The azoreductase of yeast cells: a new feature of an old enzyme

Patrícia A. Ramalho[1], Sandra Paiva[1], Artur Cavaco-Paulo[2], Margarida Casal[1], M. Teresa Ramalho[3] , M. Helena Cardoso[1]

[1] Biology Department,

[2] Textile Engineering Department,

[3] Chemistry Department,

University of Minho, Braga

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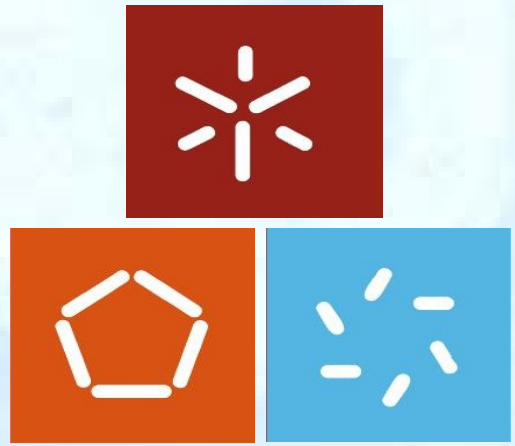
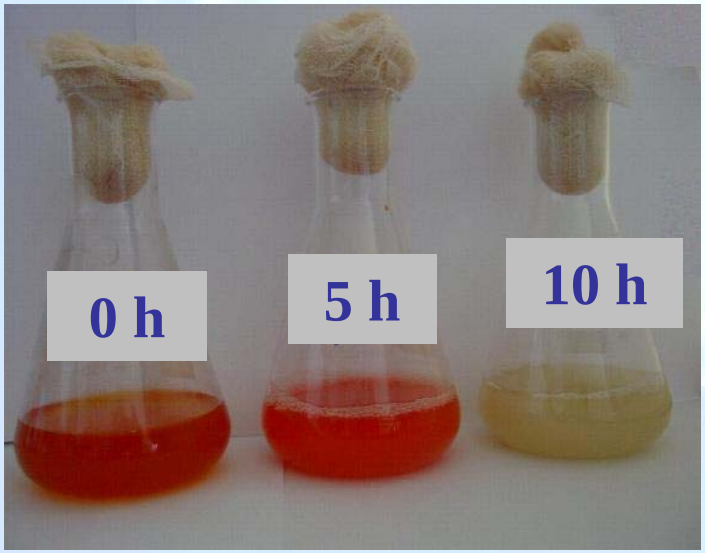


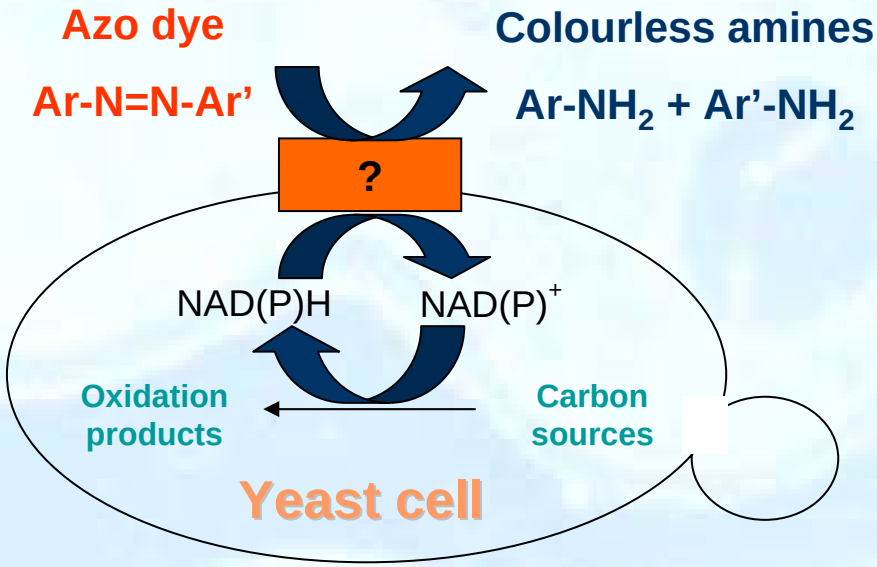
State of the art

Reduction of azo dyes removes colour

Yeasts are able to reduce azo dyes to amines

Reduction of polar azo dyes occurs extracellularly





From literature

In yeasts an externally directed redox enzyme system - located in the plasma membrane - is the ferric reductase

