Physical and Genetic Interactions Link Hox Function with Diverse Transcription Factors and Cell Signaling Proteins*

Sarah E. Bondos‡, Xin-Xing Tan§, and Kathleen S. Matthews

Positional information provided by Hox homeotic transcription factors is integrated with other transcription factors and cell signaling cascades in specific combinations to dictate context- and gene-specific Hox activity. Protein-protein interactions between these groups have long been hypothesized to modulate Hox functions, yielding a context-specific function. However, difficulties in applying interaction screens to potent transcription factors have limited partner identification. A yeast two-hybrid screen using transcription activation-deficient mutants of the *Drosophila melanogaster* Hox protein Ultrabithorax IB identified an array of interacting proteins, consisting primarily of transcription factors and components of cell signaling pathways. Interactions were confirmed with wild-type Ultrabithorax (UBX) in phage display experiments and by immunoprecipitation for a subset of partners. *In vivo* assays demonstrated that two Ultrabithorax IB partners, Armadillo, regulated by Wingless/WNT signaling, and the homeodomain protein Aristaless, inhibit UBX-dependent haltere development from the default wing development pathway. Therefore, transcription factors and cell signaling proteins that subdivide Hox-specified tissues can both alter Hox function *in vivo* and interact with the corresponding Hox protein *in vitro*. UBX may also modulate partner function: the pupal death phenotype induced by ectopic expression of the UBX partner Hairy required the presence of UBX. Thus, Hox·transcription factor complexes may integrate a variety of positional cues, generating the specificity and versatility required for context-dependent Hox function. ***Molecular & Cellular Proteomics 5:824–834, 2006.***

During animal development, transcription is regulated by a surprisingly limited number of proteins. An individual transcription factor may therefore be used in multiple tissues to regulate distinct sets of genes in a spatiotemporally specific manner. Understanding how cellular context regulates transcription factor activity is, therefore, of central importance in developmental biology and related fields. This problem is exemplified by the Hox protein family, which regulates transcription to instigate unique developmental programs in all tissue layers along the anterior/posterior axis of bilaterally symmetric animals (1–3). Alterations in Hox function are therefore also important for diversifying morphological features in development and evolution (4–8). Misexpression of Hox proteins results in dramatic alterations in body plan, appendage formation, and cell-type specification, underscoring the absolute requirement for accurate regulation of Hox function *in vivo* (2, 3, 9–11).

In a simple model describing Hox function, each Hox protein interacts with a specific regulatory sequence via its DNA-binding homeodomain to control transcription of target genes (1, 3, 12, 13). However, Hox proteins target different genes in different tissues and may even activate a gene in one context and repress it in another (1, 12, 14). Hox homeodomains tolerate significant variation in DNA sequence with little change in affinity *in vitro* (1, 2). Furthermore the same Hox protein can utilize different amino acids to bind distinct DNA target sites (15). Thus, DNA binding by an isolated Hox homeodomain does not provide the specificity necessary for context-specific gene regulation *in vivo*, a conundrum termed the "Hox paradox."

These data suggest a more complex model in which cofactors contribute to the functional specificity of Hox proteins (1, 5, 16). Interaction with the three-amino acid loop extension (TALE)[1] homeodomain protein Extradenticle (EXD in *Drosophila*, PBX (pre-B-cell leukemia transcription factor) in mammals) via the YPWM motif alters the selection of DNA binding sites and, in specific cases, may change the mode of transcription regulation (3, 17). Nuclear localization of EXD is regulated by interaction with another TALE protein, Homothorax, which can form a multiprotein complex with EXD and Hox proteins (18). Although Hox-TALE interactions are crucial for regulation of many genes, the number of TALE proteins is limited, and these factors interact promiscuously with most Hox proteins. Hox regulation of some gene targets is independent of EXD

From the Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005

Received, August 9, 2005, and in revised form, January 31, 2006
Published, MCP Papers in Press, February 2, 2006, DOI 10.1074/mcp.M500256-MCP200

[1] The abbreviations used are: TALE, three-amino acid loop extension; AL, Aristaless; APT, Apontic; ARM, Armadillo; BRD, Bearded; CBP80, cap-binding protein 80; CYCK, Cyclin K; DIP1, Disconnected-interacting protein 1; DSH, Dishevelled; EXD, Extradenticle; GLYP, glycogen phosphorylase; HSC70-4, heat shock protein cognate 4; NMO, nemo; OTU, Ovarian Tumor; SMOX, SMAD on X; TF, transcription factor; UBX, Ultrabithorax; YEMα, Yemanuclein α; ds, double-stranded; D/V, dorsal/ventral.

activity (19–22). Thus, this class of partners alone does not provide sufficient versatility to direct and differentiate all Hox functions observed *in vivo* (1, 23, 24). Few other interactions involving Hox proteins have been discovered (1, 24), and the detailed mechanism of spatiotemporal regulation remains unsolved.

The postulated need for heterologous transcription factors is supported by many experiments *in vivo*. Although a Hox protein may be expressed in a broad stripe of cells, the transcriptional and, ultimately, phenotypic cellular response may be cell-specific (14, 25). The context dependence of this response suggests that Hox activity varies within its expression domain. DNA sequences distinct from Hox binding sites have been shown to alter DNA binding by Hox proteins *in vivo*, suggesting cofactors may dictate Hox activity (23). Indeed *Drosophila* screens have detected a variety of genes that can modify the function of Hox proteins *in vivo* (26–29). In an extreme case, addition and deletion of protein interaction motifs has functionally evolved Fushi Tarazu (FTZ) from a Hox homeotic gene to a segmentation gene in *Drosophila* (30). Direct interactions between Hox proteins and proteins providing contextual information would provide a molecular mechanism for these *in vivo* data as well as resolve the Hox DNA binding specificity paradox.

Despite the hypothesized importance of Hox interactions with other proteins, few partners have been identified. One major obstacle is the difficulty in using Hox proteins in standard yeast two-hybrid screens. When fused to a DNA-binding domain, the endogenous activation domain constitutively activates transcription, whereas fusion of the Hox protein to a strong activation domain in the reverse chimera is toxic (7, 24). To circumvent these problems, yeast two-hybrid screens were conducted using an activation-deficient mutant of the *Drosophila melanogaster* Hox protein Ultrabithorax (UBX) (7). UBX promotes the formation of structures specific to the posterior thorax and a portion of the first abdominal segment, including formation of halteres, small balancing organs used during flight, from the default wing developmental pathway (2). The UBX IB isoform was selected because it contains all potential exons (31) and therefore all possible protein-interacting regions. These mutants enabled us to identify 30 known proteins that physically interact with UBX. All but one of these proteins also bound wild-type UBX in phage display assays in which we utilized the well characterized UBX-EXD interaction as a positive control. The majority of these proteins are involved in transcription regulation, nucleic acid binding, or cell signaling, similar to results from genetic interaction screens for Hox proteins (26, 29, 32). All partners have overlapping expression patterns with UBX. Three of eight of the tested transcription regulators were found to inhibit UBX activity in promoting the wing to haltere transformation *in vivo*, whereas one interaction potentiated partner activity. The preference of UBX for interaction with proteins involved in transcription regulation and cell signaling supports the hypothesis that different combinations of protein-protein interactions direct and diversify Hox function *in vivo*, allowing context-specific function.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screen—The Matchmaker LexA two-hybrid system (Clontech) was used with *Saccharomyces cerevisiae* EGY48 (MATα *ura*3 *his*3 *trp*1 $LexA_{op(x6)}$ LEU2) as described previously (24). Yeast dropout media were prepared using Clontech dropout supplement in amounts recommended by Clontech, 1.7 g/liter yeast nitrogen base without amino acids or ammonium sulfate (Difco), 20 g/liter galactose, 10 g/liter raffinose, and 5 g/liter ammonium sulfate. Liquid medium was autoclaved and used once cooled. For solid media, 20 g/liter agar was also included. Once the solid medium was autoclaved, 100 ml of 0.7 M potassium phosphate buffer, pH = 7.0, and 0.8 ml of 50 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) (Promega) were added once cooled to roughly 50 °C. Liquid dropout media (5 ml) were inoculated with a single colony from a fresh plate of EGY48 yeast harboring the reporter plasmid and grown to an optical density of 1.5 at 600 nm for subsequent transformations. The pLexA-Ubx plasmid produces a fusion between LEXA DNA-binding domain and A223P/A226P/A234D UBX, which does not activate transcription when 500 μg of DNA are transformed into culture of EGY48 yeast carrying the reporter plasmid p8op-lacZ gene (7). Two cDNA libraries, a 0–12-h *D. melanogaster* embryonic cDNA library (33) or a 0–21-h embryonic cDNA library (Clontech), fused to DNA encoding the B42 activation domain were transformed into EGY48 carrying plasmids p8op-lacZ and pLexA-Ubx. Neither UBX mutant interacted directly with the B42 activation domain. Plasmids purified from positive colonies were retransformed into yeast to confirm interaction. Specificity for UBX was gauged by measuring interactions between partner proteins with LEXA and a LEXA-Lamin C chimera.

