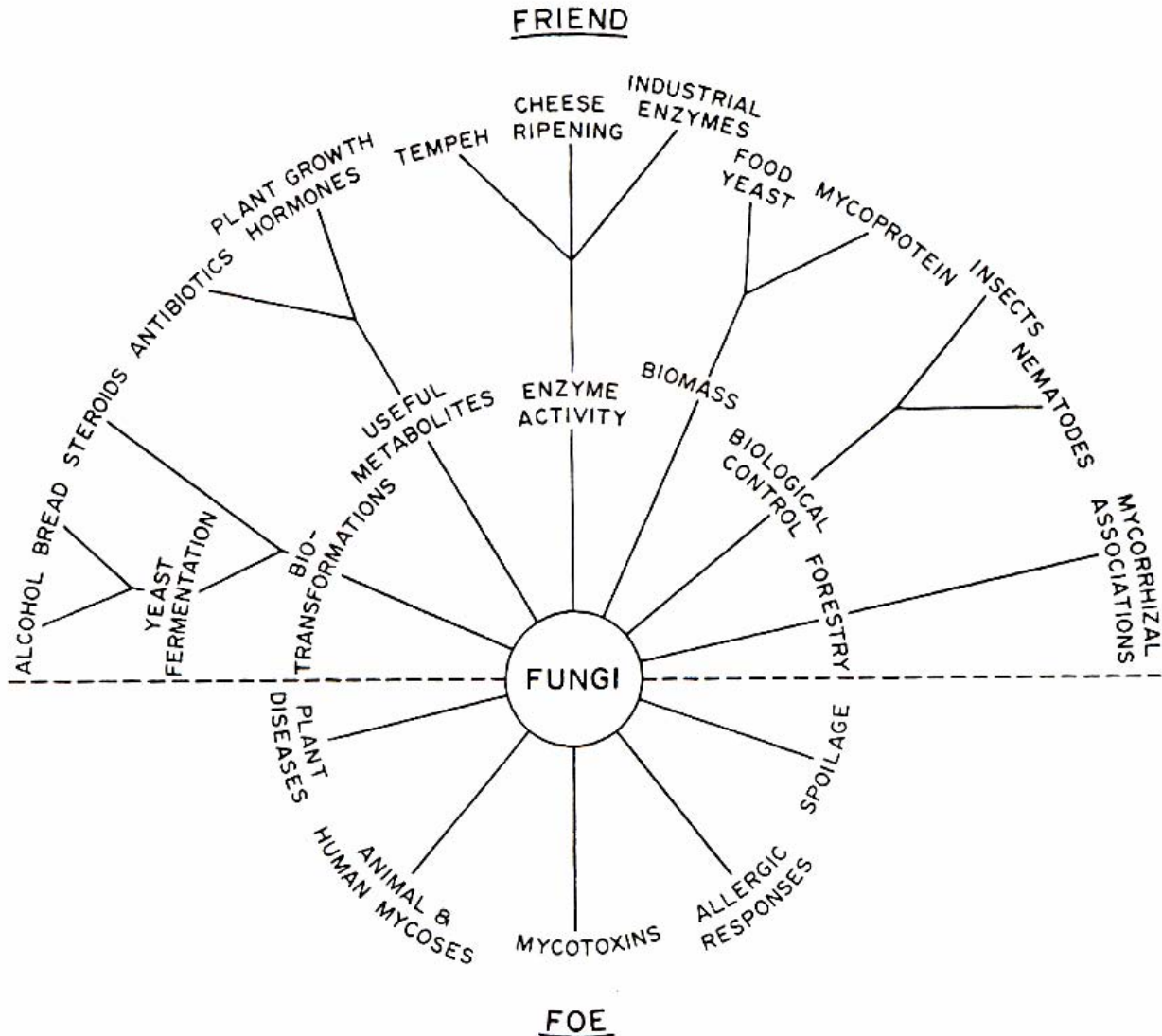


# ***Aspergillus* and biotechnology**

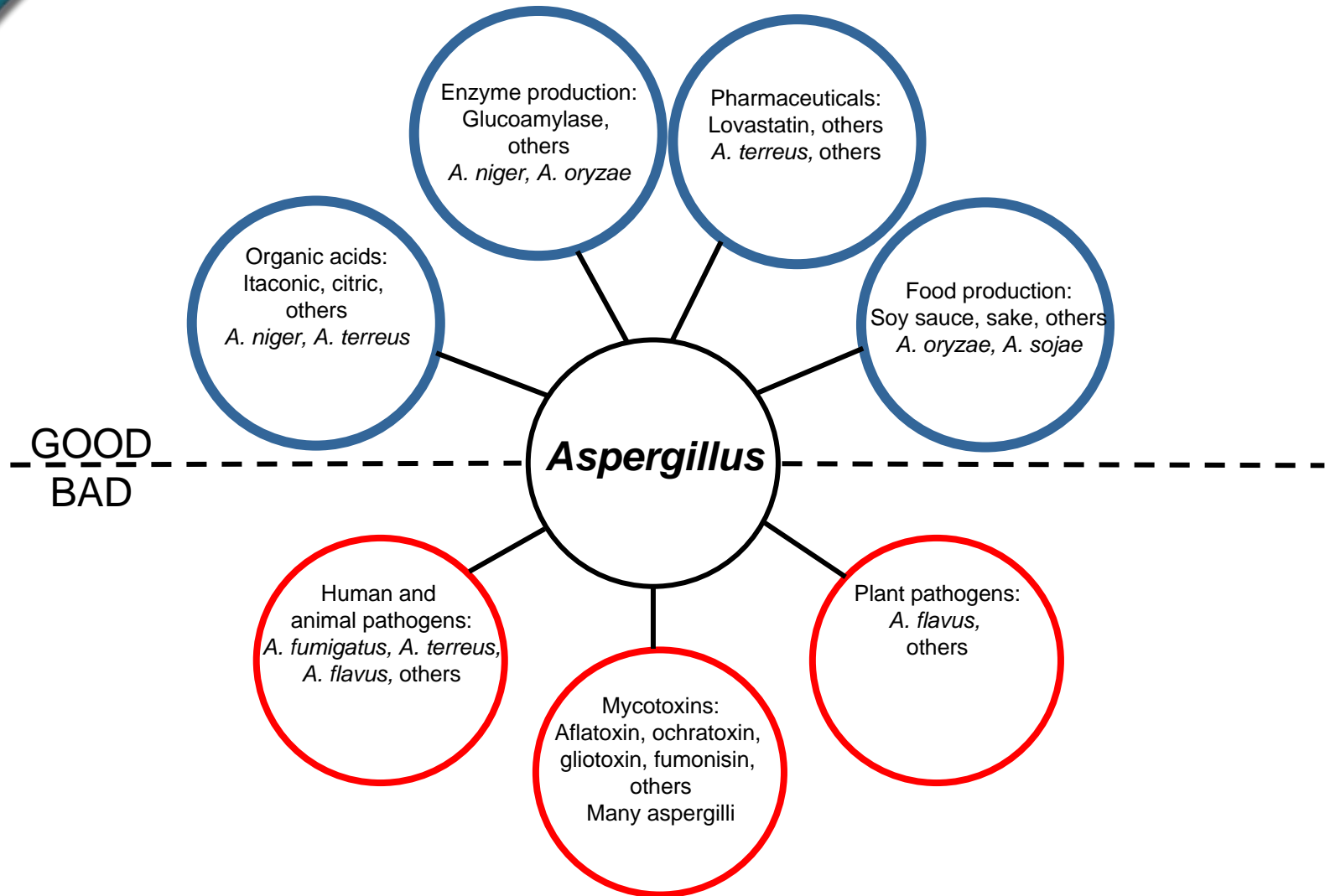
Scott E. Baker  
Pacific Northwest National Laboratory  
