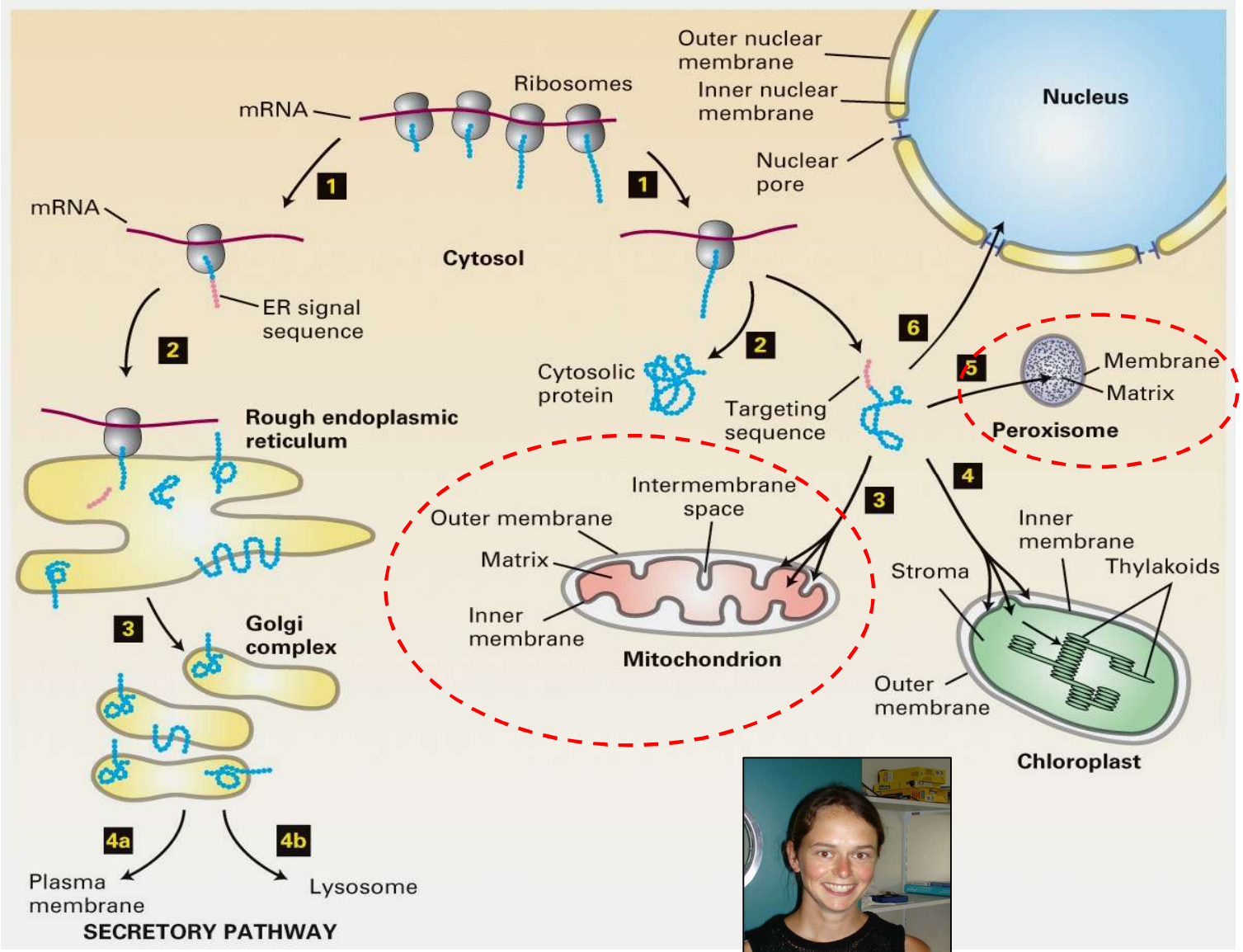
Protein Co-Import of Yeast Catalase A into Peroxisomes & Mitochondria



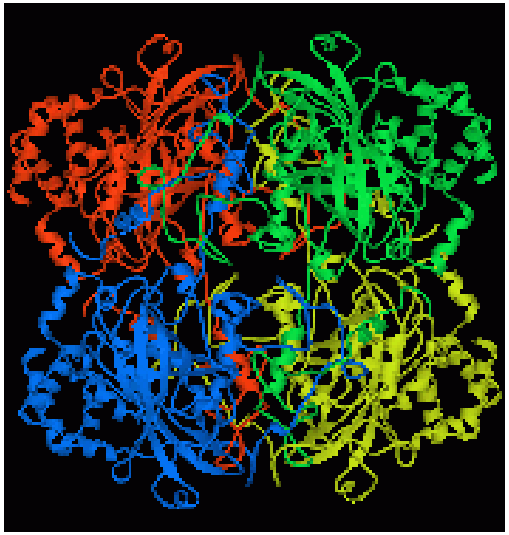
Protein Co-Import of Yeast Catalase A into Peroxisomes & Mitochondria

