Supplementary Materials and Methods

Determination of the steady state levels of hnRNP A1, SF2/ASF and PABPN1 in normal and DM1 myoblasts

Total cell extracts were prepared from normal and DM1 myoblast cell lines and equal amounts of protein (10 µg) were subjected to Western blot analyses to assess the steady-state levels of hnRNP A1, SF2/ASF, and PABPN1. The primary antibody dilutions were 1:1000 for goat anti-hnRNP A1 (Y-15) polyclonal antibodies (200 µg/ml), 1:2000 for anti-SF2/ASF (96) monoclonal antibody (200 µg/ml), and 1:2000 for goat anti-PABPN1 (G-17) polyclonal antibodies (200 µg/ml). The secondary antibody dilutions were 1:8000 for goat anti-mouse IgG (1mg/ml) and 1:4000 for donkey anti-goat IgG (400 µg/ml). To control for protein quality and loading the membranes were re-probed with goat anti-GAPDH (V-18) polyclonal antibodies (200 µg/ml) at a dilution of 1:3000. The protein band intensities were measured by densitometry analyses using Gene Tool (Syngene Inc., USA).

Standardization of Western blot analyses

Total cell extracts were prepared from normal myoblasts transfected with Flag tagged-MBNL1, MBNL2, hnRNP H, and CUG-BP1. 5, 10, and 20 µg of total protein were independently subjected to Western blot analyses using either anti-hnRNP H or anti-CUG-BP1 antibodies. In these experiments the primary antibody dilutions were 1:3000 for goat anti-hnRNP H (N-16) polyclonal antibodies (200 µg/ml), and 1:4000 for CUG-BP1 (3B1) monoclonal antibody (200 µg/ml). The secondary antibody dilutions were 1:5000 for donkey anti-goat IgG (400 µg/ml) and 1:8000 for goat anti-mouse IgG
(1mg/ml). The antibody dilutions were identical for each protein concentration tested. As a loading control, the blots were re-probed for GAPDH using GAPDH polyclonal antibodies. Western blot signals were detected using the ECL plus detection reagents (Amersham). Each film was exposed for approximately 10 seconds. The band intensities were measured by densitometry analyses using Gene Tool (Syngene Inc., USA) and the fold increment of hnRNP H and CUG-BP1 were calculated as the GAPDH normalized ratio of each over expressed protein to the endogenous protein. The experiment was carried out thrice and the fold increments and standard deviations were calculated and plotted on a graph.

**Standardization of RT-PCR analyses**

Total RNA was isolated from normal myoblasts transfected with vector alone or vector expressing Flag tagged-hnRNP H and CUG-BP1 using the RNAeasy mini kit (Qiagen) according to the manufacturer’s protocol. cDNA was synthesized from 5 µg of total RNA using the cDNA synthesis kit (Amersham Bioscience Inc., USA). PCR analyses using IR specific primers was carried out using four different cDNA concentrations (125, 150, 200 and 300 ng) for 15, 25 and 30 cycles at an annealing temperature of 60°C. The experiments were run at least three times for three independent transfections. GAPDH RNA was amplified in parallel as an internal control. The band intensities of the PCR products were measured by densitometry analyses using Gene Tool. % IR-B was calculated as (IR-B/IR-A + IR-B) X 100.
Generation of recombinant MBNL1, hnRNP H, and CUG-BP1 proteins for in vitro RNA binding analyses

The open reading frames for MBNL1, and CUG-BP1 were cloned downstream of a histidine tag [(His)₆] in the pET28 expression plasmid. The hnRNP H clone in the bacterial expression plasmid (pET15b) was a generous gift from Dr. Douglas Black, University of California, Los Angeles. *E. coli* BL21 (DE3) cells transformed with the expression plasmids were grown at 37°C in 200 ml of 2 X YT media. Following induction with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), the cells were harvested by centrifugation. The harvested cells were lysed in the lysis buffer (25 mM Tris-HCl pH 7.6, 1% Triton X-100, 1.0 mM EDTA, 100 µg/ml lysozyme, 1.0 mM DTT, and 0.1 mM PMSF) with sonication. After centrifugation the presence of the expressed proteins were verified in the supernatants and pellets by SDS-PAGE analyses. The pellets were subsequently solubilized in a solubilization buffer (25 mM Tris-HCl pH 7.6, 8.0 M urea, 1.0 mM DTT, and 0.1 mM PMSF) and clarified by centrifugation. The denatured proteins were refolded by dialysis, with several changes, against serial dilutions of urea (8M to 0.1M) in the renaturation buffer (25 mM Tris-HCl pH 7.6, 1.0 mM DTT, and 0.1 mM PMSF). The His tagged recombinant proteins (MBNL1, hnRNP H, and CUG-BP1) were purified using Nickel Sepharose 6 Fast Flow columns (Amersham Bioscience Inc., USA) according to the manufacturer’s protocol and the purity was verified by SDS-PAGE with Coomassie staining, and quantitated using a protein assay kit (Bio-Rad Inc., USA).