MEF2-dependent Recruitment of the HAND1 Transcription Factor Results in Synergistic Activation of Target Promoters*

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HAND proteins are tissue-restricted members of the basic helix-loop-helix transcription factor family that play critical roles in cell differentiation and organogenesis including placental, cardiovascular, and craniofacial development. Nevertheless, the molecular basis underlying the developmental action of HAND proteins remains undefined. Within the embryo, HAND1 is first detected in the developing heart where it becomes restricted to the atrial and left ventricular compartments, a pattern identical to that of the Nppa gene, which encodes atrial natriuretic factor, the major secretory product of the heart. We hereby report that the cardiac atrial natriuretic factor promoter is directly activated by HAND1, making it the first known HAND1 transcriptional target. The action of HAND1 does not require heterodimerization with class I basic helix-loop-helix factors or DNA binding through E-box elements. Instead, HAND1 is recruited to the promoter via physical interaction with MEF2 proteins. MEF2/HAND1 interaction results in synergistic activation of MEF2-dependent promoters, and MEF2 binding sites are sufficient to mediate this synergy. MEF2 binding to DNA is not enhanced in the presence of HAND1. Instead, cooperativity likely results from corecruitment of co-activators such as CREB-binding protein. The related HAND2 protein can also synergize with MEF2. Thus, HAND proteins act as cell-specific developmental co-activators of the MEF2 family of transcription factors. These findings identify a novel mechanism for HAND action in the heart and provide a general paradigm to understand the mechanism of HAND action in organogenesis.

The HAND1 and HAND2 proteins form an evolutionarily conserved subgroup of the tissue-restricted bHLH factors that are expressed in several neural crest and mesodermal derivatives, most notably in the heart and limbs (1–5). During embryogenesis, the two HAND genes are initially co-expressed throughout the heart tube, but their expression pattern becomes complementary as the heart develops, with HAND1 marking the atria and left ventricle while HAND2 marks the right ventricle. Genetic studies have demonstrated clearly that both proteins are required for proper cardiac cell differentiation and heart morphogenesis (6–10). Targeted inactivation of hand1 leads to embryonic lethality around embryonic day 10.5 because of cardiovascular defects that include right ventricular hypoplasia and vascular abnormalities (8, 11). In the case of HAND1, analysis of its role in heart development was initially complicated by its requirement for proper placental development; mice lacking hand1 died at embryonic day 8–8.5 of placental and extraembryonic defects, tissues where HAND1 is abundantly expressed (6, 7). Nevertheless, tetraploid aggregation experiments showed that hand1 null embryonic stem cells could not contribute to the left ventricle, suggesting a cell autonomous requirement for HAND1 in this cardiac lineage (6). Gain-of-function studies in transgenic mice showed that misexpression of HAND1 in the whole ventricles altered expression of several genes including Nppa, the gene encoding atrial natriuretic factor (ANF), and Hand2 (12). Interestingly, this study concluded that HAND1 is not a master regulator of the left ventricular myocyte lineage but rather that it acts cooperatively with other transcription factors to control dorso-ventral patterning. More recently, conditional ablation of hand1 in the heart provided support for these previous observations and revealed that absence of HAND1 in cardiac myocytes leads to structural cardiac defects, consistent with an essential role in cardiomyocyte differentiation (10). Targeted gain- and loss-of-function studies have also pointed to important roles for HAND proteins in placental (6), vascular (11, 13), craniofacial (14), and limb (15, 16) development. However, the molecular mechanisms underlying the essential role of HAND proteins in organogenesis remain largely undefined.

HAND1 was initially isolated by virtue of its ability to heterodimerize with the ubiquitous class I bHLH factors, E12/E47 (1, 4). However, several independent studies showed that, unlike the MyoD/NeuroD subfamily (17), HAND1-containing heterodimers do not bind canonical E-boxes (1, 4, 18). In vitro site selection revealed binding of HAND1/E47 to distinct DNA elements containing degenerate E-box motifs (1), but the relevance of these elements has yet to be confirmed in the natural context of HAND1 target genes. Interestingly, the ability of HAND1 to bind as a heterodimer and/or activate transcription from these sites may itself be regulated by other cofactors (18). One such cofactor may be the LIM domain-containing FHIL2 transcription factor, which was recently shown to interact with the bHLH domain of HAND1 and repress HAND1/E12-dependent transcription (19). Finally, the ability of HAND1 to form homodimers versus heterodimers with E12 can also be regulated at the level of phosphorylation; two residues within helix 1 of the bHLH domain have been identified the phosphorylation status of which alters E-protein heterodimer formation and biologic activity in the limbs (20). Other studies suggest that HAND1 may act essentially as a repressor by titrating class I bHLH factors in a manner reminiscent of that of the Id proteins (20); additionally, HAND1 was shown to contain transcriptional repressor domains capable of down-regulating GAL-