- **Yeast cells possess two types of catalase:**

  (1) **catalase T (cytosolic, Ctt1p)**

  (2) **catalase A (peroxisomal, Cta1p)**

- Function: $2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$



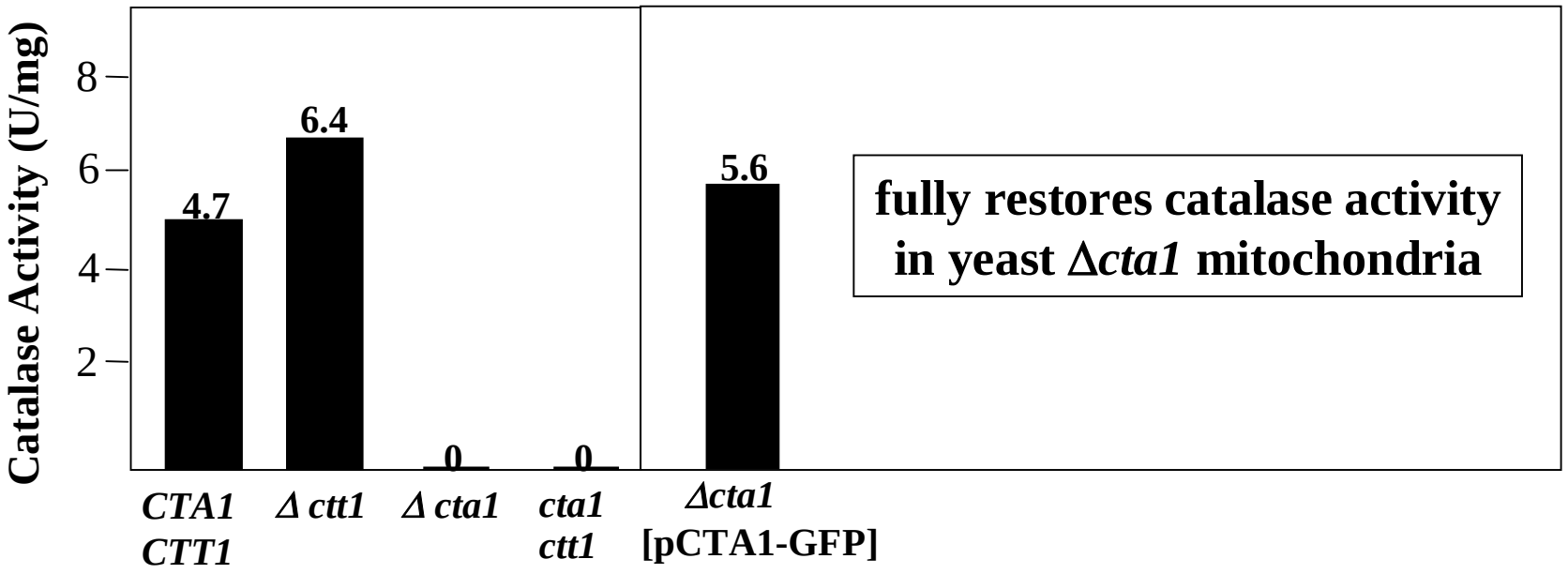
homotetrameric catalase

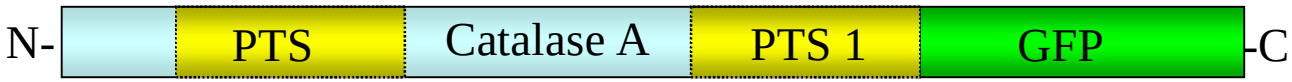
- **catalase A** contains two **P**eroxisomal-**T**argeting-**S**ignals (PTS):

  (1) **PTS1**: carboyterminal SKF motif

  (2) **internal PTS** within the N-terminal part of Cta1p

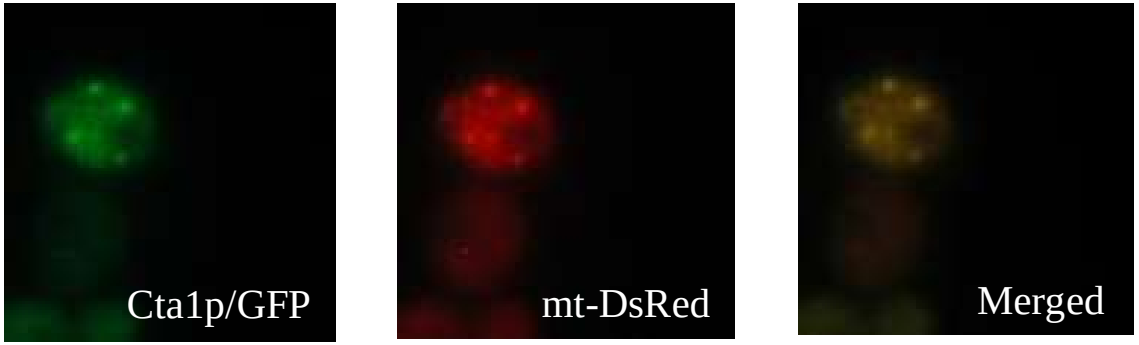
Cta1p is Responsible for Mitochondrial Catalase Activity

