring about final differences between size and shape between adult wing and haltere.*

its direct and indirect targets.

Wing is a two-layered flat structure, with one wing hair (trichome) per cell. As wing cells are larger and flatter, the trichomes are sparsely arranged. In contrast, the haltere is bulbous: the dorsal and ventral compartments do not physically oppose each other: cells too are more bulbous and bear multiple trichomes per cell, and thus, are closely arranged (Roch and Akam, 2000). Our approach was to ask how Ubx brings each one of these differences. Wing primordium is specified in the embryo and later, during larval stages, these cells organize themselves as one single continuous sheet of epithelium (termed as imaginal disc), which give rise to dorsal and ventral wing blades, hinge and vein regions and also specialized sensory bristles of the margin. These two wing imaginal discs in T2 correspond to two wings in the adult. More proximal part of each of the two wing imaginal discs specifies dorsal thorax of the adult body. The dorsal thorax is, thus, developed from two independent modules, which fuse laterally making one single continuous thorax from all sides (Fig. 1). Wing development is controlled by two major patterning events along its anterior-posterior (A/P) and dorso-ventral (D/V) axis to specify wing per se, while patterning events along the proximo-distal axis specify hinges and the dorsal thorax of the adult body (reviewed in Beira and Paro, 2016, Hariharan, 2015) (Fig. 2).

Haltere development follows a similar path, although the primordium itself is made up of fewer cells compared to the wing primordium. While there is no difference at the level of size, shape and polarity of individual epithelial cells between the third instar wing and haltere imaginal discs, Ubx-mediated modulation of patterning events eventually results in the growth and differentiation of haltere discs into structurally and thereby, functionally, into different structures as compared to wings (Makhijani *et al.*, 2007, Roch and Akam, 2000, Singh *et al.*, 2015). In this review, which we have divided into two major parts, as per the above-mentioned two questions - we summarize our current understanding of Ubx function.

Specification of haltere by Ubx

Control of patterning events

Antero-Posterior axis. In the larval discs, Engrailed (En) expressing cells constitute the posterior compartment. It induces the expression of the short-range signalling molecule Hedgehog (Hh), which diffuses to the anterior compartment to activate Patched (Ptc), Smoothened (Smo), Cubitus interruptus (Ci), Knot (Kn) and Decapentaplegic (Dpp). The posterior compartment itself does not respond to Hh signalling as En represses its receptor Ptc and also the sole mediator Ci. The role of Dpp is central to wing development; it acts as a long-range morphogen and activates several important wing patterning genes such as *spalt (Sal)* and *optomotor blind (Omb)*. Proper positioning of cells receiving varied levels of Dpp is important for cell proliferation in the wing disc (Rogulja and Irvine, 2005) (reviewed in Aza-Blanc and Kornberg, 1999, Brook *et al.*, 1996, Ingham and McMahon, 2001, Ruiz-Losada and Blom-Dahl, 2018).

In haltere discs, while expression patterns of En, Hh, Ptc and Ci are unaltered, expression of Dpp and other downstream genes are modulated by Ubx (Mohit *et al.*, 2006). Not only is *dpp* downregulated in haltere discs at the transcript level, its receptor Thickveins (Tkv) is up regulated in the A/P boundary, making Dpp protein to be internalized in the cells it is made. Additionally, Dally, which is required for Dpp to diffuse away from A/P boundary is specifically down regulated in the posterior compartment of the haltere discs (Crickmore and Mann, 2006, Crickmore and Mann, 2007, de Navas *et al.*, 2006, Makhijani *et al.*, 2007). This may result in asymmetric distribution of Dpp between anterior and posterior compartments. Indeed, in haltere discs, anterior compartment is larger than the posterior compartment (3:1 ratio in size), while in wing discs, the two are of the same size (1:1 ratio in size).

