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Bioinformatics: Bits and Bytes

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Cytochrome *c* oxidase assembly factors with a thioredoxin fold are conserved among prokaryotes and eukaryotes

Abstract Cytochrome *c* oxidase (COX) is a multi-subunit terminal oxidase of the eukaryotic respiratory chain involved in the reduction of oxygen to water. Numerous lines of evidence suggest that the assembly of COX is a multi-step, assisted process that depends on several assembly factors with largely unknown functions. Sco1/2 proteins have been isolated as high-copy number suppressors of a deletion of copper chaperone Cox17, implicating Sco1/2 in copper transport to COX subunits I or II. Here I report the similarity of Sco1/2 assembly factors to peroxiredoxins and thiol:disulfide oxidoreductases with a thioredoxin fold, suggesting that Sco-related proteins perform a catalytic rather than a copper transport function. Reported sequence similarities, together with the functional role of bacterial Sco-related proteins suggest that Sco-related proteins represent a new class of membrane-anchored thiol:disulfide oxidoreductases involved in COX maturation.

Key words Sco · Cytochrome *c* oxidase · Peroxiredoxins · Thioredoxin fold · Cox17

Abbreviations *COX*: Cytochrome *c* oxidase

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Cytochrome *c* oxidase (COX) is the terminal oxidase of the respiratory chain catalyzing the reduction of molecular oxygen to water with concomitant oxidation of cytochrome *c*. COX is a multi-subunit complex localized in the inner membrane of mitochondria or aerobic bacteria. Subunits I–III are conserved between prokaryotes and eukaryotes, and at least two of them (I and II) are required for catalytic activity [1]. Additional eukaryotic subunits may be involved in the modulation of activity, assembly, or tissue specific regulation of catalytic functions. Despite the considerable progress in elucidating the structural organization of COX [1] little is known about mechanisms of assembly of this large enzymatic complex. The presence of free subunits and partially assembled complexes suggests that COX assembly is a slow, sequential, and possibly assisted process [2, 3, 4]. More than 30 complementation groups deficient in COX activity have been identified in yeast. Most of these do not associate with known COX enzymatic activities, suggesting that assembly of mitochondrial complexes is dependent on a number of accessory proteins [5, 6]. Assembly factors may be responsible for the transport and insertion of heme (Cox10p, Cox11p), copper (Cox17p), or magnesium (Cox11p) into inactive enzymes, intermembrane subunit transport, and folding of COX subunits [7, 8, 9, 10, 11, 12].

Sco1p and the related Sco2p were originally identified as proteins required for COX assembly. Also, they are capable of partial suppressing COX17 deletion at elevated concentrations of copper [7, 8, 9, 10, 11]. Cox17p is an 8-kDa protein containing a highly labile binuclear Cu (I)-thiolate cluster, presumably involved in copper delivery to COX II subunit [4, 7]. Deletion of SCO1, but not SCO2, in yeast results in rapid degradation of both catalytic subunits of COX. The presence of CXXXC motif, similar to the CoxII copper binding site, led to the hypothesis that Sco1p is also directly involved in copper transport [10, 12]. Substitution of either of these cysteine residues results in the loss of active COX in yeast [10], demonstrating the importance of these residues. However, the requirement for elevated concentrations of copper suggests possible alternative functions of Sco1 and Sco2 in the assembly of COX.

PSI-BLAST [13] searches ($E<0.001$) against a nonredundant protein database revealed a significant similarity of Sco1p and Sco2p to several peroxiredoxines with known three-dimensional structures and bacterial

