Runt domain proteins are transcriptional regulators that specify cell fates for processes extending from pattern formation in insects to leukemogenesis in humans. Runt domain family members are defined based on the presence of the 128-amino-acid Runt domain, which is necessary and sufficient for sequence-specific DNA binding. We demonstrate an evolutionarily conserved protein-protein interaction between Runt domain proteins and the corepressor Groucho. The interaction, however, is independent of the Runt domain and can be mapped to a 5-amino-acid sequence, VWRPY, present at the C terminus of all Runt domain proteins. *Drosophila melanogaster* Runt and Groucho interact genetically; the in vivo repression of a subset of Runt-regulated genes is dependent on the interaction with Groucho and is sensitive to Groucho dosage. Runt's repression of one gene, *engrailed*, is independent of VWRPY and Groucho, thus demonstrating alternative mechanisms for repression by Runt domain proteins. Unlike other transcriptional regulatory proteins that interact with Groucho, Runt domain proteins are known to activate transcription. This suggests that the Runt domain protein-Groucho interaction may be regulated.

Runt domain proteins are new family of DNA-binding, transcriptional regulatory proteins. These proteins serve crucial regulatory functions in diverse developmental contexts. Runt, the founding member of the family, has essential functions in sex determination (5), segmentation (9), and neurogenesis (6) in *Drosophila melanogaster*. Lozenge, a second *Drosophila* Runt domain family member, plays an important role in patterning the developing eye (3). A mouse Runt domain protein variously referred to as PEBP2αB (polyoma enhancer binding protein 2, α subunit, isofrom B) or CBFa2 (core binding factor, α subunit, isofrom 2) is essential for definitive hematopoiesis (25, 34), while its human counterpart, AML1, is a frequent target for chromosomal rearrangements associated with acute myeloid leukemia (23, 24). The major conserved feature of these proteins is the Runt domain. This is a 128-amino-acid sequence that is necessary and sufficient for sequence-specific DNA binding (17); DNA binding is a conserved feature of Runt domain proteins. The Runt domain also mediates heterodimerization with members of the CBF family, proteins that stimulate the DNA binding affinity of Runt domain proteins (11, 17, 36). Runt domain proteins likely function with an associated CBFβ partner, since the phenotypes of the CBFβ (28, 35) and AML1 knockouts are indistinguishable (25, 34). The DNA-binding properties of Runt domain proteins strongly suggest that their diverse developmental functions are carried out by directly regulating the transcription of other genes. Substantial evidence exists that Runt domain proteins act to regulate transcription, but their mode of action is not well understood for any specific target gene. In general, transcriptional regulators fall into one of two classes, activators or repressors. In mammals, Runt domain proteins have been shown to activate transcription from at least 10 genes, all of which are specifically transcribed in T-, B-, or myeloid cell lineages (29). This list will presumably expand, as a number of other genes expressed in hematopoietic cells harbor Runt domain consensus binding sites. Invertebrates also utilize Runt domain proteins for transcriptional activation: the sea urchin protein SpRunt activates the CyIIIIA gene in the early embryo (2); in *Drosophila*, Runt activates transcription of several segmentation genes (22, 32) and, as a numerator element, activates the master control gene of sex determination, Sex-lethal (*Sxl*) (5). This body of evidence suggests that Runt domain proteins are inherently transcriptional activators; however, there are cases where Runt domain proteins can repress transcription. Ectopic expression of *Drosophila* Runt leads to localized repression of segmentation genes, such as *hairy* (6), *even-skipped* (*eve*), and *engrailed* (*en*) (22, 32). Additionally, one isoform of AML1 has been shown to repress granulocyte-macrophage colony-stimulating factor transcription in cultured cells (31). Thus, it is unclear whether Runt domain proteins are inherently activators, are inherently repressors, or can activate or repress in a context-dependent manner.

