Nkx2.5 (also called Csx) is an evolutionarily conserved cardiac transcription factor of the homeobox gene family. Nkx2.5 is required for early heart development, because Nkx2.5 null mice die before completion of cardiac looping. To identify genes regulated by Nkx2.5 in the developing heart, we performed differential screening in combination with suppression subtractive hybridization using RNA isolated from wild-type and Nkx2.5 null hearts at embryonic day 8.5. One gene that we found to be markedly down-regulated in the hearts from Nkx2.5 null embryos is an isoform of Mov10 like-1 (Mov10l1), a putative RNA helicase expressed in testis. We named this novel isoform as Csm (cardiac-specific isoform of Mov10l1). Csm is identical with the 3′ region of the Mov10l1 gene, but its transcript starts from the exon 16 of Mov10l1. The conceptual protein encoded by Csm cDNA contains a helicase motif as well as ATPase and RNA interaction motifs. Csm is expressed specifically in the heart, and its expression in the heart is restricted to cardiac myocytes. Csm potentiated phenylephrine-induced hypertrophic response in cardiac myocytes. Furthermore, transient cotransfection analysis showed that Nkx2.5 transactivates the Csm promoter, suggesting that Nkx2.5 is essential for embryonic Csm expression.

Csm, a Cardiac-specific Isoform of the RNA Helicase Mov10l1, Is Regulated by Nkx2.5 in Embryonic Heart*

Received for publication, January 2, 2003, and in revised form, May 13, 2003
Published, JBC Papers in Press, May 16, 2003, DOI 10.1074/jbc.M300014200

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MATERIALS AND METHODS

RNA Preparation, cDNA Synthesis, and Subtractive Hybridization—Total RNA (150 ng) from hearts of wild-type and Nkx2.5 null embryos at ED 8.5 was treated with RNase-free DNase I and used to generate cDNA using the SMART cDNA synthesis kit (Clontech, Palo Alto, CA). Subtractive hybridization was performed using a PCR-Select cDNA subtraction kit (Clontech). Briefly, two pools of RsaI-digested wild-type heart cDNA were used as tester and ligated to unique adapters. RsaI-digested Nkx2.5 null heart cDNA was used as driver without adapters. Hybridization of the tester population with excess driver and amplification of subtracted species with the two unique adapters in the tester cDNA produced a pool of PCR-amplified fragments theoretically present in wild-type heart cDNA, but not Nkx2.5 null heart cDNA (forward subtraction). Reverse subtraction was also performed using Nkx2.5 null heart cDNA as tester and wild-type heart cDNA as driver to enrich for cDNA-representing transcripts highly expressed in Nkx2.5 null hearts.

Differential Screening—Subtracted PCR fragments were subcloned, and 464 clones were recovered. cDNA inserts of the plasmid were amplified by PCR and were arrayed in duplicate onto nylon membranes. cDNAs from the forward or reverse subtractions were radioautetic factor; Csm, cardiac-specific isoform of Mov10l1; Mov10l1, isoform of Mov10 like-1; RLM, RNA ligase-mediated; RACE, rapid amplification of cDNA ends; HA, hamagglutinin; RT, reverse transcriptase; PE, phenylephrine; Ad, adenovirus; TRITC, tetramethylrhodamine isothiocyanate; CHAMP, cardiac helicase activated by MEF2 protein; NKE, Nkx2.5 binding element.
beled with [32P]dCTP by random priming. Membranes were hybridized to radiolabeled probes at 42 °C for 16 h in the presence of 50% formamide. After hybridization, the membranes were washed at 65 °C in the presence of 0.1× SSC and 0.1% SDS. Visualization was achieved by exposure to Kodak Biomax MS film (Eastman Kodak, Rochester, NY).

Cloning of Csm cdNA and the Reporter—cdNA clones representing Csm were isolated from ED 10 mouse heart cdNA libraries (Stratagene, La Jolla, CA) employing standard methodology. 5′-RNA ligase-mediated (RLM)-RACE was performed according to the manufacturer's protocol (Ambion Inc., Austin, TX). Briefly, RNase-free DNase I-treated total RNA (10 μg) was treated with calf intestinal phosphatase to remove free 5′-phosphates from ribosomal RNA, fragmented RNA, and tRNA. The RNA was then treated with tobacco acid pyrophosphatase to remove the cap structure from full-length RNA, leaving a 5′-monophosphate. The RNA adapter oligonucleotide was ligated to the RNA population using T4 RNA ligase. A random-primed reverse transcription reaction and nested PCR then amplified the 5′-end of a transcript. A 2061-bp fragment of the Csm promoter (positions –2061 to –1 in the Csm gene) was isolated from BAC clone RP23-289G24, Research Genetics, Huntsville, AL.

