**EXPANDED METHODS**

**Coimmunoprecipitation assays and western blot analysis.** V5/His tagged MyoD or NFATc3 constructs were immunoprecipitated using Ni-NTA beads (Invitrogen), followed by western blotting procedures as described in detail previously (1). Antibodies used included mouse monoclonal anti-GAPDH (Chemicon), rabbit polyclonal anti-myogenin (Santa Cruz), mouse monoclonal anti-V5 (Invitrogen), mouse monoclonal anti-Gal4 (Santa Cruz), mouse monoclonal anti-MHC (MF20, Hybridoma bank), mouse monoclonal anti-NFATc3 (sc-8405X, Santa Cruz), rabbit polyclonal anti-NFATc2 672 antiserum described previously (2).

**Cloning and real-time PCR.** MyoD constructs were inserted in frame into pBind (Promega) and pCDNA3.1/V5/His (Invitrogen) vectors. Expression vectors for an activated mutant of CnA or deletion fragments for NFATc3 were described previously (1). MyoD, NFATc2 and NFATc3 expression vectors are a kind gift from J. Molkentin and E. Olson. The NFAT9mer-Luc plasmid harbors nine copies of a high-affinity NFAT binding site from the interleukin-4 enhancer inserted upstream of a minimal TATA box and pGL3. The three NFAT binding sites were mutated in the 0.6kb myogenin-luc by directed mutagenesis using the following primers: N1 5'-GGAATCACATGTAATCCACTCCTAACGTCTTGATGTGCAGC-3'; N2 5'-GCCCCACAGGGGCTGTGGAGTAATGAAAACTAATCAAATTAC-3'; N3 5'-GGTTTAAGGTGCTGCTGAGCACCTAAGAGAAGGCTAAGTGG-3'. Detailed information about vectors and oligo sequences is available upon request. Reverse transcription and real-time PCR using the BioRad iCycler (Biorad) was described previously (1).

**Chromatin immunoprecipitations.** C2C12 cells cultured either in proliferating medium or in differentiation medium for 48 h and cross-linked with 2% formaldehyde for 20 min at room temperature. Cross-linking was stopped by the addition of glycine to a final concentration of 0.125 M. The cells were washed with cold phosphate-buffered saline (PBS), collected and resuspended in sonication buffer containing 50 mM HEPES, pH 7.9, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.5% SDS, and protease inhibitors and sonicated on ice to an average length of 200 to 1000 bp. Samples were centrifuged at 14000 rpm and precleared with protein G-Sepharose in the presence of 25 µg/mL of salmon sperm DNA and 1 mg of bovine serum albumin/ml. Precleared chromatin was immunoprecipitated with 5 µg of monoclonal anti-NFATc3 (sc-8405X, Santa Cruz) or polyclonal anti-MyoD (sc-760, Santa Cruz). The immune complexes were collected by adsorption to protein G-Sepharose. The beads were

washed twice with sonication buffer, twice with sonication buffer containing 500 mM NaCl, twice with 20 mM Tris (pH 8.0)-1 mM EDTA-250 mM LiCl-0.5% NP-40-0.5% sodium deoxycholate, and twice with Tris-EDTA buffer. The immunocomplexes were eluted with 50 mM Tris, pH 8.0-1 mM EDTA-1% SDS at 65°C for 10 min, adjusted to 200 mM NaCl, and incubated at 65°C for 5 h to reverse the cross-links. After successive treatments with 10 µg of Rnase A and 20 µg of proteinase K/ml, the samples were extracted with phenol-chloroform and precipitated with ethanol. One tenth of the immunoprecipitated DNA and input DNA (from extracts before immunoprecipitation) was analyzed using real-time PCR reactions on a MyIQ apparatus (Bio-Rad). Oligos used for the PCR amplifications were as follows: myogenin promoter amplicon 1, sense: 5'-AAGGAGAGGGAAGGGGAATC-3' and antisense: 5'-GCCAACGCCACAGAAACC-3'; myogenin promoter amplicon 2, sense: 5'-GATTTTCAAGACCCCTTCCC-3' and antisense: 5'-CCGTCGGCTGTAATTTGATTAG-3'; myogenin promoter amplicon 3, sense: 5'-TGATGTGGTAGTGGTAGGTC-3'; control region downstream myogenin, sense: 5'-TCCTGGATTACTGTCAAGC-3'.

**Nuclear extracts and electrophoretic mobility shift assay.** Nuclear extracts from HEK cells stimulated with 20 ng/mL PMA plus 1µM calcium ionophore A23187 and C2C12 cells cultured 48h in proliferating or in differentiation medium were prepared as described previsouly (3). Cells were resuspended in 600µL of ice-cold buffer A (10 mM HEPES, pH 7.6, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.75 mM spermidine, 0.15 mM spermine, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 10 mM $Na_2MoO_4$, 1 µg/mL pepstatin, and leupeptin and aprotinin at 2 µg/mL each). After 15 min on ice, cells were lysed with 0.6% (v/v) Nonidet P-40. The nuclear pellet was extracted with 50 µL of buffer C (20 mM HEPES, pH7.6, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 10 mM $Na_2MoO_4$, 1 µg/mL pepstatin, and leupeptin and aprotinin at 4 µg/mL each). Nuclear extracts were collected and stored at -80°C. Protein concentration was determined by the Bradford assay (Bio-Rad).

Gel retardation assays were performed as in Martinez-Martinez *et al.* (3). Nuclear extracts (3-5 µg) were incubated with 1µg of poly(dI-dC) in DNA binding buffer (2% (w/v) polyvinylethanol, 2.5% (v/v) glycerol, 10 mM Tris, pH 8, 0.5 mM dithiothreitol) on ice for 15 min. Then, 100 000 cpm of $^{32}$P-labelled double stranded oligonucleotides was added and incubated at room temperature for 30 min. In competition experiments, a 20-fold molar excess of unlabeled homologous oligonucleotides was added to the binding reaction mixture prior to the probe. The DNA-protein complexes were resolved by electrophoresis on a 4% non denaturing

polyacrylamide gel. The following sequences of the nucleotides used as probes in EMSAs were used: the NFAT site from the Interleukin-2 promoter (Il2: 5'-gatcGGAGGAAAAACTGTTTCATACAGAAGGCGT-3'), the Myog-N1 (N1: 5'-gatcTAATCCACTGGAAACGTCTTGA 3'), Myog-N2 (N2: 5'-gatcTGTGGAGAAATGAAAACTAATC-3'), and Myog-N3 (N3: 5'-gatcTGCTGAGCAGGAAAGAGAAGGC-3').

The pairs of complementary synthetic oligonucleotides were annealed and labeled with the Klenow fragment of the DNA polymerase I.

Supershift assays were performed by incubating nuclear extracts for 15 min. at 4°C prior to the addition of the probe with either negative controls mouse IgG1 (eBioscience) or rabbit anti-mouse collagen type IV (AB756P, Chemicon), or the following specific antibodies: mouse anti-Flag M2 (F3165, Sigma) or rabbit anti-mouse NFATc3 (sc-8321, Santa Cruz).


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2. Lara-Pezzi, E., Armesilla, A. L., Majano, P. L., Redondo, J. M., and Lopez-Cabrera, M. (1998) *Embo J* **17**(23), 7066-7077
3. Martinez-Martinez, S., Gomez del Arco, P., Armesilla, A. L., Aramburu, J., Luo, C., Rao, A., and Redondo, J. M. (1997) *Mol Cell Biol* **17**(11), 6437-6447