The Mammalian Basic Helix Loop Helix Protein HES-1 Binds to and Modulates the Transactivating Function of the Runt-related Factor Cbfa1

(Received for publication, June 3, 1999, and in revised form, October 4, 1999)

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(Drosophila) Runt is the founding member of a family of related transcription factors involved in the regulation of a variety of cell-differentiation events in invertebrates and vertebrates. Runt-related proteins act as both transactivators and transcriptional repressors, suggesting that context-dependent mechanisms modulate their transcriptional properties. The aim of this study was to elucidate the molecular mechanisms that contribute to the regulation of the functions of the mammalian Runt-related protein, Cbfa1. Here we provide the first demonstration that Cbfa1 (as well as the related protein, Cbfa2/AML1) physically interacts with the basic helix loop helix transcription factor, HES-1, a mammalian counterpart of the Drosophila Hairy and Enhancer of split proteins. This interaction is mediated by the carboxyl-terminal domains of Cbfa1 and HES-1, but does not require their respective tetrapeptide motifs, WRPY and WRPW. Our studies also show that HES-1 can antagonize the binding of Cbfa1 to mammalian transcriptional corepressors of the Groucho family. Moreover, HES-1 can potentiate Cbfa1-mediated transactivation in transfected cells. Taken together, these findings implicate HES-1 in the transcriptional functions of Cbfa1 and suggest that the concerted activities of Groucho and HES proteins modulate the functions of mammalian Runt-related proteins.

Invertebrate and vertebrate Runt-related proteins are transcription factors involved in the regulation of a variety of cell-differentiation events and aberrant functions of members of this protein family correlate with developmental abnormalities and neoplastic transformations (1–5). In particular, the mouse Runt-related protein, core-binding factor α2 (Cbfa2)1 is essential for fetal liver hematopoiesis (6–8) and its human homolog, AML1, is frequently targeted by chromosomal translocations that lead to acute myeloid leukemia (4, 6). A related mouse protein, Cbfa1, plays an essential role in osteoblast differentiation and mutations interfering with its function correlate with defects in ossification in mice and humans (3, 5, 9–11).

Studies in invertebrate and vertebrate species implicate Runt-related proteins in both transactivation and transcriptional repression, suggesting that their transcription functions may be regulated in context-dependent ways by interactions with other proteins (1, 9, 12–17). In this regard, Drosophila Runt has recently (18) been shown to interact with the protein Groucho, a general transcriptional repressor involved in a variety of gene regulatory events (19–21). In particular, genetic studies show that repression of certain Runt-regulated genes is dependent on interaction with Groucho and is sensitive to Groucho dosage (18). These results implicate Groucho in the regulation of the transcriptional functions of Runt in Drosophila.

A number of observations have suggested that the functions of mammalian Runt-related proteins are also modulated by Groucho homologs, designated as the transducin-like Enhancer of split (TLE) or Groucho-related gene products 1 through 4 (hereafter referred to as TLE1–4) (22–24). TLE proteins are co-expressed with the Runt-related proteins, Cbfa1 and Cbfa2/AML1 (AML1), in a variety of cell types (7, 14, 25–27). In addition, AML1 and TLE proteins can physically interact with each other (28). Furthermore, transient transfection studies in mammalian cells have shown that TLE proteins can inhibit the transactivation mediated by both Cbfa1 and AML1 (14, 28, 29). Together, these findings strongly suggest that TLE proteins are involved in the regulation of the transcriptional functions of mammalian Runt-related proteins.

Studies in Drosophila have also implicated a second evolutionarily conserved family of transcription factors in the regulation of the functions of Runt-related proteins. Specifically, the basic helix loop helix proteins of the Drosophila Hairy/Enhancer of split (HES) family are co-expressed with Groucho and Runt in a variety of cell types and physically interact with Groucho (18, 20, 21, 30). Moreover, genetic studies in Drosophila show that runt and HES genes contribute to common gene regulatory events important for sex determination and segmentation (30–32). Mammalian HES and runt-related genes are also co-expressed with TLE genes in a variety of cell types and their protein products participate in common developmental mechanisms (14, 27, 33, 34). Together, these findings raise the possibility that Runt-related and HES proteins may interact.

