Dermo-1, a Multifunctional Basic Helix-Loop-Helix Protein, Represses MyoD Transactivation via the HLH Domain, MEF2 Interaction, and Chromatin Deacetylation*

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Dermo-1 is a multifunctional basic helix-loop-helix (bHLH) transcription factor that has been shown to be a potent negative regulator for gene transcription and apoptosis. To understand the molecular mechanisms that mediate the function of Dermo-1, we generated a series of Dermo-1 mutants and used a MyoD-mediated transcriptional activation model to characterize the roles of its N-terminal, bHLH, and C-terminal structural domains in transcriptional repression. Both the C-terminal and HLH domains of Dermo-1 were essential for its repression of MyoD-mediated transactivation. Dermo-1 repressed, in a dose-dependent fashion, the transactivation activity of myocyte enhancer factor 2 (MEF2), a protein known to cooperate with MyoD in activating E-box-dependent gene expression. Both the N- and C-terminal domains of Dermo-1, but not the bHLH domain, were required for the inhibition of MEF2, suggesting that Dermo-1 inhibits both MyoD- and MEF2-dependent transactivation but through different mechanisms. Dermo-1 interacted directly with MEF2 and selectively repressed the MEF2 transactivation domain. An overall increase of histone acetylation induced by trichostatin A treatment reduced Dermo-1 transcriptional repression activity, suggesting that histone deacetylation is involved in Dermo-1-mediated transcriptional repression. Together, these results suggest that MEF2 is an important target in Dermo-1-mediated transcriptional repression and provide initial evidence of the involvement of histone acetylation in Dermo-1 transcriptional repression.

Mouse Dermo-1 is a member of the basic helix-loop-helix (bHLH) transcription factor family that was initially isolated using the yeast two-hybrid system with the bHLH domain of ubiquitously expressed E12 as bait (1). During embryogenesis, this gene is predominantly expressed in mesodermal- and ectodermal-derived tissues including somites, dermis, chondroblasts, limbs, teeth, and cranial structures and is believed to play important roles in the development and differentiation of these tissues and organs. Homologs of mouse Dermo-1 have been found in several other vertebrates such as humans, rats, and chicks with extensive sequence conservation during evolution (2–4). Interestingly, Dermo-1 homologs are also expressed in a subset of mesodermal- and ectodermal-derived tissues such as subectodermal mesenchyme, osteoblasts, and limb buds (2–4), which potentially act as negative regulators of differentiation. It has been shown that Dermo-1 functions as a potent transcriptional repressor for MyoD and as an anti-apoptotic agent for Myc- and p53-dependent cell death (1, 5). However, the precise biological roles of Dermo-1 during embryogenesis and its molecular mechanisms of action remain largely unknown.

In general, members of the bHLH transcription factor family are critical regulators of important biological processes such as cell lineage determination, proliferation, and differentiation (6). There is ample evidence of interplay between different members of bHLH proteins in the regulation of myogenesis, cardiogenesis, neurogenesis, and hematopoiesis (7–10). It is well documented that bHLH proteins regulate the differentiation of many other cell types during development either as transcriptional activators or repressors of gene expression (6). The bHLH proteins are characterized by two distinct motifs: (i) the basic region that mediates specific binding to the E-box consensus sequence (CANNTG) and (ii) the HLH domain that mediates heterodimerization with E proteins such as E12 (11, 12). In addition, the bHLH proteins have also been shown to form complexes with non-bHLH proteins such as the myocyte enhancer factor 2 (MEF2), SRF, and p300 (13–16). It has been established that MEF2, a MADS-domain protein, interacts directly with bHLH proteins to regulate gene transcription (13). Recently, genetic studies have shown that members of the MEF2 family, as transcription factors and coactivators for a battery of gene regulation, are critical in controlling the development of multiple tissues including heart, vasculature, neural tubes, and skeletal muscle (17–19). In tissue culture, MEF2 can induce gene expression in response to a calcium calmodulin-dependent protein kinase signal via the dissociation from HDAC factors (20). The function of MEF2 has been shown to be modulated via chromatin remodeling by recruiting HDAC 4/HDAC5/HDAC7 and their related protein MITR (20–22).