As expected, downstream components of Dpp pathway, such as,

Dad, Spalt major, DSRF and Kn are also repressed in the haltere discs (Galant and Carroll, 2002, Hersh and Carroll, 2005, Mohit *et al*., 2006, Weatherbee *et al*., 1998). Interestingly, in addition to *dpp*, *tkv* and *kn* are also directly repressed by Ubx suggesting that Ubx acts at multiple levels of a given signalling pathway to specify haltere fate, which supports the possibility that Ubx functions more like a micromanager (Akam, 1998).

Dorso-ventral axis. Similar to A/P boundary, the D/V boundary functions as the organizing centre for proper patterning and growth of the wing discs along the D/V axis (Diaz-Benjumea and Cohen, 1993). The selector gene, *apterous (ap)*, specifies the dorsal compartment in the wing discs, which cell-autonomously activates Seratte (Ser) and Fringe (Fng). These two proteins, whose expression is restricted to the dorsal compartment, potentiate Notch (N) to respond to signals coming from the ventral compartment (in the form of Delta (Dl)) only at the D/V boundary. N specifies the D/V boundary as an organizer along the D/V axis (reviewed in Brook *et al*., 1996, Ruiz-Losada and Blom-Dahl, 2018). Notch further activates Wingless (Wg), Cut (Ct) and boundary enhancer of Vestigial (Vg) (Kim *et al*., 1996). Wg functions as a morphogen activating Vein (Vn), Achaete (Ac), Distal-less (Dll) and Vg in non-DV cells in a concentration-dependent manner. The *vg* gene is a pro-wing gene. The expression of this gene is very tightly regulated since any ectopic expression of this gene leads to ectopic wings (Kim *et al*., 1996). Vn is a ligand for EGFR pathway, which together with Dll specifies wing margin (Schnepp *et al*., 1996). Ac is required to specify sensory organs of the margin (reviewed in Calleja *et al*., 2002).

In haltere discs, Ubx modulates D/V patterning differently than the way it regulates A/P patterning events. While Ap, N, Ser and D/V boundary–specific Vg are unaltered, expression of Wg is repressed, but only in the posterior compartment. However, further downstream (of Wg), Ac, Vn, Dll and non-D/V Vg are completely repressed in both anterior and posterior compartments. Repression of Wg expression in the posterior compartment could be either direct and/or through the repression of N activity. While it is not clear how Ubx represses targets of Wg in the anterior compartment of haltere discs, genetic mosaic experiments suggest a non-cell autonomous role of Ubx in repressing wing identity and specifying haltere identity (Shashidhara *et al*., 1999).

As *vg* is a pro-wing gene, its downregulation is an important factor for haltere specification. In wing discs, Vg is activated by both N (in the D/V boundary) and Wg (in non-D/V cells) pathways. Ubx appears to inhibit nuclear localization of Armadillo (Arm) in the anterior compartment (Wg itself is completely repressed in the posterior compartment), in addition to repressing events downstream of N and also directly suppress non-D/V expression of *vg*. All these indicate that D/V signalling is also modulated at multiple levels of the hierarchy of gene regulation. Perhaps, such a mechanism could be a common theme in the evolution of body plan.

Ubx control of cell number and cell size

In *Drosophila*, EGFR signal transduction via the RAS/MAPK kinase cascade has been implicated in many aspects of pattern formation as well as in control of tissue growth, cell proliferation, and apoptosis (Crossman *et al*., 2018, Pallavi and Shashidhara, 2003), (reviewed in Shilo, 2005). Ubx mediated regulation of EGFR pathway is important in specifying the haltere fate as overexpression of the positive components of this pathway such as EGFR and Vn in halteres causes significant haltere to wing transformation. Interestingly, many of the components of EGFR pathway such as *egfr, vn, pointed* and *yan* are direct targets of Ubx (unpublished data).

