Evaluation of splicing event co-variation with a strategy for the simultaneous detection of alternatively spliced MEF2C transcripts

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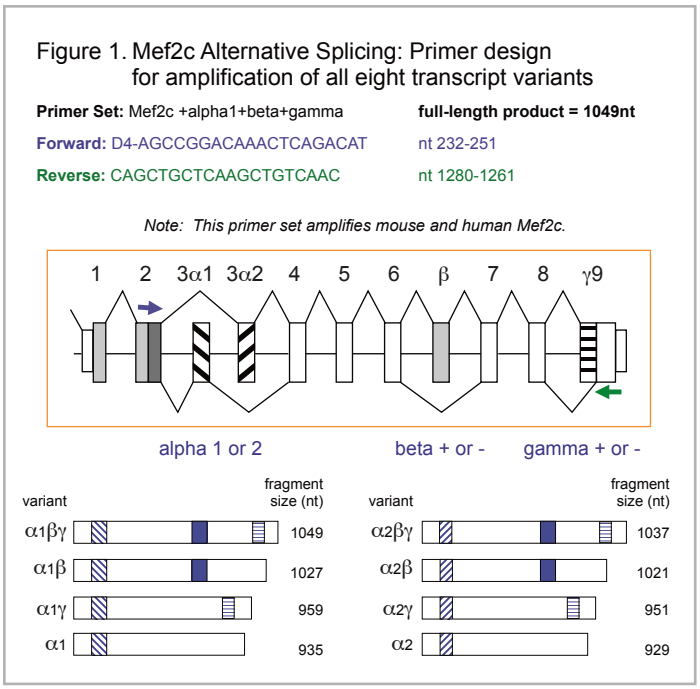
Introduction

Myocyte Enhancer Factor 2 (MEF2) proteins are transcription factors that exist in all metazoans and play pivotal roles in development and differentiation of tissues. All MEF2 proteins have an amino-terminal MADS box and adjacent MEF2 signature domain that together confer sequence-specific DNA binding and dimerization activities. The carboxy-termini of MEF2 proteins are less well conserved and harbor transcriptional regulatory and nuclear localization functions. There are 4 vertebrate *Mef2* genes, *Mef 2A, B, C* and *D,* and these have different temporo-spacial expression patterns. *Mef2A, C* and *D* encode protein variants by virtue of alternative splicing of primary transcripts, and these genes have similar structures and alternative splicing patterns that are conserved across evolution. The alternative splicing involves mutually exclusive exons (alpha1 and alpha2), a cassette exon (beta), and alternative splice acceptors that flank a short region (gamma) (Fig. 1). The corresponding short polypeptide domains encoded by these alternative segments are nested within the *Mef2* carboxy-termini and are structurally conserved across isotypes. These domains confer specific functions, including splicing variant-specific functional interactions with co-activators; potent transactivation by an “acid blob” (beta); and transrepression that is mediated by SUMOylation (gamma) and that is under control of various signaling events that modify *Mef2* and act in cis to control steady-state *MEF2* SUMOylation. As one aspect of an effort to elucidate the roles of *Mef2* alternative splicing variants, we have developed an RT-PCR long fragment assay in which all eight *Mef2C* mRNA isoforms can be simultaneously monitored in cell and tissue samples. This assay is used to confirm and extend prior observations of regulated *Mef2* alternative splicing among tissues, during development and during muscle differentiation. The technique is well suited for rapid qualitative evaluation of splicing variant expression, and could be effectively used for candidates with established splicing variants or for the validation of findings observed with “next generation” sequencing. Importantly, this strategy uniquely allows for the evaluation of co-variations in multiple alternative splicing events for primary transcripts of a given gene.