Ferric reductase activity is traditionally measured through the reduction of ferricyanide anion which is also impermeant to the plasma membrane

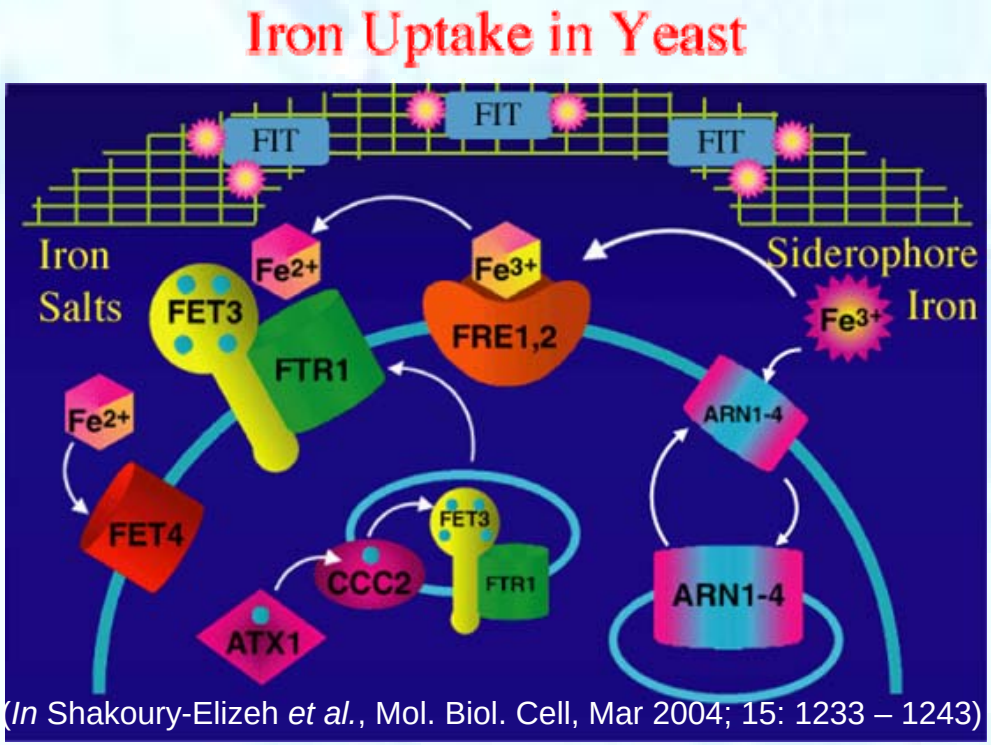
This same activity is also seen in the reduction of ferric citrate or Fe/EDTA complexes

Ferric reductase is involved in the iron uptake system of yeasts

Ferric reductase and iron uptake in yeasts

Yeasts have three systems to solubilize ferric iron from the environment:

- siderophores (Lesuisse *et al.*, 2001);
- excretion of reducing agents or organic acids (like anthranilate and 3-hydroxyanthranilate) (Bienfait *et al.*, 1987; Georgatsou *et al.*, 1994; Lesuisse *et al.* 1990);
- reduction to Fe^{2+} by a **plasma membrane reductase** (Lesuisse *et al.*, 1990; Dancis *et al.*, 1990).



(*In* Shakoury-Elizeh *et al.*, Mol. Biol. Cell, Mar 2004; 15: 1233 – 1243)

ARN1-4: siderophore transporters
FET4: low affinity ferrous iron transporter
FET3+FTR1(permease): high affinity ferrous iron transporter
ATX1: oxygen toxicity suppressor
CCC2: iron transporter in the Golgi apparatus
FIT: mannoproteins from the cell wall that retain iron

Methods

- Laboratorial *Saccharomyces cerevisiae* strain with the ability to reduce azo dyes