Plasmid Construct—A cdNA encoding Csm with a C-terminal HA epitope was cloned into pCDNA3 (Invitrogen, San Diego, CA) to generate pcDNA3-Csm. A 2061-bp fragment of the Csm promoter was ligated with pGEl2-Basic vector (Promega, Madison, WI) to construct Csm-Luc. Promoter mutations were constructed by site-directed mutagenesis with the QuickChange kit (Stratagene). Mutagenesis reactions were constructed as follows: mutated NKE1 (NKE1M), –64 to –58 (CATTGA) mutated to ACAGGT, mutated NKE2 (NKE2M), –1752 to –1746 (TCAAGT) mutated to GACCTG. All of the promoter constructs were checked by sequencing.

Cardiac Myocytes Culture—Mouse neonatal cardiac myocytes culture from 1-day-old C57B6 mice was prepared as previously described in rat with a slight modification (16). Briefly, ventricular myocytes were dissociated enzymatically and preplated for 30 min twice to enrich for dissociated enzymatically and preplated for 30 min twice to enrich for cardiac myocytes. The cells were then washed with 200 μl of reporter lysis buffer (Promega) and lysed for luciferase activity (by a Promega assay) and β-galactosidase activity. Luciferase activity was normalized against β-galactosidase activity.

Ectoporetic Mobility Shift Assay—Nkx2.5 protein was prepared by coupled in vitro transcription/translation of a T7-driven Nkx2.5 plasmid in reticulocyte lysate by using a Tnt kit (Promega). Labeled DNA probes were incubated with 3 μl of programmed lysate in 2 μl of buffer (Replication-defective Recombinant Adenovirus and Gene Transfer—HA-tagged Cam cdNA and LacZ cdNA was used to generate recombinant adenovirus expressing Cam (AdCam) and LacZ (AdLacZ) by using the Adeno-X Expression System (Clontech), respectively. Twenty-four hours after seeding, the myocytes were infected with Wt AdCam or AdLacZ diluted in the culture media at the multiplicity of infection specified in the text and incubated for 2 h. The viral suspension was removed, and cardiac myocytes were cultured with the serum-depleted culture media.

Amino Acid Incorporation into Proteins—The relative amount of protein synthesis was determined by assessing the incorporation of the radioactivity into a trichloroacetic acid-insoluble fraction as described previously (17). After 24 h in the serum-depleted culture media, cardiac myocytes were stimulated with 100 μM PE for 24 h. 0.5 μCi/ml [3H]leucine was added 2 h before harvesting. Cells were quickly rinsed twice with ice-cold phosphate-buffered saline and incubated for 30 min on ice with 5% trichloroacetic acid. After washed twice with ice-cold 5% trichloroacetic acid, cells were solubilized in 0.1 N NaOH. Total trichloroacetic acid-insoluble radioactivity was determined by liquid scintillation counting.

Measurements of Cell Size—Cardiac myocytes were infected with AdCam or AdLacZ, and 24 h later cells were stimulated with 100 μl PE for 48 h. After fixation with 4% paraformaldehyde, cells were stained with TRITC-conjugated phalloidin (Sigma, St. Louis, MO), anti-HA fluoroscein isothiocyanate (Roche Applied Science, Indianapolis, IN), and Hoechst 33258 (Sigma). Cells were imaged using a confocal microscope and measured using Image (National Institutes of Health). At least 100 cells were measured per sample.

RT-PCR—Total RNA from the heart or testis was treated with RNase-free DNase I. First strand cdNA synthesis was performed using 1 μl total RNA with SuperScript II RT and random hexamer. PCR was performed using the following primers: forward primer (5′-GCCAGATGGAAGGGCAGGCGA-3′) and reverse primer (5′-TGCGAGAATGAGGAGGGAGGGA-3′) (human MOV10L1 2830)); forward primer (5′-ACCGCGGATACCCACGACCATGGC-3′ (human MOV10L1 2534–2562)) and reverse primer (5′-ACCGCGGGTC- CAAAAGGGCAC-3′ (human MOV10L1 2580–2830)); forward primer (5′-GGAGAGGAAGGGAGGAGGGA-3′) and reverse primer (5′-GGAGAGGAAGGGAGGAGGGA-3′). PCR conditions were as follows: for rat heart cdNA, 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s; for human heart or testis cdNA, 94 °C for 3 min, 40 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min. PCR products were electrophoresed on 2% agarose gels and visualized by ethidium bromide staining.

