Genome-Scale Reconstruction of *Saccharomyces cerevisiae*

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2004 UC Systemwide Bioengineering Symposium

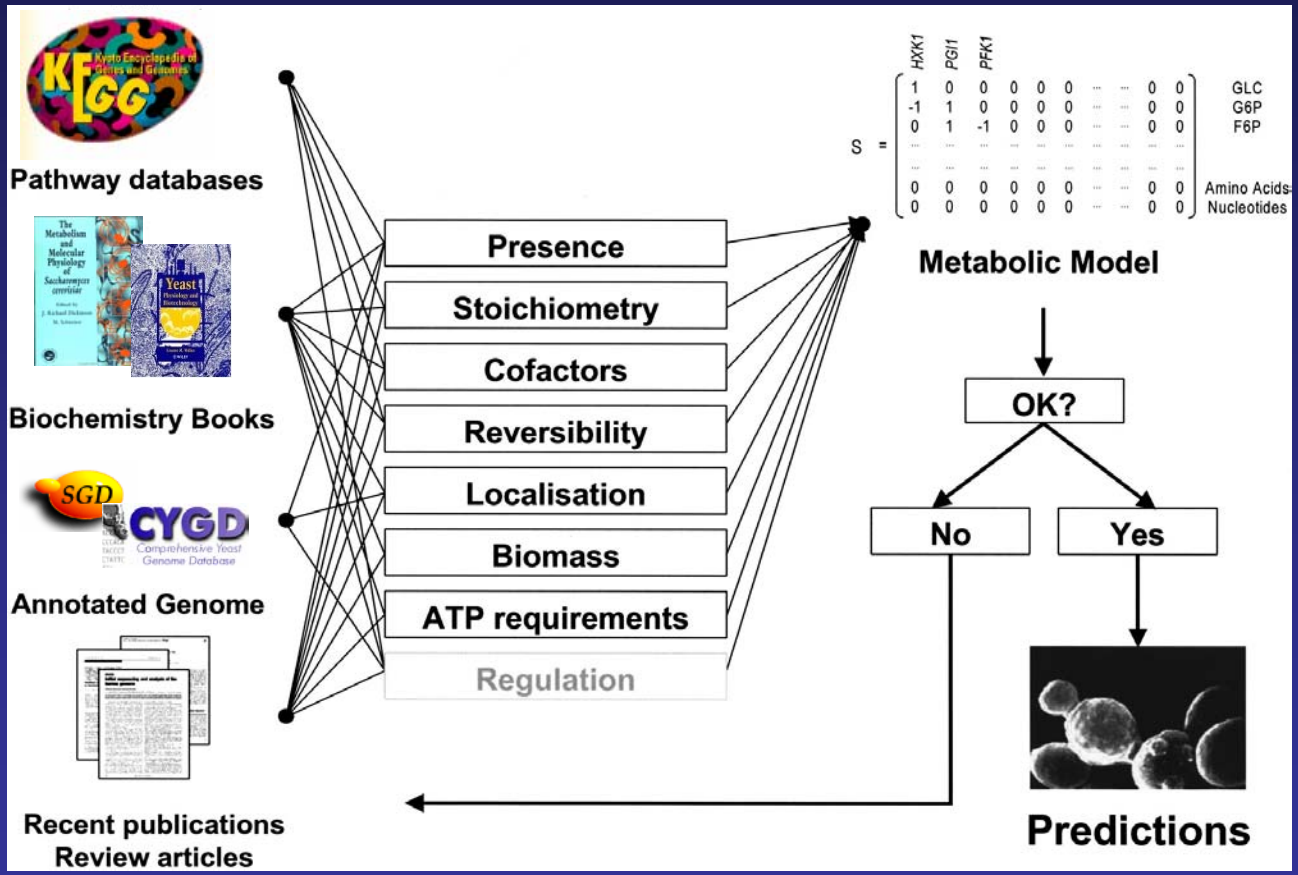
June 28, 2004

Saccharomyces cerevisiae

- Many basic cellular processes are generally conserved between yeast and higher eukaryotes, including mammals

- Yeast has many technical advantages that make it an excellent experimental system
  - Rapid growth
  - Highly versatile DNA transformation system
  - Nonpathogenic
  - Cheap and commercially available

