

In search of parsimony:
Entertaining an alternative hypothesis
for the mode of action of pyrazinamide

Anthony Baughn
Department of Microbiology
University of Minnesota

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Entertaining an alternative hypothesis
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- Brief discussion of current models and standing questions
- Introduction of alternative hypotheses
- Plan of action

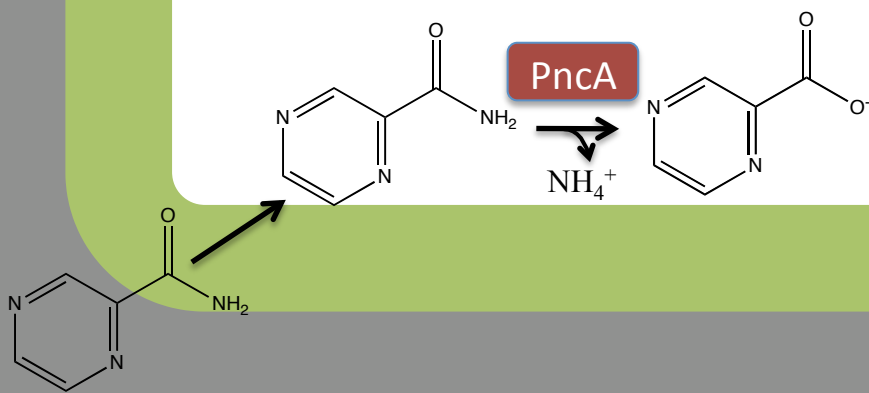
Observations that we can agree on

- PncA-mediated hydrolysis of PZA to POA by is essential for susceptibility *in vivo* and *in vitro* (Scorpio & Zhang 1996)
- PZA elicits death of *Mtb in vivo*, static at best *in vitro* (Tarshis & Weed 1953; McCune, Tompsett & McDermott 1956)
- PZA (POA) treatment compromises energy metabolism* (Zhang *et al* 2003)
- PZA (POA) susceptibility is pH dependent* (McDermott & Tompsett 1954, Zhang *et al* 2002)
- conditions that promote POA accumulation enhance susceptibility* (Zhang *et al* 1999)