Here we describe experiments designed to test whether Cbfa1 can functionally interact with the HES family member,
HES-1, which is co-expressed with Cbfα1 in mammalian skeletal cells (9, 33). Our results demonstrate that Cbfα1 can physically interact with both HES-1 and TLE proteins and identify the domains of these molecules involved in these interactions. HES-1 also interacts with AML1, in addition to Cbfα1. Our studies also show that HES-1 can potentiatively Cbfα1-mediated transactivation in transfected cells, possibly by virtue of its ability to antagonize the interaction of Cbfα1 with TLE proteins. Taken together, these results strongly suggest that the transcriptional functions of Cbfα1 are modulated by interactions with both TLE and HES family members.

EXPERIMENTAL PROCEDURES

Plasmids—The following is a summary of the names and origins of the constructs used in these studies. Additional information on cloning strategies and oligonucleotide primers used in PCR experiments is available upon request. Vent DNA polymerase was used and PCR products were routinely sequenced before subcloning into the appropriate vectors. For expression of glutathione S-transferase (GST) fusion proteins in bacteria, the pGEX1-TLE1(32-770) (near full-length TLE1 lacking the first 31 amino acids), pGEX2-TLE1(1-135) (Q domain of TLE1), pGEX3-TLE3(490-774) (WDR domain of TLE3), and pGEX1-HES1(3-281) (full-length HES-1 sequence with the presence of the SuperFect DNA) constructs were generated as described previously (35-37). The pGEX1-TLE1(290-461) plasmid was obtained by subcloning a PCR product encoding amino acids 290 through 461 (SPI domain of TLE1) into the BamHI and HindIII sites of pGEX1. The pGEX2-HES-1 C (truncated HES-1 lacking the last 6 amino acids, WRPRWN) was generated by subcloning a PCR product encoding this portion of HES-1 into the Smal site of pGEX2. The DNA construct for expression of GST-HES1 in mammalian cells, pEBG-HES1(3-281), was obtained by subcloning a PouII fragment from the rat HES1 cDNA into the filled-in ClaI site of the pEBG vector (38). Plasmids for in vitro translation reactions were obtained as follows: pcDNA3-GAL4bd-Cbfα1(1-523) and pcDNA3-GAL4bd-Cbfα1(1-442) (encoding the DNA-binding domain of GAL4 fused to either residues 241-523 or 241-442 of Cbfα1, respectively) were obtained by subcloning Xhol/XbaI fragments from the previously described (14) constructs pSG424-Cbfα1(1-523) and -Cbfα1(1-442) into the pcDNA3-GAL4 vector (36, 37). Plasmids pcDNA-GAL4bd-AMLI(1-2-453) (fusion protein of GAL4bd and AMLI) and pcDNA3-GAL4bd-AMLI(1-2-472) (fusion protein of GAL4bd and AMLI) were generated by digesting pBlueScript-AMLI or -AMLII (obtained from Y. Greener, The Weizmann Institute, Rehovot, Israel) with XhoI (followed by filling-in with Klenow DNA polymerase) and EcoRI, and by subcloning into pcDNA3-GAL4bd digested with EcoRV and EcoRI. DNA constructs for yeast two-hybrid assays were obtained as follows. pGAD424-Cbfα1(468-528) (activation domain of GAL4 fused to residues 468 through 528 of Cbfα1) was generated by subcloning a Smal fragment from pBlueScript-Cbfα1 into the Smal site of pGAD24. The plasmids pGBT9-HES1(3-281), -TLE3(444-770), and -TLE1(444-770) (GAL4bd fused to full-length TLE1, the first 435 amino acids of TLE1, or residues 444 through 770 of TLE1, respectively) were obtained as described previously (36, 37). pGBT9-HES1(193-281) (GAL4bd fused to amino acids 193 through 281 of HES-1) has been described previously (35). Plasmids pCMV5-Cbfα1 (full-length Cbfα1), pCMV5-Cbfα1(139-96) (truncated form of Cbfα1 lacking the QA domain), and pROSF260 (luciferase reporter gene under the control of six Cbfα1-binding sites, the OSE2 element) have been described previously (9, 14). The pRC1-CMV-HES1 construct (full-length HES-1) has been described previously (39). The pCMV2-FLAG-AMLI plasmid was generated by digesting pcDNA3-GAL4bd-AMLI(1-2-453) with EcoRI and XhoI, followed by filling-in with Klenow DNA polymerase and subcloning into the Smal site of pCMV2-FLAG. The pCMV2-FLAG-HES1(3-281) plasmid was obtained by subcloning a PouII fragment from the rat HES1 cDNA into the Smal site of pCMV2-FLAG. Construct pCMV2-FLAG-HES1 C (truncated HES-1 lacking the QA domain) was generated by subcloning a PCR product encoding this portion of HES-1 into the filled-in BamHI site of pCMV2-FLAG.

