Acquisition, delivery and incorporation of metals into their respective metalloproteins are important cellular processes. These processes are tightly controlled for preventing that cells are exposed to free metal concentrations that would lead to harmful oxidative damages. Copper (Cu) is one such metal that is required as a cofactor in a variety of proteins. Cytochrome c oxidases (Cox) are among the metalloproteins whose assembly and activity involves the incorporation of Cu into their main catalytic subunit. In this study, we focused on the acquisition of Cu for incorporation into the heme-Cu binuclear center of the cbb3-type Cox (cbb3–Cox) in the facultative phototroph Rhodobacter capsulatus. By genetic screens, we have identified several proteins that are involved in this process and we have started to biochemically characterize the function of these proteins and their dynamic interactions:

CooA: CooA is a member of the Major facilitator superfamily and its deletion results in cbb3–Cox deficiency that can be rescued by Cu supplementation. The total Cu content in ΔΔcCoA cells is significantly reduced, suggesting a role in Cu uptake. ΔΔcCoA strains easily acquire suppressor mutations and their genetic and biochemical characterization will be presented.

CooL: CooL is Cpx-type ATPase that is specifically required for cbb3 assembly. In the absence of CooL, only a small amount of inactive cbb3 Cox is detectable. The phenotype of the ΔΔcCoL is not rescued by additional Cu and the intracellular copper content in the absence of CooL is not different to the wild type. This indicates that CooL is not required for maintaining the general copper homeostasis in R. capsulatus. This function is instead executed by two additional ATPases CopA1 and CopA2. The deletion of these genes does not interfere with cbb3 Cox assembly, but cells become hypersensitive towards Cu.

SenC: SenC is homologous to Scol of eukaryotic cells and required for cbb3–Cox assembly in R. capsulatus. It is a copper binding protein that is upregulated in the absence of CooL. SenC interacts directly with the CcoP and CcoH subunits of cbb3 Cox and it is likely that SenC is directly or indirectly involved in the assembly of the CooL center, although we did so far not observe any cross-link between SenC and the Cu-containing CcoN subunit.

A model for the copper delivery pathway for the CooL center of cbb3 Cox will be presented.


References

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