Organ size is determined by the size and shape of its component cells. Hippo/Yorkie (Yki) pathway is implicated in organ size control and tissue homeostasis, which it accomplishes by integrating signals within the cell, cell-cell interactions and mechanical cues. It regulates growth by promoting cell proliferation, cell growth and inhibiting apoptosis (Irvine and Harvey, 2015). When it comes to Hippo pathway, all recent advances show that it could be a master of cross-talk between different growth regulatory pathways, cellular properties, growth and pattern formation, systemic hormonal signals and the final differentiated state of the organ. Growth is also influenced by environmental factors such as the nutritional status of the organism (reviewed in Shingleton, 2010). The Insulin-like (IIS/

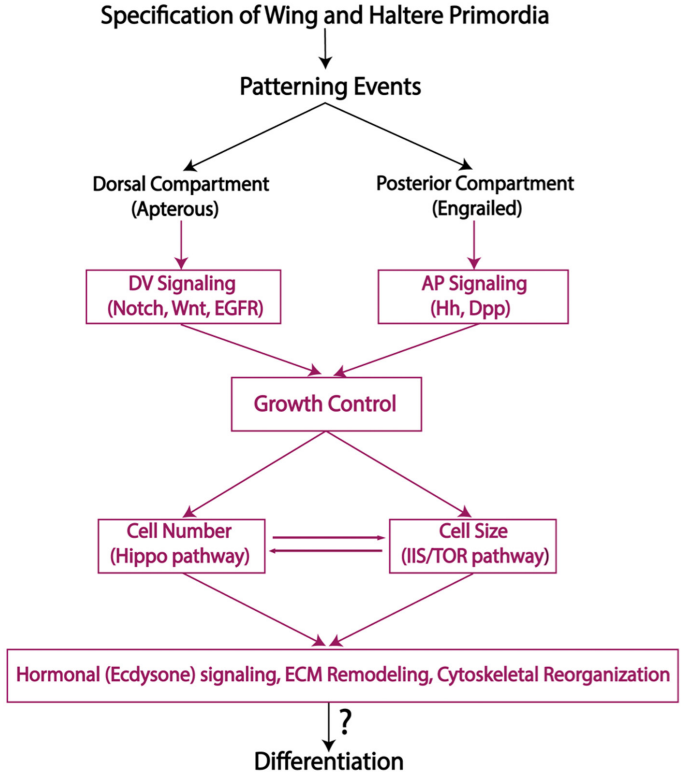

Fig. 2. Major developmental events that occur after specification of the wing and haltere primordia, which finally lead to the development of respective organs in the adult fly. *At the embryonic stage, Wg and Dpp pathways specify the appendage primordia. The primordia retain compartmental identifies as defined by the expression of Apterous (for dorsal) and Engrailed (for posterior). At the larval stages, the wing and haltere imaginal discs are patterned by the Hedgehog (Hh) signaling pathway for the anterior-posterior compartment, whereas the Notch, Wingless (Wnt) and EGFR pathways pattern the dorso-ventral region. The Hippo and Insulin/TOR pathways control the growth of the discs by influencing cell number and cell size. Patterning and growth events are followed by hormonal signaling, ECM remodeling and cytoskeletal reorganization during the pupal stages to shape the organ in the adult fly. Ubx modulates each of these developmental and growth events (from embryo to pupal stages) to repress the wing fate and specify the haltere fate in the third thoracic (T3) segment (shown in magenta). However, knowledge about its role in the terminal differentiation program still remains largely unknown.*

Akt) pathway plays an important role in sensing the nutrition status and regulating growth, primarily by regulating cell size (reviewed in Gokhale and Shingleton, 2015). Ubx downregulates function of both Yki and IIS/Akt pathways. Interestingly, while upregulation, individually, of Yki and IIS/Akt pathways in developing haltere have only marginal phenotypes, their combined upregulation show dramatic haltere to wing phenotypes, both in size and shape of the organ (Singh *et al*., 2015). Cells appear to be larger and also flatter making the transformed haltere a flattened structure like wing. Thus, it appears that the cross-talk between Hippo-Yki and IIS/Akt pathways is key in maintaining an inverse relationship between cell-size and shape (Strassburger *et al*., 2012). Further investigation is needed to understand precise mechanism, perhaps at the levels of cytoskeletal organization, by which cell size is determined (reviewed in Diaz de la Loza and Thompson, 2017, Sanchez-Herrero, 2013).