Interaction Assays in Transfected Cells and Western Blotting Analysis—ROS17/2.8 cells were cultured as described previously (9, 14, 37), and transfected using SuperFect reagent (Qiagen) according to the manufacturer’s instructions. In each experiment, cells were co-transfected with 1.0 μg of either pCMV5-Cbfα1 DNA or pCMV2-FLAG-AMLI DNA and 1.0 μg of pEBG-HES1 DNA (or pEBG as control). Approximately 24 h after transfection, cells were collected, homogenized as described previously (40), and incubated in the presence of glutathione-Sephrose beads for 60 min. The beads were then collected by centrifugation, washed five times with buffer 1 (25 mM Tris/HCl, pH 7.8, 200 mM NaCl, 0.5% Triton X-100), and immediately incubated in 2× electrophoresis sample buffer, followed by SDS-PAGE and transfer to nitrocellulose. Nitrocellulose replicas were subjected to Western blotting analysis with previously described anti-Cbfα antibodies (9, 13) or anti-FLAG-epitope monoclonal antibodies (22, 23, 25).

RESULTS

HES-1 Physically Interacts with Cbfα1 and AMLI—Based on the participation of runt and HES genes in common developmental pathways in Drosophila (30-32) and the shared ability of both HES and Runt-related proteins to bind to transcriptional corepressors of the Groucho/TLE family (14, 18, 21, 28, 36), we asked whether specific mammalian HES and Runt-related proteins might associate with each other. To address this question, we first performed coprecipitation assays using cells co-transfected with expression plasmids for HES-1 and Cbfα1. These factors are normally coexpressed in mammalian osteoblastic cells (9, 33, 34, 41, 42). Rat ROS17/2.8 osteoblastic cells were transfected with either GST or a fusion protein of GST and HES-1 that contained the entire HES-1 sequence except for the first two amino acids (GST-HES1(1-3-281)). Twenty-four hours later, cells were homogenized and the GST proteins were isolated on glutathione-Sepharose beads. Western blotting analysis of the fractions bound to the beads using anti-GST antibodies revealed that GST and GST-HES-1 were both stable and expressed at equivalent levels (Fig. 1C, lanes 2 and 4). Western blotting analysis with previously described (9, 13) anti-Cbfα antibodies revealed that full-length Cbfα1 (Cbfα11-528; refer to Fig. 1A for Cbfα1 structure) co-precipitated with GST-HES-1 (Fig. 4A, lanes 1 and 2). Consistent with this result, the transfected plasmid, AMLI, which represents the full-length form of AMLI (16, 28), coprecipitated with GST-HES-1 (Fig. 2A, lane 2), but not with GST (Fig. 2A, lane 4). Together, these results show that HES-1 can associate with both Cbfα1 and AMLI in transfected cells.

The coprecipitation of Cbfα1 proteins with HES-1 may result from either a direct interaction between these molecules or an
two-hybrid interaction assays in yeast cells previously shown to be devoid of TLE proteins (40). Co-transformation of yeast cells with plasmids encoding GAL4ad-Cbfa1(468–528), containing the last 60 amino acids of Cbfa1, and GAL4bd-HES1(193–281), containing the last 88 residues of HES-1, resulted in a specific reconstitution of GAL4 transcriptional activity (Fig. 3A). This result was indicative of an interaction between these factors in the absence of TLE proteins.

Taken together, these results show that HES-1 can interact with the mammalian Runt-related proteins, Cbfa1 and AML1, in a TLE-independent way. Moreover, they reveal that the Cbfa1/HES-1 interaction involves sequences located within the last 60 amino acids of Cbfa1 and the last 88 residues of HES-1, respectively.