Luciferase activity tended to be different between Cam and LacZ groups. The luciferase activity of Cam group was significantly higher than that of LacZ group (Fig. 3). These results suggest that Csm can enhance the expression of Cam. On the other hand, the luciferase activity of Cam-LacZ group was lower than that of Cam group, indicating that Csm-Luc can enhance the expression of Cam-LacZ. These results suggest that Csm can enhance the expression of Cam-LacZ.
Identification of Nkx2.5-dependent Gene Csm, Cardiac-specific Isoform of Mov10l1—To identify potential target genes regulated by Nkx2.5 in the developing heart, we performed differential screening in combination with suppression subtractive hybridization using RNA from hearts of wild-type and Nkx2.5 null mice at ED 8.5. Out of 17 clones examined, 9 clones were significantly down-regulated in Nkx2.5 null heart by Northern blot analysis. We chose to focus on a clone containing the 3’ region of Mov10l1 (18), a putative RNA helicase specifically expressed in testis. We name this gene Csm for cardiac-specific isoform of Mov10l1. Csm was not detectable in Nkx2.5 null heart at ED 9.5 and 10.5 (Fig. 1), indicating that the expression of Csm is strongly dependent on Nkx2.5 in the embryonic heart. Northern blotting of RNA from embryonic hearts as well as from 8 different organs of adult mice revealed the Csm transcript of 1.7 kb in the embryonic and adult hearts (Fig. 2A). A larger 4.4-kb transcript was detected only in testis, which corresponds to the Mov10l1 transcript as reported previously (18). Csm was abundantly expressed in the mouse heart at a level equivalent to Mov10l1 in the testis. When cardiac myocytes were separated from non-myocytes using primary culture, Csm was found to be expressed in myocytes but not in non-myocytes (Fig. 2B).

Using the partial cDNA fragment as a probe, we isolated a near full-length cDNA clone from a mouse 10-day embryonic heart cDNA library. 5’ RLM-RACE yielded an additional 30 bp of the 5' region. Therefore, the full-length Csm transcript, including 3’-polyadenylation consensus sequence, is 1695 bp (Fig. 3). The conceptual protein encoded by the Csm cDNA contains a helicase motif (Fig. 3, boxed) as well as an ATPase (underlined) and RNA interaction motif (dashed line) characteristic of the RNA helicase family (19). Examination of a mouse genomic data base revealed that Csm is identical with the 3’ region of Mov10l1. Mov10l1 consists of 26 exons, and Csm transcription starts within the exon 16 of Mov10l1 (Fig. 4A).

Interestingly, during the course of this study, Liu et al. (20) reported CHAMP (AF340211), a putative RNA helicase down-regulated in MEF2C null mutant hearts. Comparison of the sequences showed that Csm and CHAMP are identical except that CHAMP cDNA is about 300 bp longer than Csm cDNA at the 5’-end of the transcript. Transcription of CHAMP starts at exon 14 of Mov10l1 (Fig. 4A). To further determine whether the 5’ coding sequence of CHAMP is present in Csm transcript, we generated four probes corresponding to exons 14, 15, 16, and 17–20 of Mov10l1 from the testis cDNA and performed Northern blot analysis using mRNAs isolated from hearts of embryos at ED 10.5 and tissues of an 8-week-old mouse. A band representing 1.7 kb was observed in both embryo and adult heart (arrow). Another band was observed in testis that expressed the 4.4-kb transcript (arrowhead). RNA marker (Mr) was loaded for assessing RNA size. A cytoplasmic (Cyph) probe was used as a control for assessing RNA loading. B, blots were made with total RNA (10 μg) isolated from mouse cultured neonatal cardiac myocytes (CM) and non-myocytes (NM). A band representing 1.7 kb was observed in CM (arrow) but not in NM. A glyceraldehyde-3-phosphate dehydrogenase probe was used as a control for assessing RNA loading.

FIG. 1. Csm mRNA expression in Csx/Nkx2.5 null heart. Csm mRNA expression was detected by Northern blot analysis. Blots were made with total RNA (1 μg) isolated from hearts of wild-type embryo (+/+ ) and Nkx2.5 null embryo (−/− ) at ED 9.5 and 10.5. Csm expression was not detected in Nkx2.5 null heart. A glyceraldehyde-3-phosphate dehydrogenase probe and 28 S RNA were used as a control for assessing RNA loading. The experiments shown represent one of three independent trials, which gave nearly identical results.

RESULTS

Identification of Nkx2.5-dependent Gene Csm, Cardiac-specific Isoform of Mov10l1—To identify potential target genes regulated by Nkx2.5 in the developing heart, we performed differential screening in combination with suppression subtractive hybridization using RNA from hearts of wild-type and Nkx2.5 null mice at ED 8.5. Out of 17 clones examined, 9 clones were significantly down-regulated in Nkx2.5 null heart by Northern blot analysis. We chose to focus on a clone containing the 3’ region of Mov10l1 (18), a putative RNA helicase specifically expressed in testis. We name this gene Csm for cardiac-specific isoform of Mov10l1. Csm was not detectable in Nkx2.5 null heart at ED 9.5 and 10.5 (Fig. 1), indicating that the expression of Csm is strongly dependent on Nkx2.5 in the embryonic heart. Northern blotting of RNA from embryonic hearts as well as from 8 different organs of adult mice revealed the Csm transcript of 1.7 kb in the embryonic and adult hearts (Fig. 2A). A larger 4.4-kb transcript was detected only in testis, which corresponds to the Mov10l1 transcript as reported previously (18). Csm was abundantly expressed in the mouse heart at a level equivalent to Mov10l1 in the testis. When cardiac myocytes were separated from non-myocytes using primary culture, Csm was found to be expressed in myocytes but not in non-myocytes (Fig. 2B).