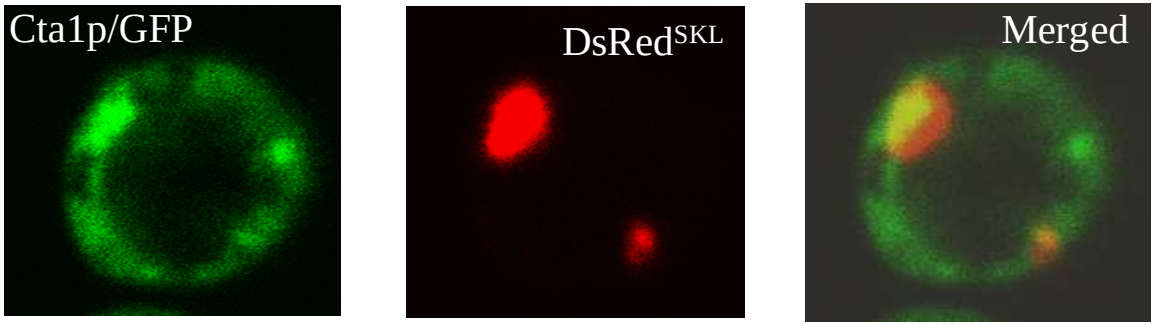




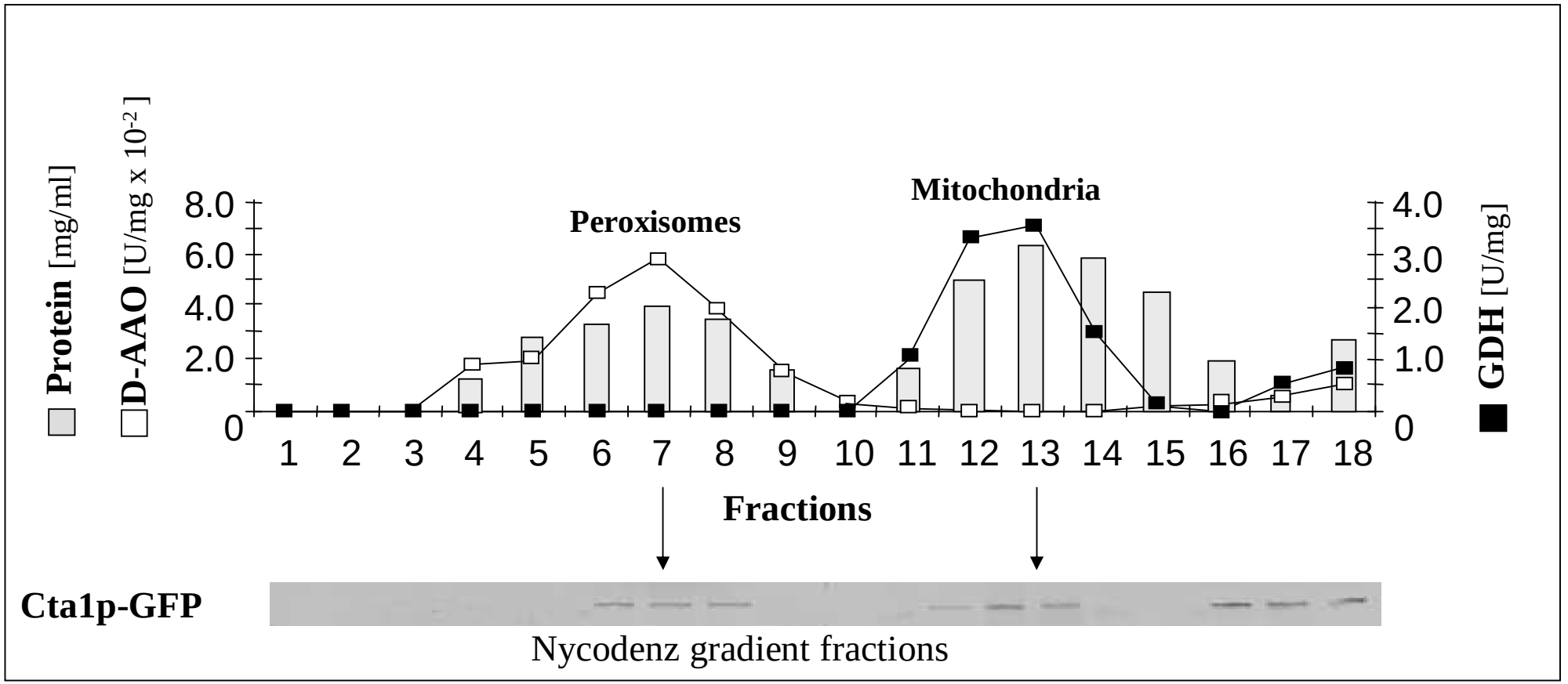
Expression of a Cta1p/GFP fusion protein in a Δ*cta1* mutant