Cbfa1 Interacts with TLE Proteins—Previous studies have shown that the carboxyl-terminal domains of Drosophila Runt and human AML1, including but not limited to the WRPY motif, mediate interactions with Groucho/TLE proteins (18, 28). Based on those results, we asked whether Cbfa1 might also physically interact with TLE proteins and, if so, whether its carboxyl-terminal domain might be involved in these interactions. Pull-down assays using in vitro translated full-length Cbfa1 and a previously described (37) fusion protein of GST and near full-length TLE1 (GST-TLE1(32–770)) showed that Cbfa1 and TLE1 can bind to each other (Fig. 4B, lane 2). Further studies with fusion proteins of GST and individual TLE domains (see Fig. 4A for description of TLE structure) revealed that Cbfa1 interacted with the first 135 amino acids (Q domain) of TLE1 (Fig. 4C, lane 2), whereas no detectable interaction was observed with residues 290 through 461 (SP domain) of TLE1 (Fig. 4C, lane 2). Cbfa1 also bound specifically to a fusion protein containing the highly conserved carboxy-terminal WDR domain shared by all Groucho/TLEs (Fig. 4C, lane 4). Since we were unable to obtain a stable preparation of a fusion protein containing the WDR domain of TLE1, these studies were performed using a fusion protein, GST-TLE3(490–774), containing the WDR domain of TLE3, which is 93% identical to the corresponding domain of TLE1 (22).

In agreement with these findings, yeast two-hybrid interaction assays demonstrated that co-transformation with plasmids encoding GAL4ad-Cbfa1(468–528) and GAL4bd-TLE1(1–770) (full-length TLE1) resulted in reconstitution of GAL4 transcriptional activity, indicative of an interaction between TLE1 and the carboxyl-terminal domain of Cbfa1 (Fig. 5A). An active GAL4 transcription complex was also reconstituted after co-transforming GAL4ad-Cbfa1(468–528) with either GAL4bd-TLE1(1–435), which contains the amino-terminal half of TLE1 excluding the WDR domain (Fig. 5B), or GAL4bd-TLE1(444–770), which contains only the WDR domain (Fig. 5C).

Further investigations revealed that the carboxyl-terminal WRPY motif conserved in all Runt-related proteins was not necessary for the interaction of Cbfa1 with TLE proteins. In particular, pull-down assays using in vitro translated Cbfa1(241–523), which extends from the end of the Runt domain to the carboxyl-terminal end but lacks the last 5 amino acids, WVRPY, showed that this protein was competent to bind to both the Q- and the WDR domains of TLEs (Fig. 5E). In contrast, Cbfa1(241–442), which lacks not only the WRPY motif but also most of the previously described carboxy-terminal repression domain of Cbfa1 (14), did not display TLE binding activity (Fig. 5F). These results are in agreement with previous studies showing that the WRPY motif of AML1 contributes to, but is not necessary for, the TLE/AML1 interaction (28). Taken together, these findings show that Cbfa1 can interact with TLE proteins and that amino acids 468–528 of Cbfa1 contain sequences that can interact with both amino- and carboxyl-ter-

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minal TLE domains.

**HES-1 Can Potentiate Cbfa1-mediated Transactivation**—The previous results show that the last 60 amino acids of Cbfa1 can mediate interaction with both HES-1 and TLE proteins. We therefore asked whether HES-1 could antagonize the interaction between Cbfa1 and TLE proteins by performing binding assays using the GST-TLE1(1–135) fusion protein, which mediates interaction with Cbfa1 (Fig. 4C) but not HES-1 (see below). In vitro translated Cbfa1 was incubated with GST-TLE1(1–135) in the absence (Fig. 6A, lane 3) or presence of an excess of either in vitro translated HES-1 (Fig. 6A, lane 5) or unprogrammed rabbit reticulocyte lysate (Fig. 6A, lane 4). The addition of HES-1 resulted in a significant decrease in the amount of Cbfa1 bound to TLE1(1–135), while the presence of the unprogrammed lysate had no effect. The competing effect of HES-1 did not correlate with binding of this protein to GST-TLE1(1–135) (Fig. 6A, lane 5), suggesting that neither HES-1 alone nor Cbfa1-HES-1 complexes were able to bind to this fusion protein. We obtained the same result when similar experiments were performed using AML1 instead of Cbfa1. In particular, addition of an excess of in vitro translated HES-1 significantly reduced the binding of the more slowly migrating form of AML1 to GST-TLE1(1–135). This effect was specific, since only a small inhibition of the binding of the faster AML1
form to GST-TLE (1–135) was observed (Fig. 6B, cf. lanes 2 and 3). These observations are in agreement with the finding that HES-1 interacts preferentially with the more slowly migrating form of in vitro translated AML1 (see Fig. 2B). Together, these results suggest that by interacting with Cbfa proteins, HES-1 may interfere with the association of the latter with TLE proteins.