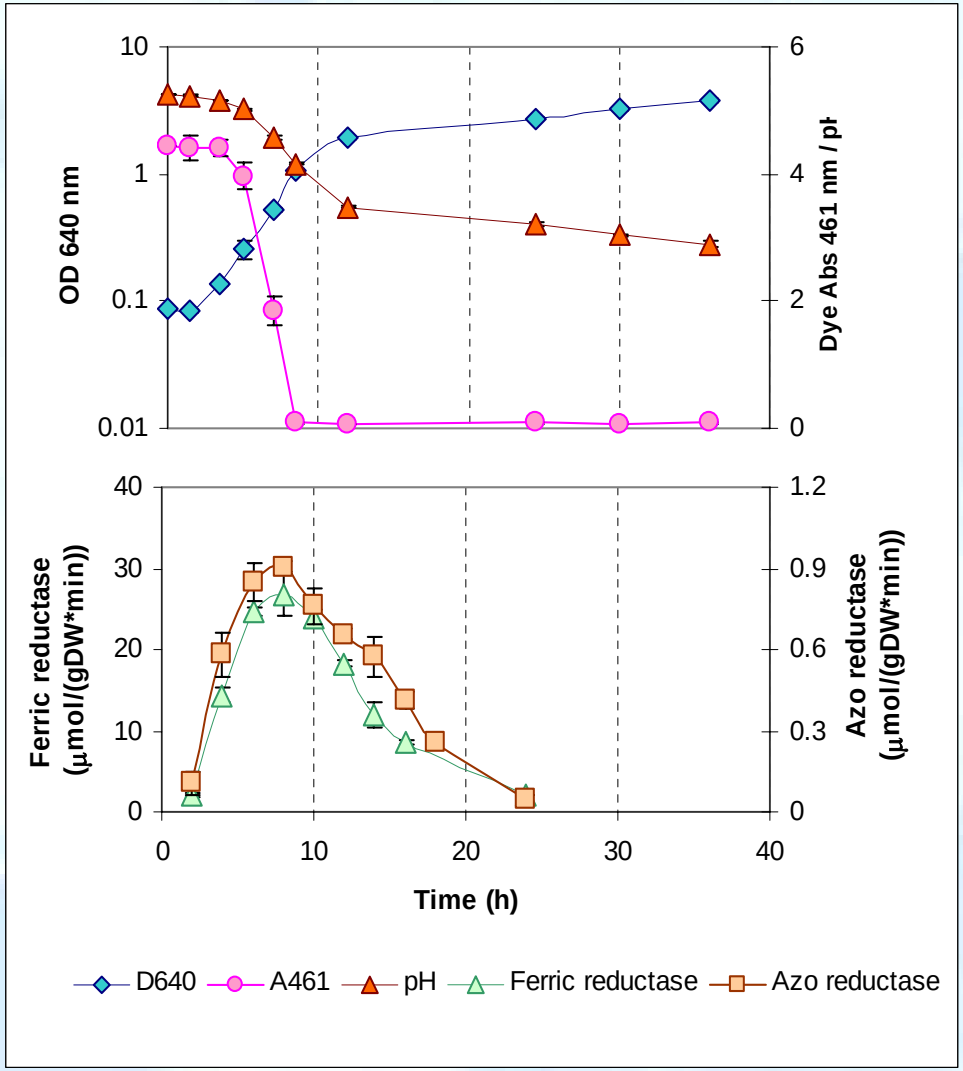
- Decolourization experiments along growth at 26ºC and 120 rpm

- Activity assays of ferric and azo reductases with cells grown for 6h in normal decolourization medium

Strategies to prove the new functionality of ferric reductase

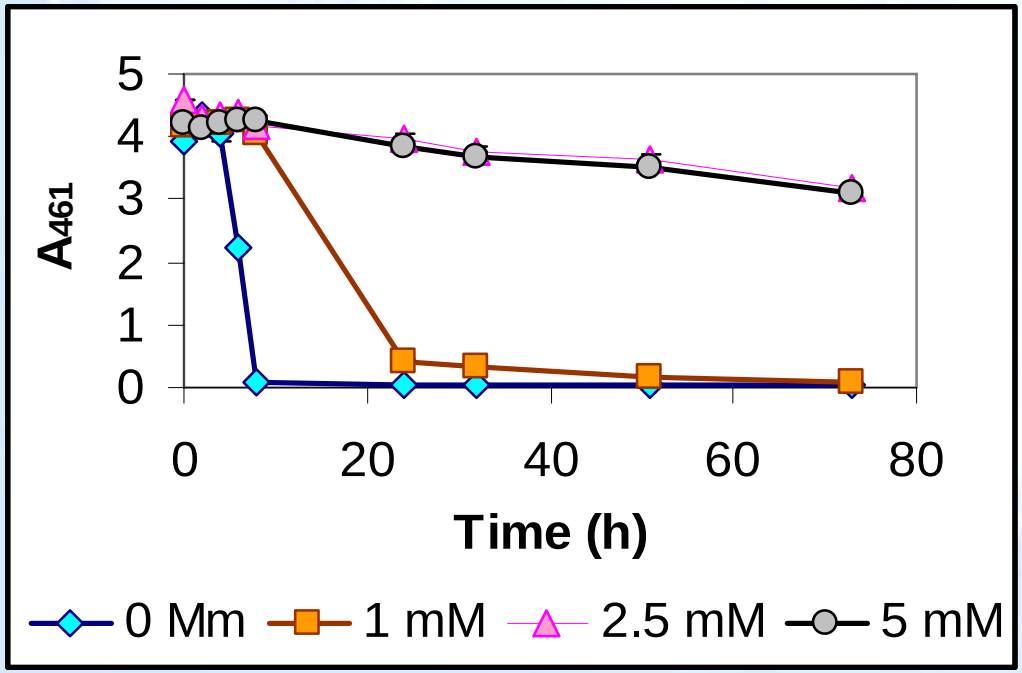
I. Measurement of both ferric and azo reductase activities along growth and decolourization:

