Identification of a new co-factor, MOG1, required for the full function of cardiac sodium channel Nav1.5*

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8The abbreviations used are: IP, immunoprecipitation; co-IP, co-immunoprecipitation; GST, glutathione S-transferase; RT-PCR, real-time polymerase chain reaction; GFP, green fluorescent protein.

The cardiac sodium channel Na,1.5 is essential for the physiological function of the heart and contributes to lethal cardiac arrhythmias and sudden death when mutated. Here, we report that MOG1, a small protein that is highly conserved from yeast to humans, is a central component of the channel complex and modulates the physiological function of Na,1.5. The yeast two-hybrid screen identified MOG1 as a new protein that interacts with the cytoplasmic loop II (between transmembrane domains DII and DIII) of Nav1.5. The interaction was further demonstrated by both in vitro glutathione S-transferase pull-down and in vivo co-immunoprecipitation assays in both HEK293 cells with co-expression of MOG1 and Na,1.5 and native cardiac cells. Co-expression of MOG1 with Na,1.5 in HEK293 cells increased sodium current densities. In neonatal myocytes, over-expression of MOG1 increased current densities nearly two-fold. Western blot analysis revealed that MOG1 increased cell surface expression of Na,1.5, which may be the underlying mechanism by which MOG1 increased sodium current densities. Immunostaining revealed that in the heart, MOG1 was expressed in both atrial and ventricular tissues with predominant localization at the intercalated discs. In cardiomyocytes, MOG1 is mostly localized in the cell membrane and co-localized with Na,1.5. These results indicate that MOG1 is a critical regulator of sodium channel function in the heart and reveal a new cellular function for MOG1. This study further demonstrates the functional diversity of Na,1.5-binding proteins, which serve important functions for Na,1.5 under different cellular conditions.

The SCN5A gene on chromosome 3p21 encodes Na,1.5, the α-subunit of the cardiac sodium channel which plays an important role in generating the cardiac action potential and mediating the conduction of cardiac electrical impulses (1,2). Numerous mutations in the SCN5A gene have been identified in long QT syndrome, Brugada syndrome, idiopathic ventricular fibrillation, cardiac conduction defects, and dilated cardiomyopathy associated with atrial fibrillation (3-7). Previous studies also suggest that alterations in the cardiac sodium channel function may be linked to two other major cardiac diseases, heart failure and atrial fibrillation. The expression of Na,1.5 protein expression was reduced about 30% in a dog model of heart failure (8), whereas in a dog model of chronic atrial fibrillation, the sodium current density/function was decreased in atrial cells (9).
Due to its critical importance in cardiac physiology and human disease, studies to define the regulatory proteins and other components of the multi-protein Na\textsubscript{v}1.5 complex have been of intense interest. The major component of the sodium channel complex is the pore-forming $\alpha$-subunit Na\textsubscript{v}1.5, which consists of four homologous domains (DI, DII, DIII, and DIV) with each domain containing six transmembrane segments (S1-S6) (1,2). The channel complex contains other subunits, for which there are at least four $\beta$-subunits identified thus far. Co-expression of $\beta$1-subunit with Na\textsubscript{v}1.5 causes a small but significant acceleration in the recovery from inactivation as well as an increase in current density which may be due to an increased targeting efficiency of the mature channel to the cell membrane (10,11). The $\beta$3-subunit causes a depolarizing shift in steady-state inactivation and a slower recovery from inactivation than the $\beta$1-subunit (12). The $\beta$2- and $\beta$4-subunits have little effect on the channel kinetics of Nav1.5 (13,14).

In addition to $\beta$-subunits, accessory proteins have been identified for Na\textsubscript{v}1.5 whose interactions have been shown to form a multi-protein complex (reviewed in (15,16)). Ankyrin-G was shown to interact with Na\textsubscript{v}1.5 and this interaction involved a 9-amino acid motif in the cytoplasmic loop II between DII and DIII, the result of which enabled for the localization of the channels to cell membrane in cardiomyocytes. The C-terminus of Na\textsubscript{v}1.5 contains 244 amino acid residues that have been shown to have important interactions with proteins such as FHF1B (fibroblast growth factor homologous factor 1B), calmodulin (CaM), Nedd4-like ubiquitin-protein ligase, dystrophin and syntrophin, Fyn, and protein tyrosine phosphatase (PTPH1) (15,17,18). In a recent study, 14-3-3 was found to interact with the cytoplasmic loop I between DI and DII and its dimerization was needed for current regulation (19). Despite this apparent diversity in accessory proteins, the complete composition of the cardiac sodium channel complex remains poorly understood. It is reasonable to expect that many more proteins are involved in the dynamic networks of protein-protein interactions with Na\textsubscript{v}1.5 and underscores the significance of multi-protein complexes that are critical for normal cardiac function.

MOG1 was initially identified as a suppressor that was able to rescue the temperaturesensitive defect of S. cerevisiae Ran, a protein involved in nucleocytoplasmic transport, microtubule and nuclear assembly, and the spatial and temporal organization of the eukaryotic cell (20,21). In vitro studies showed that MOG1 can bind to Ran-GTP (20), and release GTP (22), but its in vivo function is not clear. MOG1 has been shown to be a highly conserved protein from yeast to humans (23). Human MOG1 gene contains 5 exons and 4 introns and encodes a protein of 187 amino acids with a calculated molecular weight of 20 kDa (23). The highest expression of MOG1 was detected in the heart by Northern blot analysis (23), but the exact physiological function of MOG1 in the heart is unknown.

To identify new proteins associated with the cardiac sodium channel complex, we performed a yeast two-hybrid screen with separate intracellular domains of Nav1.5 as baits. When the cytoplasmic loop II was used as the bait, a candidate Na\textsubscript{v}1.5-interacting protein, MOG1, was identified. We further demonstrated the interaction between MOG1 and Na\textsubscript{v}1.5 by both in vitro GST-pull-down and in vivo co-immunoprecipitation assays. We examined the physiological role of MOG1 by co-expression of MOG1 and Na\textsubscript{v}1.5 in HEK293 cells and neonatal cardiomyocytes. Our results indicate that MOG1 is a co-factor for Na\textsubscript{v}1.5 and modulates the appropriate expression and function of Na\textsubscript{v}1.5.

**EXPERIMENTAL PROCEDURES**

**Construction of Plasmids and Antibodies—**

The human MOG1 gene was cloned into vector pET28a at the EcoR I and Sal I sites (pET28a-MOG1) as previously described (23). The MOG1 insert was released from pET28a-MOG1 and subcloned into pcDNA3.1C (Invitrogen) at the BamH I and Not I sites or into pCMV10, yielding mammalian expression constructs for His-tagged MOG1 (pcDNA3.1C-MOG1) and Flag-tagged MOG1 (pCMV10-MOG1), respectively.