Phage Display—The T7 Select phage display system (Novagen) was used as described previously (24). Available cDNAs for 23 potential partners were cloned into the T7 Select1-1b plasmid to create protein fusions to the C terminus of the 10B capsid protein. The encoded proteins were displayed on the surface of phage particles, and product phage were panned using biotinylated UBX immobilized on ELISA plates. The percent retention of partner-expressing phage in phage display experiments indicates the relative strength of the interaction. Positive controls using the well characterized UBX-EXD interaction had a 76% retention, and less than 10% of phage were retained in negative controls in which either no partner was expressed or the phage were biopanned in the absence of UBX.

GST Pull-down Assays—Eight full-length UBX partners (Aristaless (AL), Armadillo (ARM), cap-binding protein 80 (CBP80), Cyclin K (CYCK), Hairy, Nemo (NMO), RAD23, and SMAD on X (SMOX)) in addition to Exd as a positive control were produced and labeled with [^{35}S]methionine by *in vitro* transcription/translation (Promega). Partners were mixed with *Escherichia coli* protein extracts containing glutathione *S*-transferase-tagged UBX, and the resulting complexes were precipitated with glutathione resin. EXD and all assayed partners co-precipitated with UBX. No significant signal was observed in negative controls containing either no partner or no UBX. Therefore, partners do not interact directly with the glutathione resin, and other proteins contained within the *in vitro* transcription/translation reactions are not generating false positives in these assays. The GST pull-down experiments provide further evidence that UBX can specifically recognize and bind the selected partner proteins against a background of unrelated proteins, an indication of specific protein interactions.

Genetic Interactions and Scanning Electron Microscopy—Virgins homozygous for either the MS1096-Gal4 element, which drives *Gal4*

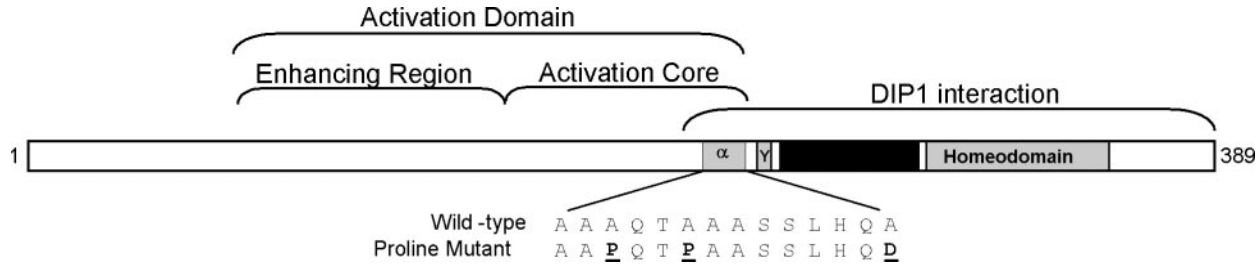

FIG. 1. **Schematics of wild-type UBX and the transcription activation-deficient mutant utilized in yeast two-hybrid screens.** The DNA-binding homeodomain (amino acids 295–354) is located C-terminal to three optional exons, all present in the UBX IB isoform (listing from N to C termini, b, mI, mII, shown as *striped boxes*). Between the activation domain and the optional exons is the YPWM motif (*Y*), a reverse turn that binds to the Extradenticle homeodomain. The interaction domain for binding DIP1, a dsRNA-binding protein, is indicated (24). The 389-amino acid UBX protein contains an activation domain, which is further subdivided into the activation core domain (amino acids 159–242) and the activation enhancing region (amino acids 68–158). The sequence for the presumptive α-helix (α, amino acids 221–234) is shown and compared with the same region of UBX A223P/A226P/A234D in which the destruction of the predicted helix abrogates transcription activation.

expression on the dorsal half of T2 and T3 imaginal discs (32), or homozygous for both MS1096-Gal4 and the Ubx^{bx-34e} gypsy insertion were mated to either wild-type flies or flies homozygous for a UAS-partner genetic element. All fly lines harboring UAS-partner constructs have been published previously (34–41). Because the MS1096-Gal4 insertion is on the X chromosome, only female progeny were analyzed to avoid effects caused by a hemizygous *versus* heterozygous element. All *Drosophila* experiments were replicated at least three times.

To prepare flies for scanning electron microscopy, adult female progeny were sacrificed by overexposure to ether and chemically dried by 12-h exposures to 50, 75, and 100% ethanol solutions in water followed by 1-h incubations with ethanol in 25, 50, 75, and 100% hexamethyldisilazane. The final hexamethyldisilazane wash was removed by evaporation, and flies were affixed to scanning electron microscopy stubs using double sided carbon tape. Stubs were coated in gold for 1 min at 100 mA and examined using a FEI-XL30 environmental scanning electron microscope. Flies only expressing partner and flies only heterozygous for the Ubx^{bx-34e} gypsy insertion served as negative controls.

Parent flies for the *Hairy* and *Ubx* genetic interaction experiments were incubated at 22, 26, 28, or 30 °C for 2 days to produce progeny, and then the adults were removed from the vials. Progeny were incubated at their respective temperatures for a further 12 days at which point all progeny had either hatched or died. The number of flies that survived to adulthood was assessed by counting by the number of empty pupa cases.

RESULTS

A major obstacle to elucidating Hox-protein interactions is the difficulty in using yeast two-hybrid screens due to cell toxicity or constitutive reporter gene activation by a Hox activation domain (24). The activation domain of the *D. melanogaster* Hox protein UBX consists of the requisite activation core domain and the enhancing region, which boosts the strength of activation by the core (Fig. 1) (7). Within the core is a putative α-helix whose structure, but not tertiary interactions, is necessary for transcription activation (7). An activation-deficient mutant was utilized to identify UBX partners by yeast two-hybrid screens. The point mutations A223P/A226P/A234D abrogate secondary structure in the predicted helix within the UBX activation domain (Fig. 1). This triple mutant, like other proline mutants tested previously (7), cannot activate transcription. With this design, ~100,000 transformants from 0–12-h (33) and 0–21-h *D. melanogaster* embryonic cDNA libraries were screened, yielding 32 known genes (Table I). Of these, only two gene products, Bearded (BRD) and YEMα, interacted directly with the yeast two-hybrid DNA-binding domain LEXA and also with the negative control LEXA-Lamin C chimera and are therefore interpreted as false positives (Table I).

Phage display experiments assessed the remaining interactions using *wild-type* UBX (Table I). Results for partner interactions were compared with the well characterized UBX-EXD interaction, controlling for both phage capacity and UBX IB activity (Table I). Available cDNAs for 23 potential partners were used to display these proteins on the surface of phage particles, and product phage were panned using biotinylated UBX IB immobilized on ELISA plates. Negative controls utilizing phage with no UBX IB or phage not expressing partner protein yielded a background of less than 10% phage retention. One yeast two-hybrid positive, glycogen phosphorylase (GLYP), could not interact with UBX in the phage display assay. ARM, CBP80, and TFIIEβ all interact, although the percent retention is lower than that observed for EXD. Phage retention comparable to EXD was observed for all of the remaining partner proteins, confirming their interaction with UBX. A second series of experiments, utilizing plates coated with purified untagged UBX IB, yielded similar results. Therefore the partner proteins are interacting with UBX IB and not the biotin tag.