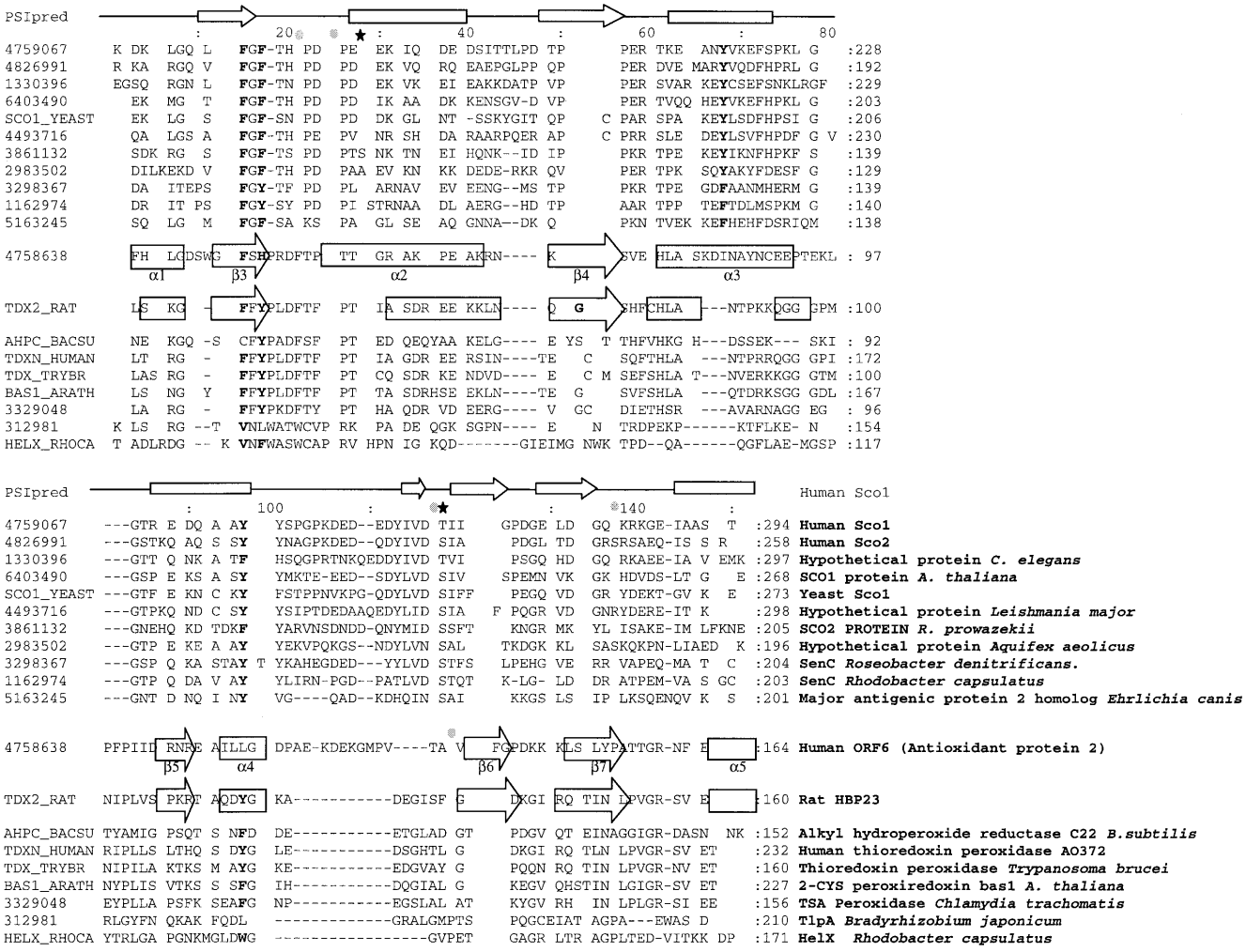

Fig. 1 Multiple alignment of Sco1-related proteins. A PSI BLAST search (E-value=0.001) of NCBI nonredundant protein database with the C-terminal portion of *Saccharomyces cerevisae* Sco1p revealed similarity to peroxiredoxins after the second iteration and bacterial thiol:disulfide oxidoreductases after the third iteration. Representative members of these groups were aligned using MULTALIN [14] with the following settings: symbol comparison table – BLOSUM 62, gap penalty 8, gap penalty at extension 0.05. Aligned sequences were prepared for publication using GenDoc [31]. Conserved amino acids were colored according to the following scheme: *dark blue* hydrophobic residues (ACFGHIKLMVWY); *light blue* aliphatic residues (ILV); *gray* aromatic residues (FHWY); *red* positively charged residues (KRH); *purple* DENQ; *green* polar (CDEHKNQRST); *yellow* small (ACDGNPSTV). *Left column* SwissProt protein names or GenBank identifier codes; *right column* amino acid positions are indicated for each protein; *on the top of the alignment* amino acid positions of aligned proteins; *above the alignment with open rectangles* secondary structure predicted with PSIpred [16], α-helixes; *open arrows* β-strands. Secondary structure of human AOP2 (ORF6) and rat HBP23 is assigned from respective crystal structures and is shown as following: *black rectangles* α-helices; *black arrows* β-strands. The annotation of secondary structure elements is as in crystal structure of ORF6 [17, 18]. *Orange circles* residues of the putative active center and Arg-119 of the ORF6 active center; *black stars above the alignment* amino acid residues in the proposed Sco active center that are mutated in patients with infantile cardioencephalomyopathy [23]