Advances Against Aspergillosis  
Miami, Florida  
19 January 2008



# The world of the mycologist...



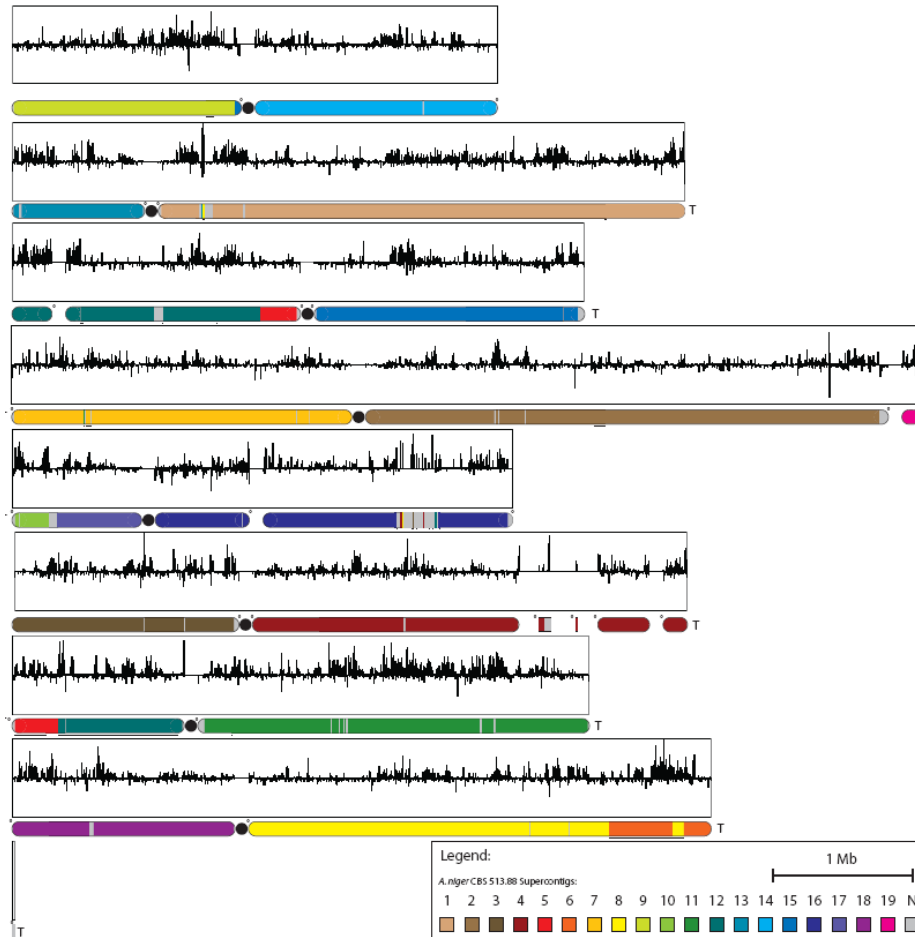
# Aspergillus...