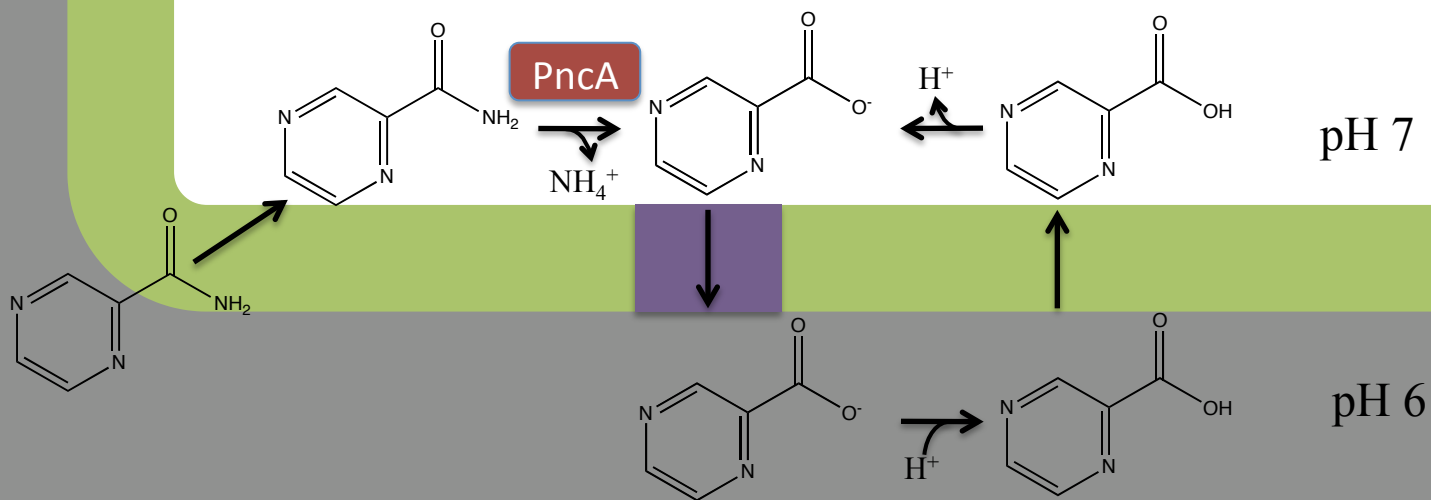
Current Models for PZA Action

- Proton Ionophore
- Inhibition of FAS-I

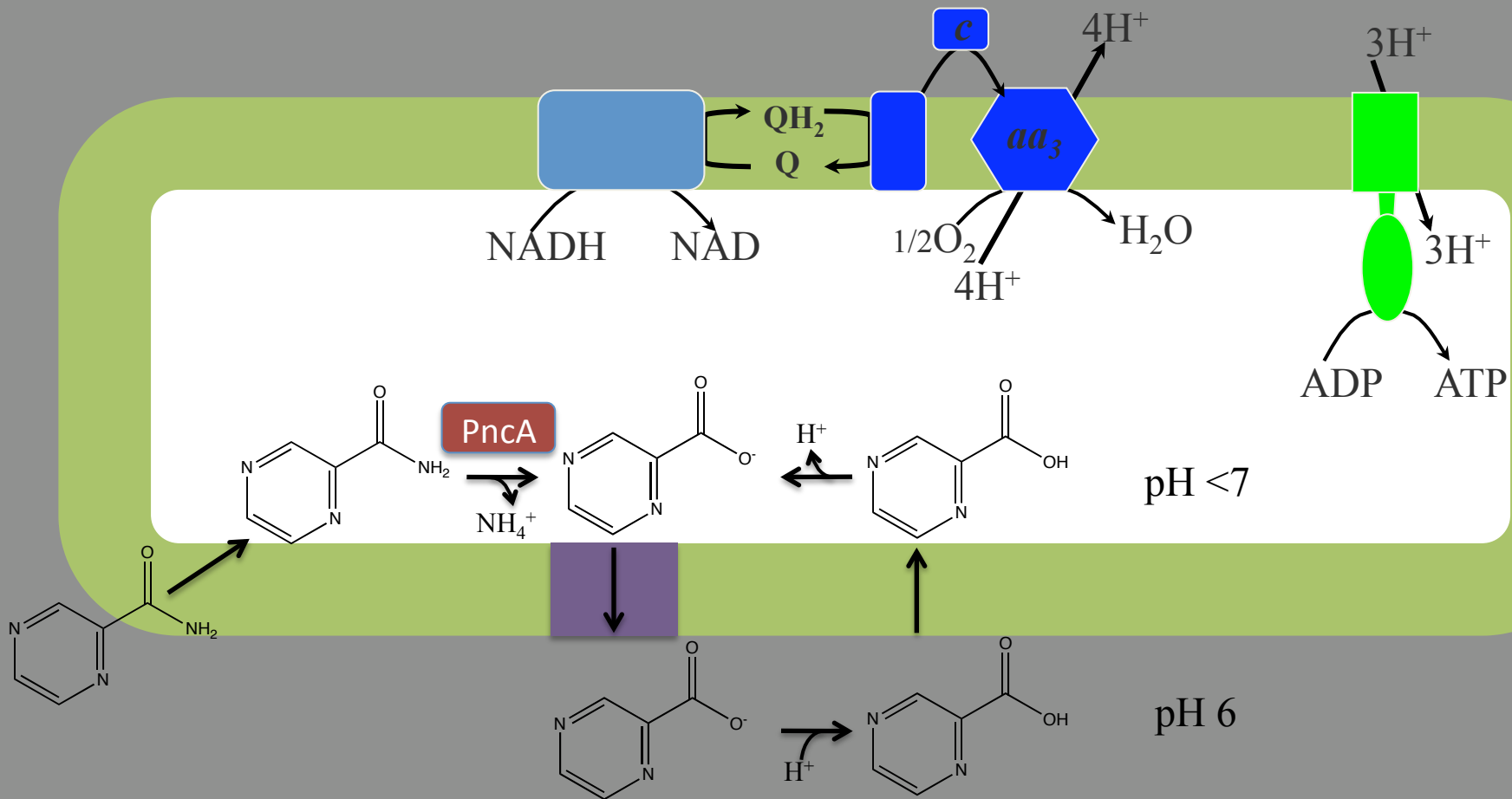
PZA gets in, gets hydrolyzed



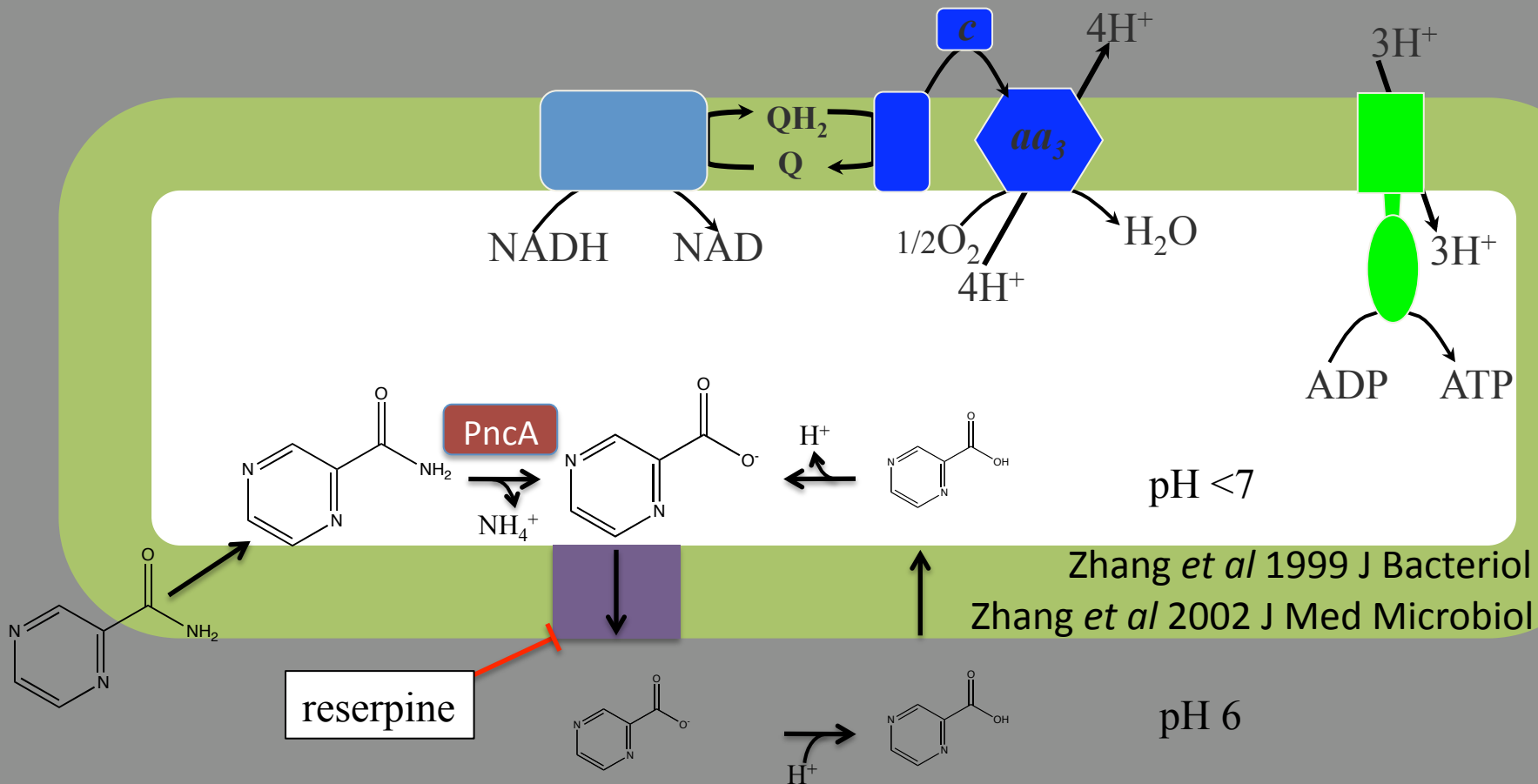
Pyrazinoic acid as a proton ionophore



