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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^{2+} for incorporation into the heme-Cu binuclear center of the cbb_3-type Cox (cbb_3-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:

CcoA: CcoA is a member of the Major facilitator superfamily and its deletion results in cbb_3-Cox deficiency that can be rescued by Cu supplementation. The total Cu content in ΔccoA cells is significantly reduced, suggesting a role in Cu uptake. Δ*ccoA* strains easily acquire suppressor mutations and their genetic and biochemical characterization will be presented.

CcoI: CcoI is Cpx-type ATPase that is specifically required for cbb_3 Cox assembly. In the absence of CcoI, only a small amount of inactive cbb_3 Cox is detectable. The phenotype of the Δ*ccoI* is not rescued by additional Cu and the intracellular copper content in the absence of CcoI is not different to the wild type. This indicates that CcoI 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 cbb_3 Cox assembly, but cells become hypersensitive towards Cu.

SenC: SenC is homologous to ScoI 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 CcoI. SenC interacts directly with the CcoP and CcoH subunits of cbb_3 Cox and it is likely that SenC is directly or indirectly involved in the assembly of the Cu_B center, although we did so far not observe any cross-link between SenC and the Cu_B-containing CcoN subunit.

A model for the copper delivery pathway for the Cu_B center of cbb_3 Cox will be presented.

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7P1

Bi-plane FPALM nanoscopy confirms that mitochondrial nucleoids play a central role in mitochondrial biogenesis

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Mitochondrial (mt) DNA is compacted in nucleoprotein complexes called nucleoids [1–4]. It has recently been reported [2] that one nucleoid harbors one mtDNA molecule, whereas previous studies had suggested that each nucleoid contains several copies of mtDNA [3]. Nucleoid structure may depend on cell type and growth conditions. Further details of mitochondrial genetics, including mechanisms of transcription and replication of mtDNA, are still poorly understood. Investigation of these processes is very difficult, since mitochondria possess two hydrophobic membranes, which must be overcome with specific probes or antibodies. Recently, new findings have pointed out that nucleoids play a major role as centers of mitochondrial biogenesis.

We focused our research on mt ribosomes. Mitochondrial ribosomal protein was fused with fluorescent proteins in inducible or lentiviral vectors. We have combined these vectors with nucleoid proteins and employed super-resolution Bi-Plane FPALM (Fluorescence Photoactivation Localization Microscopy) microscopy [4]. We have also adopted a modified dSTORM (direct Stochastic Optical Reconstruction Microscopy) technique for RNA visualization using molecular beacons that hybridize to16S rRNA.

We have revealed very close interactions between mt ribosomes and nucleoids while observing mitochondrial nucleoids as centers of ribosomal clouds. Biplane FPALM has shown that each nucleoid has its own mt ribosomes in very close vicinity. There were still some free mt ribosomes observed in the mitochondrial matrix, but their amount was very low. To test whether ribosomal RNA is also adjacent to nucleoids we applied our molecular beacon system for dSTORM *in situ* hybridization and revealed some structures corresponding to nucleoid and mt ribosome clusters.

Based on our findings we hypothesize that clusters of mitochondrial ribosomes surrounding nucleoids are centers of translation. Mt ribosomes positioned in proximity to nucleoids may enhance translation of mtRNA and forestall RNA degradation. Taken together nucleoids are major sites where mitochondrial biogenesis (replication, transcription, translation, lipid metabolism) takes place.

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References

[1] B.G. Kopek, et al., Proc Natl Acad Sci U S A 109 (2012) 6136–6141.
[2] C. Kukat, et al., Proc Natl Acad Sci USA 108 (2011) 13534–13539.
[3] T.A. Brown, et al., Mol Cell Biol 31 (2011) 4994–5010.
[4] M.J. Mlodzianoski, Opt. Express. 19 (2011) 15009–15019.

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7P2

Mechanism of Cu_A assembly in the biogenesis of cytochrome *c* oxidase

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Cytochrome *c* oxidase (COX; cytochrome aa_3) is a multi-subunit enzyme of the mitochondrial respiratory chain. Biogenesis of COX is a highly complex process involving >30 chaperones in eukaryotes. The proteins Cox17, Sco1/2, and Cox11 are necessary for copper insertion into the Cu_A and Cu_B redox centers of COX. Both Sco1 and Sco2 (Synthesis of cytochrome *c* oxidase) are strictly essential in humans, with mutations in either gene resulting in a severe respiratory deficiency and decreased COX activity. The Sco family of proteins is discussed as potential copper chaperones during the assembly of the Cu_A center in subunit II of COX [1–3]. The precise functions of the Sco proteins, however, remain unclear. The soil bacterium *Paracoccus denitrificans*, a close relative of present-day mitochondria, contains two Sco homologues and two PCu_AC like proteins named ScoA/ScoB and PCu1/PCu2. The present study aims at gaining a more detailed insight into the roles of Sco and PCu proteins in *P. denitrificans* by introducing gene deletions, to test for possible defects in the formation of the Cu_A site during the COX biogenesis process.