## **DOE Joint Genome Institute (JGI) *Aspergillus niger* Genome Project**

- ▶ Proposed to the US DOE Microbial Genome Program by the PNNL Fungal Biotechnology team
- ▶ Genome project funded by DOE SC OBER (direct to JGI)
- ▶ Collaboration with DOE's Joint Genome Institute
- ▶ Current status
  - Final draft coverage: ~7.88X shotgun
  - EST libraries constructed from RNA isolated from citric acid production and complex biomass digestion conditions~30,000 sequenced
  - Annotation: In collaboration with JGI/LANL
  - Gap closure – by Stanford-JGI
  - 454 sequencing – eight runs
  - Public release: April 2006
- ▶ Anticipated publication spring 2008

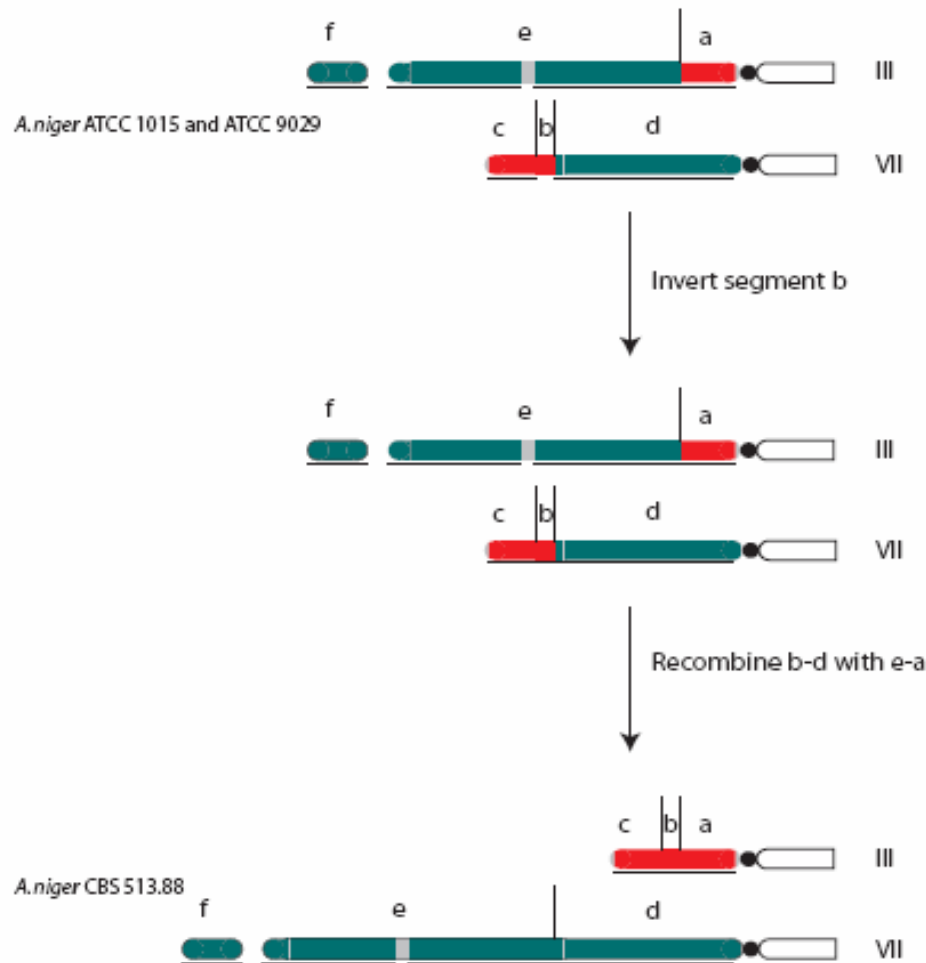
# Aspergillus niger Genomics



24 Scaffolds, no gaps

SNP level:  
CBS 513.88 and  
ATCC 9029 vs  
ATCC 1015

## Chromosomal translocation in *Aspergillus niger* strain CBS 513.88



# ATCC 1015 vs CBS 513.88

(organic acid producer vs protein producer)

