New transcription factor based technology & tools for future crop improvement

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Mendel Biotechnology, Inc.

New Phytologist Tansley Symposium
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Timberline Lodge, Mt. Hood, Oregon USA
The challenge we need to improve crop productivity!

Ag. Biotechnology Product Timelines

First generation (mid 1990s)
- single gene traits
  - herbicide and insect resistance

Next generation (early 2010s)
- complex polygenic traits
  - e.g. yield and stress
- Based on whole pathway regulation

John Stringer & Linda Horton
Floral organ identity
- e.g. MADS domain proteins

Floral symmetry
- e.g. CYCLOIDEA-related proteins
  Luo et al., 1996, Nature 383

Transcription factors: master regulators of genetic pathways

Flowering time
- e.g. CONSTANS
  Putterill et al., 1995, Cell 80

Branching pattern
- e.g. teosinte branched (CYC-family)
  Doebley et al., 1997, Nature 386

Stress tolerance
- e.g. CBF genes (AP2 family)
  Stockinger et al., 1997, PNAS, 94
Arabidopsis thaliana transcription factor complement

~ 30,000 genes in genome
~ 6% encode transcriptional regulators
~ majority of TFs fall into gene families
Screens on transcription factors have produced hundreds of leads

**Developmental Traits...**
- Branching pattern
- Flowering time

**Physiological Traits...**
- Root vigor
- Disease resistance
- N-use
- Drought tolerance

**Biochemical Traits...**
- Glucosinolate composition
- Flavonoid production

**Seed size**
- OEX line
- WT

**OEX line**

**Wild type**
Typical Ag Biotech Product Development Process

Typical development timeline & costs:
- 10 -15 years
- $50M – $100M

Discovery -> Validation
- High throughput screening; lead validation in model crops.

Phase I - Crop proof of concept
- Test gene leads in crop plants; select best candidates.

Phase II - Early Product Development
- Field testing in crop Plants. Regulatory assessment started.

Phase III - Advanced Development
- Field testing in elite germplasm. Develop Regulatory data

Phase IV - Regulatory Approvals
- Field testing in elite germplasm. Develop Regulatory data

Market Launch

MOA work supports early-mid stage development

Adapted from: Monsanto 2003 Annual report
An understanding of MOA is important for successful commercialization

**Mode of Action analysis:**
- define phenotypic basis of the trait
- define pathways through which the leads act
  - => helps prioritize among leads
  - => support the future regulatory process/acceptance
  - => identify optimization strategies
- the genetic tools in Arabidopsis make it a valuable model

Trait of interest

1^TF

1^TF

Lead 1^TF

partner proteins?

repressor proteins?

2^TF

2^TF

2^TF

2^TF

Targets

Targets

Targets

Targets
Two general trait categories are of high priority

**Intrinsic yield e.g.**
- photosynthetic performance
- enhanced growth and vigor
- modification of architecture

**Yield stability (stress tolerance)**
- drought tolerance/water use efficiency
- cold/freezing tolerance
- N utilization
- fungal disease tolerance

For recent review see:
Century et al., 2008
Plant Physiology 147, 20-29
We need a “Blue Revolution” (Kofi Annan, 2000)

• Drought is the primary limitation to agriculture

• Many TFs have been implicated in drought responses
  - Mostly from large TF families:
    AP2/EREBPs, bZIP, NAC, MYB, MYC, C2H2, WRKY
  - Recent comprehensive reviews in:
    Umezawa et al., 2006 and Bhatnagar-Mathur et al., 2008

Nuclear Factor Y (NF-Y) transcription factor family

- binds CCAAT box elements
- single copy TFs in yeast & mammals with roles in:
  - energy metabolism
  - cell cycle
- family greatly expanded in plants emerging roles in plant in:
  - flowering time
  - photosynthesis biology
  - drought responses

NF-YB1 – a promising drought tolerance technology

35S::NF-YB1

wild type

8 days drought

After Re-water
**NF-YB1** is part of a native drought response

**NF-YB1** is upregulated ~12 fold by severe drought
NF-YB1 regulates a novel genetic pathway for control of drought responses

- Microarray fingerprint shows little overlap with well characterized drought response pathways (ABA and CBF pathway).