We have constructed several deletion strains by using a double-homologous recombination approach. Activity measurements were performed in membranes prepared from deletion and complementation strains as well as with the enzymes purified from all strains with different *sco*/*pcu* deletion backgrounds. The deletions of *sco* and *pcu* show no drastic reduction of COX activity compared with wild type COX. When omitting copper from the media, the absence of *sco* and *pcu* genes has a strong effect on the function of COX. When exogenous copper amounts are low, activity measurements using membranes and purified enzyme show COX activity decreased to between 15% and 30%, respectively, in *sco* + *pcu* deletion strains. In addition we have also investigated the steady-state levels of COX subunits in these mutants under a low-copper condition by BN-PAGE (native gel electrophoresis) of solubilized membranes followed by SDS-PAGE immunoblot analysis. The steady-state levels of COX subunits were found severely reduced in mutants of Sco proteins suggesting decreased stability of the complex during the biogenesis process.

Taken together our findings show that none of the Sco/PCu proteins is important for the function of COX under normal levels of copper in the media. However mutants with *sco* deletions grown in copper-depleted media reveal a drastic effect towards COX activity.

References

[1] L.A. Abriata, et al., Mechanism of CuA assembly, Nat. Chem. Biol. 4 (10) (2008) 599–601.
[2] Audie K. Thompson, Jimmy Gray, Aimin Liu, Jonathan P. Hosler, The roles of *Rhodobacter sphaeroides* copper chaperones PCuAC and Sco (Prrc) in the assembly of the copper centers of the aa_3 and the cbb_3-type cytochrome *c* oxidases, Biochimica et Biophysica Acta 1817 (2012) 955–964.
[3] Mark Lai, C. Katherine, Diann Andrews Yam, Bruce C. Hill, Copper binding traps the folded state of the SCO protein from *Bacillus subtilis*, Biochimica et Biophysica Acta 1824 (2012) 292–302.

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7P3

Identification of the TOM complex of *Dictyostelium discoideum*

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The majority of proteins found in mitochondria are encoded in the nucleus and are synthesized in the cytosol. The efficiency of their import into mitochondria depends on a wide variety of proteins forming translocases localized in both mitochondrial membrane. One of these translocases is the TOM complex (translocase of the outer membrane). The complex is regarded as the gate into mitochondria for imported proteins as it is responsible for decoding of targeting signals, translocation of imported proteins across or into the outer membrane, and their subsequent sorting. Thus, undoubtedly the TOM complex is fundamental for mitochondrial functioning.

The subunit organization of the TOM complex has been shown to be characteristic for a given phylogenetic lineage. Beside common subunits the complex may contain subunit(s) that is(are) not present in representatives of other phylogenetic lineages. Till now the complex has been described for representatives of fungi and animals (termed *Opisthokonta* in the recent eukaryotic classification system involving six supergroups) as well as for plants (*Archaeplastida*). However, for representatives of *Amoebozoa*, *Chromalveolata*, *Excavata* and *Rhizaria* (former *Protista*) the amount of data concerning the complex is indeed small. Therefore we decided to study the TOM complex of the slime mold *Dictytiostelium discoideum* classified as a representative of *Amoebozoa* and applied as an important model for human cell biology and disease.

By expression of his-tagged form of *D. discoideum* Tom7 protein and using affinity and ion-exchange chromatography we isolated a protein complex of MW about 450 kDa that displays channel activity characteristic for the TOM complex. The complex was then analyzed by protein electrophoresis and mass spectrometry that allowed for the identification of Tom40, a channel forming subunit of the TOM complex. Thus, we proved that the isolated complex is the TOM complex and therefore it can be used for the identification of its other subunits.

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7P4

Biogenesis of alpha-helical outer membrane proteins

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Mitochondrial proteins are synthesized on cytosolic ribosomes and imported into the organelle via different proteinaceous machineries. The translocase of the outer membrane (TOM complex) forms the entry gate for the majority of precursor proteins. Subsequently, the precursors are sorted into the different subcompartments like inner membrane, intermembrane space and into the matrix. Outer membrane proteins with β-barrel structure are first transported across the TOM machinery and then inserted into the outer membrane via the sorting and assembly machinery (SAM complex). In contrast, only little is known how outer membrane proteins with α-helical membrane anchor reach their final destination. We show that different import pathways exist for protein with a-helical membrane anchor. The biogenesis of multispanning outer membrane proteins like Ugo1 requires Tom70 and Mim1. Mim1 is also required for the import of several Tom subunits with a single transmembrane span. Strikingly, the SAM machinery is a platform for the assembly of α-helical membrane-spanning Tom subunits with the central b-barrel forming Tom40. Thus, different routes exist that are used by the precursor proteins to integrate into the outer membrane of mitochondria.

References

[1] T. Becker, L.S. Wenz, V. Krüger, W. Lehmann, J.M. Müller, L. Goroncy, N. Zufall, T. Lithgow, B. Guiard, A. Chacinska, R. Wagner, C. Meisinger, N. Pfanner, The mitochondrial import protein Mim1 promotes biogenesis of multi-spanning outer membrane proteins, J. Cell. Biol. 194 (2011) 387–395.
[2] E. Schleiff, T. Becker, Common ground for protein translocation: access control for mitochondria and chloroplasts, Nat. Rev. Mol. Cell Biol. 12 (2011) 48–59.
[3] T. Becker, L.S. Wenz, N. Thornton, D. Stroud, C. Meisinger, N. Wiedemann, N. Pfanner, Biogenesis of mitochondria: dual role of Tom7 in modulating assembly of the preprotein translocase of the outer membrane, J. Mol. Biol. 405 (2011) 113–124.
[4] T. Becker, B. Guiard, N. Thornton, N. Zufall, D.A. Stroud, N. Wiedemann, N. Pfanner, Assembly of the mitochondrial protein