A third demonstration of direct interaction with UBX was provided by GST pull-down experiments for ARM, SMOX (dSMAD2), RAD23, CYCK, NMO, Hairy, AL, and CBP80 with EXD as a positive control. Radiolabeled partner proteins were generated by *in vitro* transcription/translation, although ARM and CBP80, large proteins greater than 87 kDa, both resulted in a low yield. Radiolabeled partners precipitate if they interact with a glutathione *S*-transferase-UBX chimera, which in turn is bound to glutathione-Sepharose resin. Negative controls contain cell lysate expressing GST in lieu of GST-UBX and will

TABLE I

Interaction and expression data for potential UBX partners

Activation-deficient UBX mutants alone did not interact with the B42 activation domain in the yeast two-hybrid screen (7, 24). Interaction of BRD and YEMα with LEXA and LEXA-Lamin C indicates that these proteins are false positives. A percent retention comparable to EXD (76%) in phage display experiment independently verifies the protein interactions in a manner that controls for phage capacity and UBX activity. False positives are shown in bold text. Because UBX protein is produced in all tissue layers and throughout development, no partners could be eliminated by expression analysis. Expression data were extracted from FlyBase (www.flybase.org). EF2B, elongation factor 2B; NRT, Neurotactin; P120CTN, adherens junction protein p120; RPL22, ribosomal protein L22; RPN6, protease p44.5 subunit; RPS13, ribosomal protein S13; TRN, tartan; ZF30C, zinc finger protein 30C.

Protein	Gene accession number	Interaction with		Phage display[b]	Expression overlap[c]		Nuclear localization[a,d]
		LEXA[a]	LEXA-Lamin C		Tissue	Stage	
				%			
14-3-3ϵ	U84897	N	N	72	CNS, M, Ect	E	C
AL	L08401	N	N	70	ID	E, L	Y
ALY	AF172637	N	N	N/A	N/A	N/A	Y
APT	AF050379	N	N	72	CNS	E, L	Y
ARM	X54468	N	N	65	CNS, ID, M, Ect	E, L	C
BRD	U13067	**Y**	**Y**	93			
CBP80	AJ238970	N	N	65	N/A	N/A	Y
CYCK	AF060516	N	N	76	N/A	N/A	Y
DIP1[e]	NM167772	N	N	79	CNS, ID	E, L	Y
DSH	L26974	N	N	80	CNS, ID	E, L	N
EF1γ	AF148813	N	N	80	U	U	N
EF2B	AE003781	N	N	80	U	U	N
GLYP	**AF073179**	N	N	**0**			
Hairy	X15905	N	N	72	Ect	E	Y
HSC70-4	L01500	N	N	78	U	U	Y
DMI-2	AF119716	N	N	77	CNS	E	Y
Motor protein	AF145623	N	N	N/A	N/A	N/A	U
NMO	AI124322	N	N	N/A	ID	L	U
NOC	L14009	N	N	74	CNS, Ect	E	Y
NRT	AF132188	N	N	N/A	M, ID	E, L, P	N
OTU	X13693	N	N	71	N/A	N/A	N
P120CTN	AF220496	N	N	N/A	N/A	N/A	N
RAD23	AF132147	N	N	74	U	U	Y
RPL22	U42578	N	N	81	U	U	C
RPN6	AF160939	N	N	N/A	N/A	N/A	Y
RPS13	X91853	N	N	82	U	U	C
SMOX	AF078529	N	N	77	CNS, M, ID	E, L	C
TFIIEβ	U72893	N	N	54	U	U	Y
TRN	I02078	N	N	N/A	CNS, M, ID	E, L, P	N
ZF30C	AF035275	N	N	N/A	N/A	N/A	Y
ZN72D	U73125	N	N	N/A	N/A	N/A	Y
YEMα	X63503	**Y**	**Y**	76			

[a] Y = yes; N = no.

[b] N/A, not available.

[c] CNS, central nervous system; Ect, ectoderm, ID, imaginal disc; M, mesoderm, U, ubiquitous; E, embryo; L, larva; P, pupa.

[d] C, conditional.

[e] Reported previously (24).

yield a positive result if the partner forms insoluble protein aggregates, binds to GST, or binds to the Sepharose resin. All partners tested precipitated more effectively with UBX than in negative controls, again confirming partner interaction with UBX IB (Fig. 2). In addition, another partner, Disconnected-interacting protein 1 (DIP1), has been shown previously to interact with UBX in GST pull-down experiments (24).

Parsing the UBX partners by expression patterns can evaluate potential for realistic *in vivo* significance. The putative partner and UBX must be expressed in the same tissue and at the same developmental time to interact (Table I). Partners that modulate UBX function might be expected to localize in the nucleus, although interactions in the cytoplasm may regulate UBX subcellular localization, protein production, or degradation. Unfortunately the broad spatiotemporal expression of UBX overlaps with all known partner expression patterns, preventing elimination of any UBX partners by expression pattern analysis.

The goal of the protein interaction screen was to identify proteins that could modify Hox function *in vivo*. Several partners that function as transcription factors were tested to determine whether they could inhibit UBX function during *Drosophila* haltere development. Haltere formation is instigated by UBX and reflects *in vivo* UBX activity (2, 26, 32). In

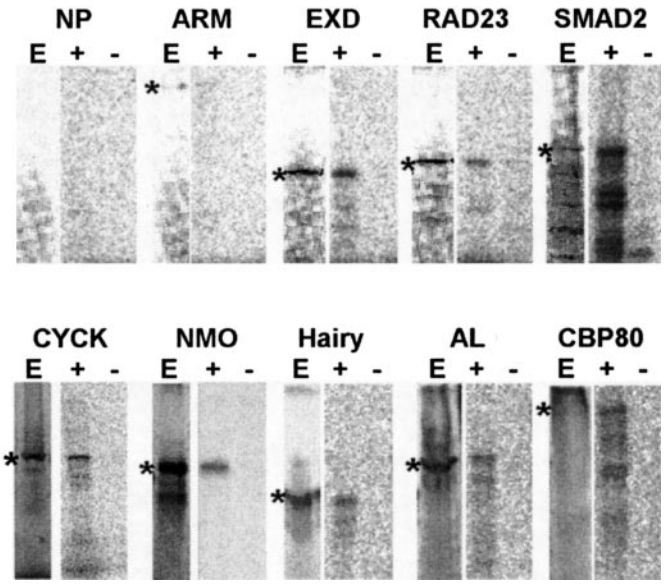

FIG. 2. **GST-UBX precipitation of partners.** ^{35}S-Labeled partner proteins were generated by *in vitro* transcription/translation (lanes marked *E*). In the no protein (*NP*) reactions, no DNA was added to the *in vitro* transcription/translation reactions. Full-length protein bands are indicated by *asterisks*. In the GST pull-down experiments, positive reactions for each partner (+) included both GST-UBX and partner protein, while the corresponding negative reactions (−) included the partner but GST instead of GST-UBX cell extracts. In each case, partner precipitated more effectively in the presence of GST-UBX, confirming the interactions.