Reconstruction of *S. cerevisiae* iFF708

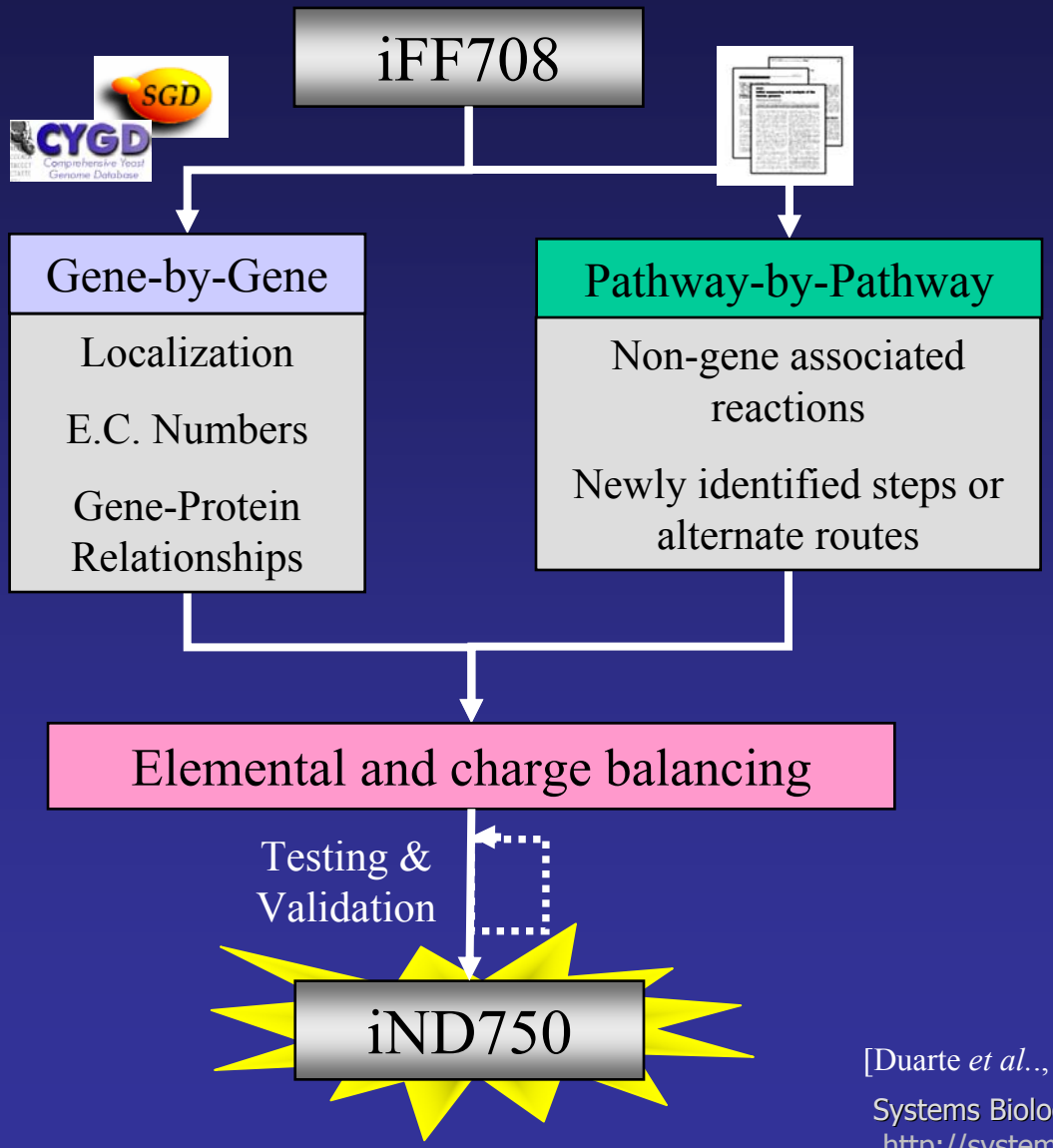


[Förster *et al*., Genome Res 2003]

