



seeding a sustainable future



EPSO Workshop on Plant Productivity for Food

Industry Perspective:
How to translate basic
findings to improve crop
productivity?

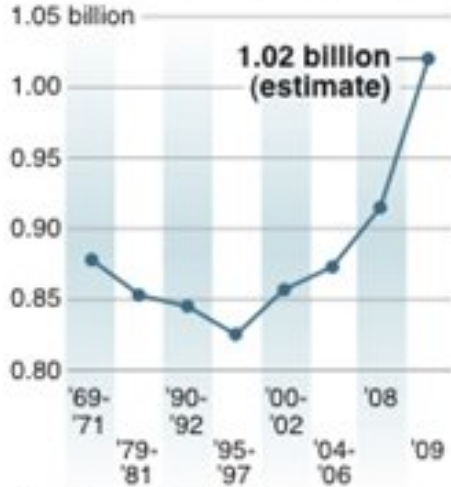
**Peter Repetti, Research Director
Mendel Biotechnology**

Ghent, Belgium
September 8, 2009

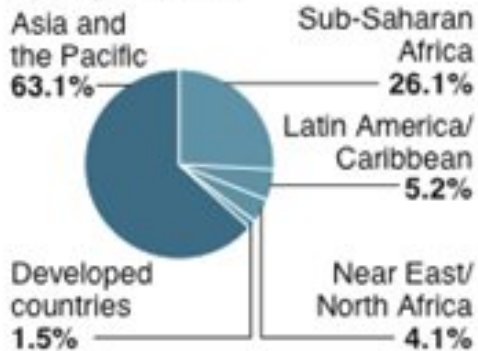
Hunger on the rise

The global number of undernourished people surpassed 1 billion. The vast majority are in Asia and Sub-Saharan Africa.

Number of undernourished



Undernourished by region, 2009



SOURCE: Food and Agriculture Organization of the United Nations AP

UN: World hunger reaches 1 billion mark
By ALESSANDRA RIZZO – Jun 19, 2009
Mendel Biotechnology 2009 – ALL RIGHTS RESERVED

The Challenge



we need to improve crop productivity!

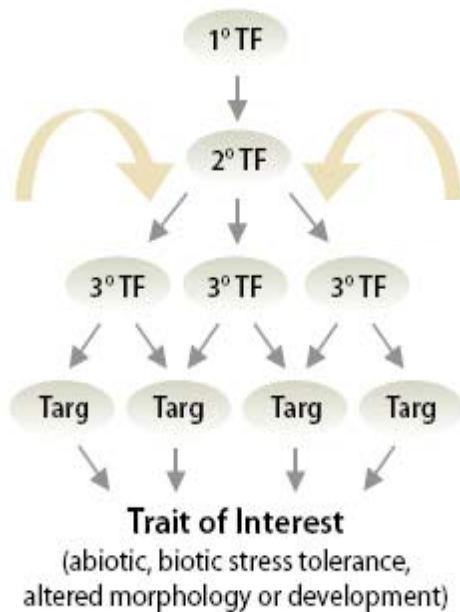


Population estimated to reach 50 billion by 2010

John Stringer & Linda Horton

Mendel was Founded Based on the Power of Transcription Factors

TFs are Master Regulators

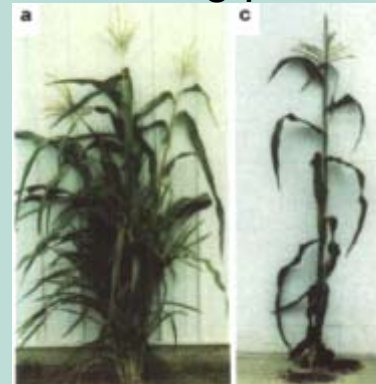


Floral symmetry



CYCLOIDEA-related proteins
Luo et al., 1996, Nature 383

Branching pattern



Doebley et al., 1997, Nature 386

Flowering time

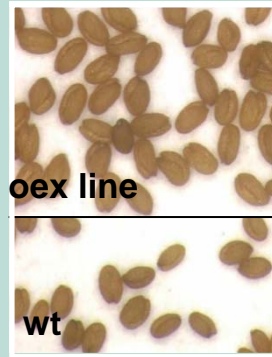


CONSTANS Putterill et al., 1995, Cell 80

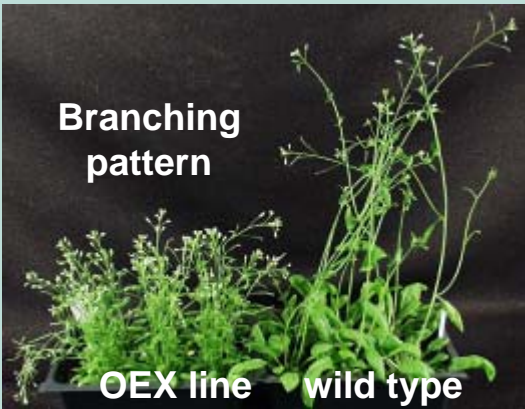
Screens on Transcription Factors Have Produced Hundreds of Leads