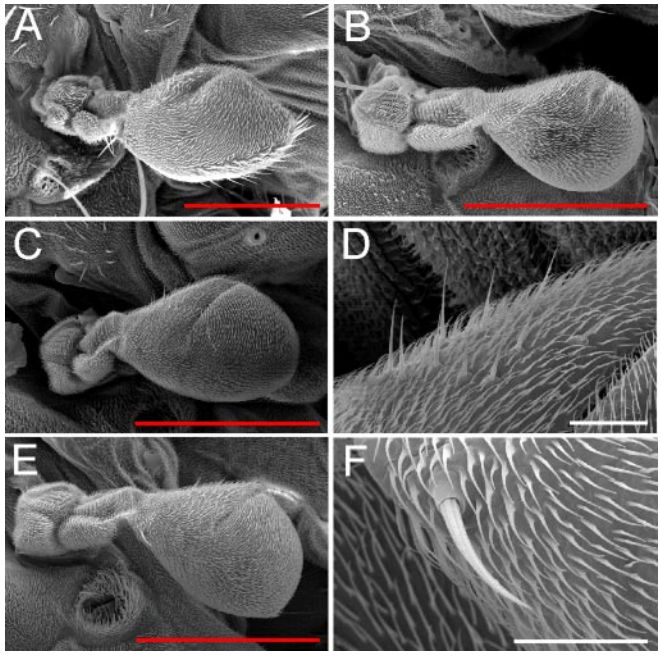

FIG. 3. **Genetic interaction of *Ubx* and *Al*.** UBX alters the default wing developmental pathway to generate halteres, the balancing organs on the third thoracic segment. Ubx$^{bx\text{-}34e}$ *homozygotes* have enlarged halteres with three rows of bristles on the D/V boundary (*A*) compared with a normal phenotype in Ubx$^{bx\text{-}34e}$ *heterozygotes* (*B*). In wild-type flies, ectopic expression of AL increases the number of capitellar sensilla (*C* and *D*), whereas expression of AL in Ubx$^{bx\text{-}34e}$ heterozygotes causes up to three winglike bristles to form along the D/V axis near the base of the capitellum (*E* and *F*). *Red bars* = 200 μm; *white bars* = 20 μm.

the absence of UBX, the haltere develops into a wing (42). Our analysis exploited a gypsy insertion line, Ubx$^{bx\text{-}34e}$, that decreases the concentration of UBX protein in the haltere in a temperature-dependent manner (43). Heterozygotes for the Ubx$^{bx\text{-}34e}$ lesion have a normal haltere phenotype but reduced UBX concentrations, and thus the halteres are poised to be more easily transformed into a winglike phenotype (Fig. 3). If a given partner inhibits UBX function, ectopic expression of partner proteins (34–41) will drive the haltere to wing transformation in Ubx$^{bx\text{-}34e}$ heterozygotes (24). Indeed decreased UBX expression combined with ectopic AL expression generates one to three large haltere bristles along the dorsoventral axis in 35% of the progeny. These bristles are morphologically similar to those found at the corresponding position on the wing blade margin (edge) (Fig. 3). Inhibition of UBX by the homeodomain protein AL via direct protein-protein interactions may therefore mirror interaction of the Hox protein Antennapedia with the homeodomain protein Eyeless, which also results in Hox inhibition (44).

The phenotype for the UBX-ARM genetic interaction pushes the haltere even further toward the wing morphology (Fig. 4). ARM (β-catenin in vertebrates) is a transcription coactivator regulated by Wingless/WNT signaling (1). When ARM is ectopically expressed, 51% of the halteres appeared to be wild type, and the remaining halteres were enlarged with one to three large ectopic bristles at the base of the capitellum. In the decreased UBX background, 48% of flies overexpressing Armadillo developed ectopic wings nearly 4 times as long and 3 times as wide as a haltere although smaller than a wild-type wing. Fine details of wing patterning and morphology, including wing veins, were maintained in the ectopic wing (Fig. 4). The different morphologies found in the triplet row of bristles along the D/V axis were also preserved in the ectopic wings. This homeotic transformation demonstrates that ARM, like AL, counters UBX function in the developing haltere.

A genetic interaction between *Ubx* and *Hairy* is observable at elevated temperatures at which the ectopic expression of the partner protein increases (45) and the expression of UBX in flies harboring the Ubx$^{bx\text{-}34e}$ genetic element further decreases (43). At room temperature, Hairy overexpression generated shriveled wings with large ectopic bristles (Fig. 5). When flies overexpressing Hairy in a wild-type UBX background were grown at 30 °C, 87% died as pupa (n = 229 for three crosses). Reducing UBX concentration with the heterozygous Ubx$^{bx\text{-}34e}$ element partially rescued the lethal Hairy overexpression phenotype: only 53% died as pupa (n = 227 for three crosses), representing a 3.6-fold increase in survival. The extent of Hairy lethality and rescue by loss of UBX is dependent on temperature, which, in turn, controls the rela-

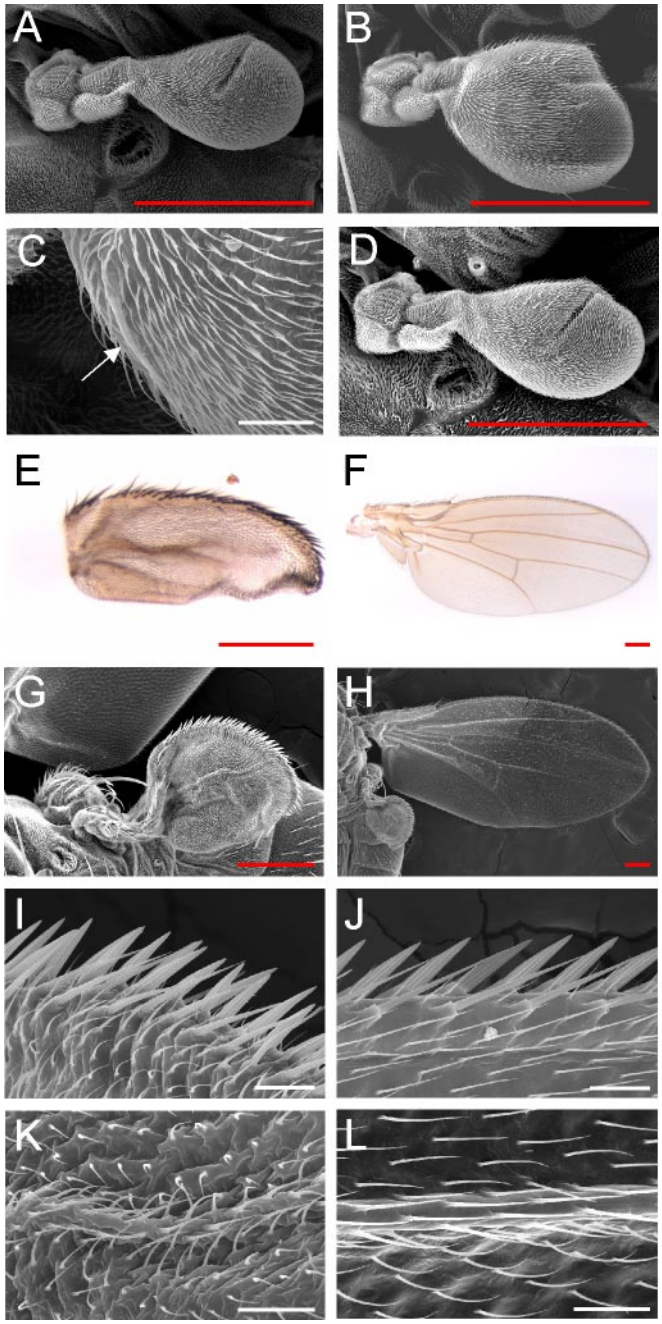

FIG. 4. **Genetic interaction of UBX and ARM.** Ectopic expression of ARM can generate a mixture of flies with normal halteres (*A*) and slightly enlarged halteres with winglike bristles along the D/V axis (*arrow*) (*B* and *C*). $Ubx^{bx\text{-}34e}$ heterozygotes with ectopic arm expression may either have normal halteres (*D*) or a transformed haltere (*E* and *G*) that resembles a wing (*F* and *H*). Bristle placement and morphology are comparable in the ectopic miniwings (*I*) and the normal wing (*J*). Likewise veins are present in both the miniwing (*K*) and the normal wing (*L*). *Red bars* = 200 μm; *white bars* = 20 μm.

tive concentrations of UBX and Hairy (Table II). Therefore, UBX contributes to the *Hairy*-mediated lethal phenotype.

