ARG5,6 encodes the mitochondrial matrix enzymes acetylglutamate kinase and N-acetyl-gamma-glutamyl-phosphate reductase. They catalyze the second and third steps, respectively, in the biosynthesis of ornithine, an intermediate in arginine biosynthesis. Mutations in ARG5,6 therefore cause arginine auxotrophy. Arg5,6p is synthesized as a single translation product, and is subsequently processed in the mitochondria into two physically separable enzymes. The kinase and reductase domains of the Arg5,6 polypeptide were identified by similarity to the ArgB and ArgC gene products from E. coli. The corresponding enzymes in S. pombeand Candida albicansare expressed as a single protein as in S. cerevisiae. Like other genes encoding arginine biosynthetic genes, ARG5,6 is transcriptionally repressed in the presence of arginine and is regulated by general amino acid control. Arginine-responsive transcription factors, including Arg80p, Arg81p, Arg82p, and Mcm1p, have been identifiedand their target upstream activating sequences in ARG5,6 have been characterized.