thiol:disulfide oxidoreductases (Fig. 1). Although overall similarity is low, several regions in Sco1-related proteins and peroxiredoxin/thiol:disulfide oxidoreductase are conserved, including cysteine residues and hydrophobic residues in the active center (Fig. 1, residues 10–30). Representative proteins were aligned with MULTALIN [14]. The secondary structure predictions of yeast Sco1p using PHD and PSIpred algorithms [15, 16] are very similar to that of peroxiredoxins (hORF6 and rat HBP23 in Fig. 1) determined by X-ray crystallography [17, 18]. The conserved regions include β-strand 3, α-helix 2, and a loop between them encompassing active center, β-strand 4, and hydrophobic residues of β-strand 6.

Peroxiredoxins and thiol:disulfide oxidoreductases reduce a wide range of substrates, including hydrogen peroxide, various organic peroxides, and disulfide bonds in proteins and low molecular weight compounds. The mechanism of substrate reduction involves an initial ionization of a cysteine residue in the active center (Fig. 1, Cys-25), followed by a nucleophilic attack of a substrate and formation of either mixed disulfide bonds or sulfenic acid [17, 19, 20]. The reduction of a mixed disulfide bond intermediate involves an attack by the second cysteine residue in the active center leading to the release

of a reduced substrate and formation of intramolecular (thiol:disulfide oxidoreductase) or intermolecular (2-Cys peroxiredoxins) disulfide bond. Regeneration of the catalytically active reduced form of an enzyme probably involves a thioredoxin/thyoredoxin reductase system. In peroxiredoxins conserved Arg-119 (Fig. 1) abstracts a proton from the catalytic sulfhydryl group [17]. In hORF6 His-17 stabilizes the ionized sulfhydryl group. This position is occupied by an aromatic residue in most analyzed peroxiredoxins and Sco-related proteins, suggesting an alternative mechanism of thiolate intermediate stabilization [18]. In HBP23 interactions between Arg-119, Glu-28, and Asp-137 coordinating a putative Cl anion in a close proximity to catalytic cysteine are implied in the activation of Cys-25 and stabilization of a reactive thiolate intermediate [18]. Although a position equivalent to Arg-119 is not conserved among Sco-related proteins, a conserved histidine residue located nearby (Fig. 1, His-117) may be involved in the activation of a sulfhydryl group in a predicted active center. Activation of the catalytic sufhydryl group by a histidine has been previously described for papain and *Yersinia* PTPase [21, 22]. Glu-28 and Asp-137 are conserved in almost all analyzed Sco-related proteins. Recent findings have implicated the substitution of Glu-28 to lysine in human Sco2 as a cause of fatal infantile cardioencephalomyopathy [23]. A second patient with mutated Sco2 in this study had Ser-118 substituted by phenylalanine. Similarly to yeast with deleted Sco1, both patients displayed marked reduction in COX activity in heart and muscles and reduction in content of mtDNA-encoded COXI and COXII subunits [23]. Thus mutations at or near residues in the predicted Sco active center detrimentally affect Sco2 function and cytochrome *c* oxidase assembly in human.

Several bacterial thiol:disulfide oxydoreductase, including *Rhodobacter capsulatus* helX, *Bradyrhizobium japonicum*u cycX, and *Bradyrhizobium japonicum* TlpA (Fig. 1), have been implicated in cytochrome *c* and COX biogenesis, presumably maintaining cysteine residues of the apocytochrome *c* heme-attachment site in reduced state [24, 25, 26]. Since cytochrome *c* biogenesis is not affected in *sco1* or *sco2* mutants, the primary target of Sco must be elsewhere. The ability of Sco1 to suppress cox17 deficiency of copper transport suggests a possible function downstream in the COX assembly pathway, probably on the step concomitant or immediately preceding copper insertion. Physical interactions of Cox17p and Sco1/2p were recently demonstrated in a high throughoutput two-hybrid screening for interacting yeast proteins [27]. Although spontaneous incorporation of copper into CoxII CuA center is possible, it requires high concentrations of copper and rather nonphysiological pH [28, 29, 30]. The formation of Cox17p/Sco1/2p could facilitate a coordinated reduction of cysteine residues in the CoxII metal-binding center with copper insertion. Therefore, when the delivery of copper to copper-binding centers of Cox II is impaired due to Cox17p deficiency, overexpressed Sco1p increases the probability of either spontaneous copper incorporation or utilization of alternative copper transporters. The ubiquity of Sco-related proteins and suggests that the mechanism of COX assembly is well-conserved between kingdoms.

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