Control of extracellular matrix and hormonal components

Ubx regulates the haltere shape by preventing the formation of flat wings, by inhibiting the cell shape changes and adhesion of dorsal and ventral wing blades. Integrins, which are required for this opposition of two blades are not regulated by Ubx, rather it has employed a very interesting mechanism to achieve bulbous shape of the haltere (De Las Heras *et al*., 2018, Diaz-de-la-Loza *et al*., 2018). At a time when basal components of the extracellular matrix (ECM), such as Viking, are degraded in wing discs due to high levels of Matrix metalloprotease 1 (Mmp1) expression in early pupal stages, Ubx-mediated inhibition of Mmp1 in haltere discs maintains a gap between dorsal and ventral layers. This gap is subsequently filled by hemocytes, thus permanently preventing close opposition of the two layers in developing halteres (De Las Heras *et al*., 2018, Pastor-Pareja and Xu, 2011).

Additional control of organ size may be mediated by growth hormones such as Ecdysone. Recently, we have observed, by ChIP-seq and RNA-seq, that many positive regulators of Ecdysone pathway are down regulated in haltere discs, while negative regulators are upregulated (unpublished results).

Mechanism of target selection by Ubx

As described above, Ubx regulates genes at multiple levels of the wing developmental pathway. Ubx is known to bind the motif "TTAATKR", the TAAT being the core sequence on which all homeodomain containing transcription factors are known to bind. None of the methods to identify direct targets of Ubx (ChIP-chip or ChIP-seq) have identified "TTAATKR"as the recognition motif that Ubx used to identify and regulate its targets. Instead many studies suggest that Ubx recognizes its targets based on what other transcription factors have already bound on the cis-regulatory regions, although its own binding to DNA is dependent on the presence of the TAAT core sequence. Its cooperative binding on DNA and/or its interactions with other DNA-binding proteins may facilitate Ubx modifying wing fate into haltere fate.

Specificity through low DNA binding sites and cofactor interactions

Ubx is a DNA-binding protein and its binding to DNA is essential for transcriptional regulation of its targets. Sequence analysis of some of the targets such as *dally* and *tkv* indicate the presence of more than one potential Ubx binding sites, which were further confirmed using ChIP and EMSA studies (Makhijani *et al*., 2007). Unpublished ChIP-seq data from our lab also suggests that Ubx may use multiple monomeric binding sites to modulate expression of many, if not all, of its target genes. This, perhaps, reflects the fact that Ubx binds to the motif "TTAATKR" with low affinity and when multiple such sites are present in close proximity, its binding may become more stable and also more specific. The role of low affinity Ubx binding sites in target gene regulation has been demonstrated for the regulation of *shaven baby* (*svb*) gene in the embryo. Although this finding has not been validated in the context of haltere specification, (Crocker *et al*., 2015) provide evidence for utilisation of low affinity sites by Ubx-Exd and Ubx-Hth complexes rather than high affinity sites for specific target selection.

Genome wide binding experiments (ChIP-ChIP) for the Ubx protein in the haltere were carried out in our lab to identify the direct targets of Ubx (Agrawal *et al*., 2011). Motif analysis of 519 targets of Ubx indicate that while binding sites for Ubx were not significantly enriched in pulled down sequences, the binding sites for other transcription factors such as GAF, Adf1 and Mad are overrepresented in pulled-down sequences as compared to the background. This indicates that association with co-transcription factors might be a possible mechanism by which Ubx recognizes and regulates its targets. Indeed, while ChIP-qPCR studies provide evidence that GAF and Ubx might be sharing more than 100 targets during haltere specification, EMSA and ChIP-western data indicate association of GAF and Ubx at the protein level (Agrawal *et al*., 2011). The importance of cofactors in Ubx mediated gene regulation is further strengthened by the fact that Ubx and Mad collaborate (but do not interact physically) to repress the *spalt major* (*salm*) enhancer in haltere discs (Walsh and Carroll, 2007).