In addition to the Runt domain, all family members share a 5-amino-acid sequence, VWRPY, located at the C terminus (Fig. 1). This conserved pentapeptide is not necessary for the DNA-binding properties of Runt domain proteins or for their ability to dimerize with CBFβ family members (11, 17, 36). The existence of such a short, highly conserved sequence element, found within a family of proteins defined by conservation of a different block of amino acids, is unusual. However, an analogous example is found in a subclass of basic helix-loop-helix (bHLH) DNA-binding proteins. The *Drosophila* Hairy-related and mammalian HES (Hairy, Enhancer of split) proteins are bHLH proteins that share three other features: a proline residue at a conserved position within the basic stretch of amino acids, a loosely conserved region located between the bHLH region and the C terminus termed the orange domain, and a tetrapeptide sequence, WRPW, located at the C terminus. The WRPW sequence has been shown to mediate the interaction of the Hairy-related/HES proteins with *Drosophila* Groucho (8,
A

<table>
<thead>
<tr>
<th>AML1</th>
<th>AML2</th>
<th>AML3</th>
<th>SpRunt</th>
<th>Runt</th>
<th>Loz</th>
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FIG. 1. Alignment of the C termini of Runt domain proteins. (A) Schematic of alignment of Runt domain proteins (2, 3, 18, 21) demonstrating the conservation of the Runt domain (shaded box) and the C-terminal VWRPY motif (solid box). The spacing between these elements is not conserved. The Runt domain of Runt spans amino acids 104 to 231. (B) The C-terminal 20 amino acids of three human, one sea urchin, and two fly Runt domain proteins are shown. Loz, Lozengze. The VWRPY sequence is the only sequence, outside of the Runt domain, that is conserved across species.

26) or the mammalian homologs, TLE1 to TLE4 (transducin-like Enhancer of split) (8, 12). Groucho and the TLEs are corepressor proteins that can repress transcription when targeted to a promoter (8). Thus, the Hairy-related/HEs proteins are thought to repress transcription by recruiting Groucho or one of the TLE proteins to promoter regions via the C-terminal VWRPY sequence (8, 26).

The similarity in position and sequence of the C-terminal regions of Hairy-related/HEs proteins and the C-terminal regions of Runt domain proteins suggests that the Runt domain proteins may also interact with Groucho or the TLEs. By analogy to the Hairy-related/HEs case, such an interaction would result in transcriptional repression by Runt domain proteins. Here, we show that Runt domain proteins do interact with Groucho; interaction between Runt domain proteins and Groucho is demonstrated by biochemical, two-hybrid, and genetic means. Biochemical and two-hybrid assays also show that the interaction between Runt domain proteins and Groucho is mediated by the VWRPY sequence. In vivo and cell culture experiments demonstrate the importance of the C terminus of Runt domain proteins in mediating repression. We also show in cell culture experiments that Runt domain proteins have the general ability to actively repress transcription; this is even the case for an isoform of AML1 which has been previously described only for its ability to activate transcription. Based on this work, all Runt domain proteins have the potential to actively repress transcription. Runt, however, also has a Groucho-independent repression activity, since the repression of one target, engrailed, is insensitive to the deletion of Runt’s C terminus and is insensitive to groucho dosage. Further, as Runt domain proteins can activate transcription, they are distinct from the Hairy-related/HEs family members, which apparently act as dedicated repressors; we suggest several models by which Runt domain proteins may activate transcription. Thus, Runt domain proteins appear to be transcriptional regulators that work in a highly context-dependent manner.

MATERIALS AND METHODS

Fly lines. The groucho gene resides within the E(spl) locus. E(spl)BP262 flies were a gift from U. Banerjee; E(spl)BP262 is a deletion that removes groucho as well as five other genes at the locus (4). E(spl)E46 flies were a gift from S. Artavanis-Tsakonas; E(spl)E46 is a point mutation within the groucho gene. For examining the effect of maternal groucho dosage on the hs/runt phenotype, E(spl)/TM6B flies (either BX22 or E48 alleles) were outcrossed to a wild-type background and sibling females [E(spl)E46 or TM6B/E] were crossed to homozygous hs/runt males. Embryos from this cross were subjected to heat shock and scored in situ hybridization. TM6B is a third-chromosome balancer. The TM6B/+ siblings provide a control for genetic background.

