



# Regulation of the TCA-cycle by overflow metabolism in *Rhizobium*

Philip Poole, David Walshaw and Mary Smith  
School of AMS University of Reading UK  
email p.s.poole@reading.ac.uk

## 1

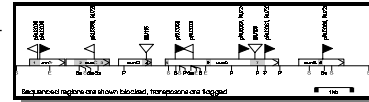
### Introduction

Oxidation of C<sub>2</sub>-dicarboxylic acids by the TCA-cycle provides the energy for N<sub>2</sub> fixation by *Rhizobium*. Regulation of the TCA cycle is therefore likely to be important in determining the partitioning of energy and electrons to nitrogen-fixation. Labelling and enzyme studies suggest that the TCA-cycle becomes blocked by a high NADH/NAD<sup>+</sup> ratio in bacteroids at the α-ketoglutarate dehydrogenase step. This leads to overflow metabolism.

## 5

### TCA-cycle genes

To enable the regulation of the TCA cycle to be studied we cloned and mutated the α-ketoglutarate dehydrogenase complex of *R. leguminosarum*. We have identified an operon consisting of *mdh-sucCDAB*, which code for malate dehydrogenase (*mdh*), α-ketoglutarate dehydrogenase (*sucA*), dihydrodipicolinate succinyl transferase (*sucB*), succinyl-CoA transferase subunit A (*sucC*), succinyl-CoA transferase subunit B (*sucD*). We also isolated regulatory mutants of the α-ketoglutarate dehydrogenase complex, which have lower enzyme activity of the complex and these mapped to *phaC*, which codes for polyhydroxybutyrate synthase.



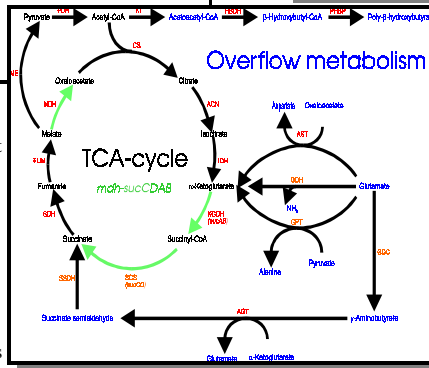
## 2

### Overflow metabolism

Overflow metabolism caused by blockage of the TCA-cycle results in the flow of carbon, energy and reductant to several pathways, including:

- Amino acid biosynthesis
- Polyhydroxybutyrate (phb) biosynthesis
- Glycogen biosynthesis
- GABBA shunt

Nitrogen fixation must compete with overflow pathways for ATP and reductant.



ferase (*sucB*), succinyl-CoA transferase subunit A (*sucC*), succinyl-CoA transferase subunit B (*sucD*). We also isolated regulatory mutants of the α-ketoglutarate dehydrogenase complex, which have lower enzyme activity of the complex and these mapped to *phaC*, which codes for polyhydroxybutyrate synthase.

## 3

### Experimental Strategies

Since pea bacteroids excrete alanine and aspartate, presumably due to blockage of the α-ketoglutarate dehydrogenase complex, we have been examining amino acid uptake and excretion in *R. leguminosarum*. This lead us to the following strategies:

- 1 Clone and mutate the general amino acid permease.
- 2 Clone and mutate the α-ketoglutarate dehydrogenase complex.
- 3 Isolate regulatory mutants of the TCA-cycle.
- 4 Examine the interaction between overflow pathways.

## 6

### Regulation of the TCA-cycle

- The genes of the *mdh-sucCDAB* operon share a promoter and are transcriptionally linked.
- *Mdh* mutants appear to be lethal.
- *Suc* mutants excrete glutamate i.e. amino acid excretion acts as an overflow pathway when the TCA-cycle is blocked.

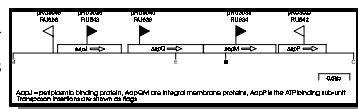
flow pathway when the TCA-cycle is blocked.

- The general amino acid permease appears to be the main excretion pathway for glutamate, while uptake is inhibited in *suc* strains.
- Blocking *phb* biosynthesis down regulates the activity of the α-ketoglutarate complex and may increase nitrogen fixation.

## 4

### General amino acid permease

The general amino acid permease is an ABC transporter consisting of four genes *aapJQMP*. It is the first prokaryotic transport system identified to transport all L-amino acids. Sequence comparison and Southern blotting has enabled us to detect similar systems in a wide range of Gram-negative bacteria and identify a new sub-class of the ABC transporter super-family. Crucially it appears to be bi-directional, allowing efflux of solutes from the cell either directly or by regulating another transporter/channel. If the *Aap* allows efflux directly this would challenge the paradigm that ABC transporters are unidirectional. Influx (uptake) is inhibited by a high intracellular concentration of glutamate, conditions where efflux occurs.



## 7

### Conclusions

- ⇒ The α-ketoglutarate complex is one of the principal control points of the TCA cycle in *Rhizobium*
- ⇒ The α-ketoglutarate complex is regulated by the overflow pathways of amino acid excretion and *phb* biosynthesis.
- ⇒ Amino acid uptake (influx) by the *Aap* is inhibited by a high intracellular glutamate concentration when the α-ketoglutarate dehydrogenase complex is blocked, allowing efflux by this pathway to proceed.
- ⇒ Altering overflow metabolism is likely to have a strong impact on nitrogen fixation. e.g. *phaC* mutants have increased rates of nitrogen-