However, a global picture for explaining the mechanism of target selection by Ubx is still lacking. Our current research is directed towards finding Hox regulatory codes to address this question. Particularly, the regulatory codes that are recognised and used by Ubx to upregulate some of its targets, and those that are used to down-regulate another set of genes. Unpublished data from our lab suggest that while such codes may exist for a group of genes, a global mechanism is either non-existent or elusive to the technology employed.

The Hox memory

Most of the genes, which are direct targets of Ubx at the third instar larval stage (based on ChIP experiments), are not differentially expressed between the wing and haltere discs. This was observed in previously published ChIP-chip data (Agrawal *et al*., 2011) as well as our recent unpublished ChIP-seq data. Interestingly, the expression of some of these genes is very different between the wing and haltere discs at the prepupal and pupal stages (Pavlopoulos and Akam, 2011). Since Ubx is continuously required throughout the development of haltere (removing Ubx at any stage leads to haltere to wing transformations), it can be speculated that it is consistently bound to the DNA, albeit loosely, or present in the vicinity of its target genes throughout development, and the regulation of its target genes is modulated by the presence of specific cofactors at different developmental stages. While most well-studied mechanisms of cellular memory are mediated by chromatin remodelling, the presence of Ubx itself on the cis-regulatory sequences of all its targets throughout the development, even though simplistic as it may appear, perhaps constitute the Hox memory.

Evolution of mechanisms

Across different insect groups, Ubx modulates wing development in T3 in different ways. While there is hardly any difference between fore- and hind-wing in *Apis, Tribolium* and *Drosophila* represent extreme modifications, albeit in opposite ways. Lepidopterans show intermediate pattern, wherein fore- and hind-wings are different in size, shape and decorative patterns. Nevertheless, Ubx appears to be functionally similar and a very large number of its targets are common across these species. However, they are not regulated in the same way in all species suggesting that evolution at the level of cis-regulatory sequences of the target genes may help Ubx orchestrating different patterns of hind wing modifications in different species.

One might attribute the presence of haltere in Dipterans as a consequence of expression of the Ubx gene. However, Ubx is expressed in the developing hindwing of various holometabolous insects. Even more interesting is the fact that although the DNA binding domain of Ubx has remained conserved across all insect species, it performs completely different functions in different lineages. In Lepidopterans, such as *Precis coenia*, Ubx regulates eyespot patterns and pigmentation in the hindwing (Weatherbee *et al*., 1999), while in Coleopterans such as *Tribolium castaneum*, it represses elytra formation and specifies a wing structure in the hindwing segment (Tomoyasu *et al*., 2005).

In Hymenopterans, such as *Apis mellifera*, it may specify a slightly smaller hindwing compared to the forewing. Interestingly, Ubx is expressed in both forewing and hindwing buds in *Apis mellifera*, although the expression levels in the forewing are lower (Prasad *et al*., 2016). ChIP-seq for the Ubx of *Apis* indicates that while a large number of direct targets are common between the hindwing and forewing buds, the number of targets was more in the hindwing. This could be due to increased levels of Ubx expression in the hindwing buds. The possibility that differential levels of the Ubx protein in the hindwing segment could give rise to different structure is interesting and adds one more dimension to the mechanisms by which Hox genes may specify developmental pathways.