To examine this possibility further, we hypothesized that if HES-1 can interfere with the Cbfa1/TLE interaction in transfected cells, then the transactivating ability of Cbfa1 might be potentiated as a result of the reduction/elimination of the repressive effect that TLE proteins can exert on Cbfa1 (14). To test this possibility, we examined the transactivating ability of Cbfa1 in rat ROS17/2.8 cells, which express high levels of endogenous TLE proteins (14, 36). These cells were transfected with a reporter plasmid containing the luciferase gene under the control of six tandem copies of a canonical Cbfa1-binding site, the OSE2 cis-acting element found in the promoter of the mouse osteocalcin gene (13). Co-transfection of increasing amounts of Cbfa1 expression plasmid resulted in a dose-dependent, but somewhat weak, activation of reporter gene expression (Fig. 7A). Importantly, this transactivation was significantly increased (approximately 15-fold) when increasing amounts of Cbfa1 were coexpressed with a fixed amount of HES-1, showing a functional cooperation between these proteins (Fig. 7A). Expression of HES-1 alone had no effect on reporter gene expression. Neither Cbfa1 alone nor the combination of Cbfa1 and HES-1 activated the expression of the reporter gene when the latter was placed downstream from a mutated OSE2 element (9) containing a 2-base pair mutation that abolishes Cbfa1 binding.2 Similar experiments were performed using human embryonic kidney 293 cells, which were shown to express relatively low levels of endogenous TLE proteins (36, 37). Unlike ROS17/2.8 cells, Cbfa1 mediated a strong transactivation of reporter gene expression when expressed in 293 cells (Fig. 7B).
We next asked whether the ability of HES-1 to promote Cbfa1-mediated transactivation was contingent on its ability to interact with TLE proteins. Since previous studies have shown that HES factors are incapable of binding to Groucho/TLEs if their carboxy-terminal WRPW motif is deleted (20, 21) we examined whether a truncated form of HES-1 lacking the last six amino acids, WRPWRN (referred to as HES-1 Δz), could potentiate the transcriptional activity of Cbfa1. As shown above in Fig. 2C, deletion of these amino acids did not impair the ability of HES-1 to interact with Runt-related proteins. More importantly, HES-1 Δz was capable of potentiating Cbfa1-mediated transactivation, albeit to a somewhat lesser extent than full-length HES-1 (Fig. 7A). Both HES-1 and HES-1 Δz were expressed at equal levels in transfected cells (Fig. 7C).

Taken together, these results indicate that HES-1 can promote the transactivating ability of Cbfa1 and that this positive effect does not depend on the formation of TLE-HES protein complexes. This suggests that HES-1 can potentiate Cbfa1 activity due to the formation of Cbfa1-HES-1 complexes that have stronger transcriptional activity than Cbfa1 alone, rather than by simply “titrating away” TLEs from Cbfa1.

**DISCUSSION**

**HES-1 Interacts with Cbfa Proteins—**Our studies have provided the first evidence that HES-1 can bind to Cbfa family members in cultured mammalian cells. The observed interactions appear to be direct and not mediated by TLE proteins.
since they can also be demonstrated in transformed yeast cells devoid of the latter. Moreover, they were observed in pull-down assays with bacterially expressed GST-HES-1 fusion proteins and preparations of AML1 and Cbfa1 obtained by in vitro translation using rabbit reticulocyte lysates devoid of detectable TLE immunoreactivity.

