In tRNA splicing, the intron is removed from the tRNA transcript by the tRNA splicing endonuclease; the resulting half-molecules are joined by the tRNA ligase encoded by TRL1 and the splice junction 2'-phosphate is removed by the tRNA 2'-phosphotransferase encoded by TPT1. The yeast tRNA splicing endonuclease consists of four subunits, Sen2p, Sen15p, Sen34p, and Sen54p, all of which are essential for viabilityand which are largely conserved across eukaryotes and archaea. Sen34p and Sen2p show sequence similarity to each other and to the catalytic subunits in other organisms. A sen34 mutation specifically blocks 3'-splice site cleavage, while a sen2 mutation specifically blocks 5'-splice site cleavage. Thus it is thought that the endonuclease complex contains two active sites, each responsible for one of the two cleavages. The endonuclease has been assumed to be nuclear, however experimental evidence shows that in S. cerevisiae, tRNA splicing occurs in the cytoplasm, with the splicing endonuclease located on the outer surface of the mitochondrial membrane, presumably via a hydrophobic segment of Sen2p. In contrast, in human cells, the endonuclease has been shown to be localized to the nucleus.