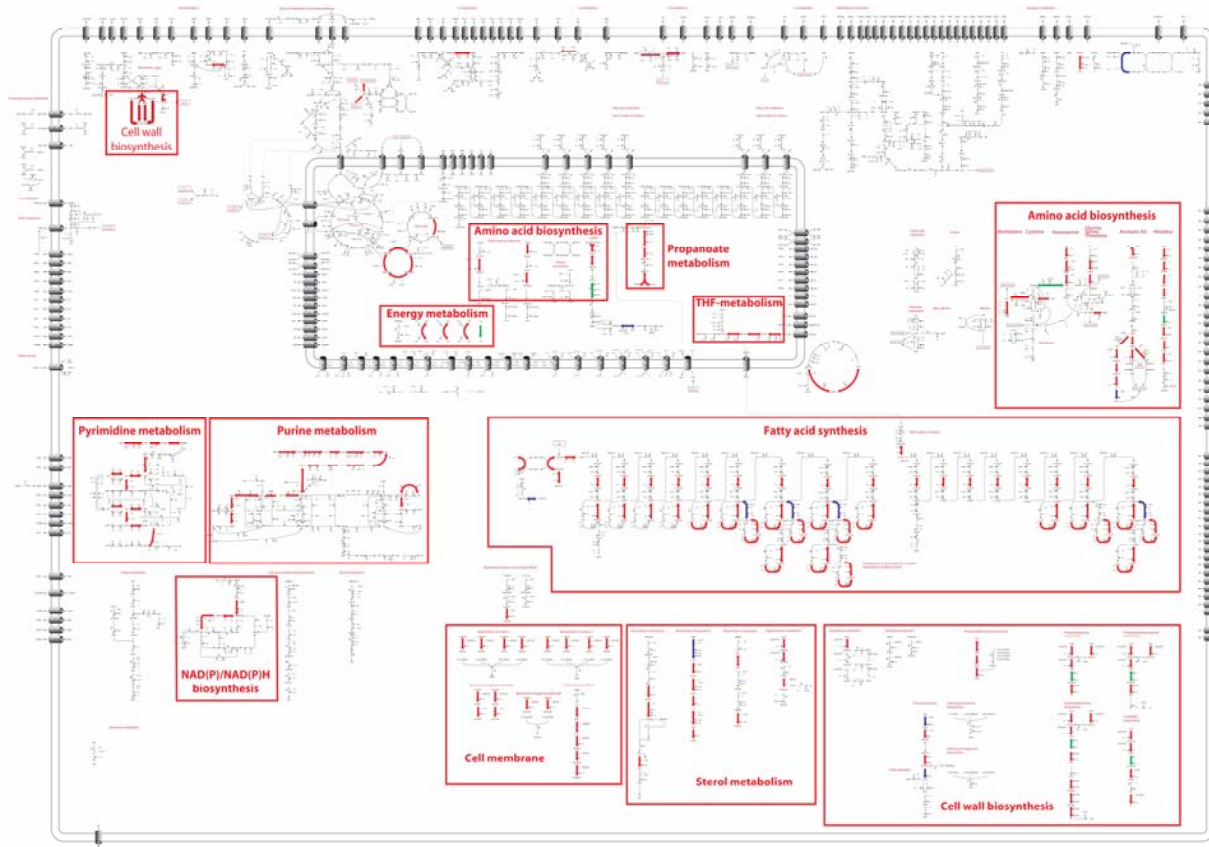
- ▶ Genomic sequence comparison
  - CBS 513.88 likely has a mutated *areA* gene
  - Relative to ATCC 1015 and ATCC 9029, two major chromosomal rearrangements in CBS 513.88
  - Codon usage is less restricted in ATCC 513.88
- ▶ Transcriptome comparison (high glucose batch fermentation)
  - ~2x more glucoamylase expression but ~6X more activity in CBS 513.88
  - Upregulation of tryptophan, serine and threonine biosynthetic pathways (glucoamylase has high tryptophan, serine and threonine content vs. the average *A. niger* protein)



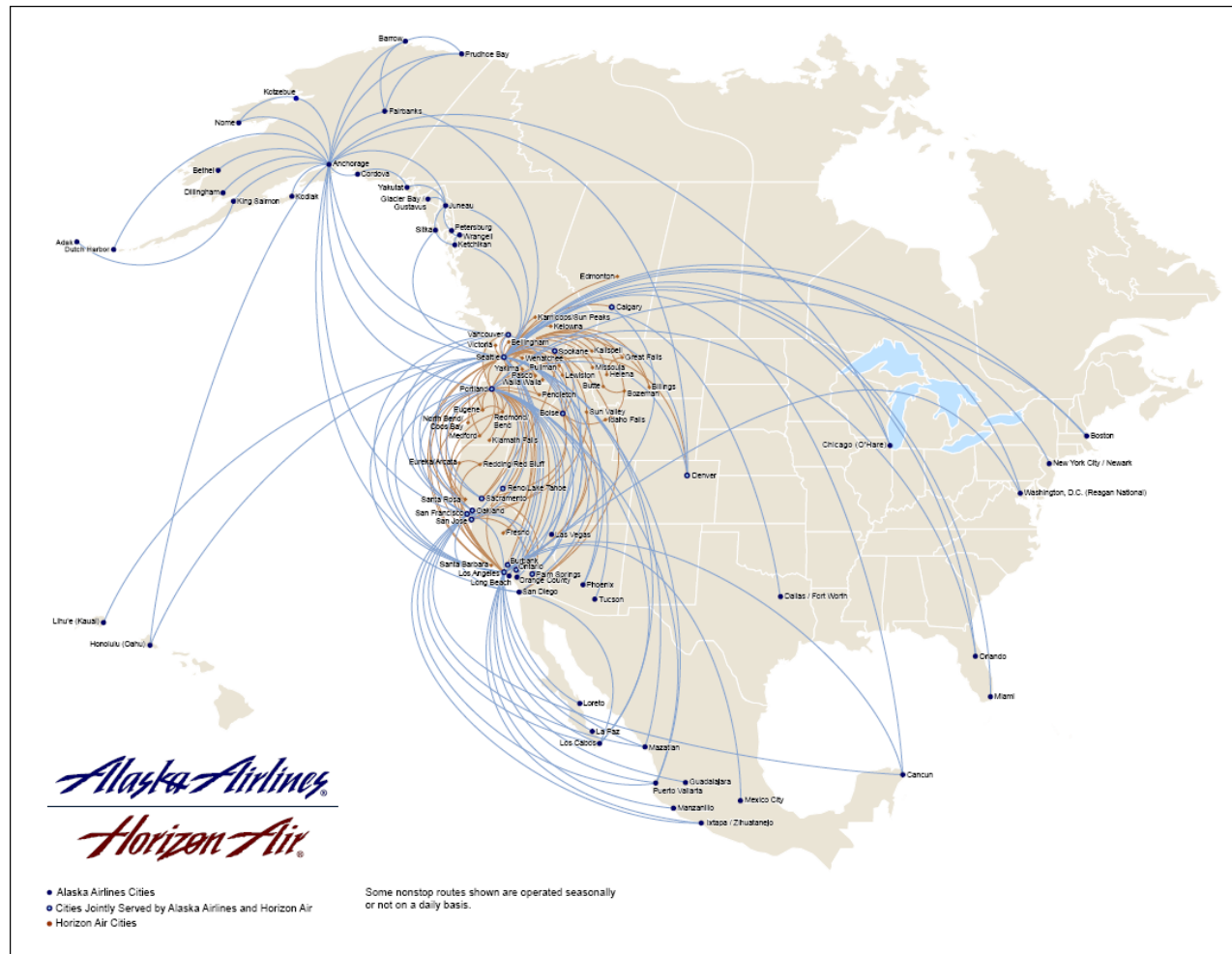
# A computational metabolic model for *Aspergillus niger*

