Abstract

Mammalian inhibitor of apoptosis (IAP) proteins were identified as homologs of a baculovirus IAP and were originally thought to function as direct inhibitors of death-inducing proteases known as caspases. More recent work has demonstrated that, in fact, most IAPs are incapable of caspase inhibition, and many IAPs perform essential cellular functions unrelated to apoptosis. IAPs have been shown to play important roles in such diverse cellular processes as receptor-mediated signaling, cytokinesis, innate immunity, and copper metabolism.

Recently, X-linked IAP (XIAP) was identified as a copper-binding protein that plays a regulatory role in copper homeostasis. Copper is an essential transition metal whose ability to exchange electrons is harnessed by many intracellular and extracellular copper-dependent enzymes to facilitate oxidation-reduction reactions in such processes as peptide amidation, mitochondrial respiration, and dismutation of superoxide. However, this ability of copper to participate in redox reactions makes it highly toxic because it can generate reactive oxygen species and directly oxidize proteins and DNA. For this reason, an elaborate system of transporters and chaperone proteins has evolved to deliver copper to copper-dependent proteins while not allowing free copper to accumulate and damage the cell.

Given the excess copper-buffering capacity of the cytosolic environment, we hypothesized that a copper chaperone might be required to deliver copper to XIAP. As

copper trafficking pathways are highly conserved evolutionarily, we performed a targeted genetic screen in *Saccharomyces cerevisiae* to identify candidate proteins involved in delivering copper to XIAP. Through this genetic screen, we identified the copper chaperone for superoxide dismutase (CCS) as a mediator of copper delivery to XIAP in both yeast and mammalian cells. We also found that XIAP and CCS physically interact in human cells, and that XIAP induced ubiquitination of CCS. Interestingly, XIAP-mediated ubiquitination of CCS did not seem to target CCS for proteasomal degradation. Instead, we found that ubiquitination of CCS by XIAP was an activating event, enhancing the ability of CCS to deliver copper to its physiologic target copper/zinc superoxide dismutase (SOD1). Collectively, our results provide valuable insights into mechanisms of regulation of intracellular copper homeostasis and redox metabolism through the XIAP-CCS complex.