Embryo manipulations. For the hs/runt repression assay, embryos were collected 1.5 to 3 h after egg laying and were heat shocked at 37°C for either 20 min (in situ hybridization with h or eve) or 1.5 min (in situ hybridization with en). The embryos recovered at 25°C for 25 min prior to dechorionation; fixation and in situ hybridization were carried out according to the method of Tsai and Iber. Groucho, embryos that were at cellular blastoderm (eve stripe 2 and h stripe 1) or midgerm band extension (en odd-numbered stripes) were scored. The wild-type (yw) control corresponds to the parental strain used for generating the transgenic hs/runt and hs/runtARPY lines. The hs/runtARPY construct was made by deleting an internal 54-bp MboI fragment from the Runt cDNA.

In the groucho dosage experiments, the heat shocks were carried out for 15 min (for hairy), 10 min (for eve), or 2 min (for en), and embryos were allowed to recover for 25 min. Scoring of the repression events was done in a blind fashion. The ranges for the trials for the E48 allele were 18 to 34% for E(spl)BP262/E versus 35 to 62% for TM6B/E for eve stripe 2, and 32 to 67% for E(spl)E46/E versus 63 to 89% for TM6B/E for stripe 1. The ranges for the trials for the BX22 allele were 6 to 34% for E(spl)BP262/E versus 47 to 53% for TM6B/E for eve stripe 2, 16 to 22% for E(spl)BP262/E versus 40 to 51% for TM6B/E for stripe 1, and 57 to 59% for E(spl)BP262/E versus 55 to 65% for TM6B/E for the odd-numbered en stripes.

GST coprecipitation assays. Coprecipitation and Western blotting were performed as in the work of Fisher et al. (8). Briefly, 5 or 20 μg of glutathione-β-transferase (GST) was immobilized on glutathione agarose beads; 1 mg of cell lysate prepared in phosphate-buffered saline with 0.5% Triton and 800 μl of binding buffer (20 mM Tris [pH 8.0], 50 mM KCl, 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 5 mg of bovine serum albumin/ml) was added, and the beads were incubated for 12 h at 4°C. The beads were washed three times with 1 ml of 1× phosphate-buffered saline before being boiled in sample buffer and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Western blotting. The anti-Groucho antibody was provided by S. Stefani (Yale University) and used at a 1:20 dilution.

Two-hybrid assays. The two-hybrid assay was performed as in the work of Golling et al. (11) except that host strain SFY 526 (Clontech) was used. The appropriate restriction fragments were PCR products (in the case of the VWRPY fusion) corresponding to Runt, PEBP2αB, or Runt-AML1 chimeric sequences were subcloned into the vector pGBT9. The Runt domain of Runt spans amino acids 104 to 231. RAM is a construct previously described (27); amino acids 112 to 224 of Runt were replaced by the corresponding sequences of AML1. The Runt-AML1 chimeric construct A5 was made by substitution of amino acids 183 to 223 of Runt with the corresponding sequence from AML1. This substitution results in a 10-amino-acid difference from the sequence of Runt; 6 of the amino acid differences are conservative. The nonconservative changes are A207F, V211P, and S217H. Full-length Groucho was cloned into pGAD GH. Acid differences are conservative. The nonconservative changes are A207F, V211P, and S217H. The fusion expression plasmid, and 1 μg of pRC-cytomegalovirus construct was made as in the work of Fisher et al. (8). Briefly, 5 or 20 μg of glutathione-β-transferase (GST) was immobilized on glutathione agarose beads; 1 mg of cell lysate prepared in phosphate-buffered saline with 0.5% Triton and 800 μl of binding buffer (20 mM Tris [pH 8.0], 50 mM KCl, 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 5 mg of bovine serum albumin/ml) was added, and the beads were incubated for 12 h at 4°C. The beads were washed three times with 1 ml of 1× phosphate-buffered saline before being boiled in sample buffer and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Western blotting. The anti-Groucho antibody was provided by S. Stefani (Yale University) and used at a 1:20 dilution.