The finding that HES and Cbfa proteins can physically interact with each other is consistent with a number of previous results. First, expression studies show that, in both invertebrates and vertebrates, HES and Runt-related proteins are coexpressed in a variety of cell types (18, 20, 21, 33, 34). Second, both of these proteins interact with Groucho/TLE family members (18, 20, 21, 28, 36), suggesting that HES- and Runt-related proteins may compete with each other for Cbfa1 binding sites for TLE proteins, raising the possibility that HES-1 and TLE proteins may compete with each other for Cbfa1 binding (see below).

The observation that the carboxyl-terminal region of Cbfa1 is involved in TLE binding is consistent with the previous identification of a transcriptional repressor function within this domain (14) and suggests that this repressor activity is due to the recruitment of the TLE corepressors. Interestingly, the region of Cbfa1 containing residues 443–516 is ~70% identical to amino acids 366 through 438 of mouse Cbfa2 (14). Since this domain of Cbfa2 also harbors a transcription repression function (17), it is possible that TLE-binding sites are present within this carboxyl-terminal region of Cbfa2.

We have also shown that binding of TLE proteins to Cbfa1 is not dependent on the presence of a carboxyl-terminal WRPY motif. This result is in agreement with previous studies that also showed that binding of TLE1 to AML1 occurs even in the absence of the WRPY motif (28). Moreover, these findings are consistent with transcription studies in transfected mammalian cells showing that TLE overexpression reduces transactivation by both Cbfa1 and a truncated Cbfa1 form lacking the WRPY motif (albeit not as effectively in the latter case) (14). These combined results differ from the previous report that binding of Drosophila Groucho to Runt requires the carboxy-terminal WRPY motif of the latter (18). However, those same studies did show a weak Groucho/Runt interaction when a truncated form of Runt lacking solely the WRPY motif was used. Only when additional sequences were deleted together with the WRPY motif did Runt fail to bind to Groucho, suggesting that other elements in addition to the WRPY tetrapeptide may mediate this interaction. It is also possible that the difference between the investigations in Drosophila (18) and mammals (28, this study) may reflect differences between Drosophila Runt and its mammalian counterparts or may derive from the use of different experimental protocols.

Finally, our studies have also revealed that Cbfa1 can interact with two separate TLE domains located within either the amino-terminal Q region or the carboxy-terminal WDR domain, both of which are highly conserved among all Groucho/TLE family members (22). The identification of the WDR domain of Groucho/TLEs as a protein-protein interaction element is not surprising given the demonstrated involvement of WD40 repeats in molecular interactions (45, 46) and the previous demonstration that the WDR domain of Drosophila Groucho is involved in the interaction with the HES protein, Hairy (20). The amino-terminal Q domain of TLE proteins has also been shown previously to mediate protein-protein interactions, including those with the PRDI-BF1/Blimp-1 (47) and UTY (37) proteins. Moreover, in agreement with our results, Cbfa1 has recently been shown to interact with the product of the Grg5 gene, which encodes a roughly 200-amino acid protein homologous to the amino-terminal Q domain of Groucho/TLEs but...
lacking the carboxyl-terminal SP and WDR regions. Thus, it appears that TLE proteins utilize both of their recognized protein-protein interaction domains to interact with Cbfa family members. Although the specific contributions of these separate TLE domains to the interaction with Cbfa proteins remain to be determined, it is worth mentioning that we have recently found that TLEs also utilize both the amino-terminal Q domain and the carboxyl-terminal WDR domain to associate with specific members of the family of winged-helix DNA-binding proteins. This suggests that the use of separate protein-protein interaction domains may be a feature underlying the association of Groucho/TLE proteins with distinct DNA-binding factors.