Using the partial cDNA fragment as a probe, we isolated a near full-length cDNA clone from a mouse 10-day embryonic heart cDNA library. 5’ RLM-RACE yielded an additional 30 bp of the 5’ region. Therefore, the full-length Csm transcript, including 3’-polyadenylation consensus sequence, is 1695 bp (Fig. 3). The conceptual protein encoded by the Csm cDNA contains a helicase motif (Fig. 3, boxed) as well as an ATPase (underlined) and RNA interaction motif (dashed line) characteristic of the RNA helicase family (19). Examination of a mouse genomic data base revealed that Csm is identical with the 3’ region of Mov10l1. Mov10l1 consists of 26 exons, and Csm transcription starts within the exon 16 of Mov10l1 (Fig. 4A).

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FIG. 2. Northern blot analysis of Csm expression in murine tissues and cells. A, blots were made with mRNA (1 μg) isolated from hearts of embryos at ED 10.5 and tissues of an 8-week-old mouse. A band representing 1.7 kb was observed in both embryo and adult heart (arrow). Another band was observed in testis that expressed the 4.4-kb transcript (arrowhead). RNA marker (Mr) was loaded for assessing RNA size. A cytoplasmic (Cyph) probe was used as a control for assessing RNA loading. B, blots were made with total RNA (10 μg) isolated from mouse cultured neonatal cardiac myocytes (CM) and non-myocytes (NM). A band representing 1.7 kb was observed in CM (arrow) but not in NM. A glyceraldehyde-3-phosphate dehydrogenase probe was used as a control for assessing RNA loading.

17–20 of Mov10l1 from the testis cDNA and performed Northern blot analysis using mRNAs isolated from the heart and testis. The probes for exon 14 or 15 did not hybridize with RNA from the heart, whereas they hybridized with RNA from testis at 4.4 kb (Fig. 5A). When the entire exon 16 was used as a probe, the 1.7-kb Csm transcript was detected in the heart, whereas the 4.4-kb Mov10l1 transcript was detected in the testis. The hybridization signal of Csm was increased when exons 17–20 were used as a probe, suggesting that the exon 16 (2202–2381) probe did not fully hybridize with Csm mRNA. This result is consistent with 5’ RLM-RACE showing that Csm transcription starts at the 2252-bp site within the exon 16 of Mov10l1 (Fig. 4B, arrow).

To further prove the 5’-end of Csm, two 50-base oligonucleotides, corresponding to antisense for 2202–2251 (which is upstream of the start site that we identified) and 2252–2301
Fig. 3. Nucleotide sequence and open reading frame of the Csm gene. The sequence of the Csm cDNA comprises 1695 nucleotides. The helicase motif is boxed. The ATPase motif is indicated by underlining, and the RNA interaction motif is indicated by dashed underlining. The polyadenylation consensus sequence is indicated by double underlining. Positions in the nucleotide and amino acid sequence are given by numbers. The sequence data of mouse Csm is available under GenBank™ accession number AY303754.

Nkx2.5 Transactivated the Csm Promoter—Examination of the mouse genomic DNA sequence (NW_000106) reveals that the putative Csm promoter has no TATA box, but two NKE, the consensus binding motif for Nkx2.5 (TYAAGTG) (21), at $64$ (NKE1) and $1752$ (NKE2) (Fig. 6A). Six additional similar sequences to the consensus motif were found within the putative Csm promoter from $2000$ to $11$. To examine whether Csm expression is regulated by Nkx2.5, we isolated a 2061-bp fragment of the Csm promoter (positions $2061$ to $1$ of the Csm gene) and performed reporter assays using the Csm promoter. Wild-type Nkx2.5 transactivated the Csm promoter by 4-fold (Fig. 6B). On the other hand, a point mutant of Nkx2.5 (I183P; i.e. Ile183 → Pro), which fails to bind DNA (22), did not transactivate the Csm promoter (Fig. 6B). Furthermore, we made NKE site mutations in the Csm promoter and examined the role of NKE for Nkx2.5 in the Csm promoter. The mutation of the NKE2, Csm(NKE2M)-Luc, did not reduce responsive-
ness to Nkx2.5, whereas the mutation of NKE1, Csm(NKE1M)-Luc, significantly reduced responsiveness to Nkx2.5 (Fig. 6C).