Based on the...

- Bibliome
- Genome
- Metabolome



# Metabolic networks and airline route maps



# Solving the airline route map



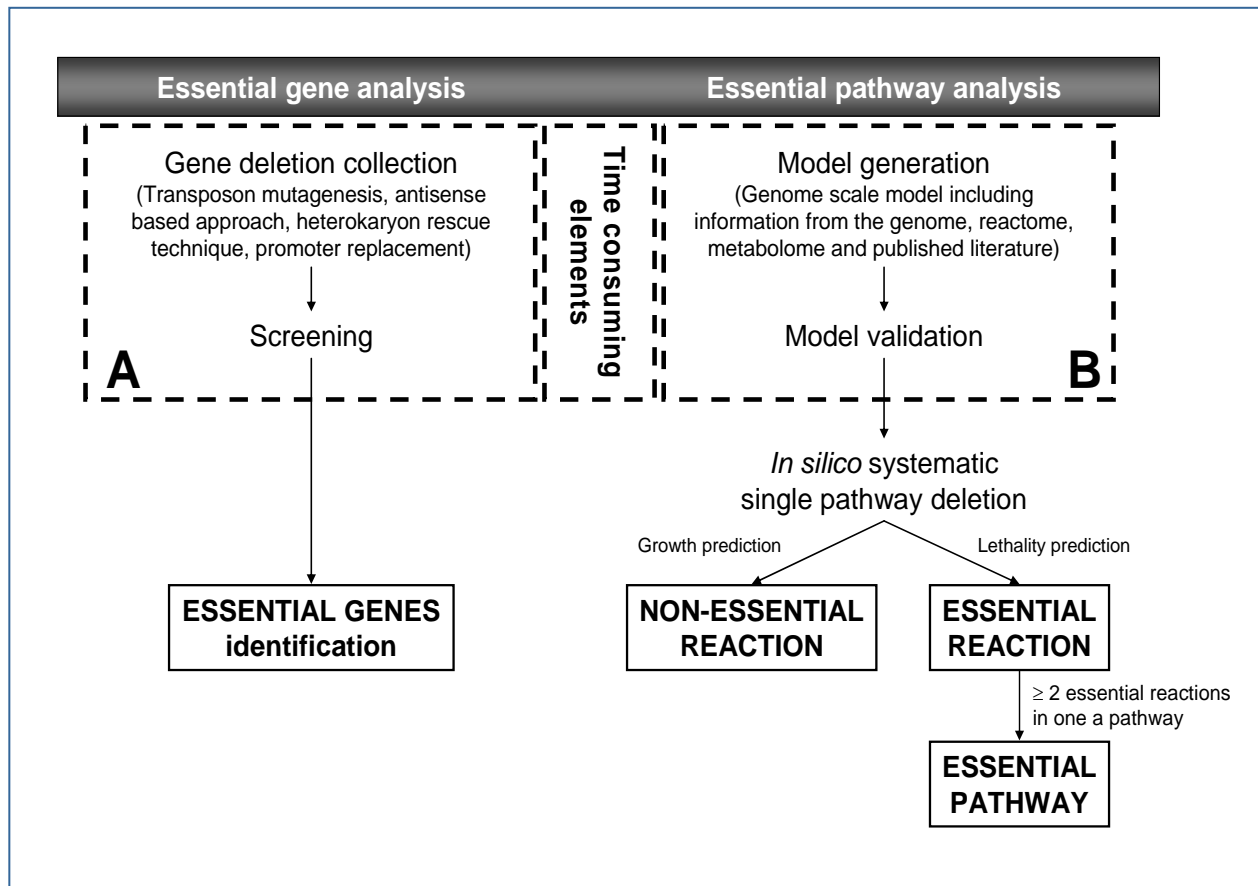
# Essential airports



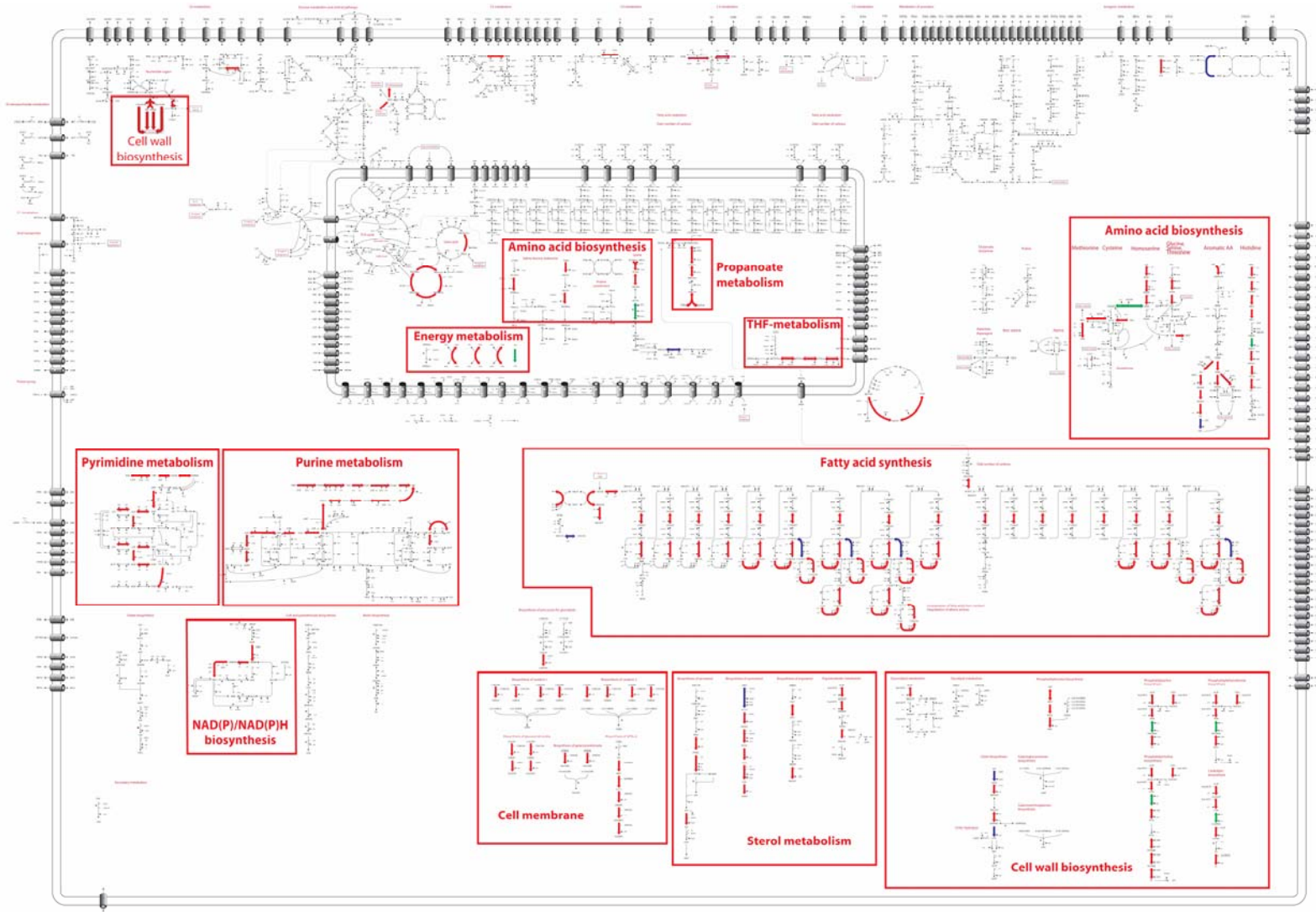