No genetic interactions were detected for SMOX, Apontic (APT), and HSC70-4. One possibility is that these proteins

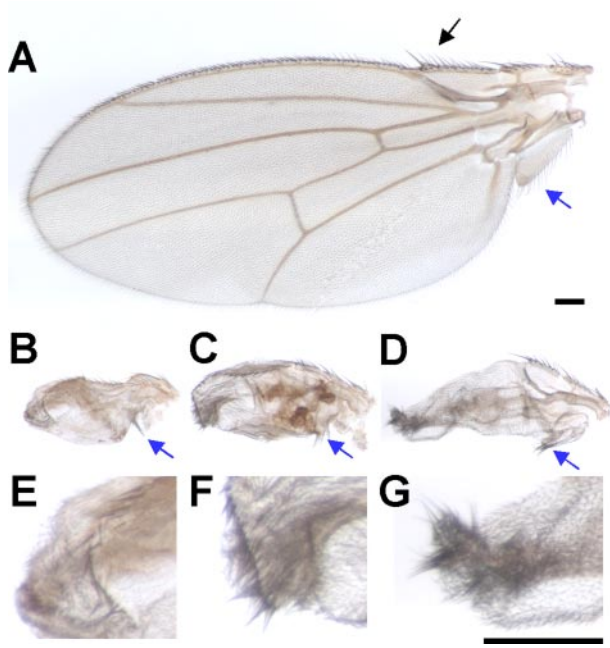

FIG. 5. **Ectopic expression of UBX, Hairy, and DIP1 result in similar phenotypes.** In contrast to a wild-type wing (*A*), ectopic expression of UBX (*B* and *E*), Hairy (*C* and *F*), and DIP1 (*D* and *G*) results in shriveled wings with additional large bristles. The anterior proximal region of the wing margin on a wild-type wing has long, dark bristles (*black arrow* in *A*), whereas the posterior proximal region (*blue arrow* in *A*) and distal tip have relatively shorter, thinner bristles. Overexpression of all three proteins using the MS1096-Gal4 driver generates large, dark bristles on the posterior proximal edge of the wing (*blue arrows* in *B–D*). Additional ectopic large bristles are found at the distal dip of wing expressing Hairy (*F*) and DIP1 (*G*) and to a lesser extent UBX (*E*).

specifically interact with the UBX IB isoform, which is not produced in the wing or haltere imaginal discs (46). These partners may contribute to isoform-specific functions *in vivo*. In addition, the assay detects an interaction that opposes UBX function, missing partners that enhance function. Interaction may also require a particular cellular context or the availability of specific DNA targets. In support of this hypothesis, genetic interactions were established only for partners that also exhibit a wing or haltere phenotype when ectopically expressed in a wild-type background. For some proteins, high endogenous expression levels may prevent detection of genetic interactions relying on ectopic partner expression.

The original protein interaction experiments utilized the UBX IB isoform to include all potential UBX coding regions. However, UBX IB is primarily expressed in the embryo (31) before UBX specifies the haltere tissue identity. Identification of genetic interactions for DIP1 (24), AL, ARM, and Hairy indicates these proteins can also interact with other isoforms present later in development.

Intriguingly the phenotypes of flies ectopically expressing UBX, Hairy, or DIP1 via the MS1096-Gal4 driver were similar (Fig. 5). At room temperature, all three phenotypes exhibited

TABLE II

A reduction of UBX protein in $Ubx^{bx\text{-}34e}$ heterozygotes partially rescues lethal phenotypes caused by Hairy overexpression

Data are listed as percent survival. Ectopic expression of partner protein only is indicated by "+," whereas ectopic expression of partner with a reduction in UBX concentration is indicated by "−." This genetic interaction is observed as a function of temperature. For each entry, 100–300 flies obtained from multiple crosses were counted.

	UBX							
	22 °C		26 °C		28 °C		30 °C	
	+	−	+	−	+	−	+	−
Percent survival	99	99	87	86	46	86	13	47

shriveled wings with large ectopic bristles. Flies with increased ectopic expression of Hairy or DIP1 due to elevated temperatures died as pupa or pharate adults. Furthermore ectopic expression of both Hairy and DIP1 in the haltere generated extra bristles. The coincident phenotypes suggest a similar mechanism of action for the three proteins in this context. Hairy is a transcription repressor protein (47), and UBX represses all of its known targets when expressed in the wing. Although the *in vivo* function of DIP1 is unknown, the presence of DIP1 specifically inhibits UBX-mediated transcription activation in yeast cell culture (24). Furthermore DIP1 ectopic expression in the eye-antennal imaginal disc alters regulation of enhancers for the *homothorax*, *spalt major*, and *distal-less* genes (36). Taken together, these data support a role for the UBX partner DIP1 in transcription repression.

DISCUSSION

The inherent lack of DNA binding specificity allows the function of Hox transcription factors to be modulated in a spatiotemporally specific manner (1, 12). Because Hox DNA binding sites are often interspersed with binding sites for other transcription factors (14, 20), complex formation with other transcription regulators is one likely mechanism to provide guiding contextual information. This hypothesis allows Hox proteins and other transcription factors to relay and integrate positional information, thus implementing corresponding spatially, temporally, and gene-specific changes in expression (26, 29, 32, 48). However, verification of this theory has been hampered by the molecular functions of Hox proteins that impede their use in traditional two-hybrid screens (24). Here we circumvented these problems by utilizing a mutant of the Hox protein UBX IB, which is deficient in transcription activation (7).

Confirmation of the UBX IB-partner interactions with complementary approaches demonstrates unequivocally that the interactions are not an artifact of UBX mutation, UBX fusion, or the presence of a bridging factor. Although a UBX IB mutant was necessary for the initial two-hybrid screen, 22 of the 23 assayed partners interacted with wild-type UBX IB in the phage display assay, and all partner proteins tested in GST pull-down experiments bound wild-type protein. Yeast two-hybrid and GST pull-down experiments required UBX IB chimeras, whereas the phage display assays utilized native, wild-type UBX IB. Therefore, the interactions did not depend on mutation or possible structural alterations of UBX IB due to fusion with another polypeptide. Interactions in the phage display system demonstrated that a bridging factor from yeast or *E. coli* is not required. The ability to form interactions in the presence of competing proteins in the yeast two-hybrid and GST pull-down assays combined with the lack of nonspecific binding to LEXA or LEXA-Lamin C indicated that the interactions are specific for UBX IB. Yeast two-hybrid experiments demonstrated that these interactions can occur within a living cell. Furthermore *in vivo* AL, ARM, and DIP1 inhibited UBX function, whereas UBX was required for the Hairy ectopic expression phenotype. Given the biochemical data, the most probable explanation for these genetic interactions is that UBX can also bind these partners in a living fly.

The identified UBX partners can be classified into five major functional categories: most are known to act in DNA binding/transcription regulation and cell signaling/cell cycle regulation, but others have functions of RNA binding/processing, DNA repair, and translation regulation (Table III). In general, few co-activators and co-repressors that mediate Hox function are known. However, the *Drosophila* Hox proteins Antennapedia and Abdominal-B both interact with the β-subunit of TFIIE (49). Human HOXA10 binds histone deacetylase 2 (21), and binding by human HOXB6 deactivates cAMP-response element-binding protein (CREB)-binding protein, a histone acetyltransferase (22). UBX also interacts with APT, a co-activator (50), and the co-repressor DMI-2, which functions as a histone deacetylase protein (51). ARM, a specific transcription regulator, and DMI-2 regulate gene targets in common with UBX (11, 51, 52), providing further opportunity for modulation of UBX function *in vivo*. Indeed *Apt* genetically interacts with the Hox genes *Deformed* and *Sex Combs Reduced* and has therefore been postulated to be a Hox cofactor (27). Together with our results, these data suggest that physical interactions with APT may be a function shared by many Hox proteins. These interactions provide significant clues to the mechanisms by which Hox proteins may implement context-specific changes in transcription regulation.

Several major signaling pathways (bone morphogenetic protein/transforming growth factor-β/activin, Ras, Wingless (WNT), and Notch/lin-12) modify Hox function *in vivo* and also subdivide UBX-specified tissue (26, 29, 32, 48, 53). However, the mechanism of action has been elusive. In some cases, a

TABLE III

Functions and gene ontology of potential UBX protein partners (www.flybase.org)

Some proteins have many functions and are thus listed multiple times. The functions of proteins not included in this table are unknown. EF2B, elongation factor 2B; RPL22, ribosomal protein L22; RPN6, protease p44.5 subunit; RPS13, ribosomal protein S13; TRN, tartan; ZF30C, zinc finger protein 30C; Pol, polymerase; TGF, transforming growth factor.