Cell culture and transfection. Transfection experiments were carried out as in the work of Fisher et al. (8). Briefly, for Schneider cells, 1 μg of achaete luciferase reporter containing 5 GAL4 upstream activation sequences (UAS), 1 μg of both Achaete and Daughterless expression plasmids, and 2 μg of the GAL4 fusion expression plasmid were transfected using calcium phosphate. After 48 h the cells were lysed and the luciferase activity was measured. The luciferase activities were standardized to a co-expressed internal control. The final activities were converted to a percentage of the activity seen with Achaete and Daughterless alone (i.e., with no repressor). A similar protocol was used in the HeLa cell experiments except that 5 μg of the modified UASαT reporter, 5 μg of the GAL4 fusion expression plasmid, and 1 μg of a pRC-cytomegalovirus construct were used. The cDNA used to make GAL4 pRBp262 corresponds to the isoform eB1 and was obtained from Y. Ito (Kyoto University); the cDNA used to make GAL4 AML1 B was a gift from S. Hieter (St. Jude Children’s Research Hospital).

RESULTS

The conserved C terminus is necessary for a subset of Runt repression events. We investigated the in vivo effects of deletions of a C-terminal region of Runt domain proteins. We chose Runt as our model protein because as a segmentation gene, runt belongs to a well-characterized genetic pathway and because there are many characterized Runt-regulated genes. Previous studies have demonstrated that ectopic expression of Runt at the blastoderm stage leads to stripe-specific repression
of two pair-rule genes, stripe 2 of even-skipped and stripe 1 of hairy (22, 32); Runt has also been shown to be involved in repressing h expression between h stripe 3 and stripe 4 (13). However, ectopic Runt expression has no effect on h expression in the region of h stripe 3/4. In addition to these stripe-specific repression events, runt represses the odd-numbered stripes of the segment polarity gene engrailed (22, 32). We measured the responses of these three targets to ectopic expression of a truncated Runt protein in which the C-terminal 3 amino acids had been deleted (hs/runt). hs/runt expression in the region of D Runt-specific antisera indicate that the Runt and Runt hs/runt expression activities of Runt; one activity is dependent on an intact VWRPY sequence, and the other is independent of this conserved C terminus.

The conserved C terminus mediates interaction with Groucho. Members of the Hairy-related family of bHLH proteins have a conserved C-terminal tetrapeptide sequence, WRPW. Previous studies demonstrated that the WRPW motif mediates a protein-protein interaction between Hairy-related family members and a corepressor, Groucho (8, 26). The similarity in both the sequence and the position within the polypeptide chain of the Hairy-related family WRPW motif and the Runt domain family VWRPY motif led us to test for interactions between Runt and Groucho. Two separate assay systems were used to demonstrate a VWRPY-dependent interaction between these two proteins. First, we utilized a coprecipitation assay to determine if Groucho protein present in a Drosophila Schneider cell extract can interact with GST-Runt fusion protein. Groucho is precipitated by full-length GST-Runt bound to glutathione beads. By contrast, there is a marked reduction in the ability of Groucho to interact with a Runt protein that lacks only the WRPY tetrapeptide (Fig. 3A). Experiments with truncated Runt derivatives indicate that the C-terminal region is necessary for this interaction. A GST fusion protein containing only the C-terminal 49 amino acids of Runt interacts with Groucho, whereas the same protein deleted for the WRPY tetrapeptide does not (Fig. 3B). Thus, Runt and Groucho interact, the C-terminal 49 amino acids of Runt are sufficient for the interaction, and the conserved VWRPY motif is necessary for this interaction.

