ROLE OF THE N-TERMINAL REGION OF CCC2, THE YEAST CU(I)-ATPASE, IN COPPER HANDLING AT ALKALINE PH IN EXTREME COPPER AND IRON CONDITIONS

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The link between copper (Cu) and iron (Fe) homeostasis, which is influenced by acid-base alterations, is known in different organisms including *Saccharomyces cerevisiae*. A key element in this link is Ccc2, the Cu(I)-ATPase, that activates apo-Fet3 – and promotes its addressing to the plasma membrane (PM) – upon transfer of its Cu(I). This ATPase has a long N-terminal region with two Cu(I)-binding domains (MBD1 and MBD2), where the conservated motif CXXC is found: ^{13}CSA^{16}C in MBD1 and ^{91}CCGS^{94}C in MBD2. In the present work we investigated the role of the N-terminal region in Cu(I) handling in combinations of extreme Cu^{2+} and Fe^{2+} concentrations (traces; intermediate; saturating concentrations) at acid and alkaline pH. Yeast strains lacking the CCC2 gene (ΔCCC2) were transformed to overexpress the wild type gene (wt) or the mutants D627A (non phosphorylating variant and, therefore, inactive control without ATPase activity), ΔN-ter (full length truncated N-terminal region), ΔMBD1 (without MBD1) and M1 (without MBD2). Ccc2 wt exhibits an alkaline pH resistance phenotype with 0.3 μM Cu^{2+} and 1.6 μM Fe^{2+}, while ΔMBD1 > M1 require the addition of 2 μM Cu^{2+} for a limited growth. The ^{13}C and ^{16}C residues are essential for the yeast survival at alkaline pH (there is no growth when serines substitute for both cysteines at pH 8.0; slight growth is seen at 10 μM Cu^{2+}). ΔN-ter does not phosphorylate and, in contrast with that seen with the other mutants, it is not located in the Golgi, thus revealing a twofold inactive enzyme. At a molecular level, the adaptative response of Ccc2 to alkaline pH is associated to a huge increase in ATPase activity. Finally, growth of yeast strains is reduced with different intensity (Ccc2 < M1 = ΔN-ter = M1(ss)) by DIDS (an inhibitor of the Cl^-:HCO_3^- exchanger) and by acetozolamide (a carbonic anhydrase inhibitor that decreases HCO_3^- supply). It is concluded: a) Ccc2 participates in the cellular response to acid-base alterations; b) the MBDs participate with different selectivity; c) the response of Ccc2 depends on Cl^- availability at the Golgi lumen, which is coupled to Cl^-:HCO_3^- exchange at the PM level.