Human cardiac sodium channel gene SCN5A was cloned into vector pcDNA3 (pcDNA3-SCN5A) as described (24,25), and used for establishing a stable HEK293 cell line with constant expression of Na\textsubscript{v}1.5, (hereafter referred to as “HEK293/Na\textsubscript{v}1.5”; a generous gift from G.E.
The cytoplasmic loop II (LII) between transmembrane domains DII and DIII of Na\textsubscript{v}1.5 was amplified by PCR using oligos containing XhoI I and EcoR I restriction sites (Forward: 5’- AGAATTCAGCTCCTTCAGTGCAAG-3’, Reverse: 5’-TCTCGAGTTAGTGGTGAGCTTT-3’) and cloned into vector pGEX4T-1-GST-tag (Novagen) (pGEX4T-1-GST-Nav1.5-LII) for expression and purification of GST-Nav1.5-LII fusion protein. The cytoplasmic loop I (LI) between DI and DII of Nav1.5 was amplified and cloned using an identical approach to generate pGEX4T-1-GST-Nav1.5-LI for expression and purification of GST-Nav1.5-LI fusion protein. Primers used to amplify the Na\textsubscript{v}1.5-LI are 5’- AGTTCAGGATCCGAGGAGCAAAA-3’ and 5’-CTTACAGCGGCCGCTTACCTCACTCCCT-3’.

Nav1.5-LII was also sub-cloned into pcDNA3.1A to express His-tagged Na\textsubscript{v}1.5-LII (pcDNA3.1A-Nav1.5-LII) in mammalian cells.

Two anti-MOG1 antibodies were developed by GeneMed Synthesis, Inc. The first MOG1 antibody (#2738) was developed with a MOG1-specific peptide, C-QPPPDNRSILGPENL at the N-terminal section, and the immunogene for the second antibody (#3350) was a peptide, C-NQQVAKDVTLHQALLRLPQYQTDL at the C-terminal section. The rabbit polyclonal anti-Nav1.5 antibody was as described previously.(26)

**Yeast Two-Hybrid Screen**

Yeast two-hybrid analysis was performed with a pre-made MATCHMAKER, human heart cDNA library constructed in S. cerevisiae host strain, Y187 (ClonTech Laboratories, Inc.). pACT2-derived constructs generate fusion proteins with the GAL4 activation domain (GAL4 AD) fused to a library of other proteins. The baits for library screening were five different segments of Na\textsubscript{v}1.5 fused to GAL4 DNA binding domain (GAL4 BD) in the pAS2 vector. As Na\textsubscript{v}1.5 is a membrane protein, the yeast two-hybrid screen with the entire Na\textsubscript{v}1.5 protein is unlikely to be fruitful because the protein may not enter the yeast nuclei. Thus, we selected five cytoplasmic segments of Na\textsubscript{v}1.5 as baits. These include the N-terminal domain (amino acids 1 to 123), cytoplasmic LI between DI and DII (Na\textsubscript{v}1.5-LI, amino acids 437 to 711), cytoplasmic LII between DII and DIII (Na\textsubscript{v}1.5-LII, amino acids 940 to 1200), the inactivation gate between DIII and DIV (amino acids 1471 to 1523), and the C-terminal domain (amino acids 1773 to 2016). A bait plasmid was transfected into yeast strain Y187 with a library of human heart cDNAs fused to GAL4-AD. Positive colonies were identified as instructed by the manufacturer (ClonTech Laboratories, Inc.). Approximately 10\textsuperscript{7} primary transformants were screened for each of the baits. DNA was isolated from each positive clone and used to transform E. coli HB101 (Leu -) to isolate only pACT2 derivative plasmids. The cDNA insert from each positive pACT2 derivative clone was amplified by PCR and sequenced by the BigDye Terminator v1.1 Cycle Sequencing kit and an ABI PRISM 3100 Genetic Analyzer. The DNA sequences were then characterized by Blast analysis against the NCBI database to determine the identity of the potential Na\textsubscript{v}1.5-interacting proteins.

**GST Pull-down Assays**

Construct pGEX4T-1-GST, pGEX4T-1-GST-Na\textsubscript{v}1.5-LI, or pGEX4T-1-GST-Nav1.5-LII was transformed into E. coli BL21 to express the GST, GST-Nav1.5-LI, and GST-Nav1.5-LII proteins. The GST and GST-fusion proteins were then affinity-purified using Glutathione Sepharose 4B beads according to the manufacturer’s protocol (Amersham Pharmacia Biotech). The proteins were eluted with 0.01 M glutathione/100 mM Tris-HCl, pH 7.5, and dialyzed in dialysis buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.1 mM EDTA, 20% glycerol, and 0.5 mM DTT).

The \[^{35}S\]-labeled MOG1 protein was prepared using a TNT Quick Coupled Transcription/Translation system (Promega). Briefly, 1 µg of plasmid DNA was mixed with TNT Quick Master mix and \[^{35}S\]-methionine, and incubated for 90 minutes at 30 °C. The \[^{35}S\]-labeled MOG1 protein was mixed with GST, GST-Nav1.5-LI, or GST-Nav1.5-LII immobilized on Glutathione Sephrose 4B beads in binding buffer (1 mM DTT, 0.5 mM PMSF, 1 mM EDTA, 20 mM Tris-HCl, 150 mM NaCl, 0.1% Triton X-100), and incubated for 2-3 hours at 4 °C. After binding, the beads were washed with binding buffer, and bound proteins were eluted with 1X SDS loading buffer, separated on a 12% SDS-polyacrylamide gel, dried, and visualized by exposing to X-ray film at -80°C for 12 hours.

**Co-Immunoprecipitation (co-IP) Analysis**

HEK293/Nav1.5 cells were maintained in Dulbecco’s minimum essential medium (DMEM) supplemented with 10% heat inactivated fetal...
bovine serum (Invitrogen) and transfected with 10 µg of pcDNA3.1C-MOG1 DNA for the expression of His-tagged MOG1 with Lipofectamine 2000 (Invitrogen). Transfection was carried out with 80% confluent cells in a 10 cm plate. Cells were harvested and lysed in TNE buffer (50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 2.0 mM EDTA, 1.0% Nonidet P40, protease inhibitor cocktail) 24 to 48 hours after transfection. 500 µg of total cell extracts was mixed with a rabbit polyclonal anti-Na,1.5 antibody (26) and incubated was continued for another 2 hours. The bound proteins were eluted by boiling the samples for 5 minutes in 1X SDS loading buffer, separated by SDS-PAGE, and transferred onto a nitrocellulose membrane. The membrane was probed with an anti-His antibody (Sigma-Aldrich) recognizing His-MOG1 fusion protein, and the protein signal was visualized by enhanced chemiluminescence according to the manufacturer's instructions (Amersham Biosciences). For reverse co-IP, a rabbit polyclonal anti-MOG1 antibody was used for immunoprecipitation and the anti-Na,1.5 antibody for Western blot analysis. Similar co-IP studies were performed to study the interaction between the cytoplasmic loop II of Na,1.5 and MOG1 by co-expressing His-tagged Na,1.5-LII and Flag-tagged MOG1 in HEK293 cells and with an anti-His antibody and an anti-Flag antibody.