Many important attributes of the bHLH factors have been initially defined in the past using muscle differentiation as a model and members of myogenic bHLH proteins as regulators (6). MyoD is the first and most well characterized bHLH protein that behaves as a transcriptional activator; it provides a paradigm for defining the function of other bHLH proteins in cell determination and differentiation during development (23). The molecular mechanisms that mediate the transcriptional activation of MyoD during myogenesis require both basic and
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The transcriptional activity of MyoD during myogenesis is also regulated by Id and Twist, members of the HLH superfamily (6). Both Id and Twist are transcriptional repressors for MyoD. However, the molecular mechanisms mediating their action are distinct. The Id proteins belong to a class of HLH proteins that lack a basic region and have a greater affinity for E proteins (26). Therefore, Id functions as a dominant negative mutant to inhibit the function of tissue-restricted bHLH proteins such as MyoD. Twist, sharing significant homology with Dermo-1, contains both basic and HLH domains and has been shown to be a potent transcriptional repressor for MyoD (1, 27).

The repression mechanisms of Twist on MyoD transactivation have been demonstrated to be mediated by direct interaction with the basic domain of MyoD, sequestering E proteins, inhibiting MEF2 activation, and inhibiting the histone acetylase activity of MyoD coactivators such as pCAF and CBP (28–30).

In the present study, we investigated the molecular mechanisms by which Dermo-1 represses transcription. Since our first report on the cloning of Dermo-1 in a mouse and its function as a transcriptional repressor for MyoD (1), no further studies have been published on the characterization of the molecular mechanisms of Dermo-1 in transcriptional repression. Here we have focused on the roles of each domain of Dermo-1 in transcriptional repression using MyoD as a model. Our results demonstrate that the HLH and C-terminal domains and not the N-terminal and the basic regions of Dermo-1 are essential for its transcriptional repression activity. Further, Dermo-1 directly associates with MEF2 sites in the promoter region normally inactivated in 10T1/2 cells. 4R-tk-luc is a simplified MyoD-dependent reporter containing four E-boxes upstream of the minimal thymidine kinase promoter. Briefly, 10T1/2 cells were grown in 6-well plates in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum until they reached 80% confluence. The next day, 10T1/2 cells were cotransfected with 0.5 µg of mutant to inhibit the function of tissue-restricted bHLH proteins (either MCK-luc or 4R-tk-luc or MEF2x3-luc or pGln5-luc (Refs. 31–33)) and 0.5 µg of activator (either EMSV-MyoD or pcDNA-MEF2C or pGa4-MEF2C or pMyoD-VPl6 or pMPEF2C-VPl6 (Refs. 32, 34)) in the presence of the regulator (Dermo-1 or a Dermo-1 mutant in pcDNA3.1FLAG vector or pcDNA-M-twist (Ref. 29) or EMSV-E12 (Ref. 35)). Empty vector (pcDNA3.1) was used to control that equal amounts of DNA were available for each transfection. 3–4 h after transfection, cells were placed in a mixture of 10% fetal calf serum in Dulbecco’s modified Eagle’s medium and incubated overnight. Then the medium was changed to 0.5% fetal calf serum for 2 days to induce differentiation. Next, the transfected cells were harvested and assayed for luciferase activity using a commercial luciferase assay kit (Promega). Luciferase activities were normalized to the protein content in each sample. To test whether Dermo-1-mediated transcriptional repression requires deacetylase activity, 10T1/2 cells were treated overnight with 330 nM deacetylase inhibitor trichostatin A (TSA), beginning 24 h after transfection. For all transfection experiments, the luciferase activities were the average of the results of three independent duplicate experiments.

To determine the ability of Dermo-1 and its mutants to inhibit MyoD transactivation, 10T1/2 cells were plated on 6-well plates and transiently transfected with 1 µg of MyoD as described above in the absence or presence of 1 µg of Dermo-1 or each mutant. Empty vectors (pcDNA3.1) were used to normalize the amounts of DNA transfected in each well. After 5 days in differentiation medium, the expression of skeletal myosin was detected by incubating cells first with an anti-myosin antibody (Sigma) for 1 h at room temperature, then with biotinylated anti-mouse secondary antibody for 30 min, and finally with a horseradish peroxidase-streptavidin conjugate (ABC system; Vector Laboratories) for 30 min. Positive cells were visualized by DAB staining for 30 min at room temperature (Roche Molecular Biochemicals). The expression level of MyoD was assayed by standard Western blot analysis.