Developmental Traits...

seed size



Branching pattern

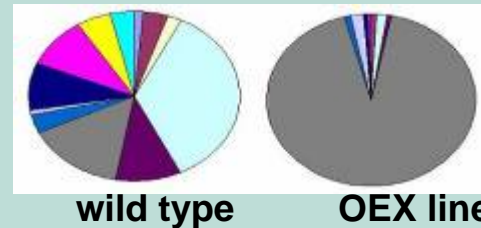


flowering time



Biochemical Traits...

Glucosinolate composition

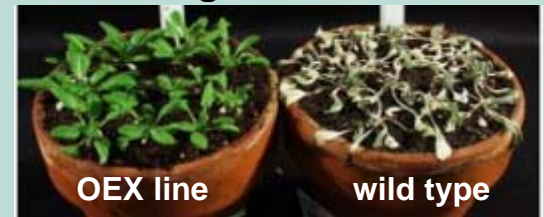


Flavonoid production

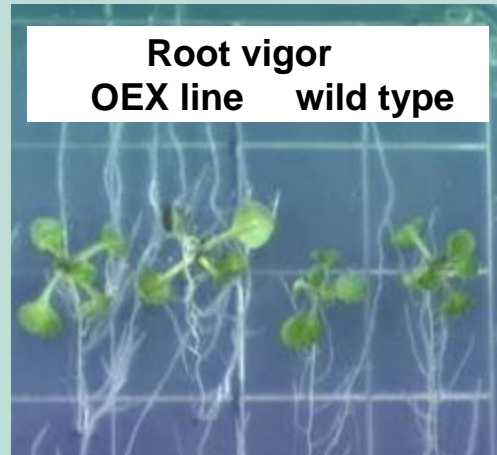


Physiological Traits...

