A Flexible Architecture for Plant Functional Genomics in Space Environments

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A Flexible Architecture for Plant Functional Genomics in Space Environments

Goals:

• Remotely measure the response of plants to any unique space condition

• Determine gene function

• Optimize plant performance under space conditions
Basic Architecture for Plant Functional Genomics

- Test Plant RNA
- DNA Microarrays
- Gene Isolation
- Gene Disruption
- Functional Analysis
- Gene Modification
- Optimized Performance
- Space Environmental Condition

Basic Architecture for Plant Functional Genomics
The “NIAC Dilemna”

“Planning for things that will be practical in 2010 - 2040 but needing to demonstrate practibility now”

Dr. Steven Dubowsky, MIT (NIAC Fellow)
Model System to Test Architecture Feasibility

Gravistimulation of Arabidopsis DNA Microarrays

Arabidopsis Genomic Database

Moss Gene Disruption by Homologous Recombination

Gravitropism Assays

Optimized Performance Arabidopsis RNA
Arabidopsis thaliana:

- model plant system
- defined gravitropic response
- genome sequence complete
- targeted gene knock-outs inefficient
Physcomitrella patens:

- gene knockout efficiencies > 90% via homologous recombination
- defined gravitropic response
- more closely related to dicots than monocots
- similar DNA usage
- used to determine cellular function of an Arabidopsis gene
Basic Architecture for Plant Functional Genomics

- Space Environmental Condition
- DNA Microarrays
- Test Plant RNA
- Image of a plant
Microarray Technology

- temporal and spatial gene expression
- Affymetrix Arabidopsis gene chips with over 8200 genes
- provides information on gene involvement in a process or pathway
Basic Architecture for Plant Functional Genomics

- Gene Isolation
- DNA Microarrays
- Space Environmental Condition
- Test Plant RNA
- Gene Isolation
Data Mining: Plant Genome Sequence Databases

The Arabidopsis Information Resource (TAIR)

The Institute for Genomic Research (TIGR)
Moss EST Sequence Databases

The Physcomitrella EST Program

University of Leeds, UK/Washington University, St. Louis, MO

Goal: 30,000 EST sequences
To Date: 14,410 moss EST sequences on GenBank

Physcomitrella Genomics Program

University of Freiburg/BASF

To Date: 120,000 EST sequences representing 22,000 different genes
Basic Architecture for Plant Functional Genomics

1. Space Environmental Condition
2. Test Plant RNA
3. DNA Microarrays
4. Gene Isolation
5. Gene Disruption
Functional Genomics

Gene function - analysis of gene malfunction

Conventional Approaches:
• manipulate the level of gene expression
• block the expression of a gene

Problems:
• non-directed integration of transgenes
• positional effects
• gene silencing due to co-suppression
• incorrect spatial and temporal expression
Targeted Gene Knock-out through Homologous Recombination
Regeneration following transformation of protoplasts

No selection Antibiotic selection
Basic Architecture for Plant Functional Genomics

Space Environmental Condition

Test Plant RNA

DNA Microarrays

Gene Isolation

Gene Disruption

Functional Analysis
Transfer strips of mutagenized tissue and allow to regenerate in gravity vector. Grow in darkness. Transfer to unilateral light source.
Feasibility of Antisense Knockouts: Can we get the oligos in?

Untransformed moss protoplasts with no fluorescein-tagged oligo

Untransformed moss protoplasts with fluorescein-tagged oligo

Moss protoplasts transformed with fluorescien-tagged oligo
Architecture Issues & Accomplishments

- Test plant *Arabidopsis thaliana* as model system to identify genes involved in the gravitropic response
- Optimize sample preparation for differential expression analysis
- Affymetrix Microarray analysis
- Identify genes whose expression increases
Establish *Physcomitrella patens* model system to determine function of genes

- propagation and maintenance
- protoplast isolation, transformation, and regeneration

Collaborative agreements with two sources for moss gene databases

Assemble molecular tools for gene knock-outs

- cDNA and genomic libraries
- two types of vectors
What’s Next?

Near Future:

- Increase repetitions for microarray analysis to confirm differential gene expression
- Construct replacement and knock-out vectors using available genomic and cDNA sequences
- Generate transformed moss and score for phenotype
The Future: 2002-2020

- Introduce additional environmental conditions into architecture
  - space-flown plant material
- Expand collaborations with Affymetrix and AVI Biopharma to develop remote technologies
- Adapt architecture to accommodate additional plant systems as data and technologies evolve
- Work with engineers to optimize architecture for space flight/habitat