Human cytomegalovirus IE1 promoter/enhancer drives variable gene expression in all fiber types in transgenic mouse skeletal muscle

ABSTRACT

The CMV IE1 promoter/enhancer is not fiber-type-restricted and can be useful for driving germ-line transgene expression in all four fiber types. However, not all fibers express the gene at high levels due in part to regional differences in overall expression levels, and to a high level of within-fiber-type variability. Given the multinucleate syncitial nature of muscle fibers, it is not likely that this variability is due to variegating heterochromatinization. The soleus muscle would make a suitable subject for near-uniform experimental gene expression driven by CMV IE1 elements.

INTRODUCTION

Background Versatile genetic manipulation of skeletal muscle through expression of experimental germ-line transgenes requires knowledge of the expression characteristics of diverse promoter/enhancer elements in the various skeletal muscle fiber types. Rodent limb and axial muscles contain four major fiber types, one slow-contracting (type I) and three fast-contracting (types IIA, IIB, and IIX (also called IID)), that differ in activity patterns and in contractile and metabolic properties. Each of the four principal fiber types expresses a distinct myosin heavy chain gene that largely determines fast or slow contractile speed and many other muscle protein gene families contain members that are differentially expressed in fast and slow fibers. Recent studies have begun to characterize the cis-regulatory elements and trans-acting factors that direct fiber-type-specific gene expression. "Universal" viral promoters, such as the cytomegalovirus (CMV) IE1 promoter/enhancer are of interest in terms of their potential for broad, high-level expression. However the transcriptional specialization of the fiber types raises the possibility of fiber-type-restricted or differential expression in skeletal muscle. Several transgenic mouse studies have shown CMV IE1-driven expression in skeletal muscle as well as in other tissues, and Baskar et al, who studied expression at the cellular level, noted a marked differential expression among skeletal muscle fibers. However, the possible contribution of fiber-type heterogeneity to this differential expression has not been addressed. The purpose of the present study was to characterize CMV IE1-driven transgene expression in skeletal muscle in relation to fiber type. Through production of transgenic mice carrying the CMV IE1/ β-galactosidase (β-gal) construct CMVLacZ, and histochemical characterization of transgene expression in hindlimb skeletal muscle, we show that CMV IE1-driven expression is not fiber-type-restricted. CMVLacZ is expressed effectively, though at varying mean levels, in all the major fiber types. However, its expression is highly variable within each of the fiber types in most muscles/regions, even among nearby fibers of the same type. We discuss the implications of our findings for the potential use of the CMV IE1 promoter/enhancer as a tool for driving experimental germ-line transgene expression in skeletal muscle. We also discuss fiber-to-fiber differences in physiological status as a possible cause of within-fiber-type expression variability, and note that variegating heterochromatinization would not be expected to generate expression variability in multinucleate syncitial cells such as skeletal muscle fibers.

CONCLUSION

The CMV IE1 promoter/enhancer is not fiber-type-restricted and can be useful for driving germ-line transgene expression in all four major skeletal muscle fiber types. It is the first promoter for which germ-line transgene expression in all major fiber types, including comparable expression in slow and fast fibers, has been documented. However, because of regional effects on expression levels, and within-fiber-type variability, not all muscle fibers express the transgene at high levels. The cause of the marked within-fiber-type variability is unknown. It does not seem likely that variegating heterochromatinization would generate expression heterogeneity in multinucleate syncitial cells such as skeletal muscle fibers - an alternative hypothesis is that within-fiber-type expression variation may reflect fiber-to-fiber differences in physiological status. Among hindlimb muscles the soleus shows comparatively little between- and within-fiber-type variation and thus would make a suitable subject for near-uniform experimental germ-line transgene expression driven by CMV IE1 elements.