Drought tolerance



Root vigor
OEX line wild type

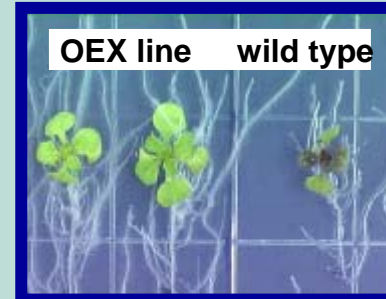


OEX line wild type



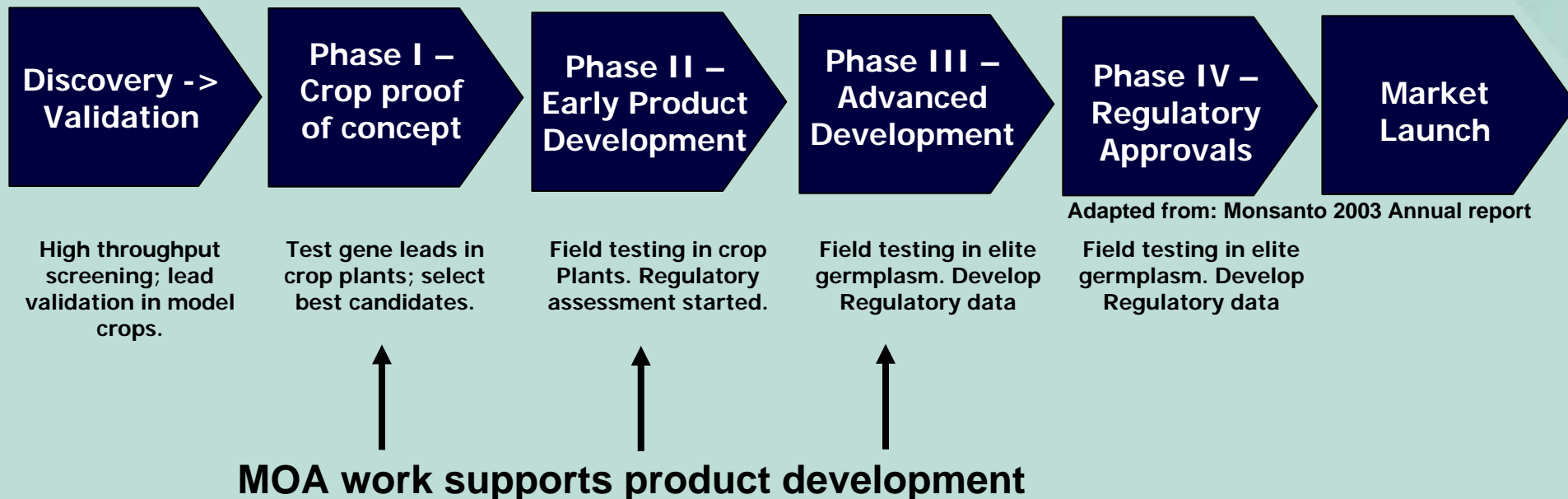
Disease resistance

N-use



Typical Ag Biotech Product Development Process

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



Mode of Action analysis:

- define phenotypic basis of the trait
 - identify optimization strategies
 - the genetic tools in Arabidopsis make it a valuable model

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**

Two General Trait Categories are of High Priority

Intrinsic yield

- photosynthetic performance
- enhanced growth and vigor

Yield stability (stress tolerance)

- drought tolerance/water use efficiency
- cold/freezing tolerance
- N utilization
- disease tolerance



For recent review see: Century et al., 2008, Plant Physiology 147, 20-29

Nuclear Factor Y (NF-Y) Transcription Factor Family

NF-YB1
a promising
drought tolerance
technology

8 days
drought

35S::*NF-YB1*



wild type



After
Re-water



Both The Genes and Control Pathways Are Conserved Across Arabidopsis....



Non-transgenic control

AtNF-YB1 Transgenic

....soybean,



Non-transgenic control

AtNF-YB1 Transgenic

cotton...