Co-expression of Cta1p/GFP and mt-DsRed / DsRed^{SKL}



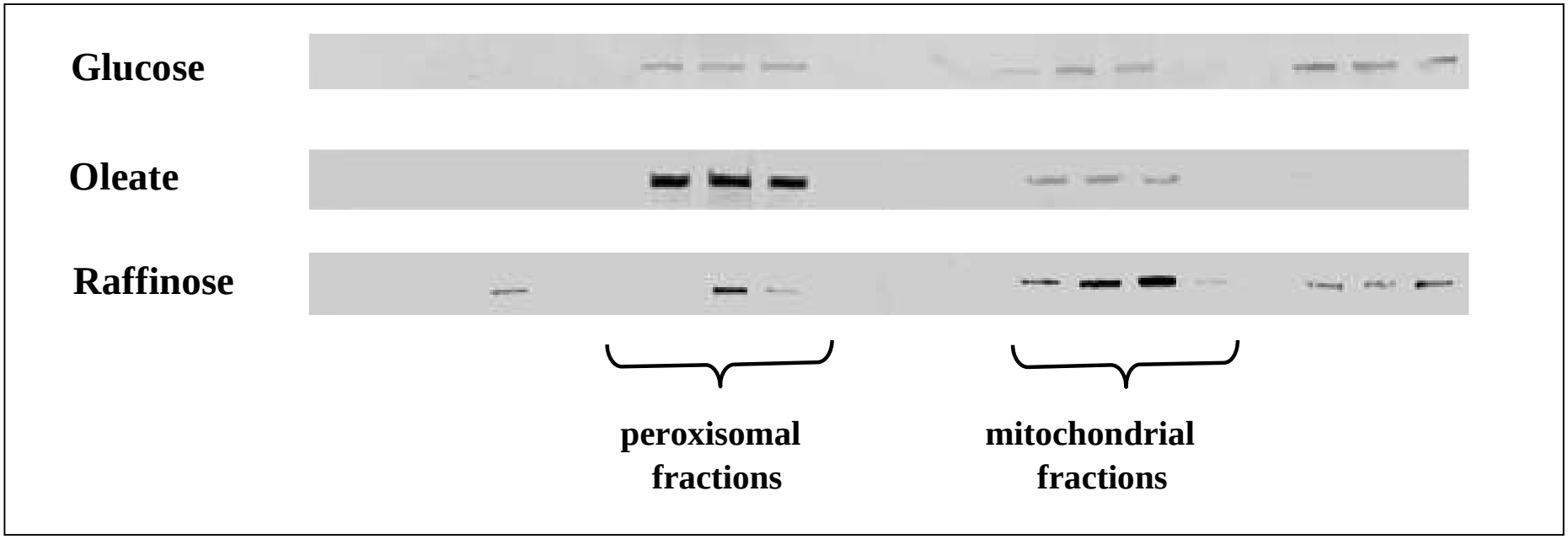


Peroxisomal & mitochondrial cotargeting of Cta1p



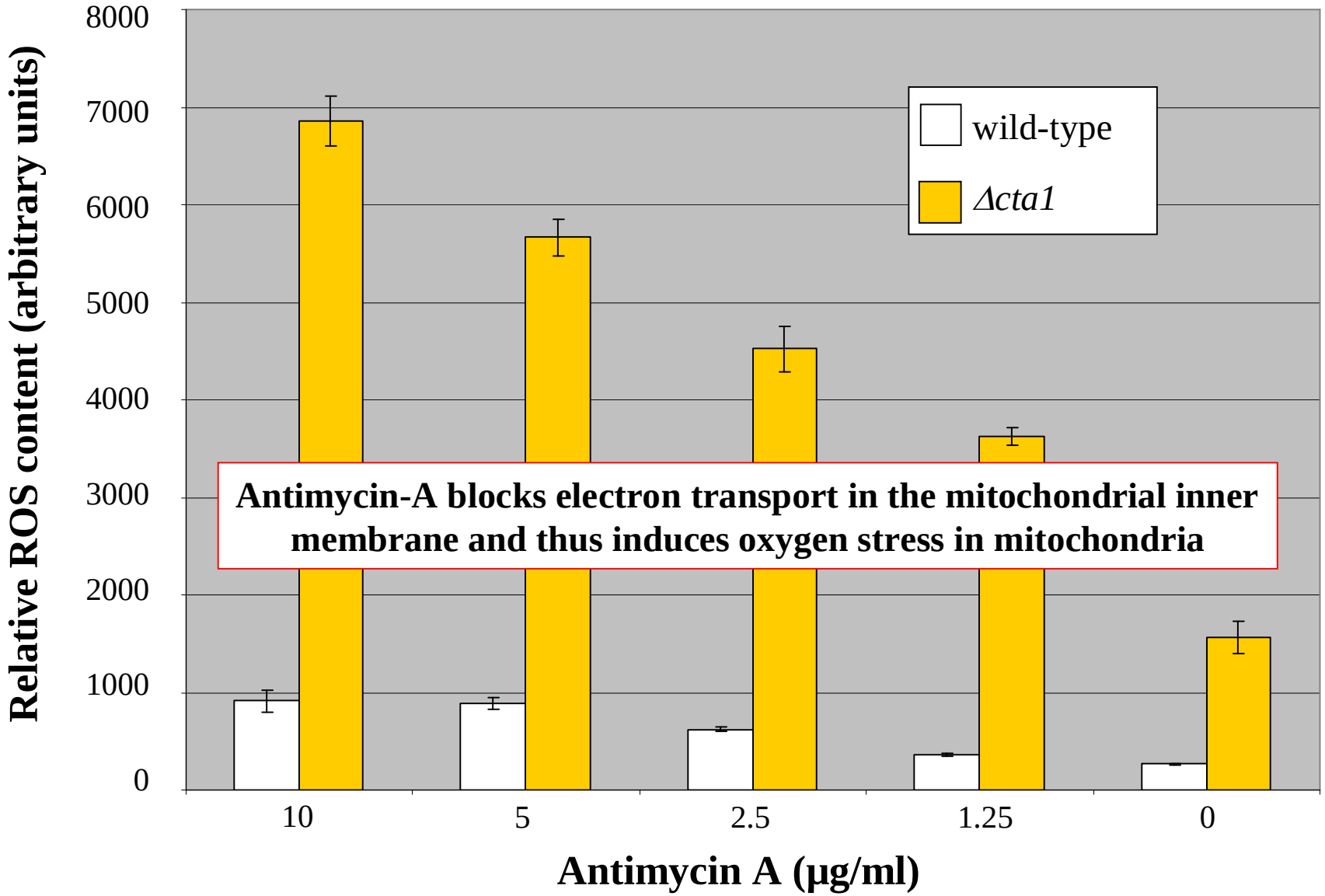
□ D-AAO (D-amino acid oxidase, peroxisomal) G■H (glutamate dehydrogenase, mitochondrial)

Cell physiology & Cta1p cotransport to different organelles



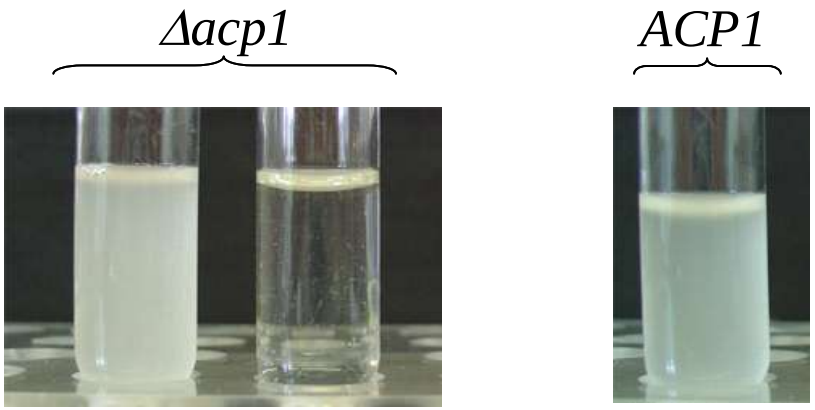
- Cta1p/GFP predominantly targets peroxisomes when cultivated on oleate
- Cta1p/GFP predominantly targets mitochondria when cultivated on raffinose

