Identification of new variant forms of the photoreceptor glutamate transporter EAAT5

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EAAT5 is the predominant glutamate transporter used by photoreceptors in the retina to recover glutamate released by their synaptic terminals. EAAT5 is unusual in having a large chloride conductance, so that recovery of glutamate may be associated with feedback regulation of release of glutamate, by modifying the membrane potential of the synaptic terminals. Accordingly any changes in the biophysical properties of the EAAT5 that is expressed might influence the functional properties of photoreceptors. Examination of Western blots of rat retinal lysate using antibodies to the amino- and carboxyl termini of EAAT5 revealed, contrary to our expectations, several bands at differing molecular weights, suggesting that smaller variant forms of EAAT5 might exist. PCR analysis was performed using primers flanking the coding region of EAAT5. Multiple bands were identified. Bands were excised, inserted into plasmids, expanded in E. Coli and 30 clones sequenced. We identified 6 forms of EAAT5, including the originally described full-length wild-type form. Five splice variant forms were identified, which skipped, either completely or partially various exons, including exon 3, exon 7 exon 8, exon 9 and exon 10. The exon 8- and exon 10-skipping forms generated frame shifts that should lead to truncated proteins. Exon-skipping forms of EAAT1 and EAAT2 have previously been implicated in human disease, so ongoing studies of EAAT5 include production of antibodies to the exon skip forms, analysis of transport properties and determination of expression profiles in the normal human retina, and in retinas with disease including macular degeneration.