Non-transgenic control

ZmNF-YB1 Transgenic

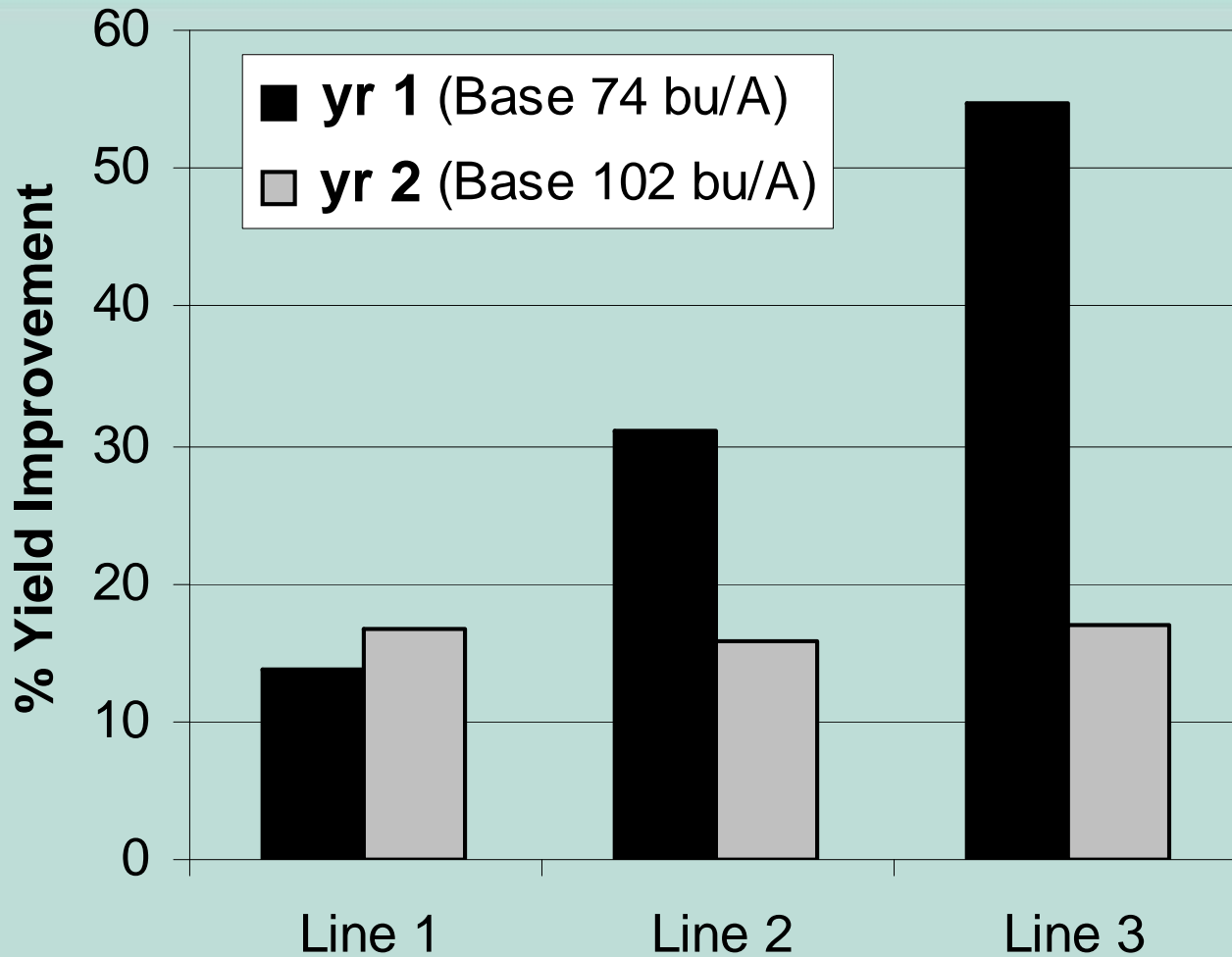
...and corn

Courtesy of Monsanto Company



Field Trial Results in Corn:

Significant Yield Advantage Obtained on Dry Acres



Courtesy of
Monsanto Company

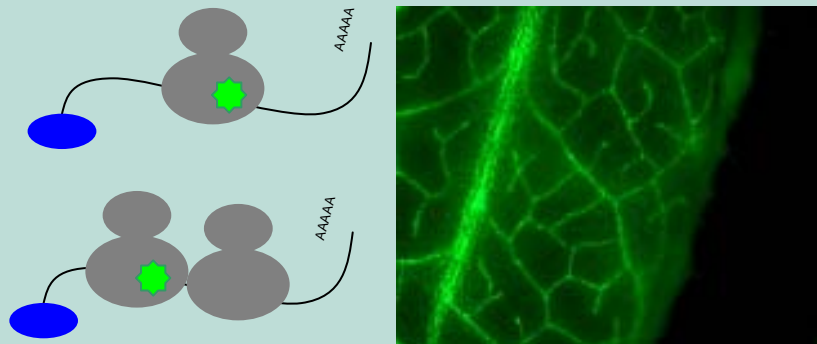
year 1: single, 6-replication drought efficacy trial using a Split-Split-Split block design in Kansas
year 2: multi-location study using group block design with 3 replicates within each location

**New tools are required to meet
the future goals of agriculture**

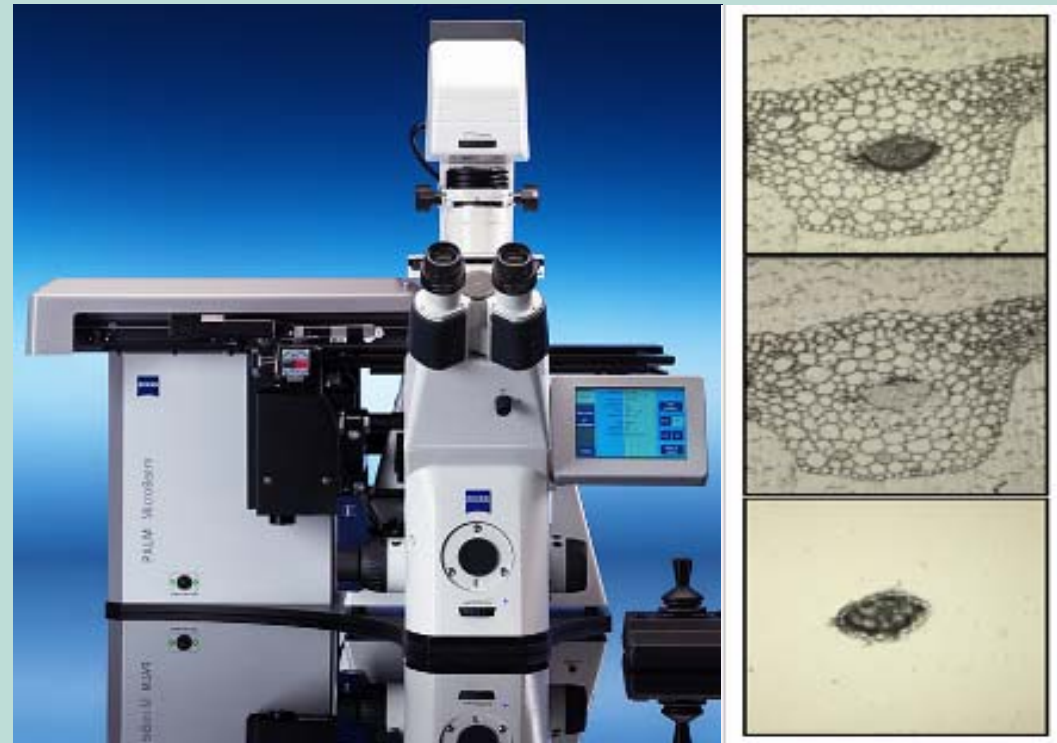
Epitope-tagged Polysomes and Laser Capture Microscopy