Cells lacking mitochondrial catalase accumulate reactive oxygen species (ROS)

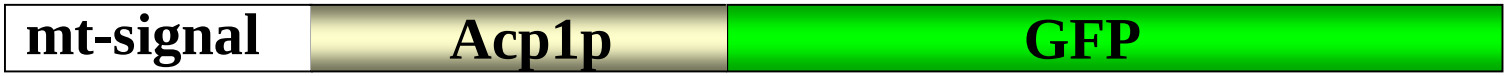


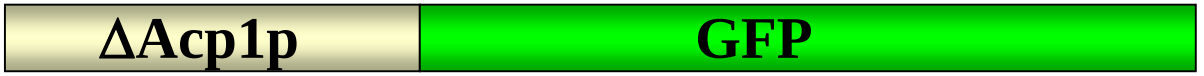
Cta1p can drive mitochondrial import of Acp1p

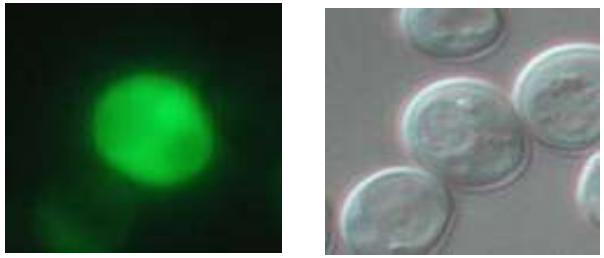
- **Acp1p** - **A**cyl **C**arrier **P**rotein **(essential yeast protein in mitochondria)**
  - component of mitochondrial fatty acid synthase
  - deletion: → reduced amount of fatty acids
    → respiratory defect, unable for growth on lactate

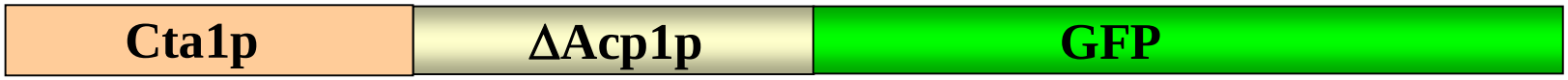


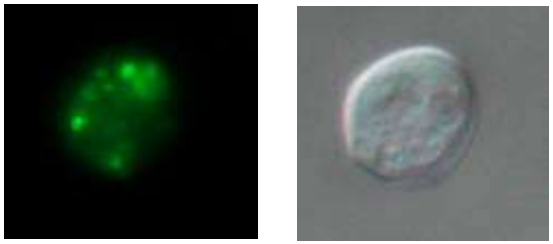
Expression of ACP variants in a yeast *Δacp1* mutant





