Copper is a necessary cofactor for several enzymes in yeast, including the iron transporter component Fet3p, superoxide dismutase Sod1p, and the mitochondrial protein Cox17p; however, copper is toxic in high concentrations, so its import must be regulated. S. cerevisiae can acquire copper through the high affinity transporters Ctr1p and Ctr3p, the low-affinity transporter Fet4p, or by mobilizing vacuolar copper via Ctr2p.CTR1 encodes a high-affinity copper transporter responsible for copper uptake in low environmental copper. Ctr1p is an O-glycosylated proteinthat binds four copperionsand forms complexes with two or three copies at the plasma membrane.The CTR3 open reading frame encodes a second high-affinity coppertransporter, but the promoter is interrupted by insertion of a Ty2 element in the widely used laboratory strain S288C. In strains where the Ty2 element is missing, such as several clinical isolates, CTR3 is functional and, when CTR1 is deleted, restores copper and iron uptake and respiration. Ctr3p forms a homotrimer on the plasma membrane.A third copper transporter, Ctr2p, assembles as a homomultimer on the vacuolar membrane and can export stored copper to the cytoplasm. CTR2 is not essential, not even in a ctr1 ctr3 background, and ctr2 mutants accumulate excess copper in the vacuole and are resistant to toxic high levels of copper. A randomly-generated mutant Ctr2p that localizes to the plasma membrane is capable of restoring copper import into a ctr1 ctr3 mutant. CTR2 and CTR3 are homologous to each other but not to CTR1.CTR1 and CTR3 transcription is activated in response to low copper levels and suppressed in response to high copper levels by the transcription factor Mac1p. Mac1p binds a copper-responsive elementin the CTR1 and CTR3 promoters. Mac1p requires copper to bind CuRE, but exogenously-added copper disrupts binding. Ctr2 is not copper-regulated and is not induced in ctr1 ctr3 mutants.