Pyrazinoic acid as a proton ionophore



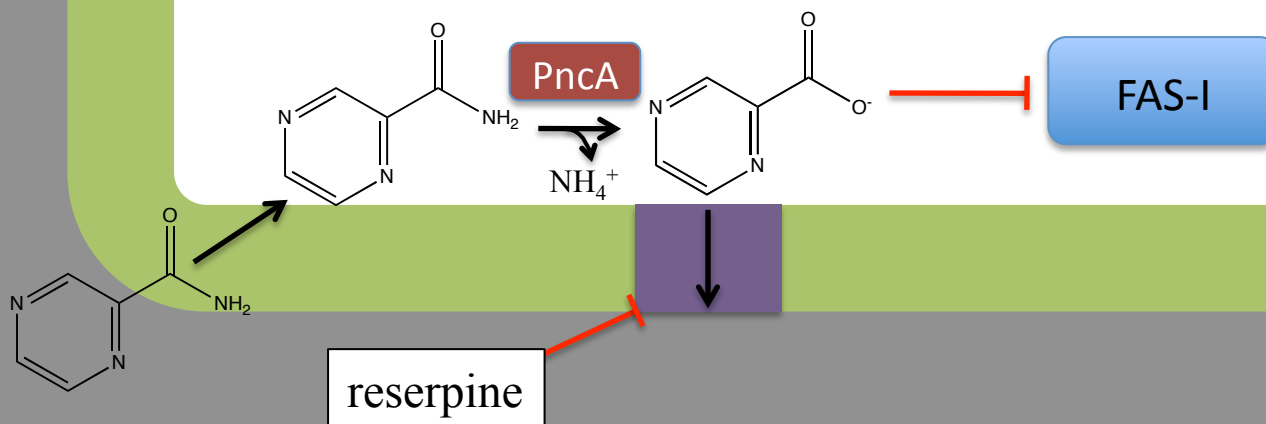
Pyrazinoic acid as a proton ionophore



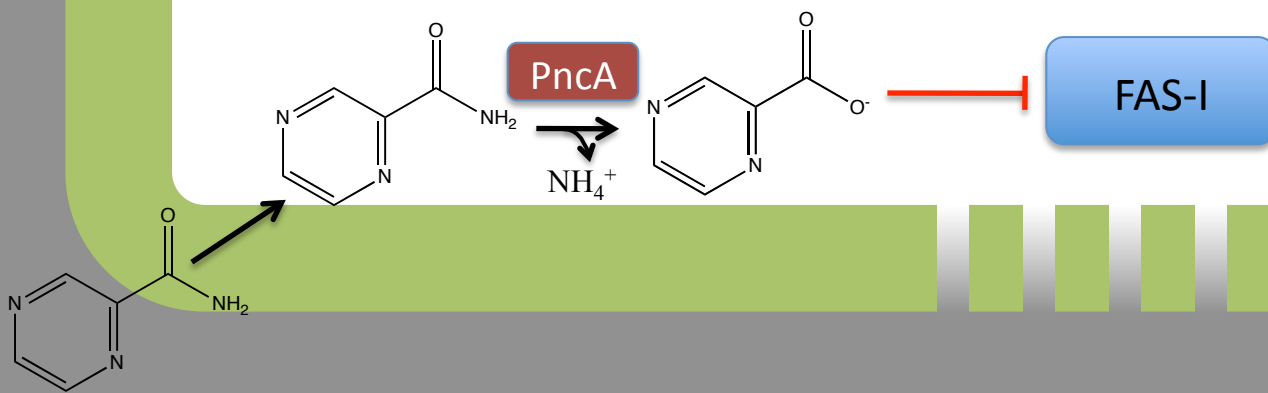
Current Models for PZA Action

- Cellular Acidification
- Inhibition of FAS-I