Western Blot Analysis—10T1/2 cells plated on 6-well plates were transiently transfected as described above but with 2 µg of pcDNA1.1FLAG-Dermo-1 or its mutants. Forty-eight hours after transfection, cells were rinsed two times in phosphate-buffered saline buffer and lysed in sample buffer (15 mM Tris HCl, 2% SDS, 4% glycerol, 1% 2-mercaptoethanol). Cell extracts were prepared by boiling for 5 min and brief centrifugation. The amounts of each sample were then subjected to 12% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore). FLAG antibody was used to detect the expression of each protein, which was visualized using a commercial chemiluminescence Western blotting kit (Roche Molecular Biochemicals).

Common precipitation Assays—For immunoprecipitation assays, Dermo-1 and MEF2C were cotransfected into COS cells and, 48 h later, harvested in lysis buffer containing 20 mM Tris Cl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 10% glycerol, and proteinase inhibitor (Roche Molecular Biochemicals). Harvested cells were then transferred to a 1.5-ml Eppendorf tube using a 21-gauge needle and centrifuged to remove debris. FLAG-tagged Dermo-1 proteins were immunoprecipitated with anti-FLAG M2 affinity gel (Sigma) at 4 °C for 4 h. After washing five times with 0.1% Nonidet P-40 lysis buffer with gentle agitation at 4 °C. Immunoprecipitated proteins were then separated out by SDS-PAGE, transferred to a polyvinylidene difluoride membrane immobiloblated with MEF2 polyclonal antibody (no. sc-313, Santa Cruz Biotechnology), and finally visualized using the chemiluminescence Western blotting kit (Roche Molecular Biochemicals). The MEF2 antibody is made against the carboxyl terminus of MEF2A and recognizes MEF2A, MEF2C, and MEF2D.

RESULTS

Dermo-1 Represses MyoD-induced Myogenic Program—Previously, we reported that Dermo-1 was able to inhibit the transactivation activities of myogenic bHLH factors (1). To determine whether Dermo-1 could repress the role of MyoD in initiating the myogenic program, we transiently transfected 10T1/2 cells with MyoD alone or MyoD plus Dermo-1 expression plasmids. As expected, MyoD converted 10T1/2 fibroblast...
cells into differentiated myogenic cells, as indicated by the expression of skeletal muscle myosin protein detected using an anti-myosin antibody (Fig. 1A). However, when Dermo-1 was cotransfected with MyoD, the number of skeletal muscle myosin-positive cells decreased about 80% (Fig. 1A). As confirmation that the reduction of myogenic cells was not due to a Dermo-1-mediated down-regulation of MyoD expression, the expression level of MyoD was examined in 10T1/2 cells transfected with MyoD alone versus MyoD plus Dermo-1. The expression level of MyoD was comparable in 10T1/2 cells transfected with or without Dermo-1 (Fig. 1B), suggesting that Dermo-1 does not affect the expression of MyoD in pEMSV vector but represses the ability of MyoD to initiate the myogenic program.

**Dermo-1 Inhibits MyoD-dependent Transactivation through a Mechanism Distinct from That Employed by Id.** 10T1/2 cells were transiently transfected with expression vectors as indicated (Fig. 2, A and B). The transactivation activities of MyoD were assigned a value of 100%. The luciferase activities were the average of results from three independent duplicate experiments.

The **C-terminal and HLH but Not the N-terminal and Basic Domains Are Essential for Dermo-1-mediated Transcriptional Repression.** To dissect the functional domains in Dermo-1 required for transcriptional repression, we generated a series of Dermo-1 mutants with mutations in its N-terminal, bHLH, or C-terminal regions (Fig. 3). The inhibitory ability of each mutant was determined using the same model system described above. Wild type Dermo-1 almost completely abolished MyoD transactivating activity, whereas deletion mutant DermoΔC-(121–160) (created by completely deleting the C-terminal region) repressed 45% of the MyoD activity (Fig. 4A). This suggested that the C-terminal region is essential for Dermo-1-mediated gene repression. However, mutants generated by limited deletions within the C-terminal region (i.e. DermoΔC-(134–160) and DermoΔC-(149–160)) did not reduce its ability as a transcriptional repressor (Fig. 4A), suggesting that the critical amino acids mediating Dermo-1 repression lie in the sequence immediately C-terminal to the HLH domain.