Isolation of Mouse Cardiomyocytes--For isolation of neonatal mouse cardiomyocytes, 10-15 mouse hearts were collected from 3-day-old CBA/B6 mouse neonates. The ventricles were excised and myocytes were isolated using the Neonatal Rat/Mouse Cardiomyocyte Isolation kit from CELLUTRON Life Technology. The cells were plated on uncoated 100 mm plates to reduce the contamination of cardiac fibroblasts. Myocytes were then cultured in the NS medium (CELLUTRON Life Technology) supplemented with 10% FBS. Isolation of adult cardiomyocytes was performed as previously described by us (27-30).

Immunohistochemistry-Immunostaining was performed on adult cardiomyocytes or frozen heart sections (6 µm) with the polyclonal antibodies, anti-Na,1.5 and anti-MOG1, as described (31,32).

Electrophysiological Analysis-HEK293/Na,1.5 cells or neonatal cardiomyocytes were transfected with 1 µg of pcDNA3.1C-MOG1 using Lipofectamine 2000 (Invitrogen) and electrophysiological recordings were performed as described previously (27,29,33). Vector pIRE-GFP DNA expressing green fluorescent GFP protein (0.25 µg) was co-transfected together with pcDNA3.1C-MOG1 to serve as an indicator.(24,25) Only GFP-positive cells were selected for recording sodium currents. Pipettes were fabricated from borosilicate glass (FHC, Inc.) and electrode resistance ranged from 2-3 MΩ when filled with pipette solution with the following composition (in mM): NaCl, 20; CsCl, 130; HEPES, 10; EGTA, 10; pH 7.2 with CsOH. Voltage command pulses were generated using the Multipatch 700B amplifier (Axon Instruments) under the control of a desktop computer with pCLAMP software (9.0, Axon Instruments). Currents were filtered at 5 kHz (-3 dB, 4-pole Bessel filter) following series resistance inhibitors, and 0.5% Triton X-100), the immunoprecipitates were subjected to SDS-PAGE and immunoblot analysis using the anti-MOG1 antibody (or anti-Na,1.5). Cell lysates incubated with protein A/G beads alone were used as a negative control. Co-IP of lysates with normal IgG was also used as a negative control in each experiment. Studies were repeated at least three times.
compensation. The holding potential for all pulse protocols was -100 mV and experiments were performed at room temperature (22°C). For HEK293 cells, the composition of the bath solution was (in mM): NaCl, 70; CsCl, 80; KCl, 5.4; CaCl₂, 2; MgCl₂, 1; HEPES, 10; glucose, 10; pH 7.3 with CsOH. For neonatal cells, the NaCl concentration was reduced to 20 mM and CsCl was increased to 120 mM for better voltage control. To reduce contaminating Ca²⁺ and K⁺ (transient outward) currents, 2 mM CdCl₂ and 2 mM 4-AP, respectively, were added to the bath.

**Cell-Attached Recording-**Seals were formed in an extracellular solution containing (mM):140 NaCl, 2 CaCl₂, 10 glucose, 10 HEPES, pH 7.2 with NaOH. After seal formation, cells were depolarized with an extracellular solution containing 140 K-aspartate, 0.62 CaCl₂, 1.06 MgCl₂, 10 glucose, 10 HEPES, 2 EGTA, pH adjusted to 7.2 with KOH. The pipette solution contained 140 NaCl, 10 TEACl, 1 MgCl₂, 10 HEPES, pH 7.2 with NaOH. Pipettes were fabricated with borosilicate glass and coated with Sylgard®. Pipette resistances were 2 MΩ. Experiments were performed at room temperature.

Currents were elicited using 100 ms step pulses in 10 mV increments from a holding potential of -120 mV. Currents were filtered at 5 kHz with a 4-pole Bessel filter and sampled at 20 kHz. Patches contained several channels, so multiple overlapping openings were generally observed near the start of the depolarization, but isolated single channel currents could be measured at later times. Events were detected using the half-amplitude threshold-crossing method, following digital Gaussian filtering to a final 2 kHz frequency and spline interpolation. Single channel current amplitudes were measured from open events lasting >0.18 ms (2× the dead time of the filter). The single-channel conductance was calculated by linear regression for currents from -80 to -20 mV.

To determine the voltage dependence of gating and the maximal conductance, peak currents at each voltage were converted to chord conductance, G = I / (V - V_R), where V_R is the extrapolated reversal potential from a linear fit to currents from -20 to 0 mV. In each experiment, G-V relations were fitted to a Boltzmann function, G = G_{Max} / (1 + e^{(V-V_S)/k}), where G_{Max} is the maximal conductance, Vs is the voltage where G = G_{Max}/2, and k is a slope factor. No significant differences in G_{Max}, Vs, or k were observed between controls and MOG1 cells.

**Western Blot Analysis-**To determine the expression level of the Na_1.5 protein in the membrane fraction, HEK293/Nav1.5 cells were transiently transfected with pcDNA3.1C-MOG1 or empty vector pcDNA3.1C as control. Transfected cells were cultured for 48 h, and washed with cold PBS. Cells were then collected and suspended in the lysis buffer (1% Triton X-100, 150 mM NaCl, 50 mM Tris-HCl, pH7.5, 1 mM EDTA, a protease inhibitor cocktail). Cell lysates were centrifuged at 14,000 rpm for 10 min at 4°C to remove cell debris, nuclei and large particulates. The supernatant portion that contains both the extracted membrane proteins and cytosolic proteins was collected and further centrifuged at 40,000 rpm to separate the membrane fraction from the cytosolic fraction. The pellet from the second centrifugation step contains the membrane protein fraction and the supernatant contains the cytosolic protein fraction. Equal amounts of protein extracts were resolved by 2% SDS containing protein loading buffer and separated on SDS-PAGE. Western blot analysis was performed as described previously (22). A rabbit polyclonal anti–Na_1.5 was diluted in 1:500 in 0.3% BSA in PBST, and used in Western blot analysis. The signal was detected using enhanced chemiluminescence (ECL kit, Amersham Biosciences, Buckinghamshire,UK). An anti-KCNQ1 antibody (Santa Cruz Biotechnology) was used as a loading control at 1:500 dilution in PBST.