Ferric and azo reductases have **parallel activity curves** with maxima in the late exponential growth phase



Strategies to prove the new functionality of ferric reductase

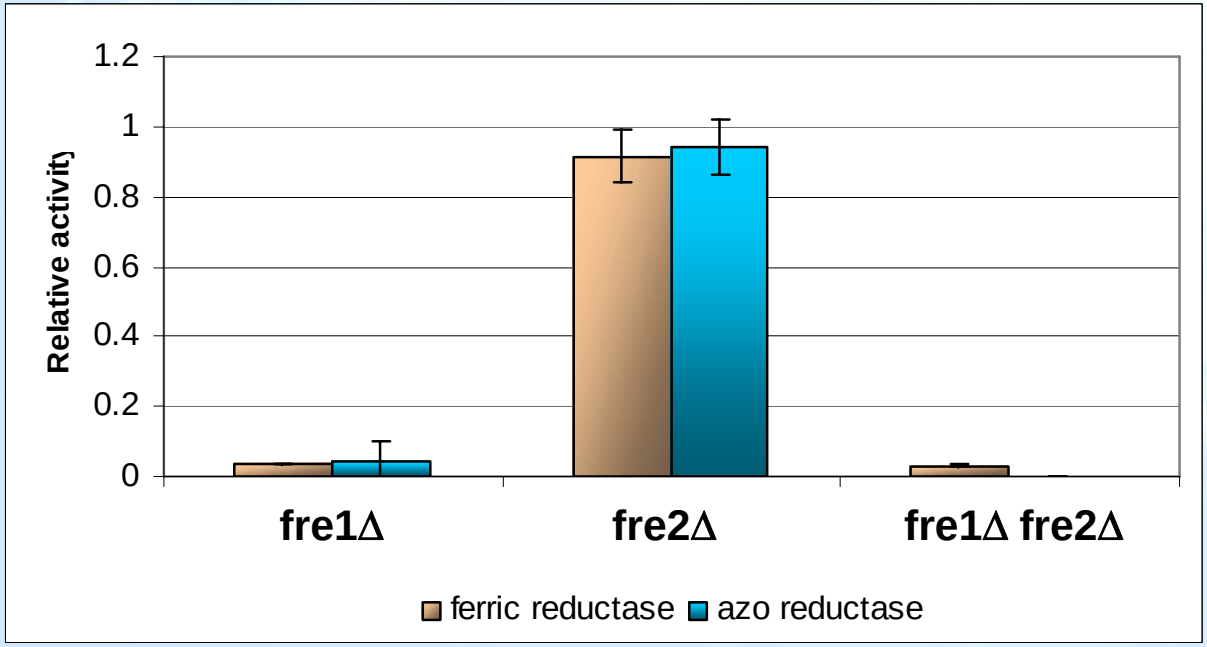
II. Addition of an known inhibitor of ferric reductase (IRON) to the decolourisation medium:



The addition of iron to the medium inhibits ferric reductase at both transcriptional and post-transcriptional levels (*Lesuisse et al. 1996*) **and delays decolorization**