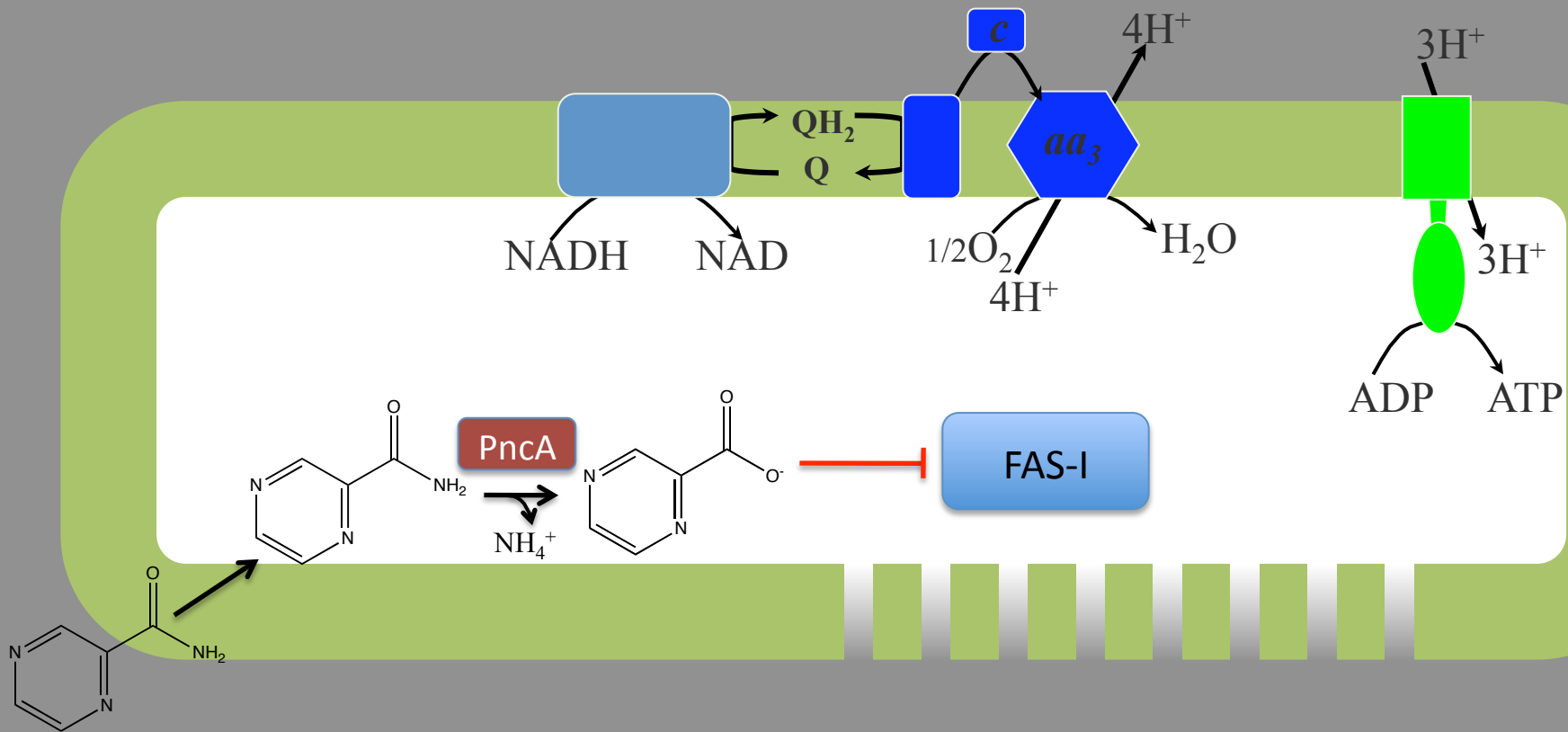
Pyrazinoic acid as a FAS-I inhibitor



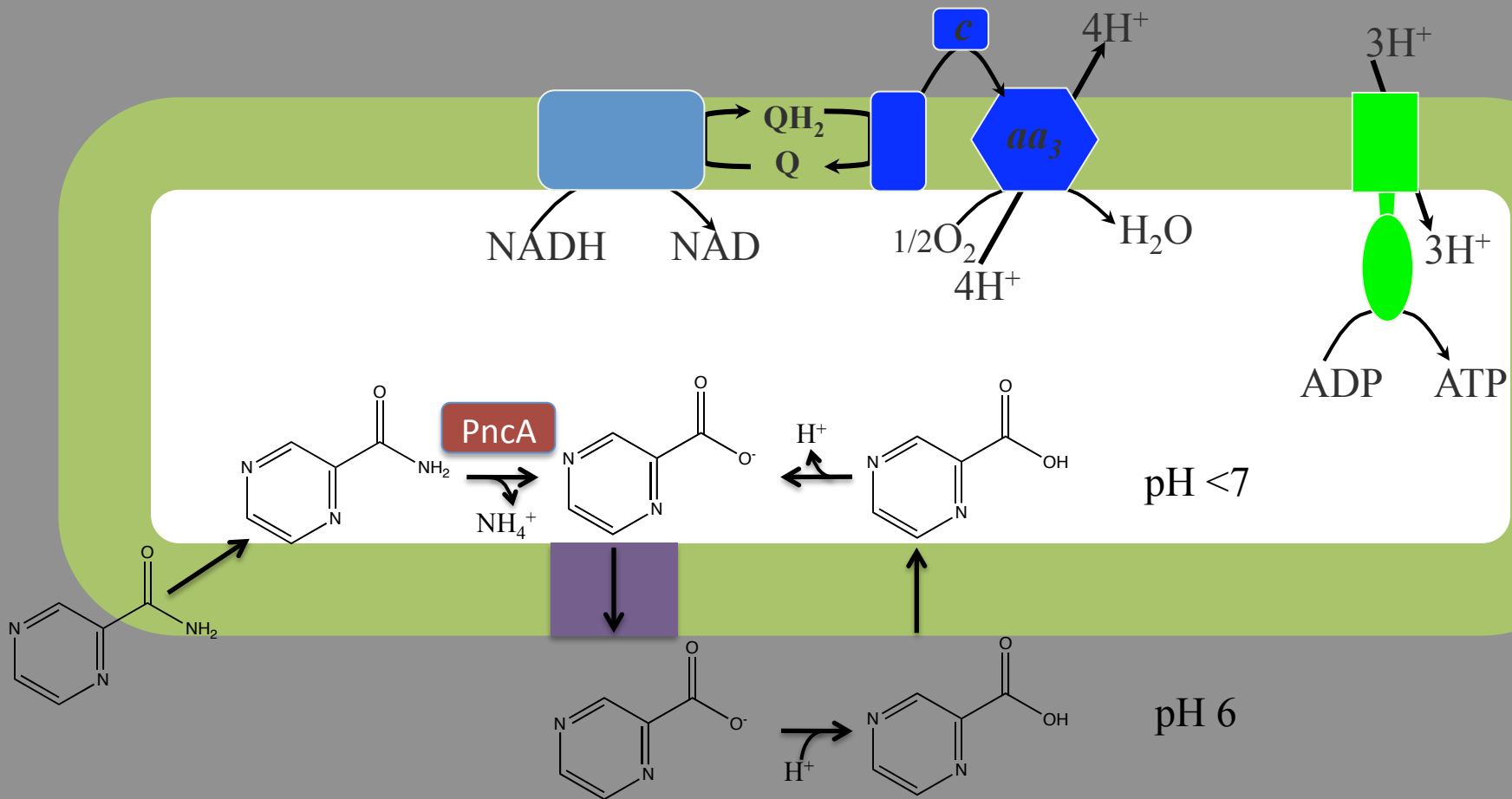
Pyrazinoic acid as a FAS-I inhibitor



Pyrazinoic acid as a FAS-I inhibitor



Pyrazinoic acid as a proton ionophore

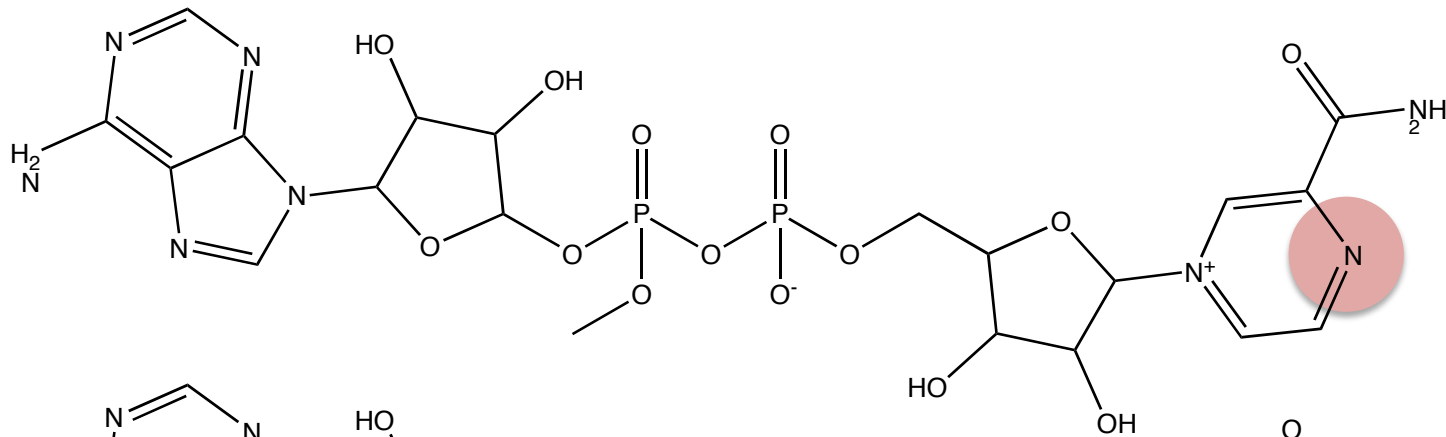