To determine whether the HLH domain in Dermo-1 was essential for its repression ability, we introduced a proline into the helix 1 region to disrupt the helix structure. The resulting mutant (DermoHLH–) completely lost its ability to repress transcription (Fig. 4A). This suggested that the HLH domain was required for the inhibitory function of Dermo-1. Consistent with this notion, a fusion protein of HLH and C-terminal do-
mains at the C-terminal of the SV40 nuclear localization signal (i.e. DermoHLHC) is sufficient to repress 80% of the MyoD transcriptional activities (Fig. 4A). However, the basic region mutant (i.e. Dermob−) and the N-terminal deletion mutant (i.e. DermoΔN-N−1−65) retained most of the repressor function (Fig. 4A). This result suggested that neither the DNA binding region nor the N terminus is essential for Dermo-1-mediated transcriptional repression.

Data obtained using MCK-luc as a reporter were similar to results obtained using 4R-tk-luc as a reporter (Fig. 4B). However, Dermo-1 was a less potent repressor of 4R-tk-luc than of MCK-luc. Because MCK-luc contains the two MEF2 sites in mediating Dermo-1 transcriptional repression. This promoter, the results suggest the potential importance of the MEF2 sites in mediating Dermo-1 transcriptional repression.

To ensure that loss of the inhibitory function by the mutants was not due to the absence of protein expression, the protein expression levels of all of the mutants described above were examined by Western blot analysis. Because all mutants were cloned in expression vectors containing the FLAG epitope tag, FLAG antibody immunostaining was used to visualize mutant proteins. Similar levels of protein expression were detected for all mutants except DermoΔC−(121−160) (Fig. 4C). Equal amounts of DermoΔC−(121−160) and wild type Dermo-1 proteins were observed when DermoΔN−N−(1−65) plasmid DNA was transfected 2.5-fold more than wild type plasmid DNA (Fig. 4C). This amount of DermoΔC−(121−160) protein could repress 45% of the MyoD activity (Fig. 4A).

To determine whether a putative NLS in the N terminus of Dermo-1 contributes to cellular localization of Dermo-1 protein, three NLS mutants (DermoNls1−, DermoNls2−, and DermoNls1&2−) were generated and transfected into 10T1/2 cells (Fig. 5A). Immunostaining for the FLAG antibody demonstrated that the NLS mutations did not significantly block the translocation of Dermo-1 protein from the cytoplasm into the nucleus (Fig. 5B). Whereas wild type Dermo-1 protein expression was exclusively nuclear, DermoNls1&2− protein expression was mostly but not completely nuclear (Fig. 5B), suggesting that amino acids other than the putative NLS sequences in the N terminus were also providing the nuclear localization signal. Mutation at both NLS sequences did not affect the expression and stability of these mutants compared with that of the wild type Dermo-1, as assessed by Western blot analysis (Fig. 5C).

**Dermo-1 Interacts Directly with MEF2 and Inhibits MEF Transactivation Activity in a Dose-dependent Manner**

Because MEF2C cooperates with MyoD to transactivate MCK and 4R-tk promoters, the effect of Dermo-1 on the transcriptional activity of MEF2C was examined. MEF2C significantly transactivated (3xMEF-luc) and activated the MEF reporter (3xMEF-luc), which contains three MEF2 binding sites upstream of the basal tk promoter. However, such a transcriptional activation was repressed by Dermo-1 in a dose-dependent manner (Fig. 6A). Dermo-1-mediated inhibition was also observed when MEF2C was replaced with MEF2A, another member of the MEF2 family (data not shown). For confirmation that Dermo-1 did not down-regulate the expression of MEF2C, the protein expression of MEF2C in the presence and absence of Dermo-1 was examined by Western
and MyoD through Distinct Mechanisms