Comparative ChIP analyses of the Ubx protein between *Drosophila melanogaster* (Dipteran), *Apis mellifera* (Hymenopteran) and *Bombyx mori* (Lepidopteran) indicate that while a considerable number of the wing patterning genes are targets of Ubx in all three lineages, most of the target genes are species specific. This would indicate that acquisition of new targets or loss of old targets may have a significant role in determining the haltere fate in Diptera (Prasad *et al*., 2016). Interestingly, certain common targets of Ubx, which are key wing patterning genes show similar expression patterns between the hindwing and forewing in *Apis* and *Bombyx*, but are differentially expressed between the wing and haltere in *Drosophila*. For example, the transcript levels of *vg*, *ct* and *wg* are similar between the *Apis* forewing and hindwing buds as detected using RNA in-situ experiments (Prasad *et al*., 2016). This is a very interesting finding since it bolsters the fact that mere binding of Ubx is not sufficient for regulation of its target genes. Vg expression in non-D/V cells is repressed in haltere discs of *Drosophila*, while it is not differentially expressed between fore- and hindwings in *Apis*. Although enhancers of *vg* of both the species have binding sites for Ubx, Adf1 may bind in a nearby region in the cis-regulatory sequence of *vg* of *Drosophila*, but not that of *Apis*. While experimental validation of this is still inconclusive, these findings suggest that changes in the cis-regulatory regions through acquisition or loss of binding sites for co-transcription factors can be an important mechanism of evolution of target gene regulation by Ubx (Prasad *et al*., 2016).

Crystallographic studies have shown that a well-conserved hexapeptide motif (HX) in Ubx is responsible for its interaction with the cofactor Extradenticle (Passner *et al*., 1999). This motif, which is towards the N-terminus, is separated from the homeodomain by a linker sequence of 50 amino acids in Ubx 1a in *Drosophila*, an isoform that is primarily responsible for haltere fate (Busturia *et al*., 1990, de Navas *et al*., 2011). Interestingly, while both the homeodomain and the HX motif are conserved across all insect species studied, the length of the linker region is shorter in other species (21 residues in *Apis*, 12 residues in *Tribolium* and 7 residues in *Bombyx*). However, overexpression of Ubx from *Apis* or *Bombyx* or *Tribolium* still could repress wing development and show similar effect on targets as *Drosophila* Ubx. Additional complexity arises from the observations that over-expression of even AbdA (perhaps AbdB), the other Hox proteins of the bithorax complex, can also induce wing-to-haltere transformations (Casares *et al*., 1996). It is possible that differences in the function of *Drosophila* Ubx as against Ubx from other species or against AbdA or AbdB of *Drosophila* could be at the binding affinity, which can be overcome by increased levels of those proteins. That is the reason for the similarity in the phenotypes when those proteins are over-expressed in developing wing discs. We are now evaluating other differences in the protein structures, if any, that might have an implication on the evolution of haltere appendages.

Conclusions

As discussed in this review, it appears that unlike specification of cell types such as neurons, muscles etc, haltere as an organ, although specified by a single gene (i.e. *Ubx*), is a multi-step regulatory process, perhaps reflecting how haltere fate is evolved from a pre-existing wing fate gradually, one step at a time (Fig. 2). We may be able to trace this by identifying targets of Ubx in other insect groups. While Ubx may regulate few growth and patterning events in other insects to modify size and shape of hind-wings, in dipterans it may have gained very large number of targets leading to complete repression of wing fate and resulting in a new organ i.e. the haltere. Signatures of these, perhaps, are elusive due to the fact that regulation of targets of Ubx is not due to the presence or absence of Ubx on cis-regulatory regions of its targets, rather it is due to its interactions with other cofactors. Nevertheless, Ubx binding on a very large number of sites, even while only a fewer genes are effectively regulated, suggest that a Hox protein may rapidly acquire new developmental functions. This is because, its function may change not due to variations in the protein or its binding sites, but due to one of the many variations that can occur around its binding sites. This is reflected in the fact that arthropods have the largest diversity in body plans, and they all have appeared to have occurred rapidly.

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