# Essential airports



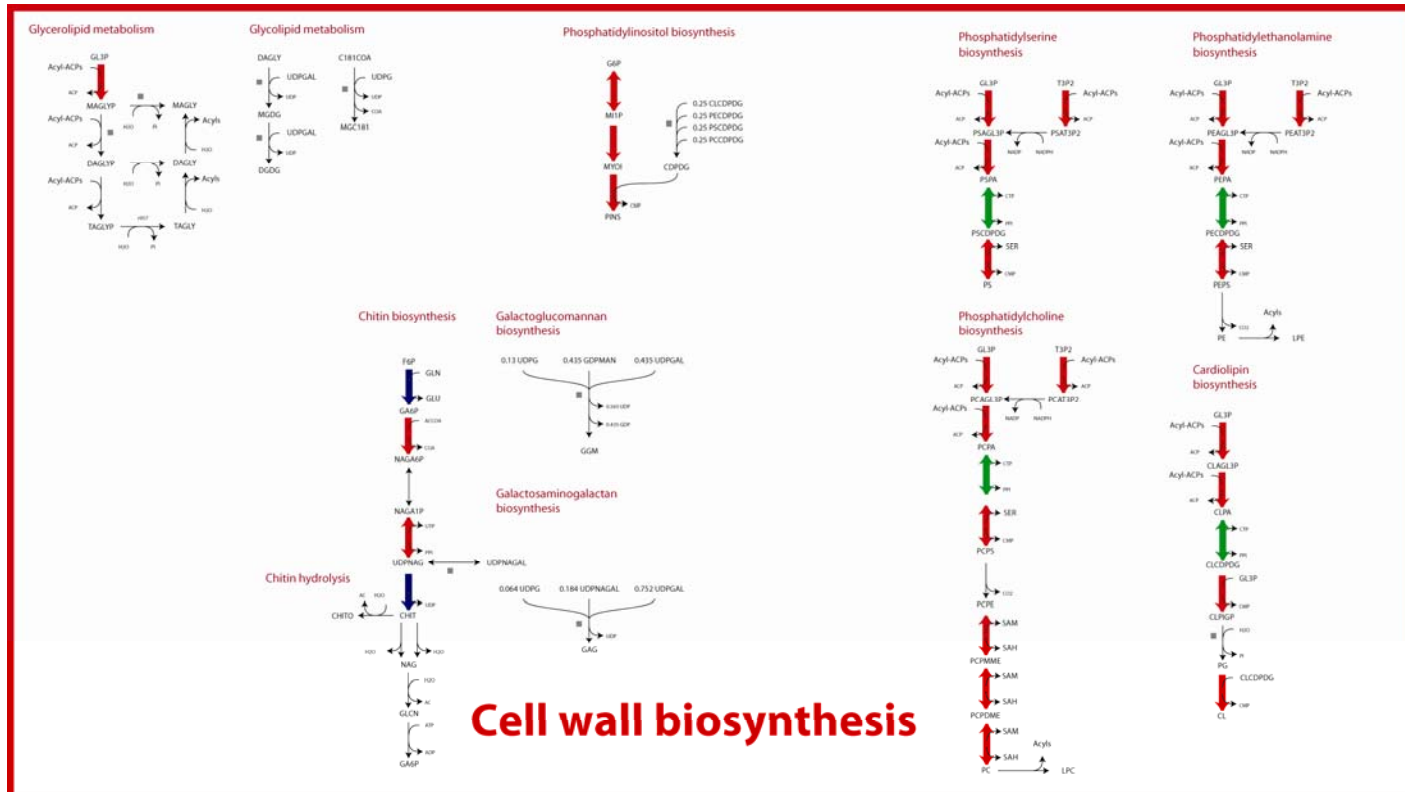
# Pathways to essentiality



# Essential reactions and pathways



# A close up of cell wall biosynthesis





# Future directions

