We show here that the introduction of a single Cys in the C-terminal region of NL1 in position 451, that is reported to be associated to the autistic spectral disorders, results in the retention of the mutated protein in the ER. The homologous mutation in two different forms of AChE (Arg 395) results in ER retention as well [4]. The mutated Cys apparently behaves in the α-β fold family proteins as an ER retention signal or a local misfolding factor resulting in retaining the protein in the cell irrespective of the oligomeric assembly of the native protein.

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Influence of the 5′ intron in the control of acetylcholinesterase gene expression during myogenesis

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Abstract

During myogenesis, marked increases in both acetylcholinesterase (AChE) and its encoding mRNA are observed. The intron in the AChE gene between non-coding exon 1 [T.L. Rachinsky, S. Camp, Y. Li, T.J. Ekstrom, M. Newton, P. Taylor, Molecular cloning of mouse acetylcholinesterase: tissue distribution of alternatively spliced mRNA species, Neuron 5 (1990) 317–327] and start-site containing exon 2 [A. Mutero, S. Camp, P. Taylor, Promoter elements of the mouse acetylcholinesterase gene, J. Biol. Chem. 270 (4) (1995) 1866–1872] appears to be responsible for the enhanced expression of the enzyme upon myoblast to myotube differentiation. Deletion of a 255 bp sequence within the first intron of the AChE gene abolishes the increase in cell-associated activity observed with differentiation. To study the involvement of the intronic region in post-transcriptional processing of AChE message, we used real time RT-PCR to quantify spliced and unspliced message levels in myoblasts and myotubes. We observe a ∼200-fold increase of the fully spliced mRNA associated with myotube formation, while the increase in the unspliced mRNA retaining either intron 1 or intron 2 is only 5 to 15-fold. We have generated knockout mice without the conserved region of intron 1. The mice show a phenotype where skeletal muscle, hematopoietic and central nervous system AChE expression differ with the greatest effect existing in the disappearance of skeletal muscle expression [S. Camp, L Zhang, M. Marquez, B. de La Torre, J.M. Long, G. Bucht, P. Taylor, Acetylcholinesterase (AChE) gene modification in transgenic animals: functional consequences of selected exon and regulatory region deletion, VIII IMC Proceedings].

1. Introduction

AChE exists in multiple molecular forms created by alternative splicing of a single transcript. The splice variants have the same catalytic domain (encoded by exons 2, 3 and 4) but different C-terminal domains [1]. The AChE variant expressed in the brain and in muscle cells of adult mammals is coded by exons 1, 2, 3, 4 and 6. AChE expression is known to be influenced by the state of myogenic differentiation [2,3]. Our previous findings revealed that, during myogenic differentiation, the increase in AChE activity parallels the mRNA increase, but the transcriptional rate is nearly identical in myoblasts and myotubes [4]. Although mechanisms that regulate the expression of the enzyme during myogenesis are not completely understood [3], here we show that the 5′intron between the cap site and the start of the open reading frame is uniquely responsible for the control of the expression of the gene.

2. Results and discussion

Using real time RT-PCR and relative quantification analysis, we calculated the fold difference in the expression of the AChE spliced message (from exon 2 to exon 3) during C2C12 cell differentiation. Our results show that the increase from myoblasts to myotubes, normalized

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to the house keeping gene cyclophillin, is around 200 fold. We were unable to quantify the splicing occurring at the E1-E2 junction, but we used absolute quantification to measure the abundance of the precursor of the mature message containing intron 1 or intron 2. Serial dilutions of a plasmid containing the whole AChE gene were used for absolute quantification. Our results show that both precursor species, containing either intron 1 or intron 2, increase with differentiation although to a lower extent (around 10-fold) compared to the spliced message. We do not observe a significant difference in the levels of two precursors, suggesting that a pre-mRNA species containing intron 1 is not preferentially retained upon myoblast differentiation.

We generated KO mice in which the 255 bp critical region has been deleted [5]. We flanked the regulatory region with loxP sites so that it could be removed by breeding with mice carrying the Cre recombinase transgene. The AChE activity levels of each construct were tested by transfecting C2C12 cells. The intron 1 deletion KO mice show a trembling phenotype and small size compared to their wild type litter mates.

AChE spliced message increases with myogenic differentiation as does unspliced precursor. The increase in the precursor forms might be explained by a possible transcriptional up-regulation of the gene during myogenesis that has been also postulated by other groups [3] although the 200-fold increase observed in the levels of the spliced message indicate that post-transcriptional regulation is also occurring. The absence of AChE activity in skeletal muscle tissue of KO mice in which the 255 bp intronic region has been deleted provides in vivo evidence of the critical involvement of the 5′ intronic region in the regulation of the AChE expression during myogenesis.

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Acetylcholinesterase genes and insecticide resistance in aphids

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1. Introduction

The peach-potato aphid (*Myzus persicae)* and the cotton aphid (*Aphis gossypii*) are important agricultural pests in many parts of the world, causing direct feeding damage and transmission of virus diseases on a range of crops. The widespread use of organophosphorus (OP) and carbamate insecticides for their control has led to the selection of resistant populations and biochemical studies of resistant strains have shown that this often results from alterations in the main synaptic AChE that renders it insensitive to these compounds. We have recently identified two mutations within the active site of the enzyme that correlate with resistance (A302S and S431F) and are now expressing the aphid AChE genes using the baculovirus system in order to: (1) confirm the functional properties of these mutations and (2) to generate a source of the enzyme for crystallography and structural studies.

1.1. Cloning of AChE genes and identification of mutations

It now seems that aphids, like most insects, have two genes that encode AChE-like proteins, termed ace-1 and ace-2. The nomenclature for these genes is highly confused because *Drosophila* has only a single ace gene, but the orthologue of this gene in other insects does not appear to be the major synaptic AChE. Hence, whilst some authors continue to refer to this gene as ace-1 because it was the first to be cloned, others have renamed

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