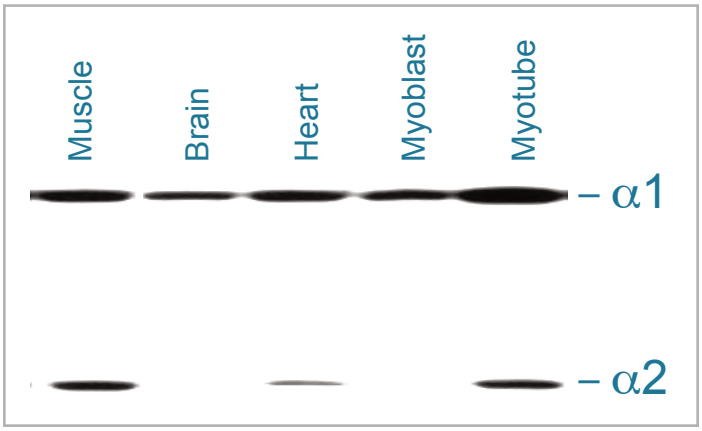


Figure 1. RNase-Protection Assay detects the expression of Mef2c alpha domain in mouse tissues and C2C12 cells. Alpha 1 is detected in all the tissues and both cell stages. Alpha 2 is only detected in muscle, heart and myotubes.

METHODS

cDNA and RNA Samples. Purified and quantitated cDNA and RNA samples were obtained from the Burnham Institute. RNA was isolated from C2C12 cell lines or mouse tissues by Trizol* extraction with DNase treatment. The C2C12 cells were untreated (blasts), treated for 1 day with either 2% Horse Serum (D1_HS) or 10% Fetal Bovine Serum (D1_FBS), or allow to differentiate for 6 days into myotubes (D6_tubes).

Primer Design. Primers for the full-length variant of Mef2c (a1,b+,g+) were designed such that all eight transcript variants would be amplified (Fig. 1). The forward primer was labeled with WellRED D4 dye for detection with the GenomeLab GeXP Genetic Analysis System (Beckman Coulter).

RT-PCR. A one-tube RT-PCR reaction (Promega Access* RT-PCR System) was performed with 200 ng of RNA or 1 ug of cDNA per reaction according to the manufacturer's instructions.

Separation by Capillary Electrophoresis (CE) and Data Analysis. PCR product separation, detection and analysis was performed with the GenomeLab GeXP Genetic Analysis System. PCR products were diluted in a mixture of Sample Loading Solution (Beckman Coulter) and MapMarker* WellRED D1-1000 (Bioventures) size standard and then separated by capillary electrophoresis at 3kV for 180 minutes. Custom analysis parameters (Dye Mobil. Calib. = PAver1, Slope Threshold = 1, Include Peaks = 1%, Size Standard 50-1000, Quartic model) were used in the Fragment Analysis module of GenomeLab Genetic Analysis System software.

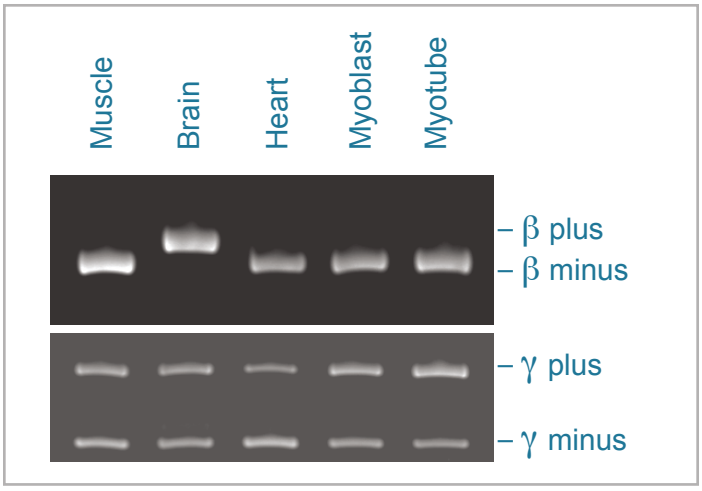


Figure 2. RT-PCR Assay detects the expression of single alternative exon site from either Mef2c beta or gamma domains in mouse tissues and C2C12 cells . Beta domain is detected while the other samples are beta-minus. Both gamma-plus and gamma-minus variants are found in the samples.