Functional class	Specific function	Gene ontology no.
Transcription regulation		
14-3-3ϵ	Transcription regulator	0030528
AL	RNA Pol II transcription factor	0003704
ALY	Transcription co-activator	0006352
APT	Transcription regulator	0003702
ARM	Transcription co-activator	0003713
CYCK	Transcription regulator	0030528
Hairy	Transcription repressor	0016565
DMI-2	Transcription repressor	0016564
NOC	Transcription regulator	0003702
SMOX	Transcription regulator	0030528
TFIIEβ	General RNA Pol II transcription factor	0016251
ZF30C	Transcription factor	0003700
DNA binding		
DIP1[a]	Chromatin binding	0003682
DMI-2	DNA helicase	0004003
RAD23	Damaged DNA binding	0003684
Signal transduction		
14-3-3ϵ	Protein kinase C inhibitor	0004863
ARM	WNT receptor binding	0016055
CYCK	Protein kinase activator	0019209
DSH	Notch and WNT signaling	0005112
NMO	Serine/threonine kinase	0004674
SMOX	TGF-β cytoplasmic mediator	0005072
Nucleotide phosphatases		
HSC70-4	ATPase	0016887
MI-2	ATPase	0016887
RNA binding/processing		
ALY	RNA-binding protein	0003723
APT	RNA binding	0003723
CBP80	pre-mRNA splicing factor	0008248
DIP1[a]	dsRNA binding	0003725
ZN72D	RNA binding	0003723
Nucleic acid binding		
RPL22	Nucleic acid binding	0003676
RPS13	Nucleic acid binding	0003676
Translation		
EF2B	Translation elongation factor	0003746
RPL22	Ribosome component	0003735
RPS13	Ribosome component	0003735
Chaperone		
ALY	Chaperone activity	0003754
HSC70-4	Chaperone activity	0003754
Other		
ARM	Cytoskeleton component	0005200
RPN6	Endopeptidase	0004175
TRN	Structural molecule	0005198

[a] Previously identified UBX-interacting protein (24).

single short enhancer synergistically implements homeotic and cell signaling inputs (54, 55). Intriguingly a subset of partners (SMOX, 14-3-3ϵ, ARM, Dishevelled (DSH), CYCK, and NMO) are regulated by these same cell signaling cascades. The identified partnerships provide evidence that direct protein-protein interactions impact transcription regulation by Hox proteins as a function of cell position. Indeed Hox-Smad interactions have been identified previously in humans where they influence both DNA binding and transcription regulation by Hox proteins (56–59). Our identification of a

UBX-SMOX interaction suggests that Hox·Smad complexes are evolutionarily conserved. Intriguingly Smad and Hox binding sites are interspersed in the *Spalt* and *Wingless* enhancers (14, 20), providing a potential locus for regulation via *Drosophila* Hox·Smad complexes.

The prevalence of transcription factors and cell signaling proteins selected by UBX also mimics results from genetic screens for genes that modify Hox function (26, 29, 32, 48). Indeed whereas less than 6% of all *Drosophila* genes with known functions regulate transcription (based on data obtained from FlyBase, www.flybase.org), 41% of UBX partners are transcription factors, demonstrating a distinct preference of UBX for this functional group. The similarity of results for protein interaction and functional modification screens supports the hypothesis that Hox proteins integrate information from different transcription regulatory pathways via protein interactions.

A few UBX interactions of note occur with proteins in other functional groups. Association with one RNA-binding/processing protein, DIP1, impedes UBX transcription activation without abrogating DNA binding (24). UBX amino acids 216–389 are required for DIP1 interaction; this region includes portions of the activation and repression domains (Fig. 1) (7, 24), implying direct interactions with regulatory domains may impede or alter their function. The similarity of ectopic expression phenotypes between DIP1 and Hairy suggests that DIP1, although it binds dsRNA, may function *in vivo* to repress transcription. Other UBX partners involved in RNA binding/processing are Ovarian Tumor (OTU) and CBP80. An increasing number of studies demonstrate interactions and functional overlap between specific transcription factors and RNA-binding proteins (60, 61). Indeed the RNA-binding protein Split Ends modulates function of the Hox protein Deformed and is required for thoracic specification by another Hox protein, Antennapedia (27, 62). Like DIP1, Split Ends is involved in transcription repression, spatial organization, and patterning (62–64).

Two partners are prominent participants in DNA repair: RAD23 and ribosomal protein L22. Physical interactions have been discovered between transcription factors and DNA repair proteins (65). In fact, TFIIH and subunits of TFIID are involved in both processes (65, 66). RAD23 is associated with the general transcription machinery in *S. cerevisiae* and binds the specific transcription factor Viviparous-1 in rice (67), providing precedent for a UBX IB-RAD23 interaction to modulate transcription in *Drosophila* as well.

Cellular differentiation and growth are coordinated processes during development. Direct interactions between the cell cycle regulator geminin and several Hox proteins have been hypothesized to link control of differentiation and growth in mice (68). Indeed Geminin binding prevents DNA binding by murine HOXB9 (68). These observations are consistent with our identification of CYCK as a UBX partner. Misexpression of Hox proteins also correlates with the progression of many types of cancer (for reviews, see Refs. 69 and 70). Hox interactions with DNA repair proteins or cell cycle-regulating proteins such as Cyclin K may, therefore, provide a mechanism for Hox malfunction in carcinogenesis.

Three proteins involved in translation were also identified. Although the very recent discovery of translation occurring within nuclei (71) raises the possibility of such regulatory interactions, ribosomal proteins often interact with unlikely partners in the yeast two-hybrid system (for example, see Ref. 72). Therefore, these interactions may well be false positives.

In summary, we utilized wild-type and activation-defective mutants of the Hox transcription factor UBX to identify and confirm interactions with many proteins. These complexes include the first physical interactions reported between Hox proteins and factors involved in the WNT, RAS/mitogen-activated protein kinase, and Notch signaling pathways as well as the first Hox-Smad interaction reported in invertebrates. Indeed UBX selects partners with similar functions: nearly half of the UBX-interacting proteins are transcription factors. Furthermore several of the remaining proteins bind nucleic acid or alter transcription regulation and patterning *in vivo* (36, 67). Genetic interactions *in vivo* demonstrate effects on both UBX and partner function. These partnerships provide evidence that combinatorial Hox protein interactions modulate gene transcription in response to positional cues from multiple cellular processes (1, 26, 48). Indeed Hox interactions with heterologous proteins may modulate both DNA binding and transcription regulation (3, 17, 24). Such protein interactions have the potential to functionally differentiate between activities of a single Hox protein in different cellular contexts, different Hox family members in a single organism, or various orthologues of the same Hox protein. Although many mechanisms may contribute to specific Hox function *in vivo*, heterologous protein interactions are expected to be key to resolving the Hox paradox.

Acknowledgments—We thank the Susan, Rauskolb, and Noll laboratories for supplying *Hairy*, *Exd*, and *Al* DNA, respectively. We thank the members of the Matthews laboratory for valuable discussions and experimental suggestions and especially Ying Liu and Michael Shih for technical assistance and Liskin Swint-Kruse and Daniel J. Catanese, Jr. for comments on the manuscript. We thank the following people for supplying flies: UAS-H, David Ish-Horowicz, London Research Institute; UAS-Al, Tetsuya Kojima, University of Tokyo; UAS-Apt, Anne Ephrussi, European Molecular Biology Laboratory; UAS-dSmox; Michael O'Connor, Howard Hughes Medical Institute (HHMI)/University of Minnesota; and UAS-Hsc70-4, Karen Palter, HHMI/University of Pennsylvania.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Biochemistry and Cell Biology, MS140, Rice University, 6100 S. Main St., Houston, TX 77005. Tel.: 713-348-4936; Fax: 713-348-6149; E-mail: bondos@rice.edu.

§ Present address: Cytogenix, Inc., 3100 S. Wilcrest, Suite 140, Houston, TX 77099.