Two different techniques to isolate cell-specific transcripts:

• Ribotag
(expressed from cell-specific promoter)

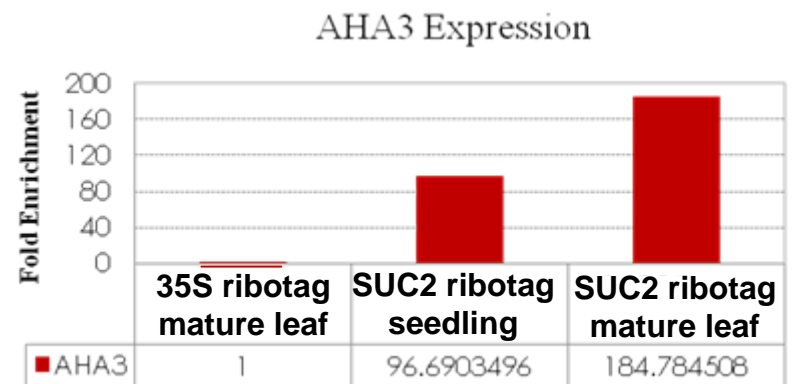
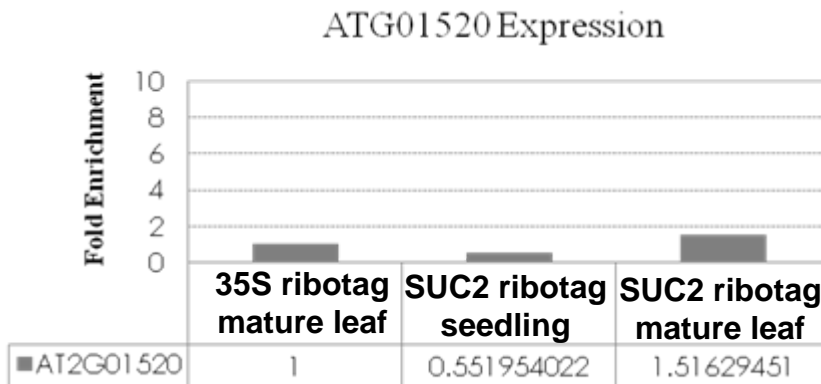
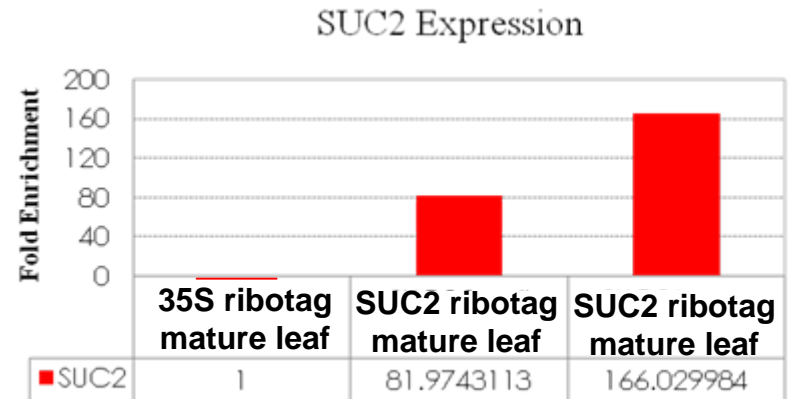
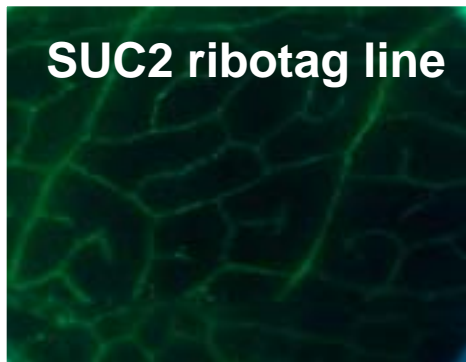