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E47-dependent transcription (1, 21). At present, the question as to whether and how HAND1 acts as a transcriptional activator is not yet settled. Evidently, analysis of HAND1 action on target promoters would provide invaluable insight into its transcriptional pathways.

In the case of the related HAND2 factor, two modes of action have been identified so far. One of them is E-box-dependent and involves DNA binding as a heterodimer with ubiquitous class 1 proteins (22). The other pathway does not depend on HAND2 binding to DNA; instead, HAND2 is recruited to the promoter through protein/protein interaction with GATA-4 (23). Interestingly, interaction with GATA proteins may be a general property of class II bHLH factors as shown previously for SCL and GATA-1 in hematopoietic cells (24, 25). Finally, HAND2 has been shown to interact with the homeodomain-containing Nkx2.5 protein and, in combination with E12, enhance its activation of the ANF promoter (26). In this study, the modest ability of HAND2 to activate the ANF promoter could be enhanced by the addition of E12, although no interaction between HAND2/E12 and any of the ANF E-boxes could be detected. In fact, all E-boxes were dispensable for HAND2/E12 action, which apparently required the Nkx2.5 binding site.

In addition to their overlapping expression pattern, two recent studies suggest that ANF may be a transcriptional target for HAND1. For example, the restricted pattern of HAND1 expression in heart atria and left ventricles is highly reminiscent of that of the Nppa gene, which encodes ANF, the major secretory product of the heart. Moreover, mice with cardiac-specific inactivated hand1 alleles have decreased ANF transcripts in the left ventricles, whereas in hand1/2 double mutant mice, ANF expression is completely abrogated (10). On the other hand, ectopic expression of HAND1 in the right ventricle was sufficient to induce transcription of the endogenous ANF gene (12). We therefore tested whether the cardiac ANF promoter may be a transcriptional target for HAND1. Our results show that HAND1 is a transcriptional activator of ANF and identify for the first time a direct transcriptional target for HAND1. The studies also unravel a novel mechanism for HAND action, which involves direct physical association and cooperative interaction with MEF2 proteins. These findings have broad implications for understanding the molecular mechanisms underlying HAND functions in cardiac and non-cardiac cells.

**MATERIALS AND METHODS**

**Cell Cultures and Transfections**—Neonatal cardiomyocytes were prepared from 4-day-old Sprague-Dawley rats and plated at a density of 250,000 cells/9.5-cm² culture dish in six-well Primaria plates as described previously (27). HeLa and NIH3T3 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Transfections were carried out using calcium phosphate as described previously (28). HeLa and NIH3T3 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Transfections were carried out using calcium phosphate as described previously (28). Transfections were carried out using calcium phosphate as described previously (28).

**Plasmids**—ANF-luciferase reporters containing various mutations and deletions of the rat ANF promoter as well as MEF2 and GATA-4 expression vectors have been described previously or were prepared using similar mutagenesis (28, 29). The endothelin-1 (pPET-1) luciferase reporter was described in Nemere and Nemere (30). The HAND1 (eHAND) expression vectors were described previously (18). The myc-Hand2 expression vector was a kind gift of Dr. Robert Schwartz.