Cause or Consequence?

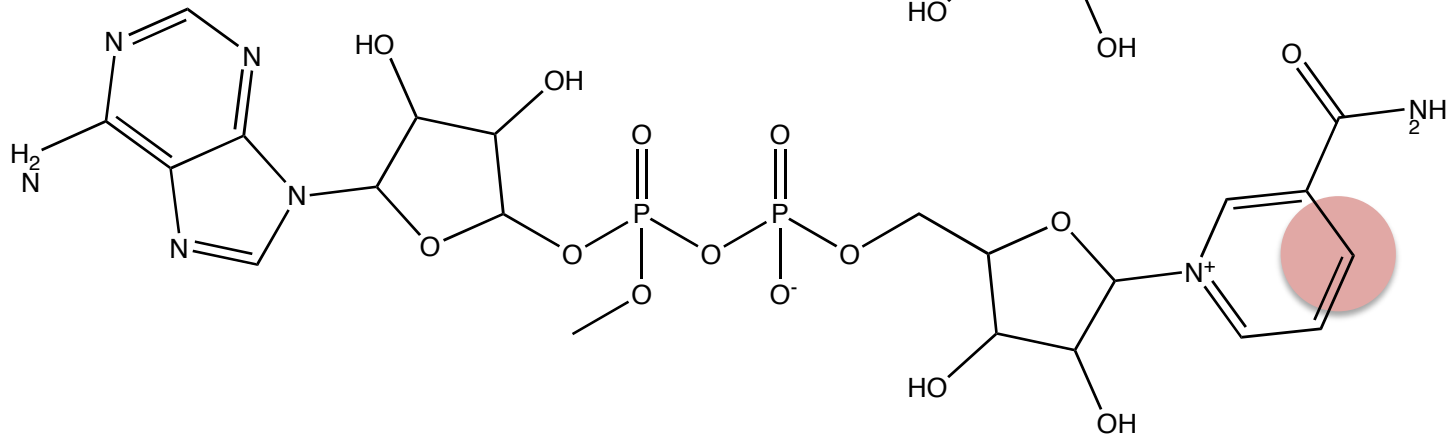


Are there any other possibilities?

- unknown products of POA (from host or Mtb)
- pyrazinamide adenine dinucleotide

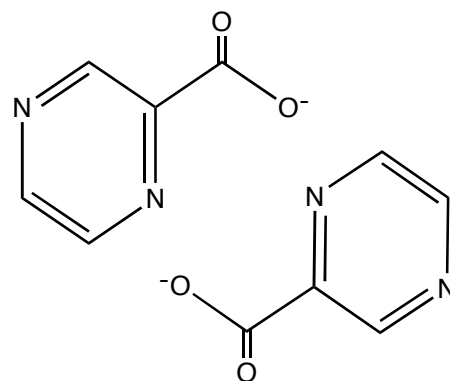
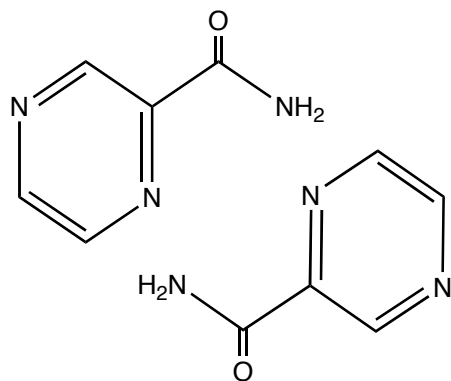