Laser capture microdissection





Validation: Dramatic Enrichment of Specific Markers of the Phloem



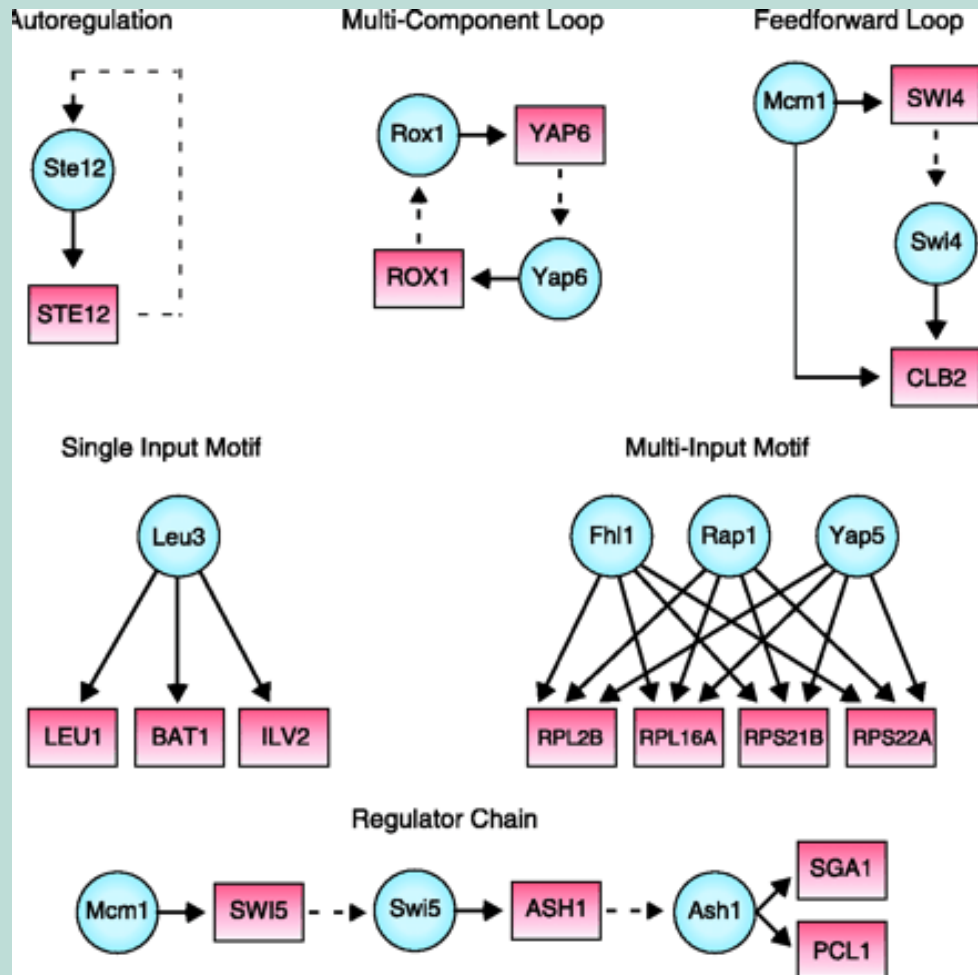
Mendel Microarray Study Revealed Several Thousand New Phloem-enriched Genes

A number of TFs are highly-enriched in this tissue type, including;

Fold	p	p-BH	Ctrl	Exp	Description
92.7	0	0	0.04	4.42	GARP/myb family
25	8.60E-27	2.10E-25	0.08	2.15	SWI/SNF
24.2	9.70E-42	5.40E-40	0.1	2.54	MYB-related
23.6	0	0	0.22	5.41	GARP/myb TF
23.2	6.70E-37	2.90E-35	0.13	3.12	NAC
22.1	0	0	0.09	1.98	homeobox-leucine zipper protein
20.3	8.20E-33	2.90E-31	0.18	3.78	GARP/myb family transcription factor
20.1	3.20E-33	1.20E-31	0.13	2.56	NAC
17.9	2.40E-41	1.30E-39	0.04	0.76	zinc finger CONSTANS-related
16.4	0	0	0.33	5.5	squamosa-like

