LEU4 and LEU9 encode alpha-isopropylmalate synthase, the enzyme that catalyzes the conversion of 2-keto-isovalerate into alpha-isopropylmalate. This reaction is the first step in leucine biosynthesis. Leu4p and Leu9p are 83% identical; Leu4p is the major isozyme, accounting for about 80% of total alpha-isopropylmalate synthase activity in wild-type cells.Like the other genes in the leucine biosynthesis pathway, LEU4 is transcriptionally regulated in the presence of isopropylmalate, a process mediated by binding of the transcriptional regulator Leu3p to the upstream activation site UASLEU. LEU4 expression is also upregulated through general amino acid control via two Gcn4p binding sites in the LEU4 promoter. The LEU4 promoter also contains a Bas Response Element, the binding site for the global transcription factor Pho2p; LEU4 gene expression decreases upon removal of the BRE or under increased concentrations of inorganic phosphate. Translation of the LEU4 transcript from both the first and second in-frame AUG codons results in two alternate gene products, a full-length and an N-terminally shortened form, both of which function as dimers. The full-length form of Leu4p contains a mitochondrial matrix targeting sequence at its N-terminus while the shorter form lacks this mitochondrial import sequence and thus remains in the cytoplasm. The majority of alpha-isopropylmalate synthesis occurs in the mitochondria, however, and it remains unknown why alpha-isopropylmalate synthase is also necessary in the cytoplasm. It has been speculated that this may be due to the loss of mitochondrial function and inaccessibility of the mitochondrially located enzymes during anaerobic growth. Leu4p enzyme activity is inactivated by the small molecules leucine and coenzyme A. Regulation by leucine is via feedback inhibition and it has been shown that amino acid Asp578 is key to this process. Inactivation by CoA is more complicated as it depends on Zn2+ as well as binding of CoA to two sites in Leu4p. In the absence of zinc, CoA binds to the substrate/product site and acts as a competitive inhibitor with acetyl-CoA. In the presence of zinc, a second CoA binding site opens up and binding of CoA to this second site causes rapid inactivation of Leu4p. Although leu4 deletion strains are still able to grow in the absence of leucine due to redundant Leu9p activity, cells lacking Leu4p are sensitive to the leucine analog trifluoroleucine and are impaired for growth on non-fermentable carbon sources. LEU4 dominant mutants are resistant to 5,5,5-trifluoro-DL-leucine, insensitive to feedback inhibition by leucine, and produce increased levels of isoamyl alcohol.