PAD



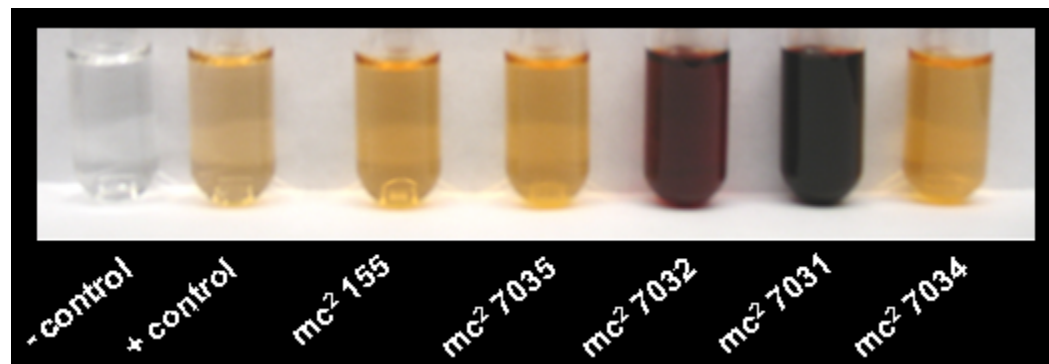
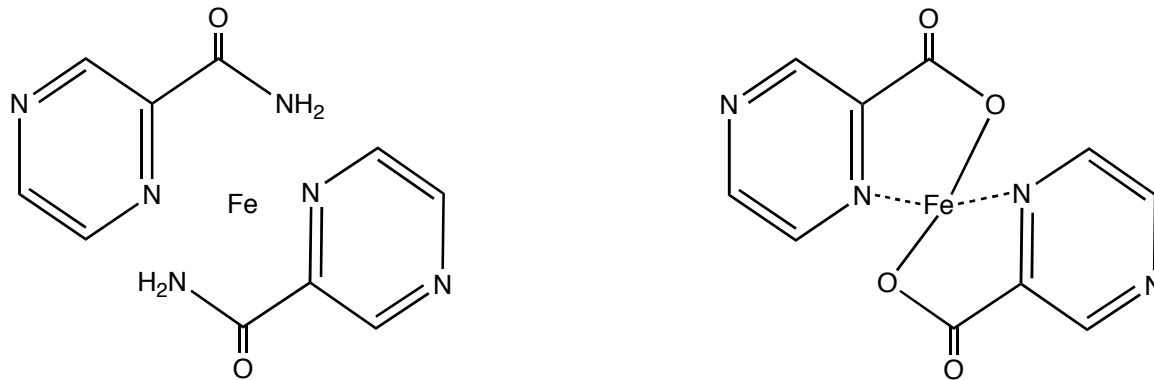
NAD⁺

Are there any other possibilities?



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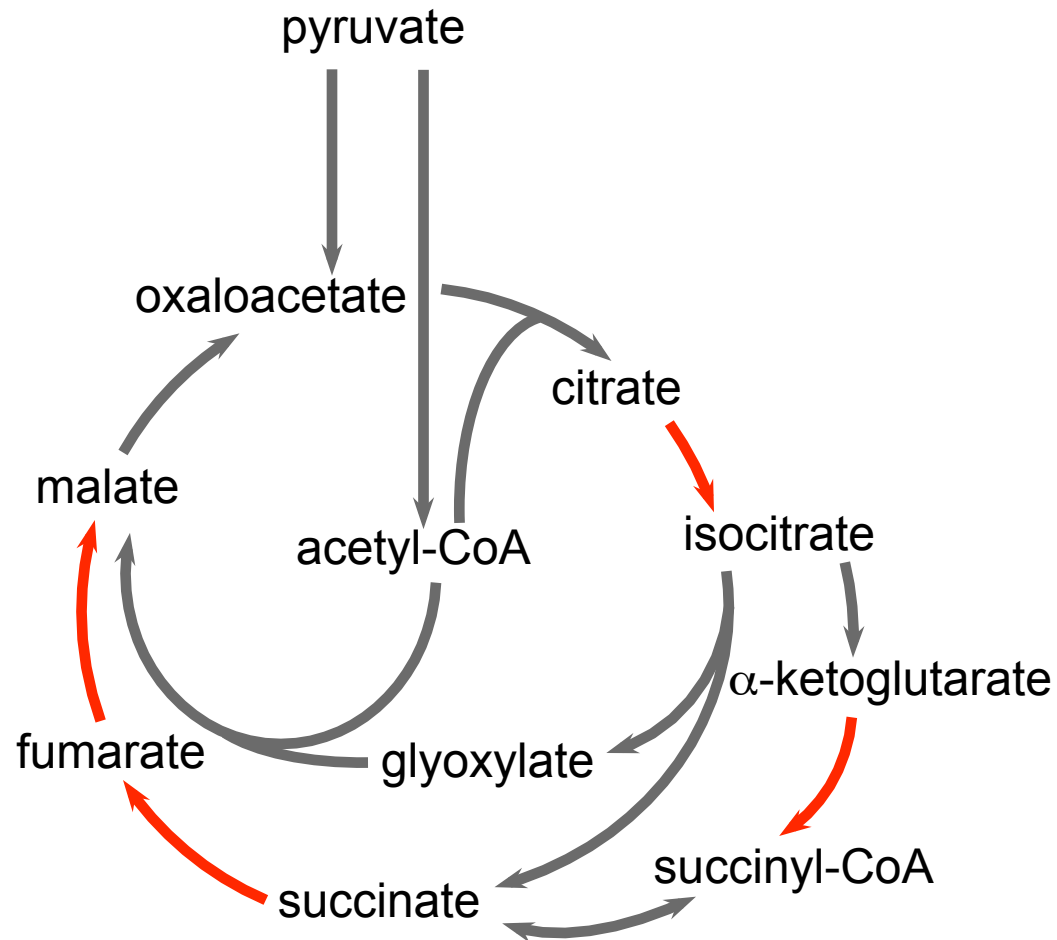
- unappreciated effects of POA
 - pyrazinoic acid is an iron chelator (Magri *et al* 1980)
 - absorbance at 468 nm (cyan) results in red color