These results were confirmed and extended by the yeast two-hybrid assay (7). As found in the experiments described above, the C-terminal 49 amino acids of Runt interact with

<table>
<thead>
<tr>
<th>Genotype</th>
<th>h *1</th>
<th>eve stripes</th>
<th>en stripes</th>
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<tbody>
<tr>
<td>Wild type</td>
<td>0 (0/110)</td>
<td>5 (3/62)</td>
<td>0 (0/33)</td>
</tr>
<tr>
<td>hs/runt</td>
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<td>68 (52/47)</td>
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<tr>
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<td>1 (1/115)</td>
<td>0 (0/24)</td>
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* Embryos were heat shocked and processed for in situ hybridization.

* Expressed as percent of embryos affected. The numbers of embryos that show the repression event and the total numbers of embryos scored are shown in parentheses.
Results of chromogenic β-galactosidase assays of lifted colonies are shown. In all cases, the Runt domain proteins and their derivatives are fusions to the GAL4 DBD; Groucho (Gro) and Brother (Bro) are fusions to the GAL4 activation domain (7). The lacZ signals are compared to the negative control in which the GAL4 activation domain carries no fusion (Act). (A) The Runt-Groucho interaction is conserved and mediated through the C-terminal VWRPY sequence. (B) The Runt domain inhibits interaction with Groucho. The numbers in parentheses are the amino acid coordinates of Runt included in the construct. RuntΔRD contains an internal deletion from amino acid 110 to amino acid 225; RAM and A5 are chimeric proteins in which the AML1 Runt domain or a subregion of the AML1 Runt domain replaces Runt’s Runt domain (27) (see Materials and Methods). (C) A single amino acid substitution at the C terminus allows for interaction of full-length Runt and Groucho. The C-terminal-most amino acid of Runt (coordinate position 509) was changed to tryptophan, the amino acid found at the C terminus of Hairy-related/HES proteins.

FIG. 4. Runt domain proteins and Groucho interact in the two-hybrid assay. One notable observation is the lack of interaction between Groucho and this interaction is dependent on Runt’s conserved VWRPY [Fig. 4A, Runt (460-509) versus Runt (460-505)]. The mouse homolog of the human Runt domain protein AML1 (referred to here as PEBP2αB1) also interacts with Groucho in this assay. Outside of the Runt domain, the only conspicuously conserved region between Runt and PEBP2αB1 is the C-terminal VWRPY motif. Deletion of the last 4 amino acids of PEBP2αB1 greatly reduces the interaction with Groucho. Finally, a two-hybrid interaction is detected between Groucho and a GAL4 DNA-binding fusion protein to which only the pentapeptide sequence VWRPY is added. Thus, this pentapeptide motif is both necessary and sufficient for interaction with Groucho in this assay system.

One notable observation is the lack of interaction between full-length Runt and Groucho in the two-hybrid assay (Fig. 4); the integrity of the full-length Runt construct is verified by the demonstration of a robust two-hybrid interaction with its partner protein Brother, the Drosophila homolog of CBFβ (11) (Fig. 4). Several observations using the two-hybrid assay suggest that full-length Runt may be in a conformation that prohibits interaction with Groucho and that amino acid replacements in both the Runt domain and the C terminus can relieve this conformational restraint. A mutant with an N-terminal deletion that removes sequences to within 31 amino acids of the Runt domain is unable to interact with Groucho, but mutants with deletions past the Runt domain show strong interaction with Groucho [Fig. 4B, Runt (73-509) versus Runt (225-509)]. An internal deletion that removes most of the Runt domain also interacts with Groucho. Further, a chimeric construct in which Runt’s Runt domain has been replaced by the AML1 Runt domain also interacts with Groucho. The region within the AML1 Runt domain that allows interaction with Groucho has been further mapped; the chimeric construct A5 has only 10 amino acid replacements relative to Runt and shows robust interaction with Groucho. These replacements are at positions in the C-terminal region of the Runt domain, and only 4 amino acid changes are nonconservative. Finally, a single amino acid change at the C terminus (Y509W) is sufficient to demonstrate an interaction with Groucho (Fig. 4C). Thus, relatively minor changes to Runt can allow the full-length protein to interact with Groucho in the two-hybrid assay.