Implications for Transcriptional Regulation by Cbfa and HES Proteins—The present demonstration that both Cbfa1 and AML1 can interact with HES-1 suggests that members of these two protein families can regulate each other's transcriptional functions. In agreement with this possibility, we have observed that HES-1 can potentiate Cbfa1-mediated transactivation in transfected cells. A number of observations suggest that HES-1 may perform this function by binding directly to Cbfa1 and inhibiting the interaction between Cbfa1 and endogenous TLE proteins, thereby reducing/inhibiting the repressive effect that TLEs can exert on the transactivating function of Cbfa1 (14). First, Cbfa1 and HES-1 can directly bind to each other. Moreover, binding sites for both HES-1 and TLE proteins are present within the same carboxyl-terminal domain of Cbfa1. In addition, HES-1 can interfere with the Cbfa1/TLE interaction in in vitro binding assays. Finally, Cbfa1-mediated transactivation can be potentiated by a truncated form of HES-1 that does not interact with TLEs due to the loss of its carboxyl-terminal WRPW motif but is still competent to bind to Cbfa proteins. Together, these observations suggest that the positive effect of HES-1 on the transcriptional activity of Cbfa1 may involve an active competition with TLEs for direct binding to Cbfa1, rather than a situation in which HES-1 simply titrates away TLEs from Cbfa1 but does not associate with the latter.

Alternative mechanisms can also be proposed. In particular, our results raise the interesting possibility that HES factors may mediate transcriptional activation, instead of repression, when they are associated with Cbfa proteins rather than with TLEs. Although a number of previous studies have shown that invertebrate and vertebrate HES proteins generally act as transcriptional repressors (20, 21, 34), recent investigations in Xenopus have implicated certain HES family members in both negative and positive feedback loop mechanisms that either repress or maintain the expression of genes of the Notch signaling pathway during embryonic somitogenesis (48). Together with our present observations, this finding suggests that, perhaps under appropriate conditions in which they escape interactions with Groucho/TLE proteins, HES factors may contribute to the transcriptional activity of other transcription factors.

The possibility that HES-1 may interfere with the Cbfa1/TLE interaction and, vice versa, that Cbfa1 may interfere with the HES-1/TLE interaction may help to explain the finding that HES-1 can repress the expression of the osteopontin gene in osteoblasts (33), whereas Cbfa1 can activate osteopontin expression (9, 44). It is possible that HES-1/TLE complexes keep the osteopontin promoter silent and that, by becoming recruited to the promoter, Cbfa1 may contribute to gene activation both directly, by providing a transactivating function, and indirectly, by interfering with the TLE/HES interaction.

These combined functions may mediate a shift from transcriptional repression mediated by DNA-bound HES-1/TLE complexes to transcriptional activation mediated by Cbfa1. In this model, the direct interaction between Cbfa1 and HES-1 may provide a way to prevent the interaction of Cbfa1 with TLEs. Specifically, by interacting with HES-1, Cbfa1 may become unavailable to TLE proteins and thus protect its transactivating ability from the repressive effect of the TLEs. This situation may provide a molecular explanation for the ability of Cbfa1 to promote transactivation of osteopontin and other osteoblast-specific genes even in the presence of TLE proteins (14, 44). This would likely not be possible if Cbfa1 were simply titrating away TLEs from HES-1, because the resulting Cbfa1-TLE complexes would probably not be able to promote transactivation (14, 28, 29).

This model is also consistent with the involvement of Drosophila Runt and Deadpan in the regulation of the Sex- lethal gene. Deadpan mediates repression of Sex-lethal and Groucho is required for this function (21, 32). Conversely, Runt can bind to the Sex-lethal promoter and stimulate its activation (43). It is possible that in males, where Runt dosage is one-half of that in females, Deadpan binds to the Sex-lethal promoter and, together with Groucho, mediates transcriptional repression. In females, Runt may be able to antagonize the Deadpan/Groucho-mediated repression by interacting with Deadpan and disrupting the repressive complexes of Deadpan and Groucho. The ensuing Runt-Deadpan complexes may then be able to promote transcription. This model would thus provide a way to regulate the Runt/Groucho interaction through the formation of Runt-Deadpan complexes, a situation that might help to explain the apparent paradox that Runt can activate Sex-lethal expression while at the same time mediating repression of other target genes in the same cells (43).

In summary, the present results implicate the activities of TLE and HES proteins in the modulation of the transcriptional functions of mammalian Cbfa proteins and suggest that the study of the interactions between these proteins may provide important information about the mechanisms underlying the transcriptional functions of Runt-related and HES proteins.

Acknowledgments—We thank Yoram Groner, Dwayne Barber, and Eseng Lai for the gift of several plasmids, George Karpati for providing access to a luminometer, and Yanling Liu for technical assistance.

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