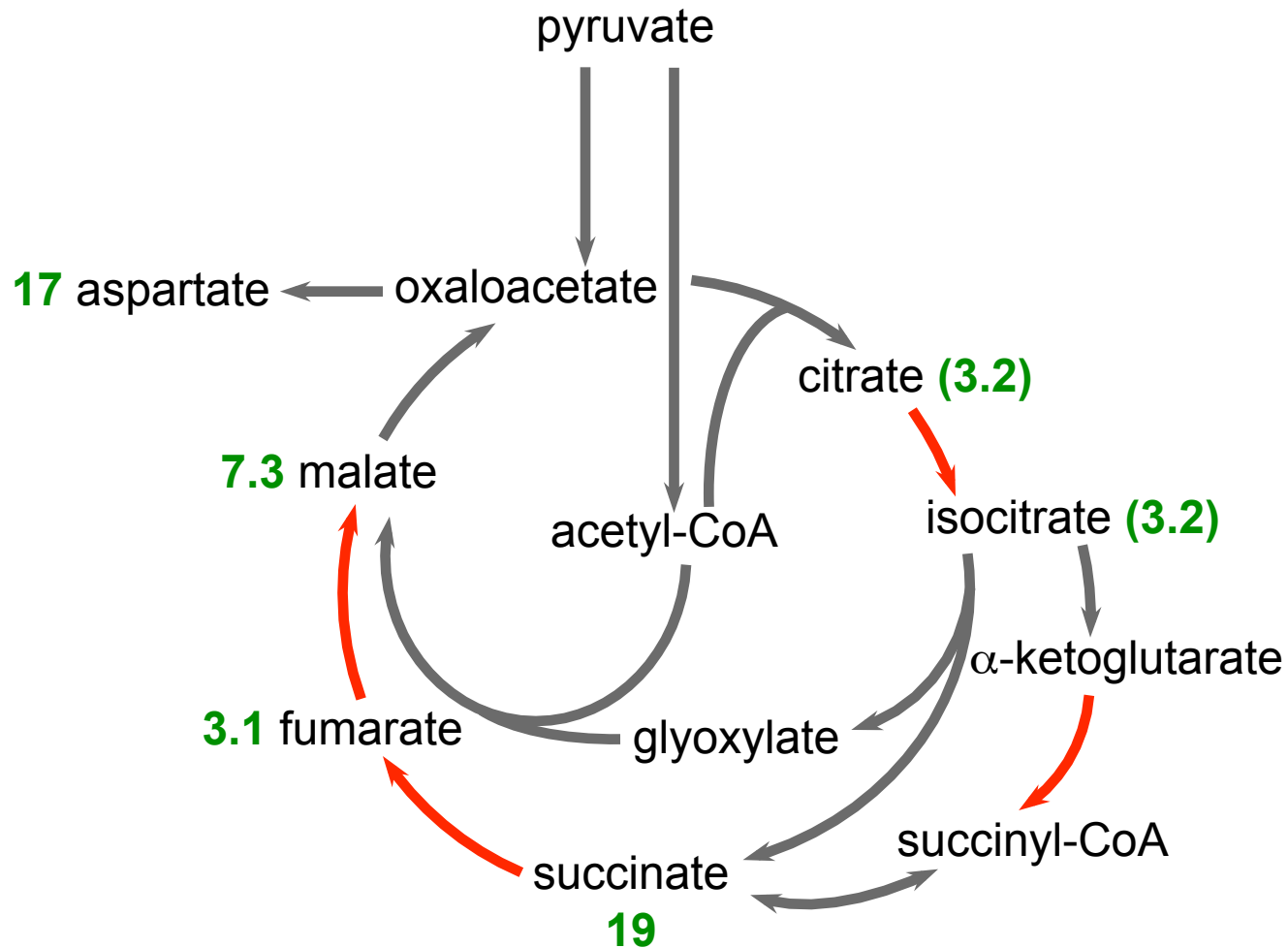
Is there any support for this model?

- *Rv0265c* Fe(III)-dicitrate transporter is induced 3.6 fold by PZA treatment (Boshoff *et al* 2004 JBC)
- Iron enhances pyrazinamide toxicity (Somoskovi *et al* 2004 JAC)