We also used Pierce Mem-PER Mammalian Membrane Protein Extraction Kit (Pierce Biotechnology, Rockford, IL) for separating the membrane fraction from the cytosolic fraction through phase partitioning. Cells (5x10^⁶) were transfected, cultured, and collected as described before and lysed with 150 µl Mem-Per Reagent A containing a mild proprietary detergent by incubation at room temperature for 10 min. 450 µl of the second diluted Mem-Per Reagent (2 parts Reagent C and 1 part Reagent B) was added to cell lysates to solubilize the membrane proteins. The mixture was incubated on ice for 30 minutes, and centrifuged at 10,000 g for 3 minute at 4°C. The
supernatant was then incubated at 37°C for 10 minutes, and then centrifuged at 10,000 g for 2 minutes to separate the membrane fraction (bottom layer) from the hydrophilic fraction (top layer). The membrane and cytosolic fractions were used for Western blot analysis with anti–Nav1.5 and anti-KCNQ1 antibodies as described above, and similar results were obtained.

Statistical Analysis—Data are represented as mean values ± SEM. Statistical analysis was performed using an ANOVA and two-tailed Student’s t-test to compare means and significance was set at the indicated P values.

RESULTS

MOG1 Was Identified as a Candidate Protein that Interacts with Na1.5 by a Yeast Two-Hybrid Screen. To identify proteins that interact with Na1.5, we carried out a yeast two-hybrid screen. We screened a human cardiac pre-transformed MATCHMAKER cDNA library (Clontech, Inc.). Five cytoplasmic segments of Na,1.5 were used as baits for the screening and these include the N-terminus, loop I (between DI–DII), loop II (between DII–DIII), loop III (between DIII–DIV), and C-terminus. The Na,1.5 C-terminus–GAL4-BD fusion protein alone activated transcription of reporter genes, thus no further screening was performed with this bait. No positive clones were obtained with the N-terminal domain and cytoplasmic loop I baits. There were 223 positive clones obtained with the cytoplasmic loop II bait, and 29 positive clones with the loop III bait. Most clones were excluded as false positives as they were derived from mitochondrial DNA or because the encoded peptides were out of frame with the GAL4 activation domain. One positive clone from the screening was found to encode the portion of MOG1 spanning amino acid residues 65 to 186 (GenBank Accession# AF265206), and was independently identified twice from the library screening.

Interaction between MOG1 and Na,1.5 as Shown by GST Pull-Down. To further demonstrate the interaction between MOG1 and Na,1.5, we carried out a GST pull-down assay. The cytoplasmic loop II of Na,1.5 (amino acids 940 to 1200) was fused to GST (GST-Na,1.5-LII), expressed in E. coli, and purified. Radioactively-labeled MOG1 protein was prepared by in vitro transcription followed by translation using 35S-methionine and this appeared as a single band of 28 kDa (Fig. 1A, lane 1). The GST-Na,1.5-LII fusion protein successfully pulled down 35S-MOG1 in the assay (Fig. 1A, lane 2), whereas two negative controls, GST alone (Fig. 1A, lane 4) and the GST-Na,1.5 cytoplasmic loop I fusion protein (GST-Na,1.5-LI, Fig. 1A, lane 3) failed to interact with 35S-MOG1. These results suggest that MOG1 interacts directly with the cytoplasmic loop II of Na,1.5.

Interaction between MOG1 and Na,1.5 as Shown by Co-Immunoprecipitation. HEK293/Na,1.5 was transfected with pcDNA3.1C-His-MOG1. Cell extracts were immunoprecipitated using an antibody against MOG1 (Fig. 2A, lane 3) or with control rabbit pre-serum IgG (Fig. 2A, lane 2). The bound proteins were then detected by Western blot analysis with an anti-Na,1.5 antibody. The anti-MOG1 antibody, but not the control IgG, precipitated a 250 kDa Na,1.5 protein. Reciprocal co-immunoprecipitation was also performed. The anti-Na,1.5 antibody was used for immunoprecipitation, and the anti-MOG1 antibody was used for Western blot analysis. MOG1 protein was successfully precipitated by the anti-Na,1.5 antibody, but not by the rabbit pre-serum control (Fig. 2B).

As MOG1 was pulled out from the yeast two-hybrid library using Na,1.5-LII, we next determined whether the loop II interacts with MOG1 using the co-immunoprecipitation assay. HEK293 cells were transiently co-transfected with expression constructs for Flag-tagged MOG1 (pCMV10-MOG1) and His-tagged Na,1.5-LII (pcDNA3.1A-Nav1.5-LII). Cell extracts were immunoprecipitated using a monoclonal antibody against His or with control IgG. The bound proteins were then detected by Western blot analysis with a monoclonal anti-Flag antibody. The anti-His antibody, but not the control IgG, precipitated Flag-tagged MOG1 (Fig. 2C). A reciprocal co-immunoprecipitation assay was performed using an anti-Flag antibody for immunoprecipitation. Na,1.5-LII fusion protein was successfully precipitated by the anti-Flag antibody, but not by control mouse serum IgG (Fig. 2D). These immunoprecipitation results illustrate
that the association of MOG1 to Na,1.5 is mediated by the cytoplasmic loop II of Na,1.5.

**In Vivo Interaction between MOG1 and Na,1.5 in Cardiac Cells.** The interaction between MOG1 and Na,1.5 was further confirmed by co-immunoprecipitation assays in vivo in mouse cardiac cells. Total protein extracts were prepared from mouse hearts. Both MOG1 and Na,1.5 proteins are abundantly expressed in cardiac cells, and can be easily detected by Western blot analysis (lanes 1 in Fig. 2E and lane 1 in Fig 2F). Two protein bands were detected for MOG1, which may represent the two MOG1 isoforms derived from alternatively spliced transcripts as reported previously by Marfatia et al.(23) Mouse cardiac protein extracts were precipitated either with a polyclonal antibody against MOG1 (Fig. 2E, lane 2) or with control IgG (Fig. 2E, lane 3). The bound proteins were then detected by immunoblot analysis with an anti-Na,1.5 antibody. The MOG1 antibody easily precipitated a Na,1.5 protein (Fig. 2E, lane 2). Similar experiments revealed that the anti-Na,1.5 antibody could precipitate MOG1 proteins from mouse cardiac cell extracts (Fig. 2E, lane 2). These results indicate that MOG1 interacts with Na,1.5 in vivo in cardiac cells.