ChIPSeq is a Powerful Technique for Analysis of Regulatory Networks Controlled by TFs

Transcriptional Regulatory Networks in *Saccharomyces cerevisiae*
Lee et al., Science 25 October 2002:



ChIP data have been critically important in assembling network maps in yeast

22 DECEMBER 2000
Science

REPORTS

Genome-Wide Location and Function of DNA Binding Proteins

Bing Ren,^{1*} François Robert,^{1*} John J. Wyrick,^{1,2*} Oscar Aparicio,^{2,4} Ezra G. Jennings,^{1,2} Itamar Simon,¹ Julia Zeitlinger,¹ Jörg Schreiber,¹ Nancy Hannett,¹ Elenita Kanin,¹ Thomas L. Volkert,¹ Christopher J. Wilson,⁵ Stephen P. Bell,^{2,3} Richard A. Young^{1,2†}

Understanding how DNA binding proteins control global gene expression and chromosomal maintenance requires knowledge of the chromosomal locations at which these proteins function in vivo. We developed a microarray method that reveals the genome-wide location of DNA-bound proteins and used this method to monitor binding of gene-specific transcription activators in yeast. A combination of location and expression profiles was used to identify genes whose expression is directly controlled by Gal4 and Ste12 as cells respond to changes in carbon source and mating pheromone, respectively. The results identify pathways that are coordinately regulated by each of the two activators and reveal previously unknown functions for Gal4 and Ste12. Genome-wide location analysis will facilitate investigation of gene regulatory networks, gene function, and genome maintenance.