Expanding and Updating the Model

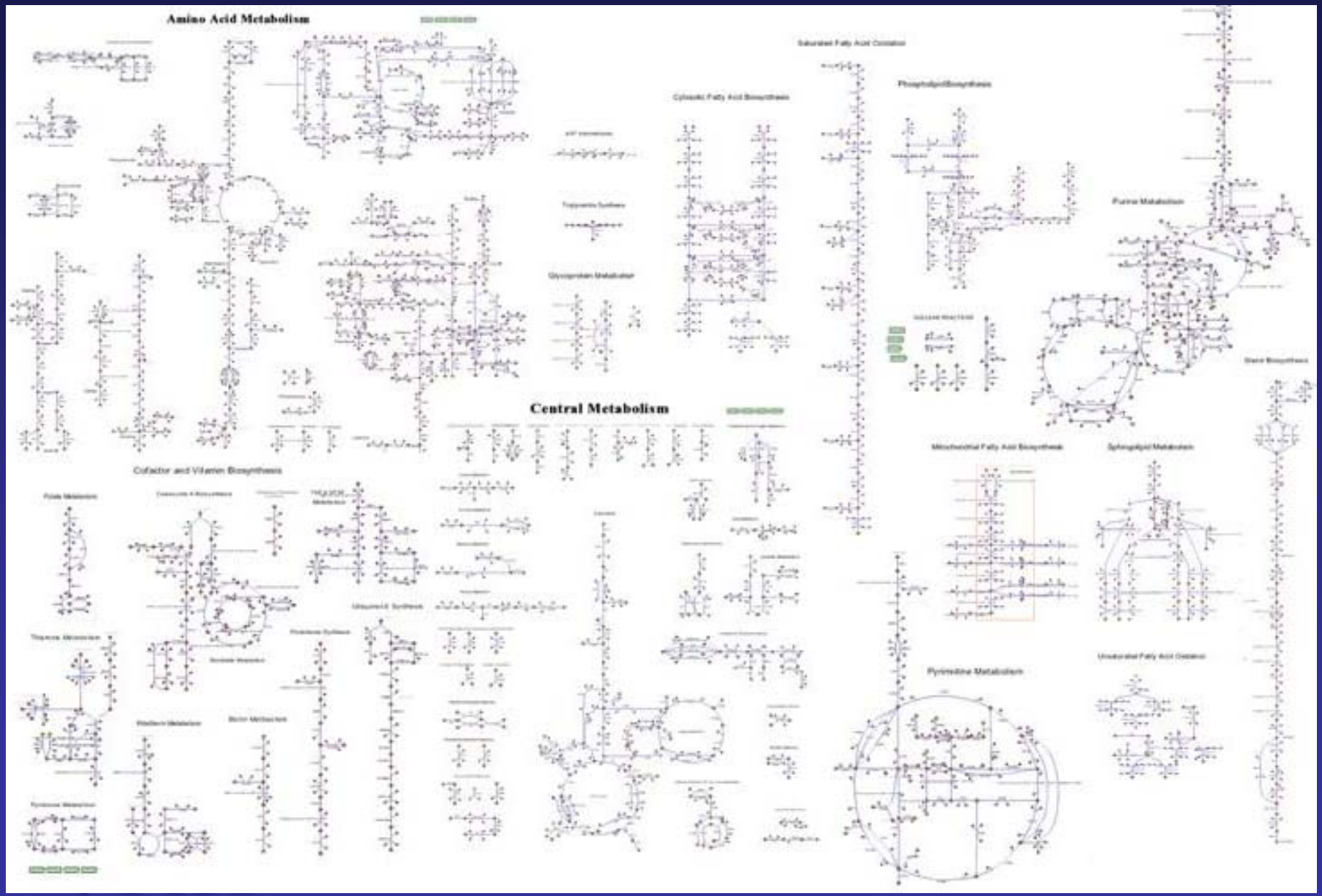
Model Evaluation