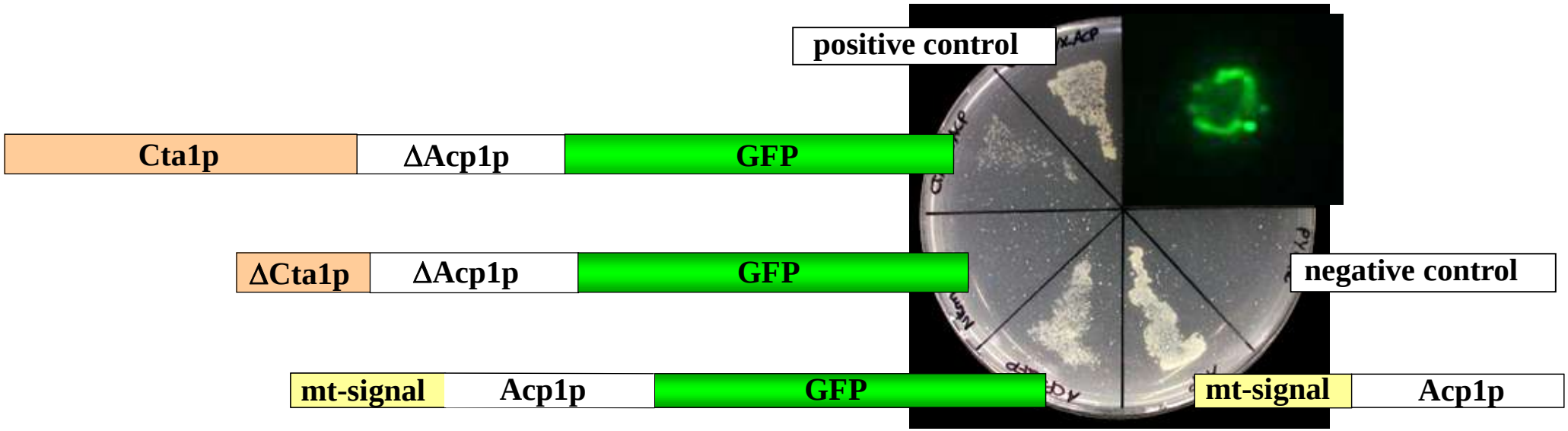




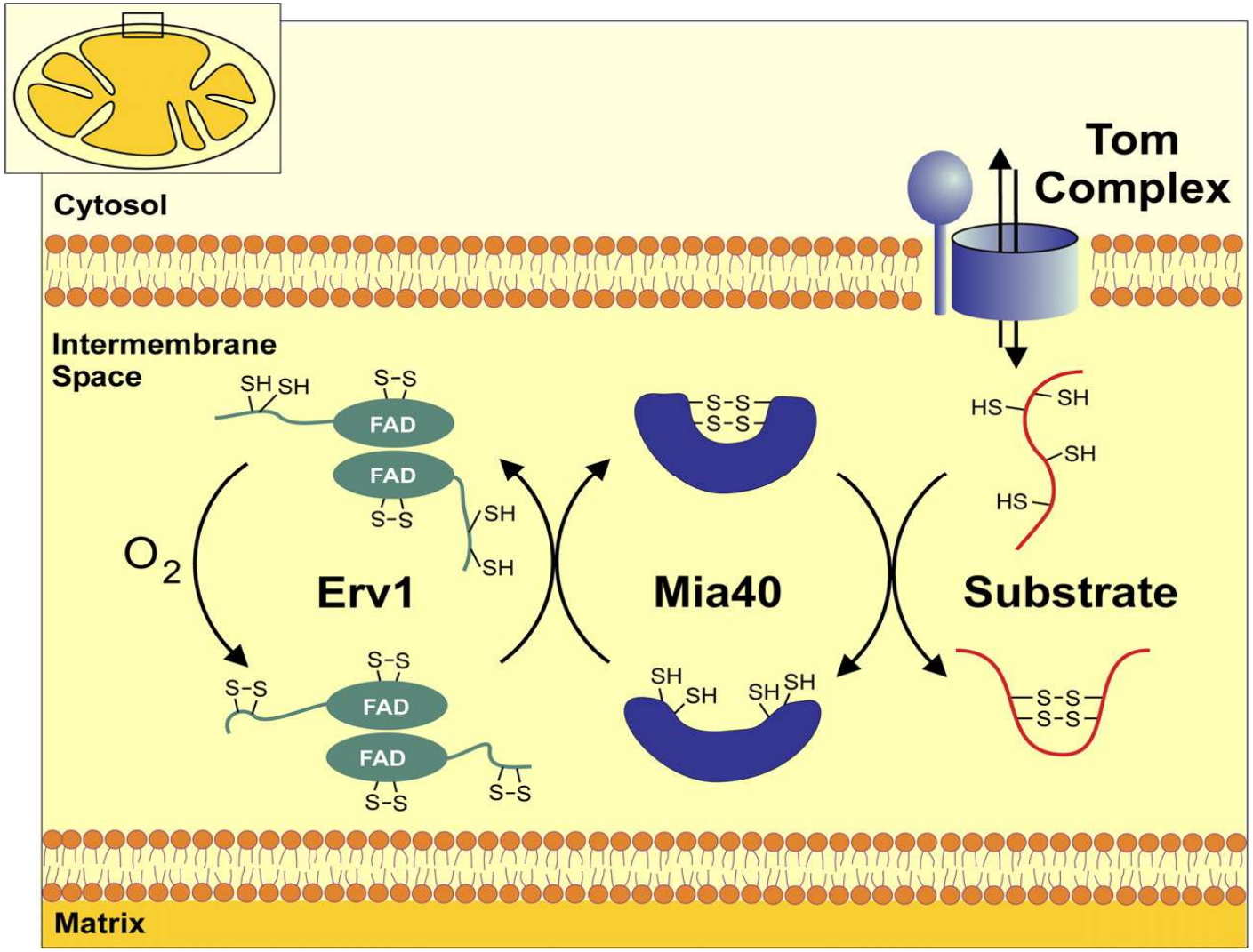


→ **Cta1p targets ΔAcp1p to mitochondria**

Cta1p contains a mitochondrial targeting signal

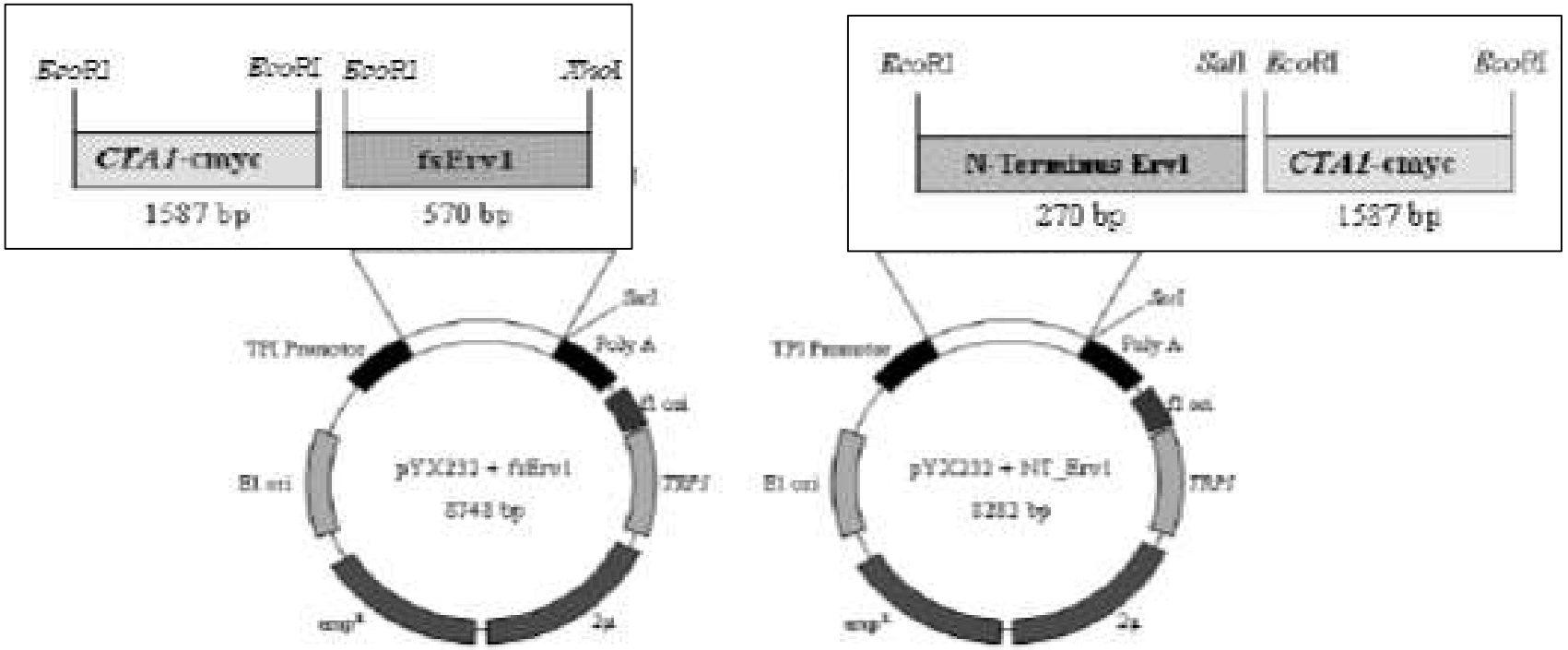


Cta1p targets ΔAcp1p to mitochondria allowing aerobic growth of a Δ*acp1* mutant on lactate