MEF2C in the cell. The predominant band was the MEF2C protein into nuclei. A, the putative NLS sequence of Dermo-1 and its NLS mutants (DermoNls1, DermoNls2, and DermoNls1&2) are shown. The mutated amino acids are indicated. B, 10T1/2 cells were transfected with FLAG-tagged Dermo-1 or DermoNls. Protein expression was detected by anti-FLAG antibody (red). Although the wild type Dermo-1 protein was expressed exclusively in nuclei, most but not all DermoNls protein was located in nuclei. C, Western analysis using anti-FLAG antibody showed comparable levels of Dermo-1 and DermoNls as indicated in the presence of 0.5 µg of the reporter gene 4R-tk-luc or MCK-luc. Luciferase activities were an average of the results of three independent duplicate experiments.

blot analysis. The expression levels of MEF2 protein were not changed (Fig. 6B), indicating that Dermo-1 did not affect the synthesis and stability of the MEF2 protein.

To determine whether Dermo-1 could form a complex with MEF2 in vivo, we coimmunoprecipitated FLAG-tagged Dermo-1 using cell lysates cotransfected with or without MEF2C. The immunoprecipitates prepared with an antibody against the FLAG-epitope tag were subjected to Western blot analysis using anti-FLAG antibody. As shown in Fig. 6C, Dermo-1 could specifically immunoprecipitate MEF2C. Conversely, in cells transfected with Dermo-1 alone (as a negative control), no MEF2 were detected under this condition. These results suggested that Dermo-1 forms a stable complex with MEF2C to exert its inhibitory effect. Interestingly, no MyoD could be coimmunoprecipitated from cells cotransfected with MyoD and Dermo-1 (data not shown). Because the anti-MEF2 antibody used here could recognize MEF2A, MEF2C, and MEF2D, the multiple bands observed in Fig. 6B and C could represent the MEF2 complex or the degradation products of MEF2C in the cell. The predominant band was the MEF2C because exogenous MEF2C plasmid was transfected in the cells.

Dermo-1 Represses the Transactivation Abilities of MEF2 and MyoD through Distinct Mechanisms—To identify the inhibitory domains of Dermo-1 required for the repression of MEF2, we cotransfected Dermo-1 mutants with MEF2C using MEF2x3-luc as a reporter. Although mutation in the basic region (DermoC) did not affect the inhibitory effect, deletions at the C terminus (DermoΔC; 121–160) and N terminus (DermoΔN; 1–65) did lose significant amounts of inhibition (Fig. 7). Interestingly, the HLH domain of Dermo-1, which had already been shown to be essential for the repression of MyoD activity, was not required for the inhibition of MEF2 transactivation by Dermo-1 (Fig. 7), suggesting that Dermo-1 was using different mechanisms to repress MEF2 and MyoD transactivation. In supporting this hypothesis, plasmids pMyoD-VP16 or pMEF2C-VP16 (in which the transactivation domain of VP16 was fused to the C terminus of MyoD or MEF2C,
respectively) were cotransfected with their respective reporters in the presence of Dermo-1 or M-twist. Both of the resulting fusion proteins (MyoD-VP16 and MEF2C-VP16) strongly stimulated transactivation of the reporters in 10T1/2 cells (Fig. 8, A–C). Cotransfection of Dermo-1, similar to M-twist, completely abolished the transactivating ability of MyoD-VP16 (Fig. 8, A and B), whereas it repressed only 30% of the transactivational activity of MEF2C-VP16 (Fig. 8C). These results demonstrated that Dermo-1 utilized distinct mechanisms to repress MyoD and MEF2 transactivation.

To further determine whether Dermo-1, like M-twist, could inhibit the transactivation domain of MEF2, we cotransfected Dermo-1 with pGAL4-MEF2C, in which the Gal4 DNA binding domain was fused with MEF2C. The resulting fusion protein bound to the Gal4 DNA binding site and strongly transactivated the Gal4-luc reporter, which carried four copies of the Gal4 binding sites in its promoter region. Dermo-1 inhibited 70% of MEF2 transactivation activity (Fig. 8D). Because the transactivation activity of this fusion protein is conferred by the transactivation domain of MEF2C, these results suggest that the transactivation domain of MEF2C is a target for Dermo-1 inhibition.

