Measurement of a possible signal for nitrogen starvation in a cyanobacterium-plant symbiosis

OUTLINE

I. Introduction

A. Green Revolution saves lives at high cost
1. Green Revolution – Plants that can exploit higher nitrogenous fertilizer
2. Great cost: making ammonia expensive
3. Great cost: Ecological damage

B. Biological nitrogen fixation: Good but limited
1. Legumes don't need nitrogenous fertilizers, fix N_2
2. Avoids costs of nitrogenous fertilizer
3. N-fixation from symbioses with rhizobia
4. N-fixation limited to legumes (**Figure: world agriculture**)

C. How to extend N-fixation to major crops?
1. Extend rhizobial to cereals? Rhizobia too specific
2. Alternative: Cyanobacterium *Nostoc* is a generalist
3. *Nostoc* achieves generality through independence, heterocysts

D. N-fixation by *Nostoc* different inside plant vs outside
1. Free-living *Nostoc* hordes N
2. Symbiotic *Nostoc* shares N
3. Symbiotic *Nostoc* fixes more N
4. Host plant modifies *Nostoc's* perception of starvation?

E. Possible key to inside vs outside: α-ketoglutarate
1. α-ketoglutarate central to N-metabolism (**Figure: pathway**)
2. Explain central role

F. α-ketoglutarate might serve as signal of starvation
1. Li et al (2003) test of α-ketoglutarate as signal for N-starvation
2. Li et al (2003) experiment
3. Li et al (2003) result
4. Li et al (2003): high level of α-ketoglutarate fools *Nostoc*

G. Central question
1. Maybe plants manipulate α-ketoglutarate in *Nostoc* to simulate starvation?
2. Does the level of α-ketoglutarate change when *Nostoc* is grown without a source of nitrogen?

II. Experiment

A. Overview of experiment
1. Measure α-ketoglutarate in *Nostoc* with biosensor
2. If α-ketoglutarate is a signal, expect higher level in *Nostoc* in plant

B. How to measure α-ketoglutarate? Biosensors
1. Principle of FRET biosensors (**Figure: FRET cartoon**)
2. Increase of distance between components decreases fluorescence
3. Presence of metabolite alters distance

C. Example of FRET use (Hires et al, 2008)
    1. Scientific purpose: detect glutamate near neuron surface
    2. Construction of glutamate-specific FRET
    3. Test of FRET with glutamate (**Figure: fluorescence +/- glutamate**)
    4. Ratio of yellow:blue emission as a measure of glutamate
    5. Actual result: release of glutamate neurotransmitter alters ratio (**Figure: time course of emission ratio change**)

D. Metabolite-specific biosensor for proposal
    1. α-ketoglutarate-specific biosensor doesn't exist
    2. Glutamate-specific biosensor
    3. Glutamine-specific biosensor instead.

E. Introduction of biosensors into *Nostoc* and *Nostoc* into plant
    1. Cloning of biosensor into plasmid
    2. Introduction of plasmid into *Nostoc* by conjugation
    3. Growth of modified *Nostoc* in plant (*Anthoceros*)
    4. Measurement of glutamate and glutamine

III. Discussion

A. Best possible results
    1. Need for fluorescence ratios to fall within useful ranges
    2. Even so, model predicts α-ketoglutarate levels, not glutamate and glutamine

B. Possibility of α-ketoglutarate biosensor
    1. Three α-ketoglutarate-binding proteins known (α-ketoglutarate dehydrogenase, PII protein, NtcA)
    2. Using binding domains from PII or NtcA may perturb regulation
    3. α-ketoglutarate biosensor outside scope of proposal

C. Problems interpreting results
    1. Cyanobacteria have endogenous fluorescence
    2. Biosensors may not sense biologically relevant levels
    3. Glutamate biosensor may be fooled by aspartate
    4. Calibration difficulties owing to unknown ionic strength in cell

D. Inspirational final words