**MOG1 Increases Sodium Current Density in a Mammalian Expression System.** We wished to determine whether the interaction of MOG1 to Na,1.5 modified channel function. For this purpose, we expressed MOG1 in HEK293/Na,1.5 cells and measured the whole-cell sodium currents. As shown in Fig. 3A and 3B, the sodium current density (expressed as peak current normalized to cell capacitance, pA/pF) across the range of test potentials was significantly increased in cells co-expressed with MOG1. The maximum current density normally measured at -30 mV for vector cells was shifted to -35 mV and increased by 61 pA/pF when co-expressed with MOG1. This is despite the fact that co-expression with MOG1 did not alter cell capacitance (16.6 ± 4.1 pF, n = 12 versus 18.6 ± 1.9 pF, n = 9, P=NS). Steady-state activation and inactivation gating properties were evaluated using the pulse protocols shown in the insets (Fig. 3C). The data for channel activation are the mean normalized conductance plotted against the test potential. MOG1 significantly shifted the voltage dependence of activation to more negative potentials, although the shift was very small, only by 4 mV (V1/2act = -49.1 ± 0.1 mV, n = 5 versus -45.1 ± 0.2 mV, n = 9, P<0.05). A two-pulse protocol was used to estimate the membrane potential dependence of inactivation. Cells were stepped to conditioning potentials for 500 ms as shown on the abscissa before depolarization to -20 mV (50 ms step) and peak current from the test potential was normalized to peak sodium current in the absence of a conditioning step. Cells expressing MOG1 showed no difference in the inactivation kinetics of Na,1.5 (Fig. 3C; V1/2inact = -84.1 ± 0.1 mV, n = 5 versus -85.5 ± 0.1 mV, n = 7, P = NS). Similarly, recovery from inactivation was not changed (Fig. 3D; t1/2 (τ1) = 4.2 ± 0.2 ms, n = 5 versus 3.8 ± 0.1 ms, n=6, P = NS) as this was assessed using a two-pulse protocol and the fractional current (P2/P1) was plotted against inter-pulse duration between P1 and P2. In summary, these results show that MOG1 increases whole-cell Na,1.5 currents.

**MOG1 and Sodium Current Density in Neonatal Cardiomyocytes.** To examine the important role of MOG1 in native cardiomyocytes, MOG1 was over-expressed in 3-day neonatal myocytes. Our results show that sodium current density was increased two-fold compared to vector-transfected cells (Fig. 4). These results suggest that MOG1 may play a critical role in the physiological function of sodium channels in native cardiomyocytes.

**Strong expression of MOG1 at intercalated discs in cardiomyocytes and heart tissues.** To further corroborate the interaction of MOG1 to Na,1.5, we studied the expression patterns of MOG1 in the heart. A peptide competition experiment was used to evaluate the specificity of the MOG1 antibody. The immunofluorescent signal was significantly eliminated when the MOG1 antibody (#2728) was pre-absorbed with the antigen peptide (compare Fig 5A and 5B), suggesting that the MOG1 antibody has a high specificity. As shown in Fig. 5C, MOG1 is widely expressed in both atrial and ventricular muscles and, interestingly, this expression was highly localized in the intercalated discs. Identical results were obtained with the second, independent anti-MOG1 antibody (#3350; data not shown), confirming the finding with antibody #2728. Immunostaining with connexin 43 antibody made it possible to easily distinguish the AV node from...
atrial and ventricular tissues (Fig. 5D). Similar to connexin 43, expression of MOG1 protein was higher in atrial and ventricular tissues compared to AV nodal tissues.

Immunostaining studies were also performed in isolated mouse ventricular myocytes and the results show that MOG1 expression was also particularly strong in the intercalated discs regions. Furthermore, MOG1 is mostly localized outside of the nucleus in cardiomyocytes. More importantly, the expression pattern of MOG1 was shown to overlap with that of Na\textsubscript{v}1.5 (Fig. 5E).

**MOG1 and Sodium Currents in HEK293/Na\textsubscript{v}1.5 Excised Patches.** To determine whether MOG1 has any effect on single channel conductance, sodium currents were recorded using the cell-attached configuration of the patch clamp technique from HEK293/Na\textsubscript{v}1.5 cells transiently transfected with MOG1 or empty vector (control). No significant effect on current amplitudes was observed (1.0 ± 0.3 nS with control vector n=5, vs. 0.8 ± 0.4 nS with MOG1 n=4) (Fig. 6A). The single-channel slope conductances were not significantly changed either (6.0 ± 0.4 pS control, n=3 vs. 6.1 ± 0.8 pS with MOG1, n=3) (Fig. 6B). These results suggest that MOG1 does not affect the conductance of single sodium channel.

**MOG1 Increases Cell Surface Expression of Na\textsubscript{v}1.5.** To investigate the potential mechanism by which MOG1 increases the sodium channel density, Western blot analysis was performed with the membrane fraction and cytoplasmic fraction of protein extracts isolated from HEK293/Na\textsubscript{v}1.5 cells transiently transfected with MOG1 or empty vector (control). As shown in Fig 7A, Na\textsubscript{v}1.5 expression in the membrane fraction was increased with overexpression of MOG1 in comparison with the control. Accordingly, Na\textsubscript{v}1.5 expression in the cytoplasmic fraction was reduced with overexpression of MOG1 (Fig. 7B). These results suggest that MOG1 increases cell surface expression of Na\textsubscript{v}1.5, which is consistent with the result that co-expression of MOG 1 and Na\textsubscript{v}1.5 increased sodium channel densities (Fig. 3 and Fig. 4).

**DISCUSSION**

Here we report the identification of a novel Na\textsubscript{v}1.5-interacting protein, MOG1. We showed that MOG1 was an important co-factor for Na\textsubscript{v}1.5 and could modulate the function of the cardiac sodium channel. Specifically, we demonstrated that MOG1 interacted with Na\textsubscript{v}1.5 and played a critical role in regulation of sodium current densities. Using a yeast two-hybrid screen, we identified MOG1 as a candidate protein that interacted with the cytoplasmic loop II of Na\textsubscript{v}1.5. Both in vitro GST pull-down and in vivo co-immunoprecipitation analyses further demonstrated this interaction (Fig. 2). Most importantly, co-immunoprecipitation studies demonstrated the interaction between endogenous MOG1 and Na\textsubscript{v}1.5 in cardiac cells (Fig. 2).

The interaction between MOG1 and Na\textsubscript{v}1.5 is important for the physiological function of Na\textsubscript{v}1.5. Co-expression of MOG1 in HEK293/Na\textsubscript{v}1.5 cells increased whole-cell sodium current density (Fig. 3). Similarly, over-expression of MOG1 in neonatal cardiomyocytes also resulted in increased whole-cell sodium current density (Fig. 4). The amplitude of the sodium current in neonatal myocytes (240 pA/pF) was slightly higher than that in HEK293/Na\textsubscript{v}1.5 cells (200 pA/pF) (Fig. 3 and Fig. 4). These results suggest that MOG1 can modulate the physiological function of Na\textsubscript{v}1.5.