**Histone Deacetylation Is Involved in Dermo-1-mediated Transcriptional Repression**—In light of increasing evidence that transcriptional repressors recruit HDAC complexes to carry out their inhibitory functions, HDAC involvement in Dermo-1-mediated repression was tested in cells treated with the deacetylase inhibitor, TSA. TSA treatment did not significantly affect the transactivating activities of MyoD and MEF2C (Fig. 9, A and B). However, in the presence of TSA, the ability of Dermo-1 to repress MyoD and MEF2 transcriptional activities was reduced 2.5- and 2-fold, respectively. In the same model system, Twist’s repression of those activities was also reduced to a similar extent (about 2–5-fold) (Fig. 9). These results suggested that Dermo-1 repressed MyoD- and MEF2-dependent transcription via a mechanism likely involved in histone deacetylation.
In the absence of Dermo-1, MyoD activates gene transcription in cooperation with E12 and MEF2 factors. When Dermo-1 is introduced into cells, its association with E12, MEF2, and a possible putative corepressor complex represses MyoD-mediated gene transactivation.

**DISCUSSION**

In this report, we characterized the roles of different domains of Dermo-1 in mediating transcriptional repression. Our mutagenesis studies showed that the HLH and C-terminal domains in Dermo-1 are essential for the transcriptional repression of MyoD and that disruption of either one of the two domains will abolish most of its inhibitory activity. However, the N-terminal and basic regions of Dermo-1 are not essential for its transcription repression activity, and mutants in these regions retain the majority of Dermo-1 repression activity. Further, we also demonstrated that Dermo-1 represses the transactivation of MyoD and MEF2 through different mechanisms. As proposed in the model (Fig. 10), the role of Dermo-1 as a transcriptional repressor for MyoD is mediated through several possible mechanisms including (i) dimerization with E12, (ii) the formation of a stable complex with MEF2 protein, and (iii) the involvement of histone deacetylase activity.

It is intriguing that the HLH domain is so critical for the Dermo-1 repression function. In general, HLH factors function as homodimers or heterodimers through their HLH domain (11, 35). Because Dermo-1 requires the presence of E12 to bind to DNA (1), it is reasonable to speculate that Dermo-1 heterodimerizes with E12 and that such a heterodimer is a potent active transcriptional repressor for MyoD transactivation. Therefore, overexpression of E12 failed to release the inhibitory effect of Dermo-1 on MyoD transactivation, suggesting that the sequestration of E12 from binding to MyoD by Dermo-1 is not the major mechanism. Whether E12 has a stronger affinity for Dermo-1 than MyoD remains to be determined. Although not many studies have reported that the HLH domain interacts directly with non-E proteins, we cannot rule out the possibility that Dermo-1 may interact with other non-E proteins through its HLH domain.

Our focus on MEF2 as a potential target for Dermo-1-mediated repression was based on the observation that Dermo-1 is a more potent transcriptional inhibitor of the MCK promoter than of the 4R-tk promoter, in which the MCK promoter contains two MEF2 sites not found in the 4R-tk promoter (36). Our results show that Dermo-1 directly targets the transactivation domain of MEF2 by forming a stable complex with it. This interaction is intriguing because MEF2 is a crucial transcription factor required for diverse biological processes. Our results raise the possibility that Dermo-1, by collaborating with MEF2, can also be integrated into the signal pathways of other developmental processes such as apoptosis, osteogenesis, and wound healing.

Although Dermo-1 can repress the transactivation activities of both MyoD and MEF2, distinct mechanisms are used in Dermo-1-mediated repression. Our coimmunoprecipitation results show for the first time that Dermo-1 forms stable complexes with MEF2, but not with MyoD. Furthermore, Dermo-1 completely overrides the transactivation activity of a VP16 domain fused with the C-terminal of MyoD, but suppresses only 30% of the transactivation activity of a VP16 domain fused with MEF2. These results suggest that Dermo-1 may target the transactivation domain of MEF2 without affecting the fusion protein's DNA binding ability. Whether Dermo-1 abolishes MyoD-VP16 transactivation activity by disrupting the fusion protein's DNA binding ability or by some other mechanisms remains to be determined.