**Recombinant Protein Production and Pull-down Assays**—HAND1 was bacterially produced as described previously (28) with few modifications. After transformation of Escherichia coli (BL21, DE3) with GST fusion vectors pGEX-3X, Amersham Biosciences, individual colonies were picked and grown in 500 ml of LB to an optical density of 0.6. Isopropyl thio-galactopyranoside was then added at a final concentration of 0.1 mM, and bacterial cultures were grown at 37 °C for 2 h. The cultures were centrifuged, and the bacteria were resuspended in 20 ml of 1% Triton X-100 cold PBS and lysed by sonication. Purification was performed using 1 ml of 50% glutathione-agarose beads (Sigma) in PBS, mixed at 4°C with agitation for 2 h, and then centrifuged at 1500 rpm at 4°C for 5 min to pellet the resin. The beads were washed three times in 50 ml of 1% Triton X-100 PBS, and the GST fusion proteins were analyzed on SDS-PAGE. Recombinant E47 and MEF2 proteins were produced in vitro using the TnT-coupled in vitro transcription translation system (Promega Corp., Madison, WI). In vitro binding studies were performed as described previously (28) with minor modifications. Briefly, 3–5 μl of 35S-labeled MEF2 and E47 proteins were incubated with 300 ng of immobilized HAND1 fusion proteins in 500 μl of IP buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 5 mM EDTA, 0.1% Nonidet P-40, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.25% bovine serum albumin (BSA)) for 2 h at 4°C with agitation and then centrifuged for 2 min at 1500 rpm at 4°C. The amount of total 35S-labeled proteins was kept constant (8 μl) in each binding reaction by adding 35S-labeled luciferase to complete. Beads were washed three times by vortexing in 500 μl of IP buffer without BSA. The protein complexes were released after boiling in Laemmli buffer and resolved by SDS-PAGE. Labeled proteins were visualized and quantified using a phosphorimaging screen and a STORM (Amersham Biosciences).

**Immunoprecipitations and Immunoblots**—Co-immunoprecipitation of FLAG-HAND1 and hemagglutinin (HA)-MEF2A (pCGN-MEF2A) was carried out using nuclear extracts of 293T cells overexpressing the relevant proteins as described previously (28). Co-immunoprecipitation reactions were carried out on 50 μg of nuclear extracts using 1 μl of anti-HA (12CA5, Roche Diagnostics) antibody in 500 μl of binding buffer without BSA, and bound immunocomplexes were washed and subjected to SDS-PAGE followed by transfer to a Hybond polyvinylidene difluoride membrane and immunoblotting.

**Electrophoretic Mobility Shift Assays**—Electrophoretic mobility shift assays were carried out essentially as described previously (28) using nuclear extracts from cells transfected with the indicated expression vectors. The probes for the MCK and ANF Mef2 sites were described previously (28).

**RESULTS**

**HAND1 Is a Transcriptional Activator of ANF**—To test whether ANF was a potential downstream target for HAND1, we carried out cotransfection studies using a luciferase reporter driven by the ~700 ANF promoter. Previous studies have established that these promoter sequences are sufficient to recapitulate spatiotemporal regulation of the endogenous ANF gene in the heart. As shown in Fig. 1A, HAND1 activated transcription from the ANF promoter in HeLa cells in a dose-dependent manner. This effect was also observed in cardiomyocytes and in other non-cardiac cells (Fig. 1B). Interestingly, the class I bHLH factors E47 and E12 also activated the ANF promoter (Fig. 1, A and B and data not shown). However, other bHLH proteins including MyoD, myogenin, Hey1, and Hey2 did not result in transcriptional activation of the promoter (Fig. 1A and data not shown). In fact, Hey 1 and 2 resulted in a dose-dependent repression of the ANF promoter especially in cardiomyocytes. Interestingly, the related HAND2 protein did not significantly activate the ANF promoter, a finding consistent with previous studies (23, 26) (Fig. 1A). These results indicate that the ANF promoter is responsive to HAND1 and provides a tool to elucidate the mechanisms of action of HAND1. Next, we carried out a limited structure-
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function study to determine the domains of HAND1 required for its transcriptional effect. Activation required the HLH domain of HAND1 but not its N terminus (Fig. 1C), a region associated previously with transcriptional repression (21). In fact, removal of the N terminus produced a superactivator (Fig. 1C). Because E12/E47 also activated the promoter, it was important to determine whether the action of HAND1 on the ANF promoter involved interaction with class I bHLH proteins or heterodimerization with other factors. A tethered HAND1-HAND1 homodimer and a tethered HAND1-ITF2 heterodimer were tested in cotransfection studies. Both failed to activate the ANF promoter (Fig. 1C), suggesting that HAND1 activation of ANF may involve interaction with other transcription factors but likely not class I bHLH.