Strategies to prove the new functionality of ferric reductase

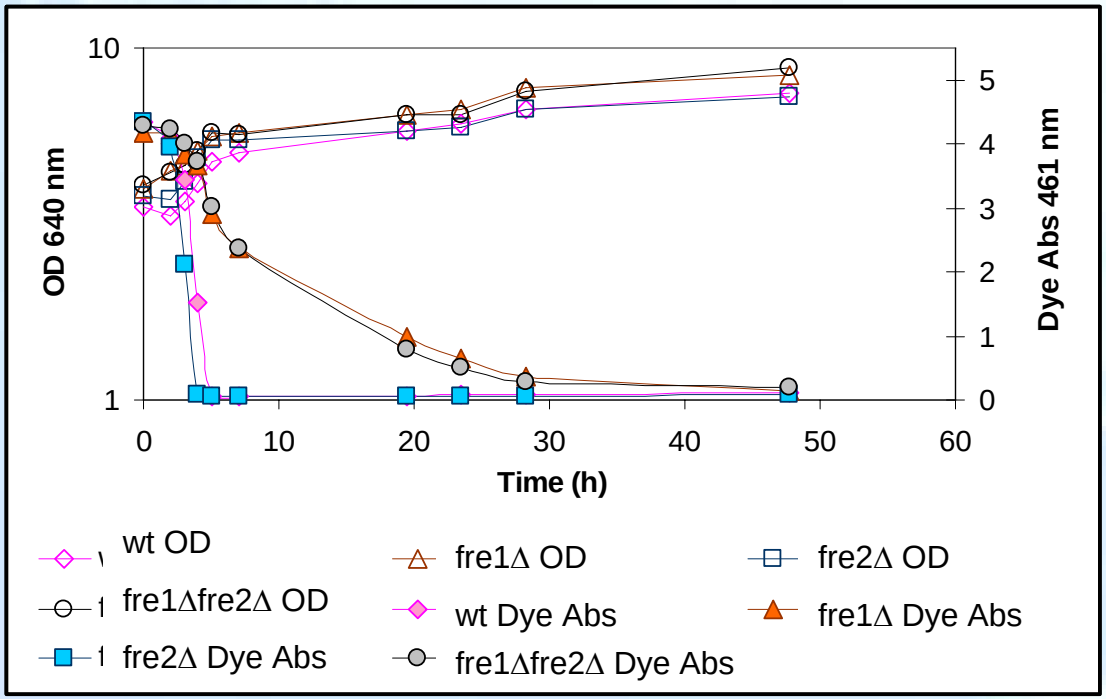
III. Deletion of the genes *FRE1* and *FRE2* in the strain: effect on azo and ferric reductase activities



The deletion of *FRE1* gene removes the decolorizing activity; *FRE2* removal does not affect the decolorizing ability, in our conditions

Strategies to prove the new functionality of ferric reductase

III. Deletion of the genes *FRE1* and *FRE2* in the strain: effect on decolourisation activity



Reduction of decolourising capacity by deletion of *FRE1* and *FRE2*

Fre1p is responsible for the major part of the azo reductase activity of intact yeast cells

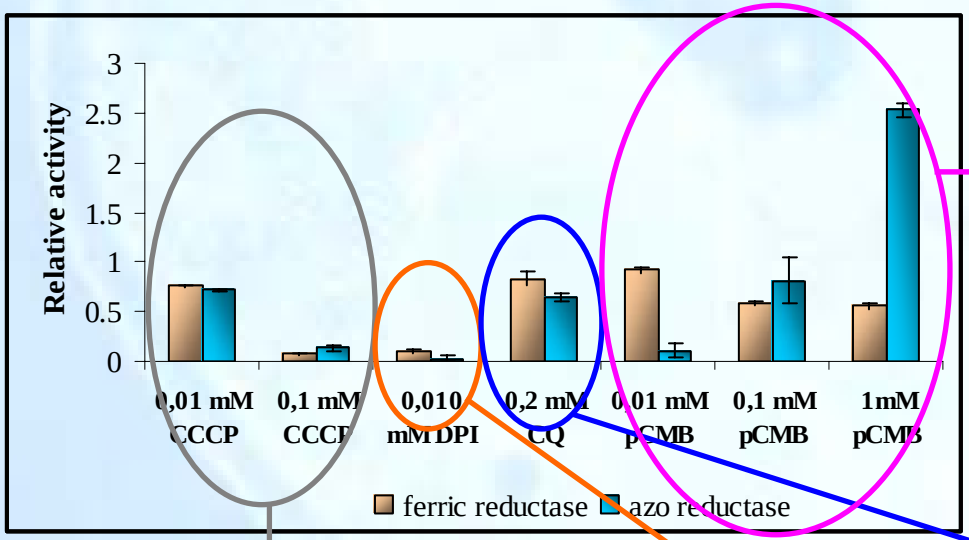
Strategies to prove the new functionality of ferric reductase