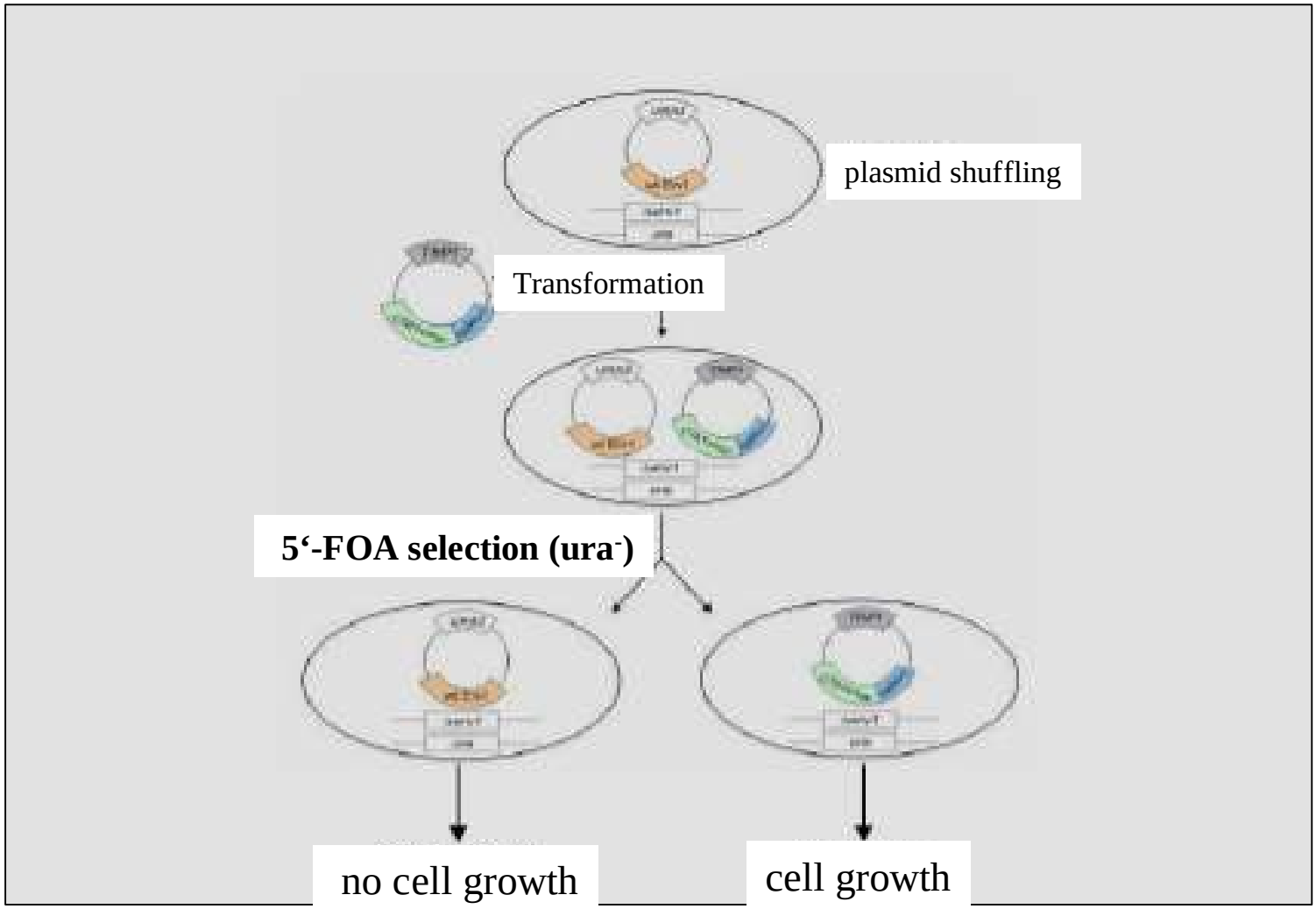


Model of the Erv1–Mia40 disulfide relay. Schematic representation of the reactions that mediate redox-driven protein import into the IMS of mitochondria. The sulfhydryl oxidase Erv1 is a dimeric FAD-binding protein that maintains an oxidized state by the use of molecular oxygen as a final electron acceptor. Erv1 directly interacts with Mia40, which functions as a redox-activated import receptor. The oxidized active state of Mia40 can interact with newly imported precursor proteins by intermolecular disulfide bonds. It has been proposed that reshuffling of the disulfide bonds releases the substrates from Mia40 in a stably folded oxidized state. Because these folded proteins cannot traverse the protein-conducting channel of the TOM complex, they remain trapped in the IMS. Alternatively, Erv1 might directly interact with some incoming substrates and pass them on to Mia40, which might function as a protein disulfide isomerase. In both cases, the Erv1–Mia40 system constitutes a folding trap that is designed to mediate the unidirectional import of proteins into the IMS of mitochondria. Reduced and oxidized thiol groups are indicated by SH and SS.