**HAND1 and E47 Target Distinct DNA Elements on ANF**—To gain further insight into the mechanism of HAND1 action, we used mutational analysis of the ANF promoter to localize the HAND1 and E47-responsive regions. Removal of sequences between −640 and −500 bp reduced HAND1 activation by 5-fold (Fig. 2A) but had no effect on the E47 activation that was lost when sequences between −500 and −480 were deleted (Fig. 2B). This region harbors a conserved E-box, which is evolutionarily conserved in ANF genes from different species. Gel shift analysis confirmed that the ANF E-box at position −490 bp is a bona fide binding site for E47 as shown in direct binding and competition experiments (Fig. 2D). These results suggested that HAND1 and class I bHLH activate the ANF promoter via distinct pathways. Next, we undertook to map the HAND1 regulatory element(s). Previously, we showed that sequences between −640 and −500 contain a Mef2 element (28). Unexpectedly, mutations of this element drastically reduced HAND1 activation (Fig. 2C), raising the possibility that HAND1 action involves functional cooperation with (endogenous) MEF2 proteins. We tested whether HAND1 affected MEF2 binding to its site using either the ANF (Fig. 2E) or MCK (data not shown) Mef2 probes. In repeated experiments using nuclear extracts expressing MEF2A alone or together with HAND1, we could not detect any increase in MEF2 binding in the presence of HAND1 (Fig. 2E). HAND1 alone did not bind to the A/T-rich Mef2 site or to an adjacent E-box (data not shown) suggesting that transcriptional activation of the ANF promoter by HAND1 does not involve direct binding of HAND1 to DNA. We also tested whether HAND1 activation could be due to interaction with endogenous GATA factors (GATA2 is abundantly expressed in HeLa cells (28)) because the related HAND2 protein was shown to interact with GATA-4 (23). Mutation of the two high affinity GATA sites in the context of the native −700 bp promoter (27) significantly reduced but did not abrogate HAND1 activation (Fig. 2C). In contrast, mutation of other regulatory elements, including the proximal SRE or the proximal NKE, had no effect on HAND1 activation (Fig. 2C and data not shown). Together, these results suggest that HAND1 is recruited to the ANF promoter via its interaction with GATA or MEF2 proteins.

**HAND1 Is a MEF2 Cofactor**—Next, we directly tested whether HAND1 and MEF2 interact functionally. This was done by cotransfecting the ANF-luc reporter with either MEF2A or HAND1 or both in NIH3T3 cells that have no detectable GATA activity. As shown in Fig. 3A, the presence of both HAND1 and MEF2A resulted in synergistic transcriptional activation. This synergy was also observed with MEF2C (Fig. 3E). Addition of the related HAND2 protein resulted in similar transcriptional cooperativity with MEF2 (Fig. 3A). The DNA binding domain of MEF2 (MEF2A DIVE) was sufficient to observe synergy (albeit to a lower extent) suggesting that MEF2 proteins acted to recruit HAND1 to target promoters. Consistent with that, HAND1/MEF2 synergy was also observed on a minimal ANF promoter driven by a multimerized Mef2 element (Fig. 3B). Given that we showed previously that MEF2 proteins could also be recruited to target promoters via GATA proteins (28), we tested whether GATA-4 could also interact with a MEF2-HAND complex. For this, we used the proximal ANF promoter that contains two GATA binding sites but is unresponsive to HAND1 or MEF2 (in the absence of GATA proteins). As shown in Fig. 3D, this promoter fragment became responsive to MEF2/HAND1 in presence of GATA-4 (left panel). Cooperativity between these three transcription factors required the presence of the high affinity GATA binding site, and its mutation significantly reduced promoter activation (right panel). Thus, it appears that MEF2 can tether HAND1 to target promoters through a DNA-dependent or independent pathway. Consistent with this, a MEF2C mutant defective in DNA binding but retaining the ability to associate physically with GATA proteins (28) and HAND1 (Fig. 4B) was as efficient as intact MEF2C in supporting synergy with HAND1 on the proximal ANF promoter in GATA-containing HeLa cells (Fig. 3E). However, in NIH3T3 cells, where synergy requires the Mef2 site, this mutant could not support synergy (data not shown).
The above results suggested that HAND1 action involves a MEF2-containing complex. We therefore tested whether these proteins interacted physically. MEF2A and HAND1 could be co-immunoprecipitated in vivo (Fig. 4A), suggesting physical interaction between the two proteins. To determine whether this interaction was direct, we performed in vitro pull-down assays using immobilized GST-HAND1 and in vitro translated 35S-labeled MEF2 proteins. E47 was included as positive control. GST-HAND1 was able to retain all of the MEF2 proteins tested, MEF2A, MEF2C, and MEF2D, as well as the positive control E47 at similar efficiency but did not retain the negative control luciferase (Fig. 4B), confirming that HAND1 and MEF2 interact directly. MEF2 proteins defective in DNA binding (MEF2C R3T and MEF2C R24L) retained the ability to bind HAND1 indicating that distinct residues are required for DNA binding and HAND1 interaction, as suggested from the functional assays (Fig. 3E). Given that HAND1/MEF2 association did not apparently enhance MEF2 binding to DNA (Fig. 2E), we tested whether functional cooperativity between the two factors may involve corecruitment of such transcriptional activators as CBP. As shown in Fig. 4C, addition of CBP resulted in further enhancement of HAND1/MEF2 as well as HAND2/MEF2 activation of the ANF promoter. Finally, HAND1/MEF2 cooperativity was not restricted to the ANF promoter. The endothelin-1 promoter that is active in vascular endothelial, endocardial, and myocardial cells was also synergistically activated by HAND1 and MEF2. Together, the data are consistent with a role for HAND proteins as co-activators of the MEF2 family of transcription factors.