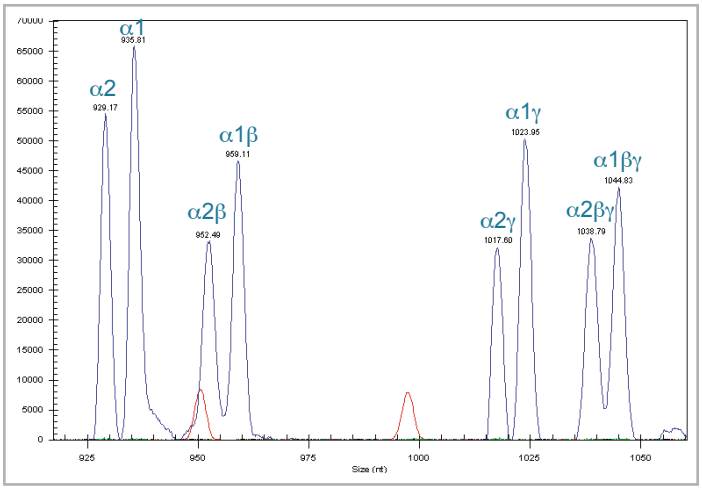


Figure 3. Electropharogram of the 8 human *Mef2c* variants amplified from cDNAs.

Mef2c Variant	Expected Size (nt)	GeXP Apparent Size (nt)
α1βγ	1049	1045
α2βγ	1043	1039
α1γ	1025	1024
α2γ	1019	1018
α1β	953	959
α2β	947	953
α1	929	936
α2	923	929

Table 1. Expected and Apparent fragment sizes for the eight *Mef2c* variants.

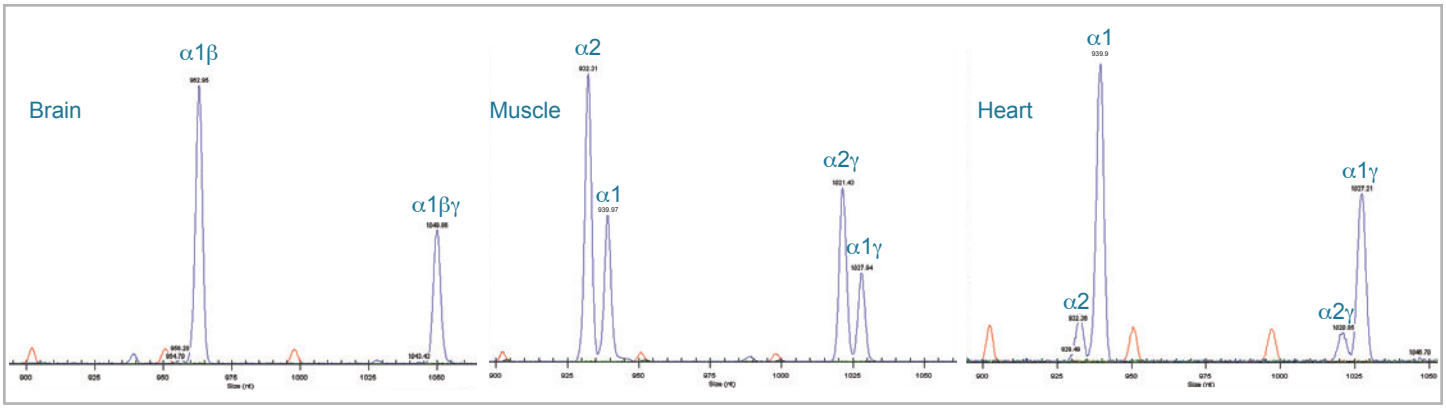


Figure 4. Electropharograms of RT-PCR products amplified with D4-*Mef2c* primer from various mouse tissue RNAs. Each tissue displays a unique expression profile, which correlates with alpha-domain RNAase Protection Assay (Fig. 1) and single exon RT-PCR (Fig.2) data from previous studies. Brain is the only sample that expresses beta domain.

