Yeast Oxidative Stress Signalling By Cytochrome C Peroxidase

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Abstract: Cytochrome c peroxidase (CCP), a heme protein, has a detoxifying function in the mitochondria of yeast (*S. cerevisiae*). It uses electrons from the electron-transport chain to reduce H_2O_2 to H_2O. The catalytic intermediate of CCP possesses an oxyferryl heme (Fe^{IV}=O) and a protein-based radical. In the absence of donor molecules, a number of protein radicals are formed in CCP, which compared to other heme peroxidases, has a remarkably high number of tryptophans and tyrosines. These residues act as good electron donors. TEMPO (2,2,6,6-tetramethylpiperidinooxy) scavenging coupled with mass spectrometry has pinpointed a number of tyrosine and tryptophan residues in CCP that are sites of radical formation. Under oxidative stress, CCP triggers the transcription of antioxidant genes, such as catalase and superoxide dismutase, *via* activation of the transcription factor Pos9. We hypothesize that oxidized forms of CCP act as sensors within the mitochondria to relay the oxidative stress signal to Pos9, which then enters the nucleus and initiates gene expression. The ability of CCP point-mutated at surface residues to activate Pos9 in the presence of excess H_2O_2 is under investigation to identify the molecular determinants of the oxidative stress signal. Activation of Pos9 is being monitored *in vivo* using a plasmid test system comprising of ?-galactosidase, a reporter gene, under control of the GAL1 promoter, and a fusion protein comprised of the Pos9 activation and the Gal4-DNA-binding domains. Activation of the fusion protein by CCP results in ?-galactosidase turnover, which produces a coloured product. Production of the latter was investigated in a wild-type yeast strain and a genetically identical strain in which the CCP gene is deleted. The results obtained so far for yeast exposed to H_2O_2 and paraquat will be presented. Research funded by NSERC.