DISCUSSION

Genetic analysis established clearly the importance of HAND proteins for heart formation, but the molecular mechanisms by which HAND proteins regulate transcription of target genes and thus, influence cardiomyocyte fate, are not fully understood. In this study, we provide evidence that the ANF promoter is a downstream target for HAND1. This finding is supported by two recent reports (10, 12) showing that ectopic expression of HAND1 in the right ventricles is sufficient to up-regulate the endogenous ANF gene and that ANF transcripts are down-regulated in mice with conditional hand1 mutated alleles. Thus, ANF appears to be a bona fide direct target for HAND1. We also show
that HAND proteins act synergistically with MEF2 factors to activate cardiac transcription. The HAND/MEF2 synergy is mediated by direct physical interaction, and MEF2 acts to tether HAND proteins to DNA either through direct binding to its Mef2 site or via a GATA-dependent pathway. This finding identifies a novel mechanism for HAND action in the heart and suggests that these proteins can alter transcription via multiple pathways and through interaction with diverse partners.

**MEF2-dependent Transcriptional Activation by Class II bHLH Factors**—MEF2 proteins have been shown to interact functionally with MyoD and with MASH1, another class II bHLH factor, and activate transcription either through a MEF2 binding site or an E-box (31–33). The finding that MEF2 also cooperates with the HAND subfamily suggests that interaction with MEF2 proteins may be a general property of class II bHLH factors. MEF2 proteins are co-expressed with class II...
bHLH factors in several cell types such as T lymphocytes, which co-express MEF2 and TAL/SCL (34), and neuronal cells, where MEF2 is co-expressed with numerous neurogenic bHLHs, such as N-SCL, NeuroD, and MASH1 (34). With respect to HAND proteins, in addition to the heart, MEF2 and HAND1 (as well as HAND2) are found in endothelial and vascular smooth muscle cells (3, 11, 35, 36). Given the established roles of MEF2 for the differentiation of these cells or for their response to various stimuli (36–39), interaction with MEF2 may provide a general paradigm for the action of tissue-restricted class II bHLHs. However, unlike MEF2 interaction with MyoD or with MASH1, HAND1/MEF2 interaction does not appear to require class I bHLH factors. This is evidenced by the MEF2/HAND1 synergistic activation of the MEF2-luc reporter in L cells, which lack class I bHLHs. Moreover, addition of varying amounts of the E47 expression vector in cotransfection experiments failed to further enhance MEF2/HAND1 activation (data not shown). Interestingly, the DNA binding domain of MEF2 was sufficient for synergy with HAND1 suggesting that HAND1 is able to support transcriptional activation. Similarly, either the MEF2 or MyoD activation domain was sufficient for MEF2/MyoD synergy (31). Thus, HAND1 may be recruited to DNA by MEF2 and may in turn recruit co-activators like p300/CBP, or it may help MEF2 corecruit such activators. In this respect, it is noteworthy that the highly homologous bHLH domain of HAND2 was shown recently to interact physically with p300 (23). On the other hand, the MADS box of MEF2 is known to bind p300 directly (40). Because this same region also binds corepressors like histone deacetylases (41, 42) and MITR (43), it is also possible that HAND1 binding serves to displace a MEF2 corepressor.