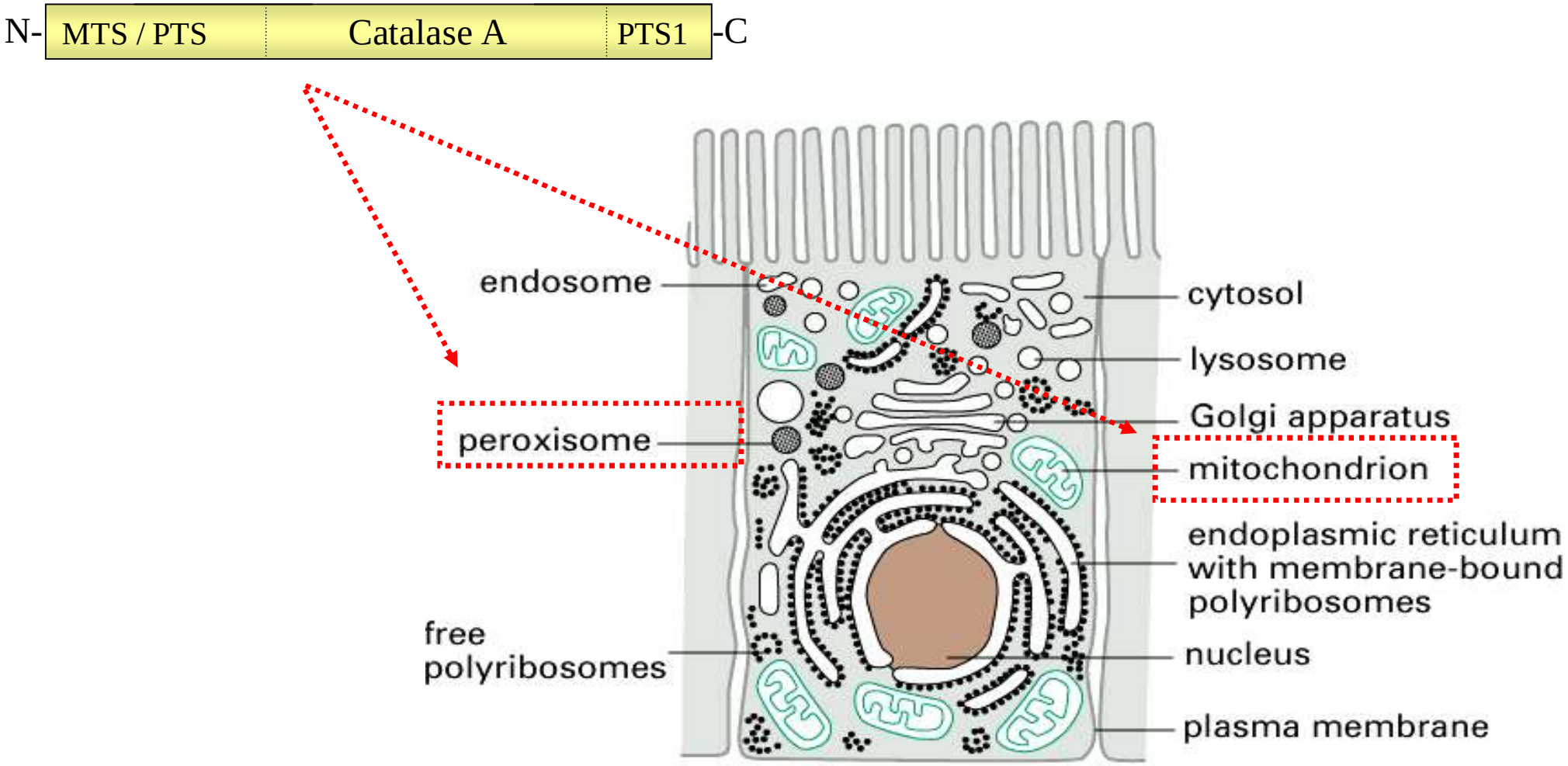
Yeast Cta1/Erv1p and Erv1/Cta1p expression constructs



Experimental strategy and set-up for Cta1/Erv1p expression



The signal for peroxisomal / mitochondrial cotargeting of catalase is localized within the N-terminal part of Cta1p



Summary & Relevance of Mitochondrial Catalase Targeting

Catalase A cotargeting to yeast peroxisomes and mitochondria depends on the physiological status of the cell:

- → **Cta1p targets peroxisomes during growth on oleate**
- → **Cta1p targets mitochondria under aerobic growth conditions** (raffinose)

Mitochondrial catalase level is significantly increased under oxygen stress (antimycin A)

- → **Cta1p might be an essential component in oxidative stress response by maintaining mitochondrial cell functions**
- → **Protein cotargeting seems a general principle in eukaryotic cell biology that has recently been designated „*eclipsed distribution*“**

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