The above result suggested that the two NKEs within −2061 bp of the Csm promoter, NKE1 is necessary for the full activation of the Csm promoter by Nkx2.5. To confirm that Nkx2.5 binds to NKE1, an electrophoretic mobility shift assay was performed with in vitro translated Nkx2.5 protein and oligonucleotide probes corresponding to NKE1 and NKE1M. As shown in Fig. 6D, Nkx2.5 bound to NKE1, but not NKE1M. These results indicate that Nkx2.5 directly regulates the Csm gene transcription in vitro, at least in part through NKE1 site.

Csm Potentiates Phenylephrine-induced Hypertrophic Response in Cardiac Myocytes—To examine the function of Csm in cardiac myocytes, we first performed Northern blot analysis to determine whether Csm is involved in the endogenous ANF mRNA expression using AdCsm. As shown in Fig. 7A, Csm alone did not induce ANF mRNA expression. However, when cardiac myocytes were stimulated with α-adrenergic agonist PE, Csm potentiated PE-induced ANF mRNA expression. We next performed the reporter assay using the ANF promoter and the Csm expression vector. Csm alone did not affect the basal transcriptional activity of the ANF promoter (Fig. 7B, row 2), whereas Csm potentiated PE-induced ANF promoter activation (compare rows 3 and 4 of Fig. 7A). We also examined whether Csm is involved in PE-induced cardiac myocytes hypertrophy. PE stimulated [3H]leucine incorporation by 50% and Csm potentiated PE-induced [3H]leucine incorporation (compare lanes 4 and 6 of Fig. 7C). A similar result was achieved using a higher multiplicity of infection (Fig. 7C, lanes 8 and 10). We then measured cell size. Csm potentiated PE-induced increase of cell size (Fig. 7D). These results indicate that Csm potentiated PE-induced hypertrophic response in cardiac myocytes.

Csm Expression in Rat and Human Heart—Generally, essential genes are conserved among different species. In the rat heart, RT-PCR revealed the presence of a transcript using primers spanning mouse Csm (1831–2090) (exons 18–20), which correspond to positions within the coding region of Csm (Fig. 8A). Sequencing of the PCR product showed 99% identity with the mouse sequence. However, when this fragment was used for Northern blotting, it did not detect Csm or Mov1011 in the rat atria or in ventricles, whereas it readily hybridized with the mouse Csm transcript (Fig. 8B).

In the human heart, a transcript was also detected by RTPCR using specific primers spanning human Mov1011(2543–2850) and (2526–3078) (Fig. 8C), which also correspond to positions within the coding region of mouse Csm. Northern blot analysis revealed that a probe for human Mov1011(2526–3078) hybridized with RNA samples from human testis but not with the RNA sample from the human heart (Fig. 8D). An oligonucleotide probe mapping within the putative coding region (antisense to human Mov1011(2497–2546)) hybridized with RNA samples from human testis, but it did not hybridize
with the RNA sample from the human heart. These findings indicate that the level of expression of Csm and/or Mov10l1 in rat and human heart is below the detection level of Northern blot analysis and is far below the level of Csm expression in the mouse heart.

Examination of human genomic DNA sequence (AL080347 and NT_019197) reveals that human MOV10L1 has 27 exons and that the 5′-end of MOV10L1 is about 2 kb in Northern blot analysis (20). However, we detected a single 1.7-kb transcript in heart. We demonstrate in Fig. 5 that the 1.7-kb mRNA does not contain exons 14 and 15 of mouse Mov10l1 and that the 5′-end of Csm is located within exon 16 of Mov10l1. These findings raise the possibility that Mov10l1 mRNA and CHAMP mRNA are not expressed in mouse heart. Therefore, we further examined whether Mov10l1 is directly or indirectly downstream of Nkx2.5 at this stage of embryonic development. We found two consensus binding motifs for Nkx2.5 (TYAAGTG) (21) within the putative Csm promoter (325 to 2206) and reduced responsiveness to Nkx2.5. Values are expressed as mean ± S.E. (*, p < 0.05 compared with Csm-Luc).