- Key targets observed in 35S::NF-YB1 profiles
  • photosynthesis components
  • pathways for synthesis of protective pigments
  • components of energy metabolism

- Key components of phenotype
  • increased WUE
  • increased chlorophyll levels
  • maintenance of turgor during stress
  • higher rate of photosynthesis during stress
Both The Genes and Control Pathways Are Conserved Across Arabidopsis....

Non-transgenic control  AtNF-YB1 Transgenic  Non-transgenic control  AtNF-YB1 Transgenic  Non-transgenic control  ZmNF-YB1 Transgenic

....soybean,  cotton...  ...and corn

Courtesy of Monsanto Company
Field trial results in corn

Significant Yield Advantage obtained on dry acres

- yr 1 (Base 74 bu/A)
- yr 2 (Base 102 bu/A)

% Yield Improvement

Line 1
Line 2
Line 3

year 1: single, 6-replication drought efficacy trial using a Split-Split-Split block design in Kansas
year 2: multi-location study using group block design with 3 replicates within each location

Nelson et al., 2007, PNAS 104: 16451
New tools to meet the future goals of plant biotech

Excerpt from Monsanto press release from June 04, 2008

“... Monsanto will double yield in its three core crops of corn, soybeans and cotton by 2030, compared to a base year of 2000...”
Chemical approaches: complementary to genetics for modulating transcription factor (TF) regulated pathways

Genetics
Transgene regulation via constitutive, tissue-specific or conditional promoters

Chemistry
Pathway regulation via inhibition or activation of signaling components

Trait of interest
(e.g. abiotic stress tolerance, disease resistance, enhanced growth)
Reporter lines enable quantification of pathway induction

Promoters are selected using
- Public data (genetic pathways)
- Proprietary data (transcriptional profiling (TxP) analysis)

An ideal promoter
- Conserved across species
- Induced specifically in the pathway of interest
- Expressed in leaf/cotyledon tissue
- Downstream of multiple regulatory pathways
Compound imparts drought tolerance on soil-grown plants

Arabidopsis

DMSO

Compound (350µM)

Survival following drought treatment and rewater

Barley

CONTROL

H144217
(600g/ha)

H144217
(1000g/ha)

Courtesy Bayer CropScience AG
Systems biology will be important in delivering future technology.

Integrated systems biology map of molecular interaction networks

Network knowledge directs future rounds of discovery work

High resolution maps depend on high quality in-vivo TF x DNA and TF x protein interaction data at the level of the individual cell-type.

e.g. combination of 1 + 2 + 3 gives fitness peak
Ribotag: an emerging tool for cell-type specific expression studies

RNA isolation and expression analysis

IP of polyribosomes

Total lysate

Stable transformation of Arabidopsis

Reference: profiling of RNA associated with polysomes via tagging of RPL18

prom LexA:Gal4 E9

opLexA HFRPL18 ocs-t

opLexA GFP term3

in cis in same construct
Validation: dramatic enrichment of specific markers in the phloem

SUC2 ribotag line

35S ribotag line

**SUC2 Expression**

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<th>35S ribotag mature leaf</th>
<th>SUC2 ribotag mature leaf</th>
<th>SUC2 ribotag mature leaf</th>
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**ATG01520 Expression**

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**AHA3 Expression**

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Projected timelines for biotechnology products

- **Impact of molecular biology**
  - first generation (mid 1990s)
    - single gene traits – herbicide and insect resistance

- **Impact of Genomics**
  - next generation (early 2010s)
    - complex traits – yield and stress
    - initial lead technologies identified from screens

- **Impact of Systems Biology & Chemistry**
  - later generations (> 10 years hence)
    - super-enhanced complex traits – yield and stress
    - optimal intervention points in regulatory pathways
      - rationalized from “holistic” knowledge of entirety of pathways
Concluding remarks

- TF-based technologies are excellent candidates for enhancement of crop yield and stress tolerance.
- Genetics and chemistry offer complementary approaches to pathway regulation.
- Systems biology will enable future generations of super-enhanced products.
- Plant biologists have a critical role to play in ensuring a sustainable future.