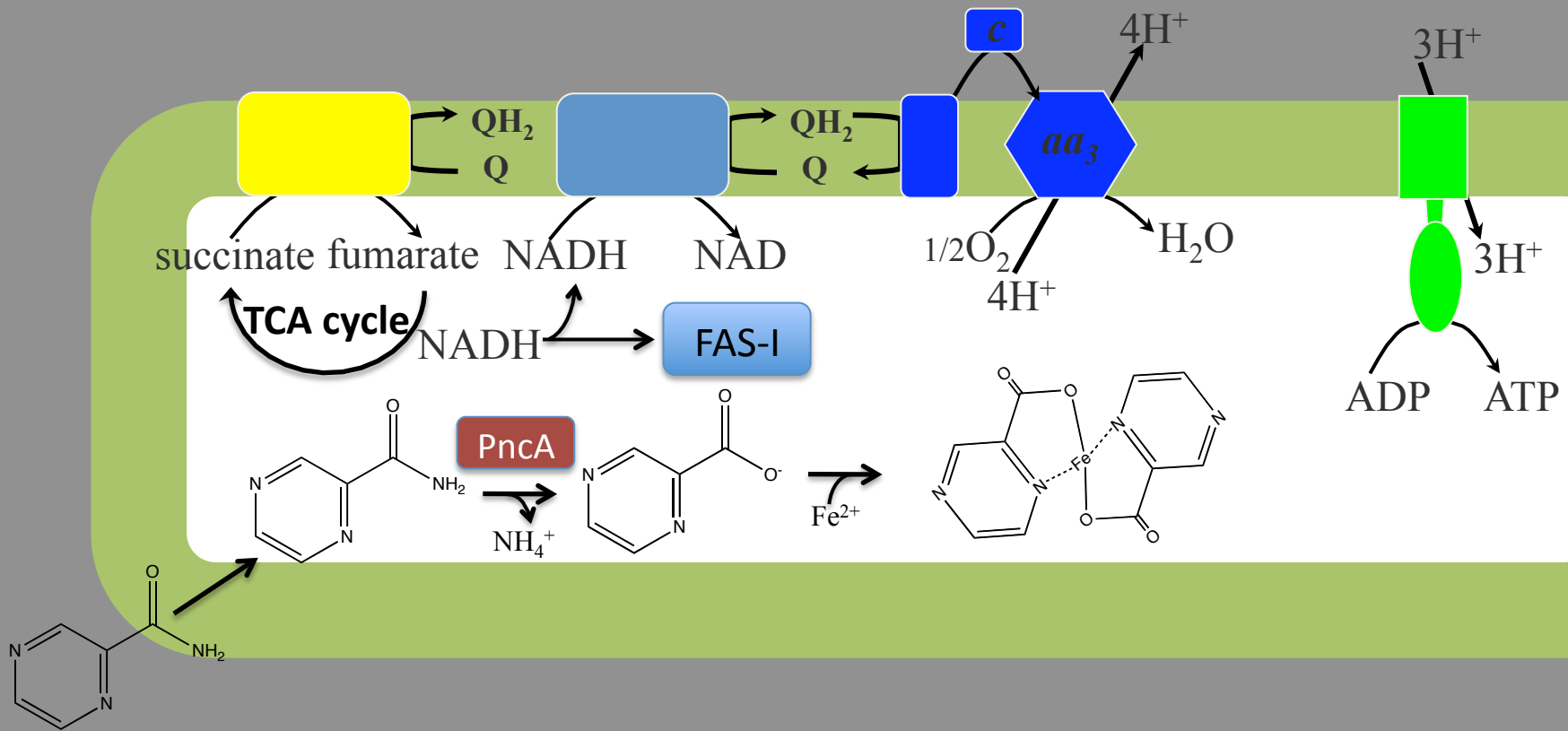
Iron and the TCA cycle



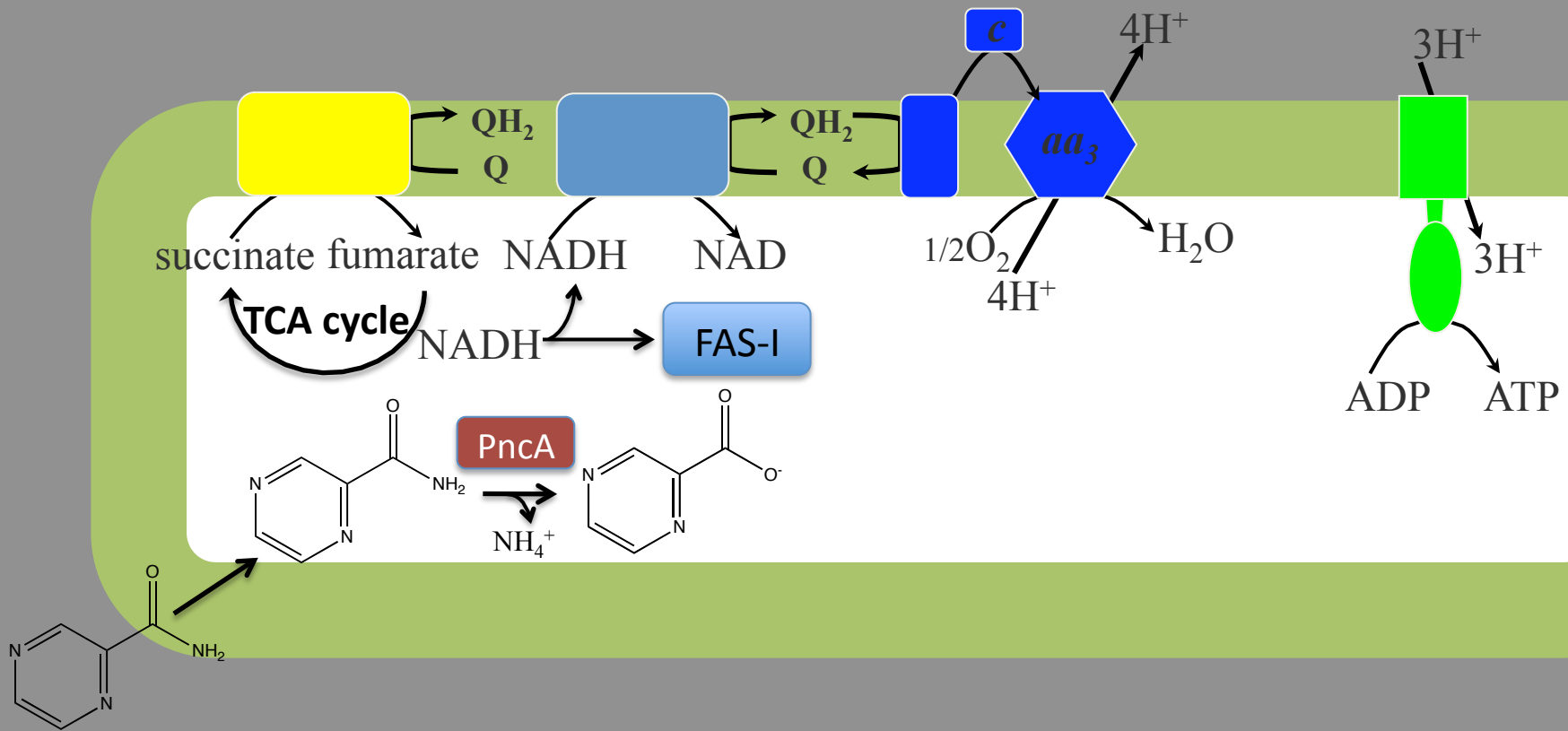
Effect of pyrazinamide treatment on TCA cycle intermediates



PZA-mediated metabolic arrest



There's always a caveat



What should we do?

- Ask the cells what is going on
 - Time course of events
 - Re-evaluate expression arrays
 - Metabolomics and flux analysis
 - Assess activities of Fe and Mn dependent enzymes following PZA treatment
- Test ROS-susceptibility of PZA treated *Mtb*
- Susceptibility of mutants with iron acquisition defects
- Test susceptibility of *Mtb* to PZA in *PHOX*^{-/-} mice, hemochromatosis model
- Assess other pro-iron chelators for anti-TB activity

Acknowledgements

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