IV. Use of inhibitors

- CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) – protonofore (Schröder *et al.*, 2003);
- DPI (diphenyleneiodonium chloride) – flavoenzyme inhibitor (Wright and Kuhn 2002);
- Chloroquine – antagonist of CoQ (Santos-Ocana *et al.*, 1995);
- *p*-chloromercuribenzoate (pCMB) – thiol reagent susceptible of reacting with intra-membrane protein groups; inhibits NADH:ferricyanide reductase (Löw et al., 1978).

Strategies to prove the new functionality of ferric reductase

IV. Use of inhibitors



The stimulation of the azoreductase activity points to the existence of two alternative paths and inhibition of the most favourable one.

Both activities depend on a proton gradient

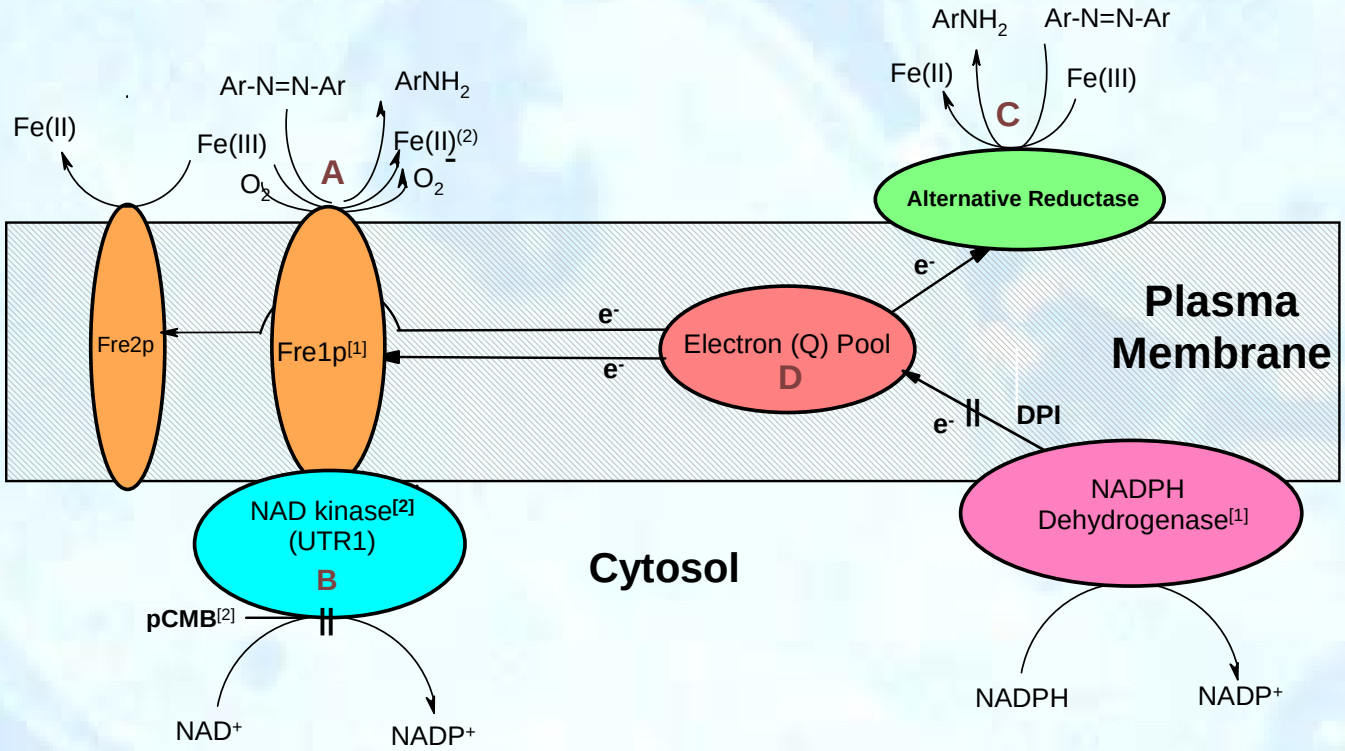
Both depend on flavoenzymes

Apparently CoQ_x is more important in the azo reductase activity

Conclusions

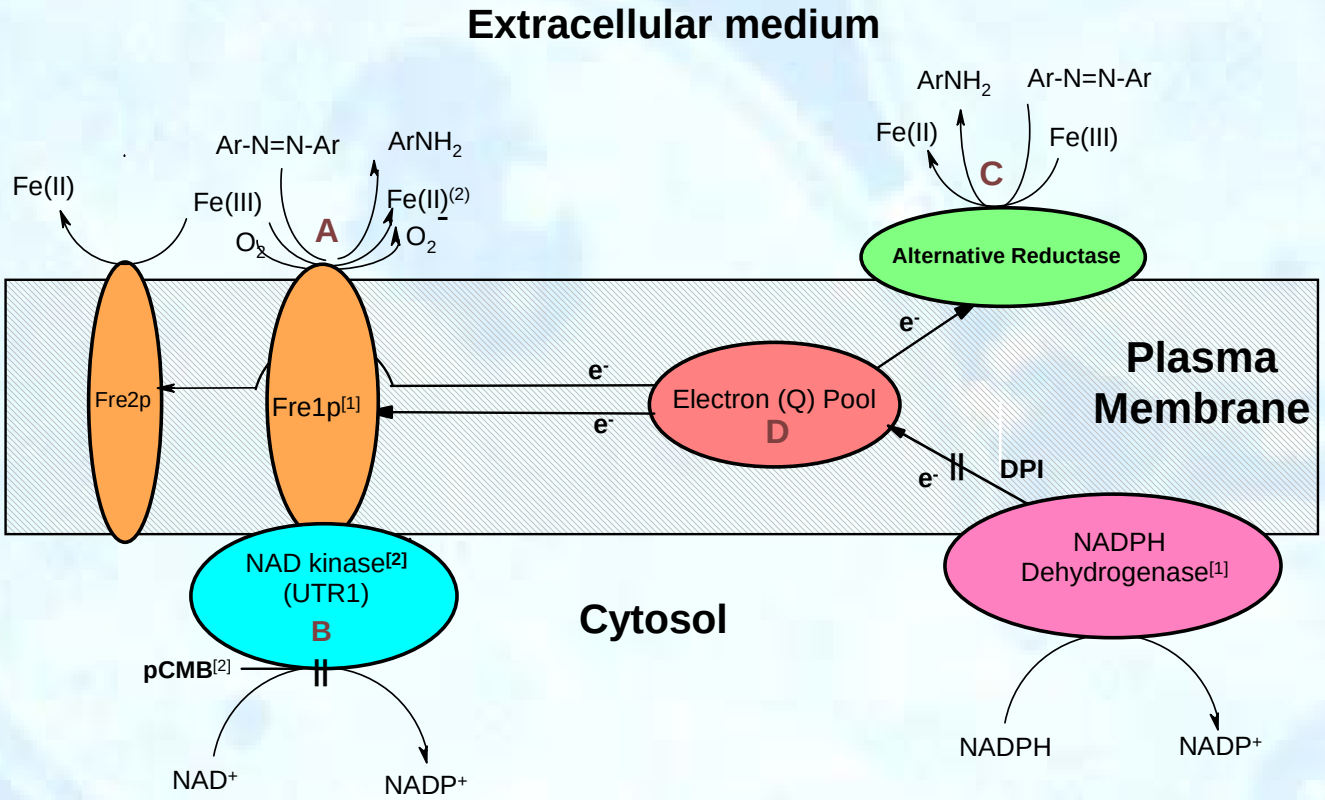
- The ferric reductase is the main activity involved in extracellular azo dye reduction by yeasts (fre1p mainly).
- However there is an alternative redox system to ferric reductase in the plasma membrane still unknown and that is able reduce some extracellular compounds (azo dyes).

Proposed model



- **The major fraction of azo reductase activity depends on Fre1p [1; this work] and on a NADPH dehydrogenase [1] A**
- **Activity of Fre1p depends on a cytosolic NAD kinase [2] B**
- **Azo dye reduction (an presumably ferric iron reduction) must occur at an alternative site [this work] C**

Proposed model



- **pCMB stimulates azo reductase activity at higher concentrations [this work]**
- **These observations are consistent with the existence of membrane transporters capable of switching electrons between two external reduction sites (electron or Q pool) D**

[1] E Lesuisse, M Casteras-Simon and P Labbe 1996 JBC 271, 13578-13583
[2] S Kawai *et al.* 2001 FEMS Microbiol Lett 200, 181-184

Thanks for your attention!