It is well recognized that the dynamics of histone acetylation and deacetylation are closely related to the regulation of gene transcription (37). Because Dermo-1-mediated repression is sensitive to TSA, we speculate that histone deacetylase is involved in the Dermo-1 repressor function. Consistent with this possibility, overexpression of HDAC further increases the Dermo-1 repressive function. However, the involvement of HDAC is only one of the repression mechanisms, considering that TSA only partially relieves Dermo-1 transcriptional repression activity. Histone acetylation can be regulated through several mechanisms such as the inhibition of HAT activity or the recruitment of corepressors possessing histone deacetylase activities. The accumulating evidence suggests that HDAC recruitment is a strategy commonly used by numerous transcriptional repressors, including nuclear receptor corepressor N-CoR and SMRT (38-41), the tumor suppressor protein Rb (42, 43), and even some transcriptional activators such as MEF2 (20, 44). Recently, another bHLH protein named SHARP-1 has been reported to employ an HDAC-dependent inhibitory mechanism in executing its repressor function (45). We have yet to determine whether Dermo-1 recruits HDAC directly or indirectly.

Dermo-1 belongs to the same family as *Drosophila* Twist (1). Genetic studies have defined the essential role of *Drosophila* Twist in mesoderm formation and the activation of a set of well defined signal pathways for mesodermal migration, heart, and skeletal muscle formation and differentiation involving genes such as the FGF receptor, tinman, and *MEF2* (46-50). In vertebrates there are two members in this family, Dermo-1 and Twist, which share considerable homology at the bHLH and C-terminal regions. However, it is noteworthy that these two factors differ substantially at the N-terminal regions (1). During development, Dermo-1 and Twist also exhibit overlapping temperospatial expression patterns (1, 2). Functionally, both Dermo-1 and Twist have been found to be potential oncoproteins in promoting colony formation of E1A/Ras-transformed mouse embryo fibroblasts in soft agar (5). Furthermore, they also inhibit Myc- and p53-induced apoptosis (5). During myogenesis, both of them are expressed in somites and diminished in myotomes as cell differentiation occurs (1, 28, 51). Consistent with those results, both Dermo-1 and Twist have been shown to be potent MyoD-mediated transcriptional repressors (30) (1, 28), which is consistent with the mechanism for *Drosophila* Twist in myogenesis (52).

Despite the similarity in transcriptional repression and anti-apoptosis, Dermo-1 and Twist clearly play distinct roles at different stages of differentiation and development. During osteogenesis, Dermo-1 inhibits the differentiation of preosteoblasts, whereas Twist is required for the maintenance of osteogenitor cells (2). The functional difference between Twist and Dermo-1 is best illustrated in the Twist knockout mouse in which the absence of Twist cannot be compensated by Dermo-1 and leads to embryonic lethal phenotypes due to defects in head mesenchyme, with extensive apoptosis at the somites (53). Furthermore, M-twist in different tissues acts differently and targets to diverse downstream genes. For example, in head, branchial arch, and limb bud mesenchyme, M-twist acts in a cell-autonomous manner, whereas in somites, it acts as a non-

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cell-autonomous manner. It remains to be determined whether M-twist functions in different tissues by forming diverse heterodimers or heteromers with different E proteins or other bHLH or non-bHLH proteins. Similarly, recent analyses of Dermo-1 knockout mice reveal remarkable phenotypes with abnormalities in the development of vertebrae, wound healing, and hair regeneration; the mice eventually die of cachexia.\(^3\) M-twist apparently fails to substitute for the function of Dermo-1.

The significance of understanding the molecular mechanisms mediating Dermo-1 function during development has become apparent because of its role in anti-apoptosis and the remarkable phenotypes in Dermo-1 knockout mice. In this report, we focused on defining the role of each domain in Dermo-1-mediated transcripational repression and demonstrated that Dermo-1 can repress MyoD to initiate myogenesis using similar yet distinct regulatory mechanisms compared with M-twist. Previously, substantial evidence has been reported that M-twist directly interacts with both MyoD and MEF2 proteins (28, 29). Whereas the basic domain of M-twist is required to repress MyoD transactivation activities, its N-terminal domain interacts directly with pCAF and p300 to inhibit the HAT activities of pCAF and p300 (29, 30). However, in this report we showed that mutations in the basic region and N-terminal domain of Dermo-1 retain the majority of its repression activity. It remains to be determined whether the sequence difference in these regions in Dermo-1 and M-twist accounts for their functional difference. These studies provide the groundwork to further the mechanisms of Dermo-1 in other biological processes such as apoptosis, hair regeneration, and wound healing.

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