Increased sodium current densities in HEK293/Na\textsubscript{v}1.5 cells caused by the over-expression of MOG1 suggest that there is either an increase in the number of sodium channels on the cell surface or an enhancement in single channel conductance. Kinetic analysis of the steady-state activation and inactivation kinetics of Na\textsubscript{v}1.5 in HEK293/Na\textsubscript{v}1.5 cells over-expressed with MOG1 revealed a small 4 mV hyperpolarizing shift in activation with little or no effects on channel inactivation. This, by itself, did not reveal the underlying basis for the increase (~60 pF/pA) in the current density in HEK293/Na\textsubscript{v}1.5 cells with overexpression of MOG1. Following examination of excised patches from HEK293/Na\textsubscript{v}1.5 cells over-expressed with MOG1, we further revealed no effect on single channel conductance (Fig 6B). These results suggest that an enhancement in single channel conductance cannot account for the effect of MOG1 on Na\textsubscript{v}1.5.

The above analysis prompted us to examine the possibility that an increase in the number of...
sodium channels on the cell surface may be the underlying mechanism for the finding of increased sodium current densities by MOG1. This hypothesis was tested by Western blot analysis, which revealed an increase in Na$_{1.5}$ expression in the plasma membrane (Fig 7A), and a decrease in Na$_{1.5}$ expression in the cytoplasm of HEK293/Na$_{1.5}$ cells over-expressed with MOG1 (Fig 7B). These results suggest that the channel number on cell surface was increased. Taken together, the available evidence supports a model in which MOG1 increases the number of sodium channel and/or availability on cell surface, which then results in an increase in sodium current density.

MOG1 is highly conserved from yeast to humans, suggesting that it is an essential protein for cellular functions. However, the specific physiological function (s) of MOG1 is obscure. MOG1 was shown to interact with Ran GTPase, a protein required for the trafficking of proteins and RNA in and out of the nucleus, and mediates the release of GTP from Ran in vitro (20,21). Thus, MOG1 was proposed to play a regulatory role in nuclear import and export by maintaining the Ran-GTP gradient from the nuclei to the cytoplasm (22). During the nucleocytoplasmic transport, MOG1 was proposed to shuttle between the cytoplasm and the nucleus (22). Indeed, in HEK293 cells, human MOG1 is localized throughout the cell (23). Due to its cytoplasmic localization, the function of MOG1 may not be restricted to the nucleus, on the contrary, it may function outside of the nucleus. Lu et al. showed that yeast MOG1 may interact with an osmotic stress sensor Sln1p and regulates the SLN1-SKN7 signal transduction in yeast (34). It is interesting to note that Sln1p is a plasma membrane protein, specifically a two-transmembrane domain sensor of the high-osmolarity glycerol (HOG) response pathway (35). These results suggest that MOG1 can interact with a plasma membrane protein. Thus, our finding of the interaction between MOG1 and membrane protein Na$_{1.5}$ is no surprise. Oki et al. recently showed that yeast MOG1 can also interact with Cid13 (a poly(A) polymerase for suc22 mRNA encoding a subunit of ribonucleotide reductase) with a potential role in regulation of cell cycle S-M transition (36). In addition, genetic suppression studies in yeast implicated that MOG1 could be required for membrane localization of Opi3p, a phospholipid methyltransferase required for membrane formation, and might play a role in Ssp1-mediated stress response pathway (36). To date, all studies that explored the roles of MOG1 were either in vitro studies or involved yeast. The results from the present study, for the first time, reveal a novel physiological role for MOG1 in mammalian cells in vivo and suggest that this small protein is a critical component in the multi-protein Na$_{1.5}$ complex with a modulator role in determining the amplitude of cardiac sodium currents. The function(s) of mammalian MOG1 may not overlap completely with yeast MOG1 as human MOG1 failed to fully replace the yeast MOG1 in a complementation test of growth defects of a MOG1 deletion yeast strain (23).

Increased cell surface localization of Na$_{1.5}$ by MOG1 may be achieved through two potential mechanisms, increased transport of Na$_{1.5}$ to cell surface or reduced turnover of Na$_{1.5}$ when localized on plasma membrane. Trafficking of membrane proteins like Na$_{1.5}$ is a highly regulated process (reviewed by Herfst et al. (37)). The first key step of the process is the transition from the ER to Golgi compartment, which is governed by the ER retention motifs and ER export signals. The cytoplasmic loop I of Na$_{1.5}$ contains several ER retention motifs (the RXR motif) that are involved in mediating the increase of sodium currents by protein kinase A (38). There is a potential ER export signal (DXE) in the C-terminus of Na$_{1.5}$ that may regulate the exit of fully folded and assembled Na$_{1.5}$ out of the ER (37). Selective Golgi export or transport of Na$_{1.5}$ from the Golgi to plasma membrane is another key step for trafficking of Na$_{1.5}$ (37). The trafficking of Na$_{1.5}$ may be regulated by its interacting proteins. The PDZ domain located at the C-terminus of Na$_{1.5}$ can associate with dystrophin and syntrophin proteins (18). In dystrophin-deficient mice, expression of Na$_{1.5}$ was reduced by 50%, and the sodium current from cardiomyocytes was reduced by 29% (18). As PDZ-domain interacting proteins can affect the membrane protein trafficking (39), dystrophin and syntrophin proteins may regulate the appropriate expression of Na$_{1.5}$ by controlling the transport of Na$_{1.5}$ to plasma membrane. Co-expression of the β1 subunit with Na$_{1.5}$ caused a small acceleration in the recovery from inactivation and
an increased density of sodium currents (10, 11). Further studies showed an increase of Na\(_{\text{v}1.5}\) localization to plasma membrane by the β1 subunit (10, 11), however, it remains to be determined how β1 increases trafficking of Na\(_{\text{v}1.5}\) to plasma membrane. The function of MOG1 is similar to that of the β1 subunit and may regulate the trafficking of Na\(_{\text{v}1.5}\) to plasma membrane. Co-expression of MOG1 with Na\(_{\text{v}1.5}\) caused a small but significant shift of the activation curve to a more negative potential and an increased density of sodium currents (Fig. 3 and Fig. 4). Western blot analysis showed increased expression of Na\(_{\text{v}1.5}\) on plasma membrane (Fig. 7). As with β1, the molecular mechanism by which MOG1 increases targeting of Na\(_{\text{v}1.5}\) on plasma membrane is not clear. As studies from yeast suggest that MOG1 can interact with the nucleocytoplasmic transport machinery, a hypothesis that can be tested in the future is that MOG1 may interact with the transport complex involved in the transport of Na\(_{\text{v}1.5}\) from the Golgi to plasma membrane or from the ER to Golgi.

