

Supplementary information

Cooperation between two periplasmic copper chaperones is required for full activity of the *cbb*₃-type cytochrome *c* oxidase and copper homeostasis in *Rhodobacter capsulatus*

Petru-Iulian Trasnea^{a,b}, Marcel Utz^a, Bahia Khalfaoui-Hassani^c, Simon Lagies^a, Fevzi Daldal^c
and Hans-Georg Koch^{a,*}

^aInstitut für Biochemie und Molekularbiologie, ZBMZ, Stefan-Meier-Strasse 17 and ^bFakultät für Biologie; Albert-Ludwigs-Universität Freiburg, 79104 Freiburg, Germany; ^cDepartment of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.

Running title: PccA-SenC interactions determine *cbb*₃-Cox biogenesis

Key words: *cbb*₃-type cytochrome *c* oxidase biogenesis, periplasmic copper chaperones, copper homeostasis, respiration, *Rhodobacter capsulatus*

*Corresponding author: Phone 0049-761-203-5250

Fax 0049-761-203-5289

Email: Hans-Georg.Koch@biochemie.uni-freiburg.de

Supplementary Table 1: Strains and plasmids used in this study

Strain or plasmid	Description	Phenotype	Reference
<i>Escherichia coli</i>			
DH5 α			(Hanahan, 1983)
HB101	F ⁻ $\Delta(gpt-proA)62 leuB6 supE44 ara-14 galK2 lacY 1 \Delta(mcrC-mrr) rpsL20$ (Str ^R) <i>xyl-5 mtl-1recA13</i>	Str ^r	(Sambrook & Russel, 2001)
BL21(DE3)			Stratagene Inc.
<i>Rhodobacter capsulatus</i>			
MT1131		Wild-type, NAD ^I ⁺	(Daldal <i>et al.</i> , 1986)
SB1003		Wild-type NAD ^I ⁺	(Yen <i>et al.</i> , 1976)
GK32	$\Delta ccoNO::kan$	NAD ^I ⁻	(Koch <i>et al.</i> , 1998)
Y262		GTA overproducer	(Yen <i>et al.</i> , 1979)
CW1	$\Delta ccoGHIS::spe$	NAD ^I ⁻	(Koch <i>et al.</i> , 2000)
LS01	$\Delta senC$	NAD ^I ⁻	(Swem <i>et al.</i> , 2005)
IT1	$\Delta PccA::kan$	Km ^r	This work
IT10	$\Delta senC \Delta(PccA::kan)$	Km ^r	This work
<i>plasmids</i>			
pRK2013	Conjugation helper plasmid	Km ^r	(Ditta <i>et al.</i> , 1985)
pRK415	Broad host-range vector	Tet ^r	(Ditta <i>et al.</i> , 1985)
pBluescript II KS+	Cloning vector	Amp ^r	Stratagene Inc.
pBS_PccA	PccA cloned into SacI and KpnI sites of pBlueScript	Amp ^r	This work
pRK415_PccA	PccA cloned into SacI and KpnI sites of pRK415	Tet ^r	This work
pBS_ΔPccA	2,2 SacI-KpnI fragment carrying the $\Delta(PccA::kan)$ allele on pBlueScript	Amp ^r	This work
pRK415_ΔPccA	2,2 SacI-KpnI fragment carrying the $\Delta(PccA::kan)$ allele on pRK415	Tet ^r	This work
pET22b_PccA Strep	PccA cloned in pET22b for in vivo overexpression	Amp ^r	This work
pET22b_PccA StrepΔM	PccA cloned in pET22b with the mutated copper binding motif, for in vivo overexpression	Amp ^r	This work
pTFD342	<i>rnfA</i> '160:: <i>phoA</i> '	Tet ^r	(Kumagai <i>et al.</i> , 1997)
pBK86	CcoAH249:: <i>PhoA</i> in pTFD342	Tet ^r	This work
pBK88	CcoAR281:: <i>PhoA</i> in pTFD342	Tet ^r	This work

Supplementary Table 2. Primers used in this work

Primer name	Sequence 5'- 3'	Description	
PccA sacI for	ccagcttgaagagctcgatcaccacatc	Deletion of PccA with the downstream and upstream regions from MT1131	
PccA KpnI rev	gcaacatctatgggtaccgcaaccag		
pB_circ_upII	ttctctcatatgatcgcgcc	Deletion of PccA from pBS-PccA plasmid	
pB_circ_downIII	gaacctggctcctcgagcatg		
pKD_kan_for	ctactggcatatgtggaaa	Amplification of Kanamycin cassette from pKD4 plasmid	
pKD_kan_rev	aagctcgagctttcatagaa		
PccA strep For	cagttcgaaaagtgagctgctaacaaagcccg	Strep tag addition for <i>in vivo</i> expression	
PccA strep Rev	cgggtggctccactcgaggggggtcatctt		
PccAM76C Mut1_for	gcgatggcgtgatgcattcgagc	Insertion of four cysteine residues at the PccA positions 76, 93, 109 and 117	
PccAM76C Mut1_rev	ggcgaaatcgacctgaccgc		
PccAV93C Mut2_for	accgggatgatcgattcgggcgcg		
PccAV93C Mut2_rev	cacgcccgggcatcggt		
PccAG109C Mut3_for	aaactggcgccgctgcccggctg		
PccAG109C Mut3_rev	caccgtgcccccgcccggc		
PccAG117C Mut4_for	gcgccgatcgccgaaggggtg		
PccAG117C Mut4_rev	ggtcaggcacatgaacatcac		
PccA M90L For	cccggctgatcgatgctggc		Replacement of two methionine residues from the copper binding motif of PccA
PccA M90L Rev	tcacgcccgcggcatcggt		
PccA M114L For	cgggctgcatgtgctgttcatg		
PccA M114L Rev	cccggcgccagtttccac		
CcoA-XhoI-F	cgcgccagcgcctcgagatagcgcacct	<i>ccoA::phoA</i> fusion	
CcoA-BamHI249-R	cgatgctggcggcattcggatccgtgtggcccagcccacca		
CcoA-BamHI281-R	aaaagaccaatgccgacaatgggatcccggccaaagcgggcaa		

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