FIG. 5. Expression of Mov10l1 in mouse heart. A, Mov10l1 RNA is expressed in mouse heart, the amount is below the detection limit of Northern blot analysis. B, Mov10l1 mRNA is expressed in mouse heart. Mov10l1 mRNA and CHAMP mRNA are not expressed in mouse heart. Therefore, we further examined whether Mov10l1 mRNA is expressed in mouse heart by using RT-PCR. A PCR product corresponding to Mov10l1 was detected in the mouse heart cDNA (data not shown). Taken together with the results of Northern blot analysis, this finding suggests that, although Mov10l1 RNA is expressed in mouse heart, the amount is below the detection limit of Northern blot analysis. Similarly, al-

FIG. 6. Transactivation of the Csm promoter by Nkx2.5. A, schematic representation of the genomic structure of the mouse Csm promoter. Consensus binding motifs for Nkx2.5 are shown by solid boxes. B, COS cells were transiently cotransfected with Csm-Luc and with wild-type Nkx2.5(WT) or Nkx2.5(I183P) expression vectors. Wild-type Nkx2.5 activated Csm-Luc, but Nkx2.5(I183P) did not. Values are expressed as mean ± S.E. C, COS cells were transiently cotransfected with Csm-Luc, Csm(NKE2M)-Luc, or Csm(NKE1M)-Luc and with wild-type Nkx2.5(WT) expression vectors. Csm(NKE1M)-Luc markedly reduced responsiveness to Nkx2.5. Values are expressed as mean ± S.E. (*, p < 0.05 compared with Csm-Luc). D, NKE1 and NKE1M were incubated with in vitro translated Nkx2.5 protein. NKE1 had a high binding affinity of Nkx2.5 protein, and mutations into NKE1 dramatically reduced the binding affinity of Nkx2.5 protein.

FIG. 7. Potentiation of phenylephrine-induced hypertrophic response by Csm. A, cardiac myocytes were infected with AdCsm or AdLacZ. Cardiac myocytes were stimulated with 100 μM PE for 24 h. Blots were made with total RNA (5 μg) isolated from rat cultured neonatal cardiac myocytes. Csm potentiated PE-induced ANF mRNA expression. ANF mRNA expression was normalized against glyceraldehyde-3-phosphate dehydrogenase mRNA expression (*, p < 0.05). B, cardiac myocytes were transiently cotransfected with ANF-Luc and with or without Csm expression vectors. Cardiac myocytes were stimulated with 100 μM PE for 24 h. Csm potentiated PE-induced ANF promoter activation (*, p < 0.05). C, cardiac myocytes were infected with AdCsm or AdLacZ. Cardiac myocytes were stimulated with 100 μM PE for 24 h. Csm potentiated PE-induced [3H]leucine incorporation (*, p < 0.05). D, cardiac myocytes were infected with AdCsm or AdLacZ. Cardiac myocytes were stimulated with 100 μM PE for 48 h. Csm potentiated PE-induced increase of cell size. Values are expressed as mean ± S.E. (*, p < 0.05).

Mov10l1, was markedly down-regulated in Nkx2.5 null heart. Csm mRNA was readily detected in wild-type heart at ED 9.5 and 10.5, whereas it was undetectable in Nkx2.5 null heart. This finding indicates that Csm expression depends on Nkx2.5 expression in embryonic heart, suggesting that Csm is either directly or indirectly downstream of Nkx2.5 at this stage of embryonic development. We found two consensus binding motifs for Nkx2.5 (TYAAGTG) (21) within the putative Csm promoter from −2061 to −1. We showed transactivation of the Csm promoter by Nkx2.5 in vitro and reduced responsiveness of mutated NKE1 in the Csm promoter to Nkx2.5. These findings suggest that Nkx2.5 directly regulates Csm expression at the stage of embryonic development.

Csm is identical to CHAMP except the 5′-end of CHAMP is 300 nucleotides longer. CHAMP mRNA has been reported to be about 2 kb in Northern blot analysis (20). However, we detected a single 1.7-kb transcript in heart. We demonstrate in Fig. 5 that the 1.7-kb mRNA does not contain exons 14 and 15 of mouse Mov10l1 and that the 5′-end of Csm is located within exon 16 of Mov10l1. These findings raise the possibility that Mov10l1 mRNA and CHAMP mRNA are not expressed in heart. Therefore, we further examined whether Mov10l1 mRNA is expressed in mouse heart by using RT-PCR. A PCR product corresponding to Mov10l1 was detected in the mouse heart cDNA (data not shown). Taken together with the results of Northern blot analysis, this finding suggests that, although Mov10l1 RNA is expressed in mouse heart, the amount is below the detection limit of Northern blot analysis. Similarly, al-

DISCUSSION

The homeodomain protein Nkx2.5 is one of the earliest cardiac markers from fly to vertebrate, and its expression continues through adulthood (1, 2, 4, 5, 23–28). In the present study, we attempted to identify genes regulated by Nkx2.5 in the developing mouse heart using suppression subtractive hybridization. We showed that Csm, a cardiac-specific isoform of
though CHAMP mRNA may be expressed in the mouse heart, the amount seems below the detection limit of Northern blot analysis. These results indicate that Csm is the predominant isof orm of Mov10l1 in the mouse heart.