REFERENCES

1. Bondos, S. E., and Tan, X.-X. (2001) Combinatorial transcription regulation: the interaction of transcription factors and cell signaling molecules with homeodomain proteins in *Drosophila* development. *Crit. Rev. Euk. Gene Express.* **11,** 145–171
2. Hughes, C. L., and Kaufman, T. C. (2002) Hox genes and the evolution of the arthropod body plan. *Evol. Dev.* **4,** 459–499
3. Mann, R. S., and Morata, G. (2000) The developmental and molecular biology of genes that subdivide the body of *Drosophila. Annu. Rev. Cell Dev. Biol.* **16,** 243–271
4. Grenier, J. K., and Carroll, S. B. (2000) Functional evolution of the Ultrabithorax protein. *Proc. Natl. Acad. Sci. U. S. A.* **97,** 704–709
5. Mann, R. S., and Affolter, M. (1998) Hox proteins meet more partners. *Curr. Opin. Genet. Dev.* **8,** 423–429
6. Ronshaugen, M., McGinnis, N., and McGinnis, W. (2002) Hox protein mutation and macroevolution of the insect body plan. *Nature* **415,** 914–917
7. Tan, X.-X., Bondos, S., Li, L., and Matthews, K. S. (2002) Transcription activation by Ultrabithorax Ib protein requires a predicted α-helical region. *Biochemistry* **41,** 2774–2785
8. Tomoyasu, Y., Wheeler, S. R., and Denell, R. E. (2005) Ultrabithorax is required for membranous wing identity in the beetle *Tribolium castaneum. Nature* **433,** 643–647
9. Brodu, V., Elstob, P., and Gould, A. P. (2002) *Abdominal A* specifies one cell type in *Drosophila* by regulating one principal target gene. *Development* **129,** 2957–2963
10. Rozowski, M., and Akam, M. (2002) Hox gene control of segment-specific bristle patterns in *Drosophila. Genes Dev.* **16,** 1150–1162
11. Weatherbee, S. D., Halder, G., Kim, J., Hudson, A., and Carroll, S. (1998) Ultrabithorax regulates genes at several levels of the wing-patterning hierarchy to shape the development of the *Drosophila* haltere. *Genes Dev.* **12,** 1474–1482
12. Graba, Y., Aragnol, D., and Pradel, J. (1997) *Drosophila* Hox complex downstream targets and the function of homeotic genes. *BioEssays* **19,** 379–388
13. Hersh, B. M., and Carroll, S. B. (2005) Direct regulation of knot gene expression by Ultrabithorax and the evolution of cis-regulatory elements in *Drosophila. Development* **132,** 1567–1577
14. Grienenberger, A., Merabet, S., Manak, J., Iltis, I., Fabre, A., Bérenger, H., Scott, M. P., Pradel, J., and Graba, Y. (2003) Tgfb signaling acts on a Hox response element to confer specificity and diversity to Hox protein function. *Development* **130,** 5445–5455
15. Remacle, S., Shaw-Jackson, C., Matis, C., Lampe, X., Picard, J., and Rezsöhazy, R. (2002) Changing homeodomain residues 2 and 3 of Hoxa1 alters its activity in a cell-type and enhancer dependent manner. *Nucleic Acids Res.* **30,** 2663–2668
16. Hayashi, S., and Scott, M. P. (1990) What determines the specificity of action of *Drosophila* homeodomain proteins? *Cell* **63,** 883–894
17. Pinsonneault, J., Florence, B., Vaessin, H., and McGinnis, W. (1997) A model for extradenticle function as a switch that changes HOX proteins from repressors to activators. *EMBO J.* **16,** 2032–2042
18. Rieckhof, G. E., Casares, F., Ryoo, H. D., Abu-Shaar, M., and Mann, R. S. (1997) Nuclear translocation of extradenticle requires homothorax, which encodes an extradenticle-related homeodomain protein. *Cell* **91,** 171–183
19. Beslu, N., Krosl, J., Laurin, M., Mayotte, N., Humphries, K. R., and Sauvageau, G. (2004) Molecular interactions involved in HOXB4-induced activation of HSC self-renewal. *Blood* **104,** 2307–2314
20. Galant, R., Walsh, C., and Carroll, S. B. (2002) Hox protein repression of a target gene: extradenticle-independent, additive action through multiple monomer binding sites. *Development* **129,** 3115–3126
21. Lu, Y. F., Goldenberg, I., Bei, L., Andrejic, J., and Eklund, E. A. (2003) Hox A10 represses gene transcription in undifferentiated myeloid cells by interaction with histone deacetylase 2. *J. Biol. Chem.* **278,** 47792–47802
22. Shen, W., Chorbak, D., Krishnan, K., Lawrence, J., and Largman, C. (2004) HOXB6 protein is bound toe CREB-binding protein and represses globin expression in a DNA binding-dependent, PBX interaction-independent process. *J. Biol. Chem.* **279,** 39895–39904
23. Li, X., Veraksa, A., and McGinnis, W. (1999) A sequence motif distinct from Hox binding sites controls the specificity of a Hox response element. *Development* **126,** 5581–5589
24. Bondos, S. E., Catanese, D. J., Jr., Tan, X.-X., Bicknell, A., Li, L., and Matthews, K. S. (2004) Hox transcription factor Ultrabithorax Ib physically and genetically interacts with disconnected interacting protein 1, a double-stranded RNA-binding protein. *J. Biol. Chem.* **275,** 26433–26444
25. Lohman, I., and McGinnis, W. (2002) Hox genes: it's all a matter of context. *Curr. Biol.* **12,** R514–R518
26. Florence, B., and McGinnis, W. (1998) A genetic screen of the *Drosophila* X chromosome for mutations that modify Deformed function. *Genetics* **150,** 1497–1511
27. Gellon, G., Harding, K. W., McGinnis, N., Martin, M. M., and McGinnis, W. (1997) A genetic screen for modifiers of *Deformed* function identifies novel genes required for head development. *Development* **124,** 3321–3331
28. Harding, K. W., Gellon, G., McGinnis, N., and McGinnis, W. (1995) A screen for modifiers of *Deformed* function in *Drosophila. Genetics* **140,** 1339–1352
29. Merabet, S., Catala, F., Pradel, J., and Graba, Y. (2002) A green fluorescent protein reporter genetic screen that identifies modifiers of Hox gene function in the *Drosophila* embryo. *Genetics* **162,** 189–202
30. Löhr, U., and Pick, L. (2005) Cofactor-interaction motifs and the cooption of a homeotic Hox protein into the segmentation pathway of *Drosophila melanogaster. Curr. Biol.* **15,** 643–649
31. López, A. J., Artero, R. D., and Pérez-Alonso, M. (1996) Stage, tissue, and cell specific distribution of alternative Ultrabithorax mRNAs and protein isoforms in the *Drosophila* embryo. *Roux's Arch. Dev. Biol.* **205,** 450–459
32. Boube, M., Benassayag, C., Seroude, L., and Cribbs, D. L. (1997) Ras1-mediated modulation of *Drosophila* homeotic function in cell and segment identity. *Genetics* **146,** 619–628
33. Finley, R. L., Jr., Thomas, B. J., Zipursky, S. L., and Brent, R. (1996) Isolation of *Drosophila* Cyclin D, a protein expressed in the morphogenetic furrow before entry into S phase. *Proc. Natl. Acad. Sci. U. S. A.* **93,** 3011–3015
34. Boyle, M., Bonini, N., and DiNardo, S. (1997) Expression and function of clift in the development of somatic gonadal precursors within the *Drosophila* mesoderm. *Development* **124,** 971–982
35. Brand, A. H., and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fate and generating dominant phenotypes. *Development* **118,** 401–415
36. DeSousa, D., Mukjopadhyay, M., Pelka, P., Zhao, X., Dey, B. K., Robert, V., Pélisson, A., Becheton, A., and Campos, A. R. (2003) A novel double-stranded RNA-binding protein, disco interacting protein 1 (DIP1), contributes to cell fate decisions during *Drosophila* development. *J. Biol. Chem.* **278,** 38040–38050
37. Elefant, F., and Palter, K. B. (1999) Tissue-specific expression of dominant-negative mutant *Drosophila* HSC70 causes developmental defects and lethality. *Mol. Biol. Cell* **10,** 2101–2117
38. Filardo, P., and Ephrussi, A. (2003) Bruno regulates gurken during *Drosophila* oogenesis. *Mech. Dev.* **120,** 289–297
39. Marquez, R. M., Singer, M. A., Takaesu, N. T., Waldrip, W. R., Kraytsberg, Y., and Newfeld, S. J. (2001) Transgenic analysis of the Smad family of TGF-β signal transducers in *Drosophila melanogaster* suggests new roles and new interactions between family members. *Genetics* **157,** 1639–1648
40. Myat, M. M., and Andrew, D. J. (2002) Epithelial tube morphology is determined by the polarized growth and delivery of apical membrane. *Cell* **111,** 879–891
41. Tsuji, T., Sato, A., Hiratani, I., Taira, M., Saigo, K., and Kojima, T. (2000) Requirements of Lim1, a *Drosophila* LIM homeobox gene, for normal leg and antennal development. *Development* **127,** 4315–4323
42. Lewis, E. (1978) A gene complex controlling segmentation in Drosophila. *Nature* **276,** 565–570
43. Martínez-Laborda, A., Serrano-Cartagena, J., and Micol, J. L. (1996) A genetic analysis of bx bxd cis double mutants in the *Drosophila* Ultrabithorax gene. *Mol. Gen. Genet.* **250,** 540–546
44. Plaza, S., Prince, F., Jaeger, J., Kloter, U., Flister, S., Benassayag, C., Cribbs, D., and Gehring, W. J. (2001) Molecular basis for the inhibition of *Drosophila* eye development by *Antennapedia. EMBO J.* **20,** 802–811
45. Duffy, J. B. (2002) GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife. *Genesis* **34,** 1–15
46. Subramaniam, V., Bomze, H. M., and López, A. J. (1994) Functional differences between Ultrabithorax protein isoforms in *Drosophila melano-*

