

System I

As thoroughly explained elsewhere [1], the cytochrome *c* maturation System I apparatus comprises a variety of proteins which are finely orchestrated to perform the seemingly simple task of cytochrome *c* assembly. Nonetheless, not all protein components can be used as reliable computation biomarkers towards the identification of this maturation system. CcmA and CcmB both belong to the ATP-Binding Cassette (ABC) transporters superfamily, which exhibits high sequence conservation within its members. Most notably, their ATP-binding domains include two short motifs (Walker motifs) associated with many nucleotide-binding proteins [2] and, thus any annotation attempt would result in several non-specific hits. CcmG is a specific thiol-oxidoreductase but, as such, it adopts a *trx*-like fold which classifies it as a thioredoxin [3] and makes it completely inappropriate as a signature gene, considering the ubiquitous nature of these proteins in all living cells. CcmH from *E.coli* is, in most organisms, divided into two proteins, CcmH and CcmI, with the former being a thiol-oxidoreductase albeit with an unusual structural fold [4]. Although inability to detect CcmH during an annotation project does not necessarily exclude System I from being the dedicated cytochrome *c* maturation pathway of the organism [5], identification of CcmH can offer supportive indication towards that conclusion. CcmI, on the other hand, contains TPR repeat domains and, therefore cannot be used as a biomarker [1]. Detection of the system-specific heme-handling genes in the genome of an organism can offer strong evidence for the presence of cytochrome *c* maturation System I. CcmE, a heme chaperone, represents a novel class of covalently heme-bound proteins [6] and contains a highly conserved motif (C/H)XXX_Y. CcmC and CcmF, like CcsA in System II, contain a typical tryptophan-rich WWD domain, flanked by conserved histidine residues [7]. CcmD, at last, is not the most suitable biomarker due to its small size and poor sequence conservation, although it can

further validate the possible presence of CcmC, since it is always downstream of the latter and adopts a well preserved domain structure [8].

System II

In silico identification of cytochrome *c* maturation System II proceeds in a heuristic manner that is based on two indispensable Ccs proteins (CcsA and CcsB) that form a tight complex and perform most of the tasks required for cytochrome *c* holoform assembly. Although in some organisms a fused CcsBA is the dedicated cytochrome *c* synthetase [9], in most cases two separate gene products are identified. CcsA exhibits high sequence conservation and, in resemblance with CcmC and CcmF from System I, contains a WWD domain and flanking histidines [1]. CcsB, which usually proceeds or follows CcsA in the genome, is poorly conserved at the sequence level, even though its conserved secondary structure could be of help. The fused CcsBA also exhibits highly conserved features [10].

System III

Cytochrome *c* maturation System III has so far been detected only in eukaryotes and seems to have the simplest protein composition of all. The prototypical cytochrome *c* heme lyase (CCHL) together with a related type of it (CC₁HL; responsible for cytochrome *c*₁ maturation in some organisms) [11] are the only defining components of this system described up to now [12]. Together with these, a dedicated flavoprotein (Cyc2p), suggested to be involved in the redox pathway of System III cytochrome *c* assembly in fungi [13] and the human cytochrome *c* heme lyase (HCCS) were also included in our dataset.

References

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