In addition to increased trafficking of Na\(_{\text{v}1.5}\) to the plasma membrane, MOG1 may reduce the turnover of Na\(_{\text{v}1.5}\) localized on plasma membrane. Any change in the internalization and degradation of Na\(_{\text{v}1.5}\) will affect the expression level of Na\(_{\text{v}1.5}\) on cell surface. The ubiquitin-protein ligase Nedd4 is another Na\(_{\text{v}1.5}\)-interacting protein that may mediate the ubiquitin-dependent internalization and subsequent degradation of Na\(_{\text{v}1.5}\). Accordingly, co-expression of Nedd4 and Na\(_{\text{v}1.5}\) led to reduction of the sodium current density by 40% (40) and accelerated internalization of Na\(_{\text{v}1.5}\) (41). It is unlikely that MOG1 is involved in the ubiquitin-dependent internalization and subsequent degradation of Na\(_{\text{v}1.5}\). On the other hand, the direct interaction between MOG1 and Na\(_{\text{v}1.5}\) may stabilize the Na\(_{\text{v}1.5}\) protein, and reduce its degradation, which may explain the finding of increased cell surface expression of Na\(_{\text{v}1.5}\) by MOG1.

A previous study with Northern blot analysis revealed that the heart was the tissue with the highest expression level of MOG1 (23), suggesting that MOG1 plays an important role in cardiac function. The results from the present study further define the role of MOG1 in the heart. We provide strong evidence that MOG1 regulates the proper expression and function of Na\(_{\text{v}1.5}\) in cardiomyocytes. Further studies with immunostaining revealed that the expression of MOG1 was concentrated in the intercalated discs of both atrial and ventricular cells (Fig. 5C). Moreover, the expression pattern of MOG1 was similar to the pattern shown by connexin 43, the primary gap junctional protein in cardiomyocytes, whereby the expression intensities were localized to the intercalated discs regions (Fig. 5D). As intercalated discs are essential for regulating electrical coupling between cells in the myocardium, co-localization of MOG1 and Na\(_{\text{v}1.5}\) in these regions suggests that MOG1 may play an important role in establishing and regulating electrical connections between cardiomyocytes.

We found that in cardiomyocytes, MOG1 was mostly localized on cell membrane and outside of the nucleus (Fig. 5E). In HEK293 cells, it was reported that MOG1 was expressed throughout the cell, including the nucleus and cytoplasm (23), however, the study could not distinguish whether MOG1 was also localized on plasma membrane due to complication of strong signal of MOG1 in cytoplasm. The cell-specific subcellular localization of MOG1 in cardiomyocytes vs. HEK293 cells is an interesting observation, but the molecular mechanism warrants further investigations. Cell- or tissue-specific proteins that interact with MOG1 may be a rational explanation for the observation. The interaction with Na\(_{\text{v}1.5}\) or other cardiac specific membrane proteins is expected to recruit MOG1 to the plasma membrane.

Marfatia et al. identified two alternatively spliced MOG1 transcripts, hMOG1a and hMOG1b (23). Consistent with this finding, our Western blot analysis of cardiac cell extracts revealed two isoforms of the MOG1 protein. Since the hMOG1a was the MOG1 isoform identified in our yeast two-hybrid screen, functional studies for MOG1 in this study were carried out with hMOG1a only. Searches of databases including the UCSC Genome Browser database (http://genome.ucsc.edu/) revealed three alternatively spliced MOG1 transcripts. The functional significance of the other alternatively spliced MOG1 transcript(s) or protein isoform(s) remains to be established in the future.

Na\(_{\text{v}1.5}\) mutations that affect its cell surface expression (trafficking) have been
associated with Brugada syndrome and other types of lethal arrhythmias. Thus, genetic mutations in MOG1 may affect the expression and function of Na\textsubscript{1.5}, leading to similar cardiac diseases. On the other hand, identification of proteins like MOG1 that regulate expression and function of Na\textsubscript{1.5} may serve as interesting targets for developing interventional options to manage lethal arrhythmias associated with Na\textsubscript{1.5} mutations or abnormalities. Furthermore, the present findings provide new insights into the physiological role of MOG1 in vivo in mammalian cells, and future studies of MOG1 will likely offer more insights on its role and its relevance in myocardial function.

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Acknowledgments—We thank Dr. A. H. Corbett at Emory University School of Medicine for the kind gift of the bacterial expression construct for MOG1, Dr. G. E. Kirsch and Dr. X. Wang for the stable HEK293 cells with constant expression of Nav1.5, and Dr. S. Karnik for advice on isolation of membrane proteins.

FOOTNOTES:
This work was supported by the National Heart, Lung, and Blood Institute grants R01 HL66251 (Q.K.W.), the China National Basic Research Program (973 Program 2007CB512000 and 2007CB512002, Q.K.W.), an American Heart Association Established Investigator Award 0440157N (Q.K.W.), and an American Heart Association Scientist Development grant 0630193N (Q.C.).

FIGURE LEGENDS

FIGURE 1. GST pull-down assays for the interaction between Nav1.5 and MOG1. In vitro-translated 35S-MOG1 bound strongly to Nav1.5 cytoplasmic loop II fused to GST (GST-Nav1.5-LII; lane 2), but not to cytoplasmic loop I fused to GST (GST-Nav1.5-LI; lane 3) or GST alone (lane 4). Lane 1, one-tenth of the input (35S-MOG1).

FIGURE 2. Co-immunoprecipitation assays for the interaction between Nav1.5 and MOG1. A, Whole cell lysates prepared from HEK293/Nav1.5 cells transiently transfected with a pCMV-10-MOG1 construct were immunoprecipitated with an anti-MOG1 antibody (lane 3) or pre-serum (lane 2) and analyzed by Western blotting with a polyclonal anti-Na1.5 antibody. The anti-MOG1 antibody successfully precipitated Na1.5. Lane 1, one-tenth of the input (cell extracts). B, Reciprocal co-immunoprecipitation with an anti-Na1.5 antibody for immunoprecipitation and the anti-MOG1 antibody for Western blotting. Lane 3, one-tenth of the input (cell extracts). C, Interaction between His-tagged loop II of Na1.5 and Flag-tagged MOG1 co-transfected into HEK293 cells. An anti-His antibody successfully precipitated Flag-tagged MOG1 (lane 2), but the control mouse serum failed to precipitate Flag-tagged MOG1. Lane 1, 1/50 of the input (cell extracts). D, Reciprocal co-immunoprecipitation for C. Lane 1, control mouse serum failed to precipitate His-tagged Na1.5-LII; lane 2, an anti-Flag antibody successfully precipitated His-tagged Na1.5-LII; lane 3, 1/50 of the input (cell extracts). E, Protein lysates extracted from mouse cardiac cells were immunoprecipitated with an anti-MOG1 antibody (lane 2) or control serum (lane 3). Lane 1, one-fifth of input. Signals were detected with an anti-Na,1.5 antibody. F, Reciprocal co-immunoprecipitation with an anti-Na,1.5 antibody for immunoprecipitation and the anti-MOG1 antibody...
for Western blotting. Lane 1, one-fifth of input. Lane 2, immunoprecipitation with the anti-Na,1.5 antibody. Lane 3, immunoprecipitation with control serum. Each experiment was repeated at least three times and similar results were obtained.