gaster: evidence from elimination, substitution and ectopic expression of specific isoforms. *Genetics* **136,** 979–991
47. Bianchi-Firas, D., Orian, A., Delrow, J. J., Vazquez, J., Rosales-Nieves, A. E., and Parkurst, S. M. (2004) Hairy transcriptional repression targets and cofactor recruitment in *Drosophila*. *PLoS Biol.* **2,** 975–990
48. Howard, R. M., and Sundaram, M. V. (2002) *C. elegans* EOR-1/PLZF and EOR-2 positively regulate Ras and Wnt signaling and function redundantly with Lin-25 and the Sur-2 Mediator component. *Genes Dev.* **16,** 1815–1827
49. Zhu, A., and Kuziora, M. A. (1996) Homeodomain interaction with the β subunit of the general transcription factor TFIIE. *J. Biol. Chem.* **271,** 20993–20996
50. Liu, Q.-L., Jindra, M., Ueda, H., Hiromi, Y., and Hirose, S. (2003) *Drosophila* MBF1 is a co-activator for Tracheae Defective and contributes to the formation of tracheal and nervous systems. *Development* **130,** 719–728
51. Kehle, J., Beuchle, D., Treuheit, S., Christen, B., Kennison, J. A., Bienz, M., and Müller, J. (1998) dMi-2, a Hunchback-interacting protein that functions in polycomb repression. *Science* **282,** 1897–1900
52. Lawrence, N., Langdon, T., Brennan, K., and Arias, A. M. (2001) Notch signaling targets the Wingless responsiveness on the Ultrabithorax visceral mesoderm enhancer. *Curr. Biol.* **11,** 375–385
53. Saleh, M., Rambaldi, I., Yang, X.-J., and Featherstone, M. S. (2000) Cell signaling switches HOX-PBX complexes from repressors to activators of transcription. *Mol. Cell. Biol.* **20,** 8623–8633
54. Szüts, D., Eresh, S., and Bienz, M. (1998) Functional intertwining of Dpp and EGFR signaling during *Drosophila* endoderm induction. *Genes Dev.* **12,** 2022–2035
55. Tremml, G., and Bienz, M. (1992) Induction of labial expression in the *Drosophila* endoderm: response elements for Dpp signaling and for autoregulation. *Development* **116,** 447–456
56. Bai, S., Shi, X., Yang, X., and Cao, X. (2000) Smad6 as a transcriptional corepressor. *J. Biol. Chem.* **275,** 8267–8270
57. Shi, X., Bai, S., Li, L., and Cao, X. (2001) Hoxa-9 represses transforming growth factor-β-induced osteopontin gene transcription. *J. Biol. Chem.* **276,** 850–855
58. Shi, X., Yang, X., Chen, D., Chang, Z., and Cao, X. (1999) Smad1 interacts with homeobox DNA-binding proteins in bone morphogenic protein signaling. *J. Biol. Chem.* **274,** 13711–13717
59. Yang, X., Ji, X., Shi, X., and Cao, X. (2000) Smad1 domains interacting with Hoxc-8 induce osteoblast differentiation. *J. Biol. Chem.* **275,** 1065–1072
60. Cassiday, L. A., and Maher, L. J., III (2002) Having it both ways: transcription factors that bind DNA and RNA. *Nucleic Acids Res.* **30,** 4118–4126
61. Nakamura, T., Mori, T., Tada, S., Krajweski, W., Rozovskaia, T., Wassell, R., Dubios, G., Mazo, A., Croce, C. M., and Canaani, E. (2002) ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. *Mol. Cell* **10,** 1119–1128
62. Wiellette, E. L., Harding, K. W., Mace, K. A., Ronshaugen, M. R., Wang, F. Y., and McGinnis, W. (1999) spen encodes an RNP motif protein that interacts with Hox pathways to repress the development of head-like sclerites in the *Drosophila* trunk. *Development* **126,** 5373–5385
63. Lin, H. V., Doroquez, D. B., Cho, S., Chen., F., Rebay, I., and Cadigan, K. M. (2003) Split ends is tissue/promoter specific regulator of Wingless signaling. *Development* **130,** 3125–3135
64. Mace, K., and Tugores, A. (2004) The product of the *split ends* gene is required for the maintenance of positional information during *Drosophila* development. *BMC Dev. Biol.* **4,** 15
65. Martinez E., Palhan, V. B., Tjernberg, A., Lymar, E. S., Gamper, A. M., Kundu, T. K., Chair, B. T., and Roeder, R. G. (2001) Human STAGA complex is a chromatin-acetylating transcription coactivators that interacts with pre-mRNA splicing and DNA damage-binding factors *in vivo*. *Mol. Cell. Biol.* **21,** 6782–6795
66. Kohn, K. W. (1999) Molecular interaction map of the mammalian cell cycle control and DNA repair systems. *Mol. Biol. Cell* **10,** 2703–2734
67. Schultz, T. F., and Quatrano, R. S. (1997) Characterization and expression of a rice RAD23 gene. *Plant Mol. Biol.* **34,** 557–562
68. Luo, L., Yang, X., Takihara, Y., Knoetgen, H., and Kessel, M. (2004) The cell-cycle regulator geminin inhibits Hox function through direct and polycomb-mediated interactions. *Nature* **427,** 749–753
69. Cillo, C., Faiella, A., Cantile, M., and Boncinelli, E. (1999) Homeobox genes and cancer. *Exp. Cell Res.* **248,** 1–9
70. Owens, B. M., and Hawley, R. G. (2002) *Hox* and *non-Hox* genes in leukemic hematopoiesis. *Stem Cells* **20,** 364–379
71. Iborra, F. J., Jackson, D. A., and Cook, P. R. (2001) Coupled transcription and translation within nuclei of mammalian cells. *Science* **293,** 1139–1142
72. Lehner, B., Semple, J. I., Brown, S. E., Counsell, D., Campbell, R. D., and Sanderson, C. M. (2004) Analysis of a high-throughput yeast two-hybrid system and its use to predict the function of intracellular proteins encoded with the human MHC class II region. *Genomics* **83,** 153–167