**The conserved C terminus is important for transcriptional repression in cell culture.** The functional significance of the VWRPY-mediated interaction between Runt domain proteins and Groucho was investigated by using transient transfection assays. Targeting of Groucho to DNA with a GAL4 DNA-binding domain (DBD) represses transcription of a reporter gene containing UASG (8). Consistent with the idea that Runt can repress transcription by recruitment of Groucho, we find that a full-length GAL4-Runt fusion is able to repress transcription from this same reporter gene in Drosophila Schneider cells which contain endogenous Groucho (Fig. 5). This repression activity maps to the C-terminal region of Runt, as a GAL4 fusion containing amino acids 460 to 509 of Runt is a potent repressor in this assay. Furthermore, removal of the WRPY sequence decreases the potency of both the full-length GAL4-Runt fusion and the GAL4-Runt (460-509) fusion as transcriptional repressors (Fig. 5). We also examined the activity of mammalian Runt domain proteins in HeLa cells. GAL4 fusions of human AML1 and mouse PEBP2αB1 proteins both act to repress transcription in this assay; as was found for Runt, deletion of the C-terminal 4 amino acids compromises the repression activity, though significant repression activity remains (Fig. 6). These experiments demonstrate that the ability to function as transcriptional repressors is a conserved property of Runt domain proteins, and they indicate the importance of the C-terminal VWRPY motif for this activity.

**Genetic interaction between Runt and Groucho.** The experiments described above establish that the VWRPY sequence mediates an interaction with Groucho and demonstrate that the C terminus contributes to the activity of Runt domain proteins as transcriptional repressors in cultured cells. Thus, the VWRPY-dependent repression of h stripe 1 and eve stripe 2 by Runt in Drosophila embryos is likely to involve interaction with Groucho. In order to test this, we examined the penetrance of the phenotypes produced by ectopic Runt expression in embryos with reduced levels of maternally provided groucho activity. This approach is necessary because the severe defects in embryos that contain no Groucho protein (26) interfere with the interpretation of the hs/runt phenotype. In
particular, we compared the response of h stripe 1 and eve stripe 2 in embryos with wild-type (2×) dosage of maternal groucho to the response in embryos that have half (1×) the normal maternal dosage of groucho. Induction conditions were empirically derived such that expression of the single copy of hs/runt produced clear but not fully penetrant repression of the target genes. Under these conditions, Runt’s repression of both of these targets is less effective in embryos with reduced Groucho dosage (Table 2). This result is observed with the two groucho mutations tested, a deletion of groucho [E(spl)BX22] as well as a point mutation that behaves as a null allele [E(spl)E48] (4). This data establishes a genetic interaction between Runt and Groucho and provides in vivo evidence that interaction with Groucho is important for the VWRPY-dependent repression activities of Runt.

By contrast, the maternal dosage of groucho does not affect the ability of runt to repress the odd-numbered en stripes (Table 2). This reconfirms the distinction between the VWRPY-dependent and -independent activities of Runt. Runt regulation of ensembled, and perhaps of other Runt-regulated genes, occurs through a separate, distinct mode of repression.