Sequence analysis reveals that Csm contains a helicase motif as well as an ATPase and RNA interaction motif. Helicases are grouped into major superfamilies of proteins (SFI and SFII) based on the occurrence of seven conserved motifs (19). Csm belongs to SFI, and is most closely related to the Upf1p, which is involved in RNA metabolism (29). Csm is very similar to CHAMP except in the 5′ region. Overexpression of CHAMP in primary neonatal cardiac myocytes has been reported to block hypertrophic growth (30). Therefore, we examined the function of Csm on hypertrophic response in cardiac myocytes. The hypertrophic response in neonatal cardiac myocytes is characterized by a series of phenotypic changes such as an increase in cell size, increased protein synthesis, an induction of specific genes such as ANF, and increased organization of contractile proteins into sarcomeric units (31). We examined these hypertrophic responses. We showed here that Csm potentiated ANF mRNA expression, amino acid incorporation, and increase of cell size by PE. In contrary to the previous report about CHAMP (30), Csm has promoting effects on hypertrophic response. CHAMP has another 188 amino acids in the N-terminal region compared with Csm. There are five repeated motifs of the sequence (TRNDXGSITN(V/I)) and two ATPase motifs in this region. Therefore, the different function between CHAMP and Csm on hypertrophic response is likely attributed this region.

Although Csm is relatively abundant in the mouse heart, its expression in rat and human hearts is below the detection level of Northern blot analysis. The putative human Csm promoter in intron 16 of MOV10L1 has one similar sequence of consensus binding motif for Nkx2.5 (CWTAAATG) (21). On the other hand, the putative mouse Csm promoter from −2000 to −1 has several as described above. Subtle differences in promoter sequences likely explain the difference in Csm expression between human and mouse. This also raises the possibility that Csm is regulated differently by Nkx2.5 between human and mouse. Therefore, our findings may give a caution for future studies of the Nkx2.5-regulated molecular pathway in human analyzed by using mouse models.

In summary, Csm is a putative RNA helicase that is markedly down-regulated in Nkx2.5 null hearts. Csm is the predominant isof orm of Mov10l1 in the mouse heart. Csm potentiated PE-induced hypertropic response in cardiac myocytes. However, in humans Csm and/or MOV10L1 expression in the heart is very low. Csm regulation by Nkx2.5 may be quite different between human and mouse.

Acknowledgments—We thank W. Pu and P. Jay for critical reading of the manuscript.

REFERENCES

1. Komuro, I., and Izumo, S. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8145–8149
2. Lints, T. J., Parsons, L. M., Hartley, L., Lyons, I., and Harvey, R. P. (1995) Development 119, 419–431
3. Harvey, R. P. (1996) Dev. Biol. 178, 203–216
4. Bodmer, R. (1990) Development 110, 718–729
5. Kasahara, H., Bartunkova, S., Schinke, M., Tanaka, M., and Izumo, S. (1998) Circ. Res. 82, 936–946
6. Lyons, I., Parsons, L. M., Hartley, L., Li, R., Andrews, J. E., Robb, L., and Harvey, R. P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1654–1658
7. Tanaka, M., Chen, Z., Bartunkova, S., Yamashita, T., Ishido, S., Hotta, H., and Yokoyama, M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 11868–11873
8. Bao, Z. Z., Tanaka, M., Schott, J. J., Izumo, S., Cepko, C. L., Bruneau, B. G., Bao, Z. Z., Tanaka, M., and Izumo, S. (1998) Development 126, 1269–1280
9. Benson, D. W., Silberbach, G. M., Kavanagh-McHugh, A., Cottrill, C., Zhang, Y., Riggs, S., Smalls, O., Johnson, M. C., Watson, M. S., Seidman, J. G., Seidman, C. E., Plowden, J., and Kugler, D. J. (1999) J. Clin. Invest. 104, 1567–1573
10. Biben, C., and Harvey, R. P. (1997) Genes Dev. 11, 1357–1369
11. Biben, C., Palmer, S., Elliott, D. A., and Harvey, R. P. (1997) Cold Spring Harbor Symp. Quant. Biol. 62, 395–403
12. Zou, Y., Evans, S., Chen, J., Koo, H. C., Harvey, R. P., and Chien, K. R. (1997) Development 124, 783–804
13. Bruneau, B. G., Bao, Z. Z., Tanaka, M., Schott, J. J., Izumo, S., Cepko, C. L., Seidman, J. G., and Seidman, C. E. (2000) Dev. Biol. 237, 266–277
14. Christoffels, V. M., Habets, P. E., Franco, D., Campione, M., de Jong, F., Lamers, W. H., Bao, Z. Z., Palmer, S., Biben, C., Harvey, R. P., and Seidman, J. G. (2000) Dev. Biol. 233, 266–278
15. Palmer, S., Groves, N., Schindeler, A., Yesh, T., Biben, C., Wang, C. C., Sparrow, D. B., Barnett, L., Jenkins, N. A., Copeland, N. G., Keentgen, F., Mohan, T., and Harvey, R. P. (2001) J. Cell. Biochem. 81, 985–998
16. Sadahira, J., Jahn, L., Takahashi, T., Kukk, T. J., and Izumo, S. (1992) J. Biol. Chem. 267, 10551–10560
17. Ueyama, T., Kawashima, S., Sakoda, T., Rikitake, Y., Ishida, T., Kawai, M., Yamashita, T., Ishido, S., Hotta, H., and Yokoyama, M. (2000) J. Mol. Cell. Cardiol. 32, 947–960
18. Wang, P. J., McCrary, J. R., Yang, F., and Page, D. C. (2001) J. Clin. Invest. 107, 422–426
19. de la Cruz, J., Kressler, D., and Linder, P. (1999) Trends Biochem. Sci. 24, 192–198
20. Liu, Z. P., Nakagawa, O., Nakagawa, M., Yangaswar, H., Passier, R., Rich, J., Sadoshima, J., Jahn, L., Takahashi, T., Kulik, T. J., and Izumo, S. (1997) J. Biol. Chem. 272, 10515–10520
21. Chen, C. Y., and Schwartz, R. J. (1996) J. Biol. Chem. 271, 15628–15633
22. Kasahara, H., Usheva, A., Ueyama, T., Aoki, H., Horikoshi, N., and Izumo, S. (2001) J. Biol. Chem. 276, 4570–4580
23. Apstein, A., and Frasch, M. (1993) Trends Dev. Biol. 7, 1325–1340