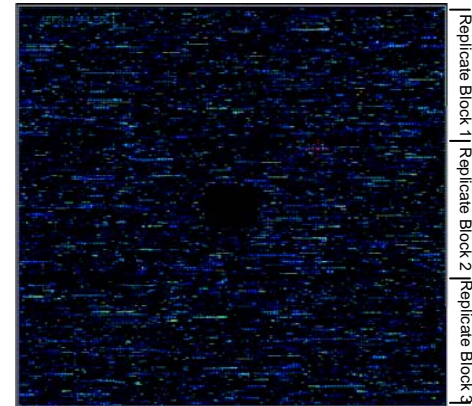
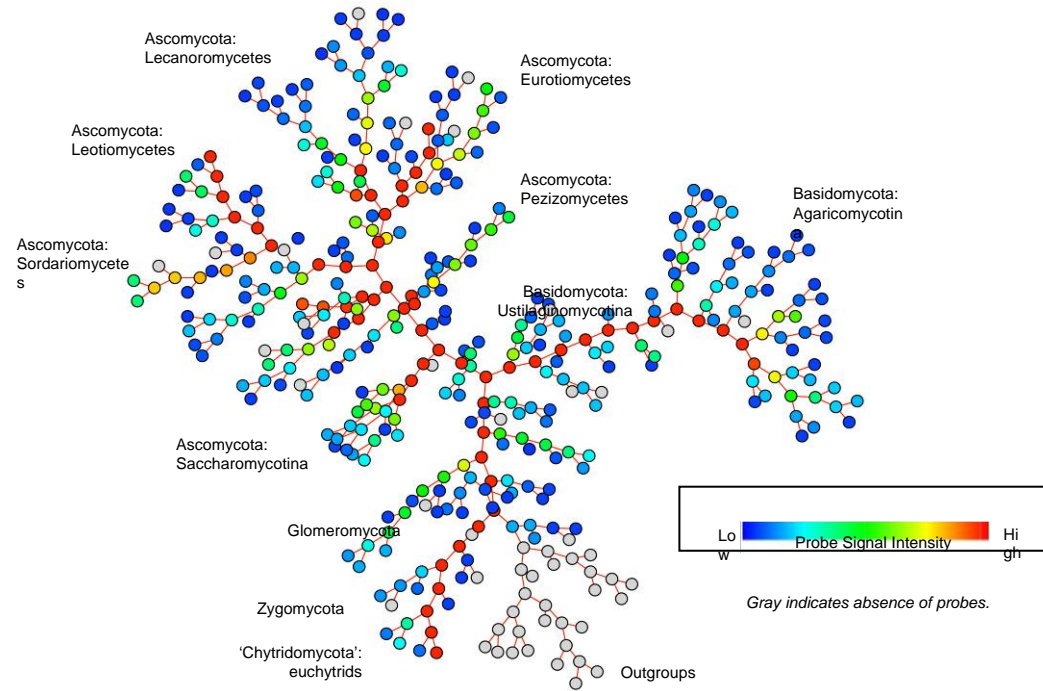
- ▶ *Aspergillus fumigatus* specific model
- ▶ Deletion studies of predicted essential genes
- ▶ Tests of deletion strains in mouse model