**Combinatorial Interactions between HAND and Non-HLH Transcription Factors**—As stated earlier, HAND1 can bind to and activate a GGNNTCTGG-containing element (termed Th1). The ~700 bp ANF promoter contains no consensus Th1 elements, but two GC-rich regions centered around ~530 bp and ~180 bp have a TCTGG motif. Although we cannot formally exclude a role for these elements in HAND1 transcriptional activation, our mutational analysis does not point to any significant role for either element in HAND1 action. Additionally, our results do not suggest a requirement for E47 or other class I bHLHs in HAND1 transcriptional effects on the ANF promoter. Rather, our work and two other studies (23, 26) that examined the effect of HAND2 on ANF suggest that transcriptional activation by HAND proteins involves interaction with non-HLH factors. In fact, although both HAND2 studies reported a positive effect on the ANF promoter, the mechanism by which this occurred was controversial. One study suggested that the HAND2 effect was mediated by GATA-4 (23). The other study failed to detect synergy between GATA-4 and HAND2 and suggested that HAND2 acted as a (modest) co-activator of the homeodomain protein Nkx2.5 (26). Additionally, Dai et al. (23) did not test the combinatorial effects of HAND1/GATA-4, but Thattaliyath et al. (44) reported that although HAND1 interacted physically with Nkx2.5,

**FIGURE 4.** HAND1 interacts physically with MEF2. In vivo (A) and in vitro (B) evidence for protein/protein interactions. A, co-immunoprecipitation (IP) of HA-tagged MEF2A and HAND1 in 293 cells. B, in vitro pull-down assays using GST-HAND1 and in vivo 35S-labeled translated E47, MEF2A, -2C, and -2D; for a detailed description of the experiments, please see “Materials and Methods.” C, effect of CBP on HAND1/MEF2 synergy. Cotransfections were carried out in NIH3T3 cells as described above. D, cooperative interaction between HAND1 and MEF2A over the ANF and the preproendothelin-1 (ppET-1) promoter. Cotransfections were carried out in HeLa cells as described above. The data are the mean of n = 4.
it did not activate the ANF promoter either alone or in combination with Nkx2.5. In contrast, our results and those of Togi et al. (12) indicate clearly that HAND1 is an activator of ANF transcription. Moreover, although we could not detect any cooperativity between Nkx2.5 and HAND1, the MEF2/HAND1 synergy over the ANF promoter could be further potentiated by the addition of Nkx2.5 suggesting that MEF2 may be necessary for a functional Nkx2.5/HAND1 interaction (data not shown). Thus, we believe that HAND1 alters transcription of ANF and that of other target genes such as ET-1 through combinatorial interactions with non-HLH transcription factors, notably GATA and MEF2. Our study revealed that HAND1 activation of ANF in MEF2- and GATA-expressing cells was decreased when either the MEF2 or GATA element was mutated. However, HAND/MEF2 synergy did not require the GATA sites. HAND1 alone or in combination with MEF2 potentiated GATA-4 activation of the ANF promoter in the absence of Mef2 sites. These observations suggest that HAND1 can interact independently with GATA and MEF2 and activate transcription via their respective sites. They also suggest possible combinatorial interaction among all three factors. The specific HAND1 collaborator may vary depending on developmental stages or physiologic status of the cell. For example, the ANF MEF2 binding site contributes differentially to ANF promoter activity in embryonic but only 50% in postnatal myocytes (4). Because MEF2 activity is also regulated developmentally (45) and given that HAND1 expression is predominantly embryonic, it is tempting to suggest that HAND1 may represent a stage-specific MEF2 cofactor that contributes to positive modulation of MEF2 activity.

In conclusion, the present study reveals that HAND1 is a transcriptional activator and a MEF2 cofactor. Given the importance of MEF2 proteins in cardiovascular development and homeostasis (36–38, 45, 46), further studies on the regulation of their collaborators, including HAND1, are warranted. Finally, the identification of the ANF and ET-1 promoters as targets for HAND1 should facilitate further dissection of the HAND1 regulatory pathways in organogenesis and pathophysiology.

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