FIG. 8. Csm expression in rat and human heart. A, RT-PCR was performed using rat heart cDNA as a template with (+) or without (−) RT. 300 bp of PCR products were obtained with RT. B, blots were made with total RNA (30 μg) isolated from hearts from mouse and rat. Lanes are as follows: mouse whole heart RNA (lane 1); mouse atrium RNA (lane 2); mouse ventricle RNA (lane 3); rat atrium RNA (lane 4); and rat ventricle RNA (lane 5). A band representing 1.7-kb mRNA was detected in RNA from mouse heart, indicating that this band was Csm (arrow). However, no band was detected in RNA from rat heart. A cyclophilin (Cyph) probe was used as a control for assessing RNA loading. C, RT-PCR was performed using human heart (lanes 1 and 2) and testis (lanes 3 and 4) cDNAs as templates with the following primers: Lanes 1 and 3, human MOV10L1-(2543–2562) as a forward primer and human MOV10L1-(2850–2853) as a reverse primer; lanes 2 and 4, human MOV10L1-(2526–2548) as a forward primer and human MOV10L1-(3075–3055) as a reverse primer with (+) or without (−) RT. 300 bp (lanes 1 and 3) and 550 bp (lanes 2 and 4) of PCR products were obtained with RT. D, blots were made with total RNA (20 μg) isolated from human heart and testis. A probe corresponding to human MOV10L1-(2526–3055) was hybridized with RNA samples (arrowhead) from human testis, but it did not hybridize with RNA samples from human heart. An oligonucleotide probe corresponding to the antisense sequences for human MOV10L1-(2497–2548) also hybridized with RNA samples from human testis, whereas it did not hybridize with RNA samples from human heart. Arrowheads indicate human MOV10L1. Positions in the nucleotide are given by numbers from human MOV10L1 (GenBank™ AF285604). A cyclophilin (Cyph) probe was used as a control for assessing RNA loading.
24. Tonissen, K. F., Drysdale, T. A., Lints, T. J., Harvey, R. P., and Krieg, P. A. (1994) Dev. Biol. 162, 325–328
25. Schultheiss, T. M., Xydas, S., and Lassar, A. B. (1995) Development 121, 4203–4214
26. Chen, J. N., and Fishman, M. C. (1996) Development 122, 3809–3816
27. Turbay, D., Wechsler, S. B., Blanchard, K. M., and Izumo, S. (1996) Mol. Med. 2, 86–96
28. Shiojima, I., Komuro, I., Mizuno, T., Akawa, R., Akazawa, H., Oka, T., Yamazaki, T., and Yazaki, Y. (1996) Circ. Res. 79, 920–929
29. Culbertson, M. R. (1999) Trends Genet. 15, 74–80
30. Liu, Z. P., and Olson, E. N. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 2043–2048
31. Thorburn, J., Frost, J. A., and Thorburn, A. (1994) J. Cell Biol. 126, 1565–1572
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J. Biol. Chem. 2003, 278:28750-28757. doi: 10.1074/jbc.M300014200 originally published online May 16, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M300014200

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