We Have Recently Applied the “ChIPSeq” Technique to TF-Overexpressing Plants

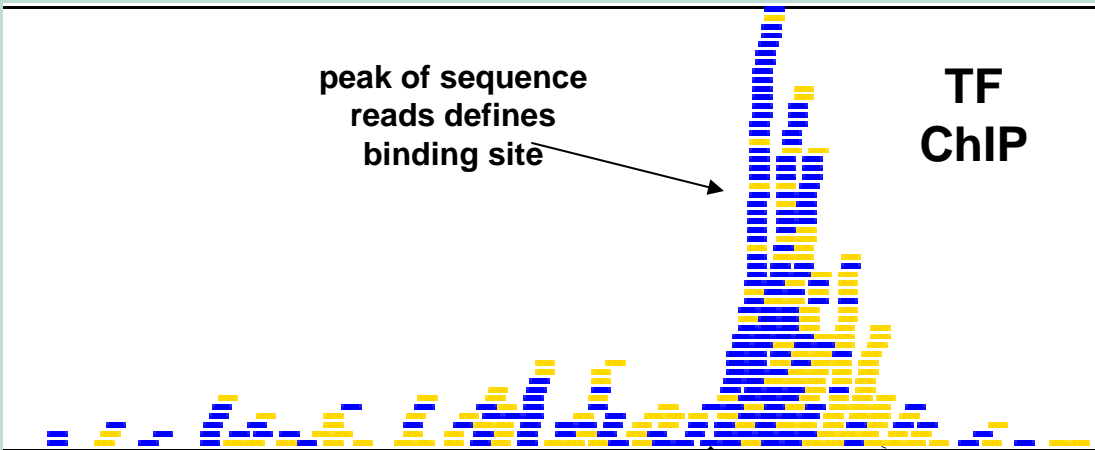
Mendel data

Input

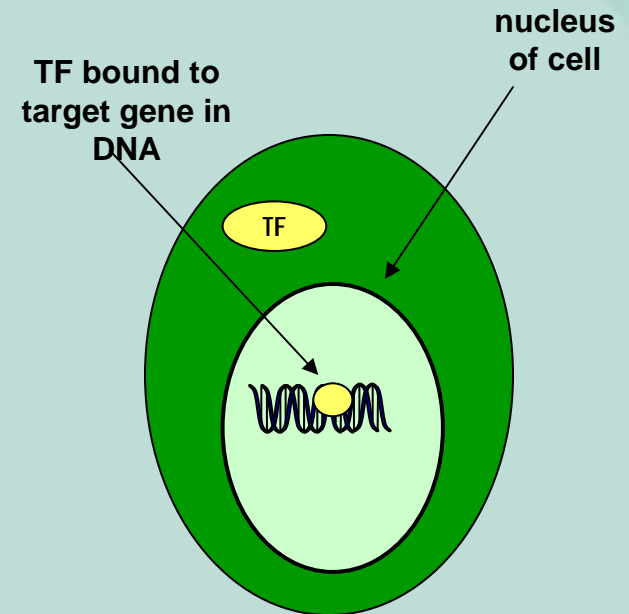


peak of sequence reads defines binding site

TF
ChIP

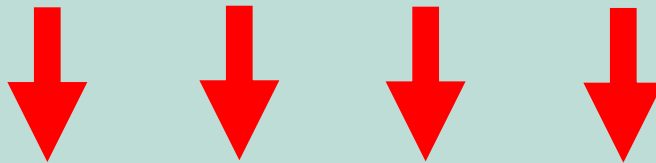
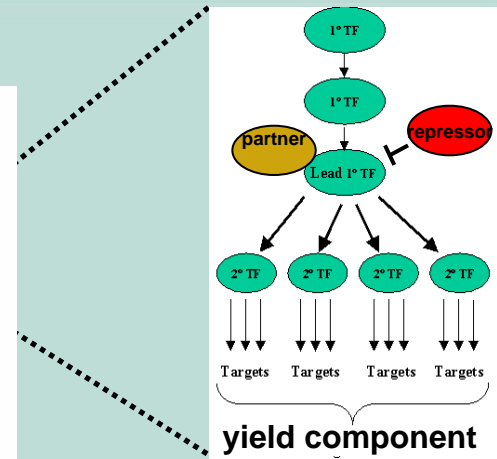
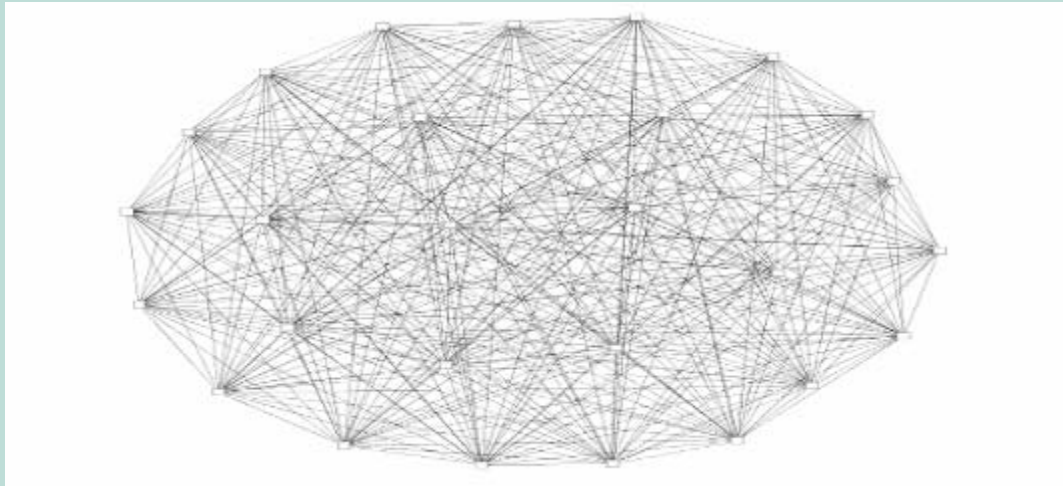


Promoter Target gene



identifies binding sites of TFs in DNA

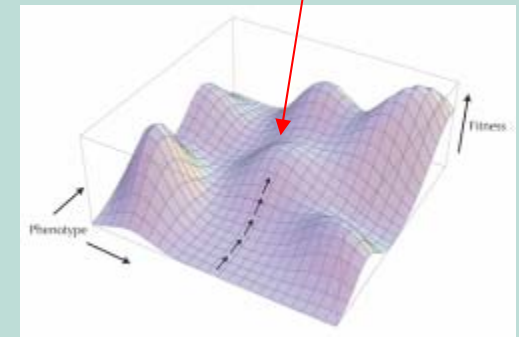
Systems Biology Will be Important in Delivering Future Technology



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



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**

A stylized green leaf icon consisting of three curved lines, positioned to the left of the word "END".

END



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

Ref: Pennisi, 2008. The Blue Revolution, Drop by Drop, Gene by Gene. Science 320: pp. 171 - 173