# Predicted *A. fumigatus* essential genes (without close human BLAST hits)

EC no.	Essential enzyme	<i>A. fumigatus</i> gene ID	Additional info
<b>Amino acids biosynthesis</b>			*
5.4.99.5	Chorismate mutase	Afu5g13130	Aromatic
2.4.2.18	Anthranilate phosphoribosyl transferase	Afu4g11980	Aromatic
2.1.1.17	Phosphatidylethanolamine N-methyltransferase	Afu2g15970	Aromatic
4.2.1.51	Prephenate dehydratase	Afu5g05690	Aromatic
2.5.1.54	3-deoxy-7-phosphoheptulonate synthase	Afu1g02110	Aromatic
4.2.1.19	Imidazoleglycerol-phosphate dehydratase	Afu6g04700	His
3.5.4.19	phosphoribosyl-AMP cyclohydrolase	Afu1g14570	His
3.1.3.15	Histidinol phosphatase	Afu4g04030	His
1.1.1.23	Histidinol dehydrogenase	Afu1g17660	His
2.4.2.17	ATP phosphoribosyltransferase	Afu7g04500	His
1.1.1.3	Homoserine dehydrogenase	Afu3g11640	Lys, Gly, Ser, Thr
2.7.2.4	Aspartate kinase	Afu5g05590	Lys, Gly, Ser, Thr
1.2.1.11	aspartate-semialdehyde dehydrogenase	Afu3g06830	Lys, Gly, Ser, Thr
2.3.3.14	Homocitrate synthase	Afu4g10460	Lys
1.1.1.86	Ketol-acid reductoisomerase	Afu3g14490	Val, Leu, Ile
2.3.1.31	homoserine O-acetyltransferase	Afu5g07210	Met
2.1.1.13	Methionine synthase	Afu4g07360	Met
<b>Propanoate metabolism</b>			
4.1.3.30	2-methylisocitrate lyase	Afu6g02860	
4.2.1.79	2-methylcitrate hydrolyase	Afu6g03730	
<b>Fatty acid biosynthesis</b>			*
2.1.1.71	Methylene-fatty-acyl-phospholipid synthase	Afu1g09050	
2.3.1.38	[ACP]acetyltransferase	Afu3g04220	
2.3.1.85	Fatty-acid synthase	Afu3g04210	
<b>Pyrimidine and purine metabolism</b>			
1.8.1.9	Thioredoxin reductase	Afu6g09740	
2.4.2.10	Orotate phosphoribosyltransferase 1	Afu2g11290	
4.1.1.21	Phosphoribosylaminoimidazole carboxylase	Afu4g12600	
<b>Cell wall biosynthesis</b>			*
2.4.1.34	1,3-beta-Glucan synthase	Afu5g05770	
3.1.3.12	Trehalose-phosphatase	Afu3g05650	
<b>Sterol biosynthesis</b>			*
2.5.1.21	Squalene synthase	Afu7g01220	
2.7.4.2	Phosphomevalonate kinase	Afu5g10680	
<b>NADH/NADPH metabolism</b>			
2.4.2.19	Nicotinate mononucleotide pyrophosphorylase	Afu3g05730	
<b>Urea and metabolism of amino groups</b>			
2.3.1.35	Glutamate N-acetyltransferase	Afu5g08120	
<b>Manitol biosynthesis</b>			
1.1.1.17	Mannitol-1-phosphate 5-dehydrogenase	Afu2g10660	

# Fungal Tree of Life microarray

Target Sequence	GTAGGTGAACCTGCGGAAGGATCATTACTGAGTGCGGGCTGCCTCCGGGC
Probe 1	CATCCACTTGGACGCCCTCCTAGTA
Probe 2	ATCCACTTGGACGCCCTCCTAGTAA
Probe 3	TCCACTTGGACGCCCTCCTAGTAA
Probe 4	CCACTTGGACGCCCTCCTAGTAA
Probe 5	CACTTGGACGCCCTCCTAGTAA
Probe 6	ACTTGGACGCCCTCCTAGTAA
Probe 7	CTTGGACGCCCTCCTAGTAA
Probe 8	TGGACGCCCTCCTAGTAA
Probe 9	TGGACGCCCTCCTAGTAA
Probe 10	GGACGCCCTCCTAGTAA

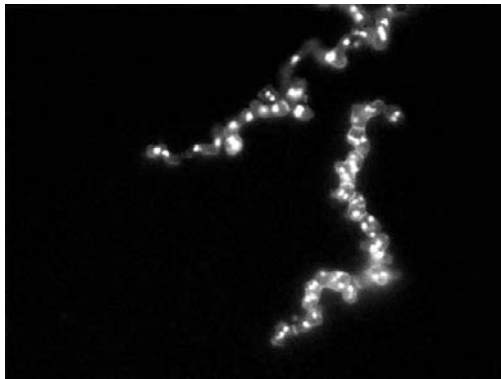


# Acknowledgements

## Essential Pathway Analysis

Jette Thykaer (DTU)

Mikael Andersen (DTU)



## PNNL Tree of Life Array Team

Jon Magnuson

Jim Collett

Jason McDermitt

David Culley

Rumin Tan

## JGI Aspergillus niger genome team

Mikael Andersen, DTU

Diego Martinez (JGI-LANL)

Igor Grigoriev (JGI-PGF)

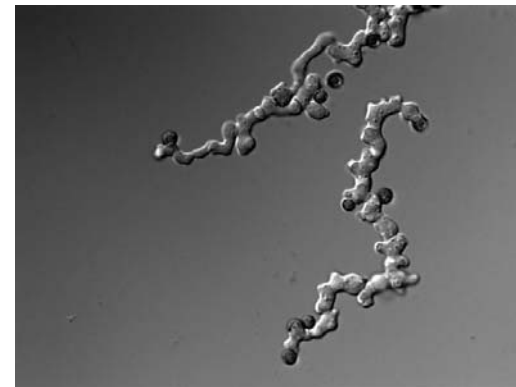
Jane Grimwood (Stanford-JGI)

Ziyu Dai (PNNL)

Erika Lindquist (JGI-PGF)

Hans Roubos (DSM)

Many, many, many others



US DOE Office of the Biomass Program

US DOE Office of Biological and Environmental Research

Danish Research Council