**DISCUSSION**

Runt domain proteins interact with Groucho. We have shown that Runt domain proteins interact with Groucho to repress transcription. The ability of Runt domain proteins to interact with Groucho is a conserved property; we have demonstrated an interaction between the *Drosophila* proteins Runt and Groucho and between the mammalian Runt domain protein PEBP2aB (AML1) and Groucho. This conserved feature of Runt domain proteins is mediated by a conserved motif, VWRPY, that maps outside the Runt domain. The two-hybrid assay results suggest that the pentapeptide sequence alone is the primary sequence that determines the interaction; there is no cross-species sequence conservation at the C terminus of Runt domain proteins other than the VWRPY sequence, nor do predictive algorithms suggest a common secondary structure at the C terminus. The pentapeptide sequence alone was sufficient to detect an interaction in the two-hybrid assay, while deletion of the C-terminal 3 amino acids from Runt resulted in loss of interaction.

The distinctions between the Runt domain consensus (VWRPY) and Hairy-related/HES consensus (WRPW) raise the question of whether the C termini of these families of proteins are interchangeable; that is, whether there is a functional significance to the amino acid differences. One possible explanation for the distinct Groucho-recruiting C termini is suggested by the apparent difference in transcriptional regulatory potential between Hairy-related/HES proteins and Runt domain family members. Unlike the Hairy-related/HES proteins, which are known only to repress transcription, the Runt domain proteins interact with Groucho to repress transcription. The ability of Runt domain proteins to interact with Groucho and provide in vivo evidence that interaction with Groucho is important for the VWRPY-dependent repression activities of Runt.
domain proteins have the additional ability of activating transcription. The ability of Runt domain proteins to activate transcription suggests that the interaction with Groucho is regulated; when Runt domain proteins assemble on a promoter that is to be activated, Groucho must be absent or in a context where it cannot exert its repressive effects. Therefore, the difference between the Groucho-recruiting C termini of the Hairy-related/HES family and the Runt domain family may be the difference between a constitutive Groucho interaction and one that is regulated.

Our two-hybrid assay results provide a hint of a regulated interaction between Runt and Groucho. It appears that in this assay Runt is poised to interact with Groucho but cannot. Runt can interact with Groucho when alterations as discrete as those of the A5 chimeric construct (10 amino acid changes relative to Runt) are introduced. Similarly, more drastic alterations, such as deletion of the Runt domain or of the N-terminal 460 amino acids, also result in a form of Runt that interacts with Groucho. It is noteworthy that alterations in the Runt domain induce an interaction with Groucho; the interaction of this domain with Brother (11) induces a conformational change that stimulates the DNA-binding properties of Runt. Perhaps such a conformational change could lead to an interaction with Groucho. This leads to the possibility that the Runt domain is acting to modulate the Groucho interaction, perhaps through interaction with the VWRPY sequence, since the Y509W mutation allows for Groucho interaction. It will be important to determine whether the Runt-Groucho and Runt-Brother interactions are exclusive or perhaps interdependent. Finally, a role for the Runt domain in the regulation of the Runt-Groucho interaction could explain the coevolution of Runt domain proteins and the VWRPY sequence.

The VWRPY motif mediates transcriptional repression. Both repression assays used in this work, the ectopic expression assay in Drosophila embryos and the active repression assay in cultured cells, demonstrate the importance of the conserved C-terminal sequence in transcriptional repression. The conservation of the VWRPY sequence in Runt domain proteins, the conservation of the interaction with Groucho, and the evolutionary and functional conservation of Groucho and the mammalian TLE proteins strongly suggest that the mammalian proteins will repress transcription through the C-terminal motif but also through alternative modes of repression.

Transcriptional activation and repression by Runt domain proteins. The results presented here demonstrate that Runt domain proteins can repress transcription through the C-terminal motif but also through alternative modes of repression. A second repression activity of Runt is defined by the VWRPY-independent, maternal groucho dosage-independent repression of en. However, the cell culture repression assay does not define a second repression domain of Runt. It is possible that repression at en works by a passive mechanism, that is, by competing with an activator for a common or overlapping binding site. This activity would not be detected by our assay. Alternatively, factors not present in Schneider cells may be necessary for the en repression. Regardless, these observations establish a precedent for alternative pathways of transcriptional repression by Runt domain proteins.

