

The protozoan parasite *Toxoplasma gondii* differentially expresses two distinct enolase isoenzymes known as ENO1 and ENO2, respectively. To understand differential gene expression during tachyzoite to bradyzoite conversion, we have characterized the two *T.gondii* enolase promoters. No homology could be found between these sequences and no TATA or CCAAT boxes were evident. The differential activation of the ENO1 and ENO2 promoters during tachyzoite to bradyzoite differentiation was investigated by deletion analysis of 5'-flanking regions fused to the chloramphenicol acetyltransferase reporter followed by transient transfection. Our data indicate that in proliferating tachyzoites, the repression of ENO1 involves a negative distal regulatory region (nucleotides -1245 to -625) in the promoter whereas a proximal regulatory region in the ENO2 promoter directs expression at a low level. In contrast, the promoter activity of ENO1 is highly induced following the conversion of tachyzoites into resting bradyzoites. The ENO2 promoter analysis in bradyzoites showed that there are two upstream repression sites (nucleotides -1929 to -1067 and -456 to -222). Furthermore, electrophoresis mobility shift assays demonstrated the presence of DNA-binding proteins in tachyzoite and bradyzoite nuclear lysates that bound to stress response elements (STRE), heat shock-like elements (HSE) and other cis-regulatory elements in the upstream regulatory regions of ENO1 and ENO2. Mutation of the consensus AGGGG sequence, completely abolished protein binding to an oligonucleotide containing this element. This study defines the first characterization of cis-regulatory elements and putative transcription factors involved in gene regulation of the important pathogen *T.gondii*.