Network Assembly

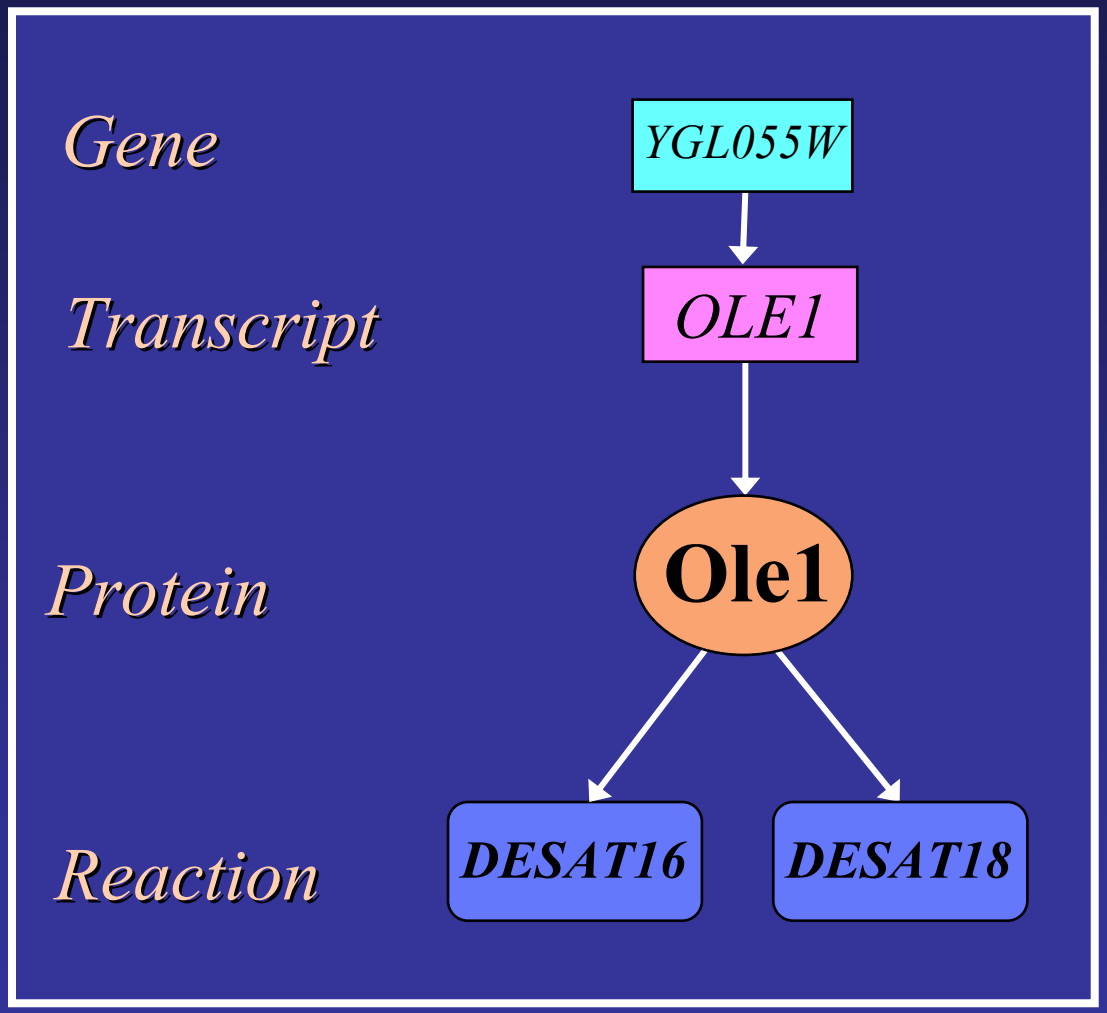


[Duarte *et al.*., Genome Res 2004]