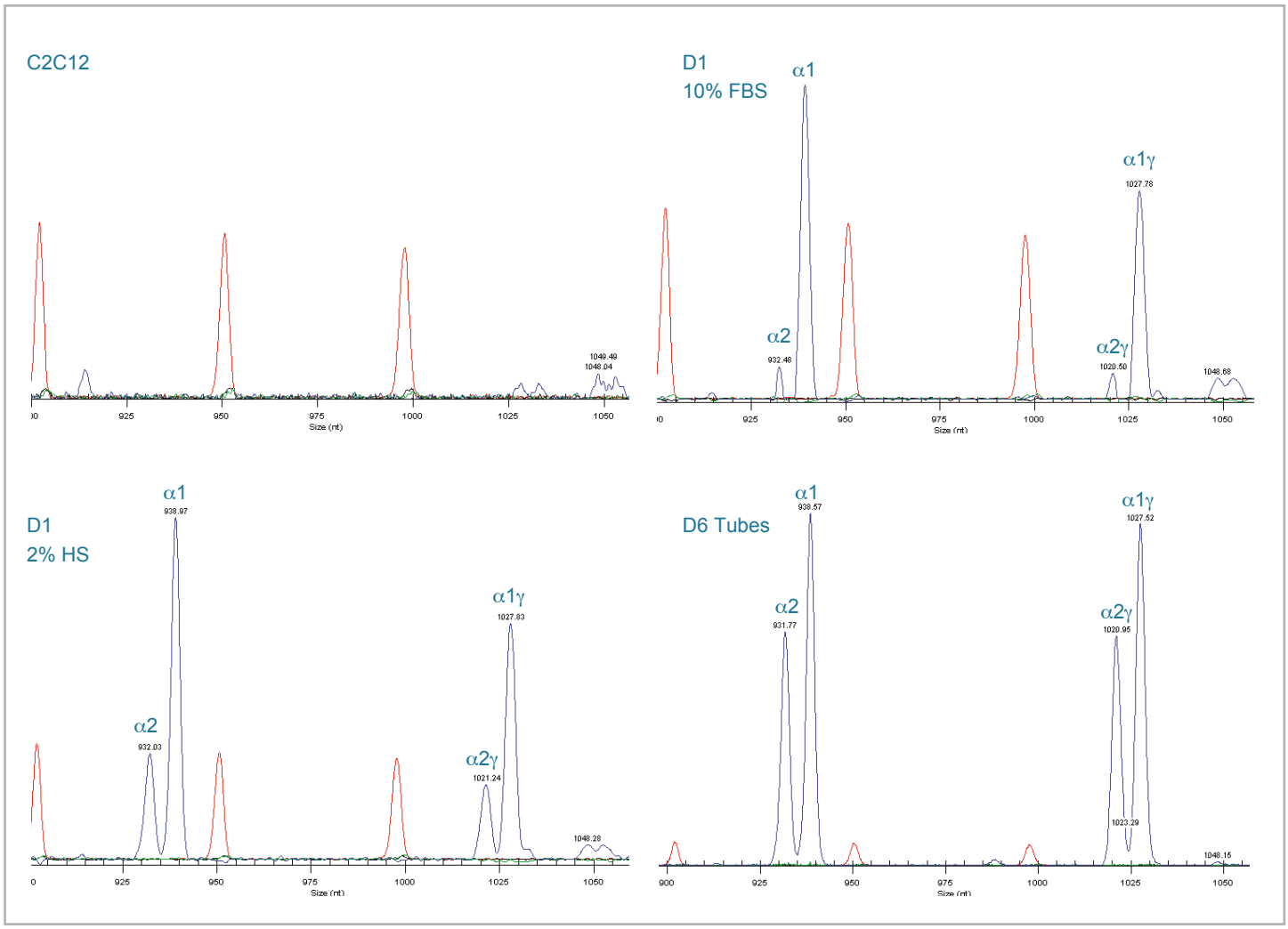


Figure 5. Electropharograms of C2C12 cells. Cell were left untreated as myoblasts (blasts), or treated with either 10% fetal bovine serum (FBS) or 2% horse serum (HS) for 1 day (D1), or differentiated for six days (D6) into myotubes (tubes). No expression of *Mef2c* is detected in undifferentiated myoblasts, where as increasing amounts of the α1, α2, α1γ and α2γ transcript variants were detected in the cells treated with 10% FBS or 2% HS with maximal expression of these four variants detected in the differentiated myotubes.

CONCLUSION

The GenomeLab GeXP Genetic Analysis System is capable of resolving fragments as long as 1050 nucleotides with sizes differing as little as six nucleotides. This allows for simultaneous amplification of transcript variants that contain multiple, alternatively spliced exons, to monitor and compare the expression pattern of alternatively spliced genes in tissues and cell lines. This strategy uniquely allows for the evaluation of co-variation in multiple alternative splicing events for primary transcripts of a given gene with greater sensitivity than traditional RPA or RT-PCR assays.

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