The finding that Runt domain proteins, like the Drosophila Hairy-related and mammalian HES proteins, can interact with the corepressor Groucho is particularly striking in two developmental contexts in Drosophila, sex determination and segmentation. In both of these pathways Runt shows an opposing, antagonistic effect relative to that of a Hairy-related protein (5, 33, 38): Hairy in the case of segmentation and Deadpan (Dpn) in the case of sex determination. The antagonistic inputs of Runt and Hairy-related proteins in these pathways may be explained by competitive interactions for Groucho. For example, consider the regulation of Sxl in sex determination: Runt, a numerator element, serves to activate the embryonic promoter of Sxl (SxlPE) in female embryos, while in males Dpn, the only defined denominator in this pathway, serves to repress SxlPE. Dpn is thought to repress SxlPE by recruiting Groucho to the SxlPE; there are four known Dpn binding sites in the promoter region (15). In females, where Runt dosage is twice that in males, Runt may effectively compete with Dpn for Groucho, reducing or eliminating Dpn-Groucho repression complexes from the SxlPE, thus allowing activation. Alternatively, Runt may activate SxlPE by assembling on the promoter in the absence of Groucho or in a context where Groucho’s repression activity is neutralized. Whether Runt’s participation in sex determination requires the VWRPY C terminus or Groucho is an important issue to address in the future.

A similar competition for Runt and Hairy for Groucho could explain Runt’s activation of ftz; however, in this case it is clear that the VWRPY sequence is not absolutely necessary for activation of ftz by Runt, since the ectopically expressed RuntARPY shows reduced but clear activation of ftz in the interstripe regions (data not shown). Furthermore, the issue of whether Runt directly activates ftz is controversial (16). While the potential for Runt domain proteins to activate transcription at other promoters by competing with Hairy-related/HES proteins for Groucho exists, it is unlikely to be a universal mechanism. Indeed, competition for Groucho/TLE cannot explain the ability of AML1, a protein capable of interacting with AML1, AML3, and LEF-1, to activate transcription at other promoters by competing with Hairy-related/HES–TLE complexes were the sole mechanism for activating transcription, and they provide evidence that Runt domain proteins can activate transcription by assembling on DNA without Groucho. Recent identification of ALY, a protein capable of interacting with AML1, AML3, and LEF-1, is intriguing; ALY can stimulate the DNA-binding activities of these proteins and interacts with their conditional activation domains (1). Alternatively, cooperative interactions between AML1 and Ets-1 (37) and AML1 and c-Myb (14) have been described. These cooperative interactions lead to synergistic transcriptional activation at the TCR α (10), TCR β (30), and TCR δ (14) enhancers. Perhaps cooperative interactions with
DNA-binding, transcriptional activators and/or interactions with accessory proteins such as ALY provide contexts in which Runt domain proteins can assemble on DNA and activate rather than repress transcription.

We have shown a Groucho- and VWRPY-dependent repression activity of Runt domain proteins. This implies that all Runt domain proteins, previously considered to be primarily transcriptional activators, have the potential to recruit Groucho (or the mammalian TLEs) and repress transcription. The observation that Runt represses en in a Groucho- and VWRPY-independent manner is one example of the multiple modes of transcriptional regulation by Runt domain proteins. The model whereby Runt domain proteins can activate transcription through their ability to compete for the interaction with Groucho, and the cases where transcriptional activation is apparently independent of Groucho binding, are further indications of the remarkable adaptability of their ability to compete for the interaction with Groucho, and the transcriptional regulation by Runt domain proteins. The model for independent manner is one example of the multiple modes of expression activity of Runt domain proteins. This implies that all with accessory proteins such as ALY provide contexts in which regulation by helix-loop-helix proteins. Nucleic Acids Res. 23:3441–3448.


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