FIGURE 3. Over-expression of MOG1 in HEK293/Na,1.5 cells increased sodium current density. A, Raw traces for sodium currents with (right) and without (left) over-expression of MOG1 that were elicited with the current protocol depicted in the inset. B, For activation, cells were held at -100 mV and depolarized in 5 mV increments. The current-voltage relationship for both cell groups is summarized with current amplitudes normalized to cell capacitance (pA/pF, abscissa). Both steady-state activation and inactivation were determined by fitting the peak currents with a Boltzmann distribution: $I/I_{\text{max}} = 1/(1+e^{(-V-V_{1/2})/s})$, where $I$ is the current at test potential $V$, $I_{\text{max}}$ is the maximum current, $V_{1/2}$ is the potential giving the half-maximum current; see Text for fitting parameters. C, Steady-state activation (right) and inactivation curves (left). Steady-state activation was plotted over the indicated voltage range and expressed as the current at the test potential over the maximum current ($I/I_{\text{max}}$, abscissa). A two-pulse protocol was used to estimate the membrane potential dependence of inactivation. Cells were stepped to conditioning potentials for 500 ms as shown on the abscissa before depolarization to -20 mV (50 ms step) and peak sodium current from the test potential was normalized to peak sodium current in the absence of a conditioning step. D, Recovery from inactivation was assessed for both cell groups utilizing a two-pulse protocol and the fractional current (P2/P1) was plotted against interpulse duration between P1 and P2. The fraction of channels that had recovered following various time intervals was calculated by dividing the peak current measured during a test pulse to -20 mV. The average data was fitted with a bi-exponential function: $I/I_{\text{max}} = A_1(1-e^{-t/\tau_1})+A_2(1-e^{-t/\tau_2})$. In cells over-expressing MOG1, no effects in the inactivation kinetics were observed but a hyperpolarizing 4 mV shift was detected in channel activation. All studies were repeated at least three times, and the same results were obtained (data not shown).

FIGURE 4. MOG1 over-expression increases sodium current densities in neonatal cardiomyocytes. Maximum currents were elicited using a single pulse from -100 to -20 mV and peak currents were expressed as current densities (pA/pF) and averaged for the cell group. Cell capacitances ($C_m$) = 32.2 ± 4.7 pF (+ MOG1), 29.3 ± 2.8 pF (-MOG1). The study was repeated three times, and the same results were obtained (data not shown). “*” indicates $P=1.5\times10^{-5}$.

FIGURE 5. Expression profile of the MOG1 protein in heart tissues and isolated ventricular myocytes. A and B, The specificity of the MOG1 antibody (#2728) used for the study was examined by immunostaining of mouse atrial tissue in two adjacent tissue slides without (A) or with the peptide immunogen (B). Scale bar=100 µm. C, Strong expression of MOG1 in the right atrium (RA) and right ventricle (RV) with relatively lower expression in the distal common bundle or atrio-ventricular node (AV). D, Immunostaining showing expression profile of connexin 43 (CX43). Scale bar=100 µm. E, Co-localization of MOG1 and Na,1.5 at intercalated discs in a cardiomyocyte. Adult ventricular myocytes were isolated and double-stained with polyclonal anti-MOG1 (red) and anti-Na,1.5 (green) antibodies. Nuclei were stained with DAPI (blue). Right panel indicates the overlay of MOG1 and Na,1.5 images. Scale bar=5µm.

FIGURE 6. Effect of MOG1 on sodium currents in cell-attached patches. A, Sample current records during 100 ms depolarizations to -70 mV from a holding potential of -120 mV: a vector control cell (left) and a MOG1-transfected cell (right). B, Single-channel current-voltage relationships for vector control cells (filled squares) and MOG1-transfected cells (open circles). Slope conductances were $6.0 \pm 0.4$ pS (control, n=3) and $6.1 \pm 0.8$ pS (MOG1, n=3).
FIGURE 7. Co-expression of MOG1 increased cell surface expression of Na,1.5. A, Western blot analysis with an anti-Na,1.5 antibody with the membrane fraction of protein extracts from HEK293/Na,1.5 cells transfected with a MOG1 expression plasmid (+) or the empty control vector (-). The membrane filter was later immunoblotted with an anti-KCNQ1 antibody to ensure that an equal amount of proteins extracts (- and + MOG1) were used. Over-expression of MOG1 increased the expression of Na,1.5 on cell membrane. B, Similar studies as in (A), but the cytosolic fraction of protein extracts was analyzed.
Figure 1

\[ \begin{array}{cccc}
{^35}\text{S-MOG1} & + & + & + \\
\text{GST-Na}_{v1.5-\text{LII}} & - & + & - \\
\text{GST-Na}_{v1.5-\text{LI}} & - & - & + \\
\text{GST} & - & - & + \\
\end{array} \]
Figure 3

(A) Current-voltage (I-V) plots for Na$_{v}1.5$ and Na$_{v}1.5$ + MOG1. 

(B) Voltage-dependence of the activation of Na$_{v}1.5$ and Na$_{v}1.5$ + MOG1. 

(C) Voltage-dependence of the inactivation of Na$_{v}1.5$ and Na$_{v}1.5$ + MOG1. 

(D) Time course of the inactivation of Na$_{v}1.5$ and Na$_{v}1.5$ + MOG1.
Figure 4

![Graph showing current density comparison between MOG1 and Vector]

- MOG1
- Vector

Current Density (pA/pF)

- MOG1
- Vector

n = 11
Figure 6
Fig 7

A

MOG1

250kD

Na\textsubscript{v}1.5

75kD

KCNQ1

50kD

B

MOG1

250kD

Na\textsubscript{v}1.5

75kD

KCNQ1

50kD
Identification of a new co-factor, MOG1, required for the function of cardiac sodium channel Nav1.5

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J. Biol. Chem. published online January 9, 2008

Access the most updated version of this article at doi: 10.1074/jbc.M709721200

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