iND750 Metabolic Network



Gene-Protein-Reaction Associations



Genomics
ORF annotation

Transcriptomics
mRNA levels

Proteomics
protein levels

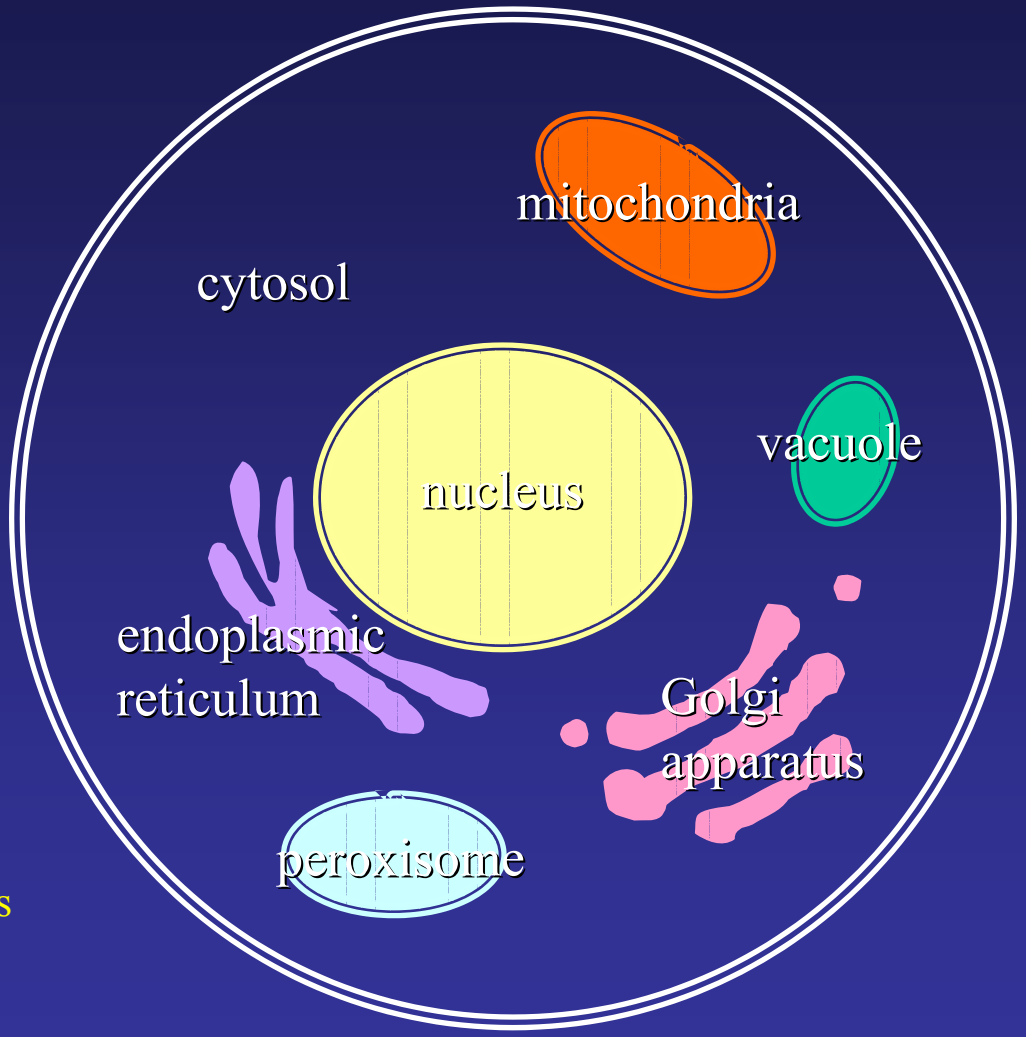
"Fluxomics"
flux measurements

Compartmentalization

Mitochondria
Oxidative phosphorylation

Nucleus
DNA replication, transcription, RNA processing

Endoplasmic Reticulum
Protein synthesis and modification, some lipid synthesis



Peroxisome
Toxic compound degradation

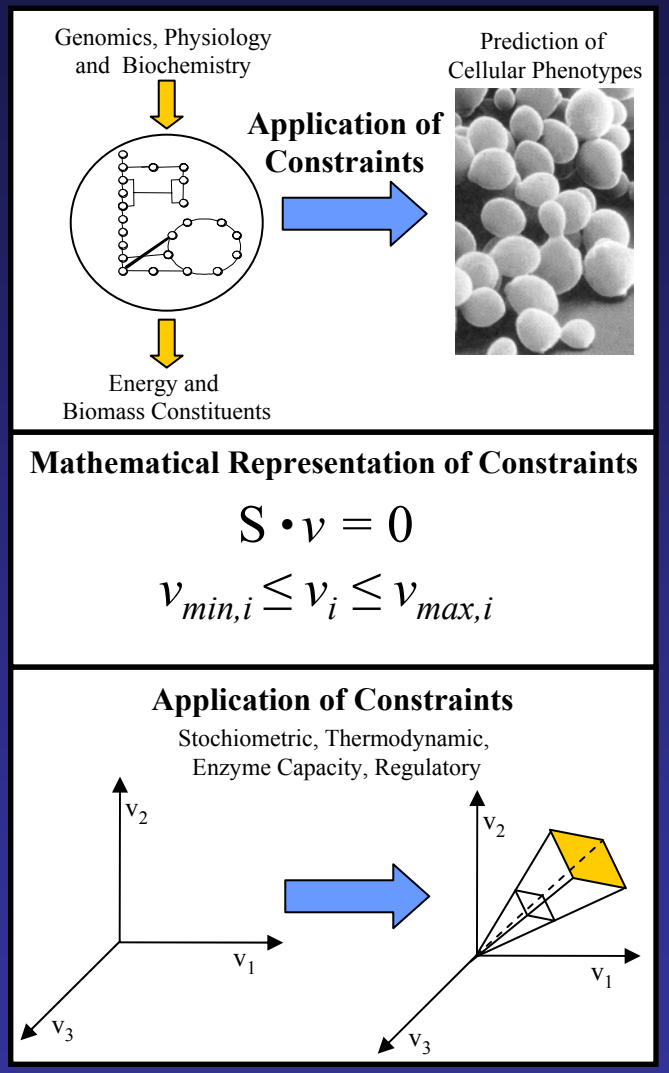
Vacuole
Storage component for food particles, water, and other components

Golgi Apparatus
Protein and lipid modification, storage, and packaging

Constraint-Based Analysis

How often have I said to you that when you have eliminated the impossible, whatever remains, however improbable, must be the truth?

–Sherlock Holmes,
A Study in Scarlet



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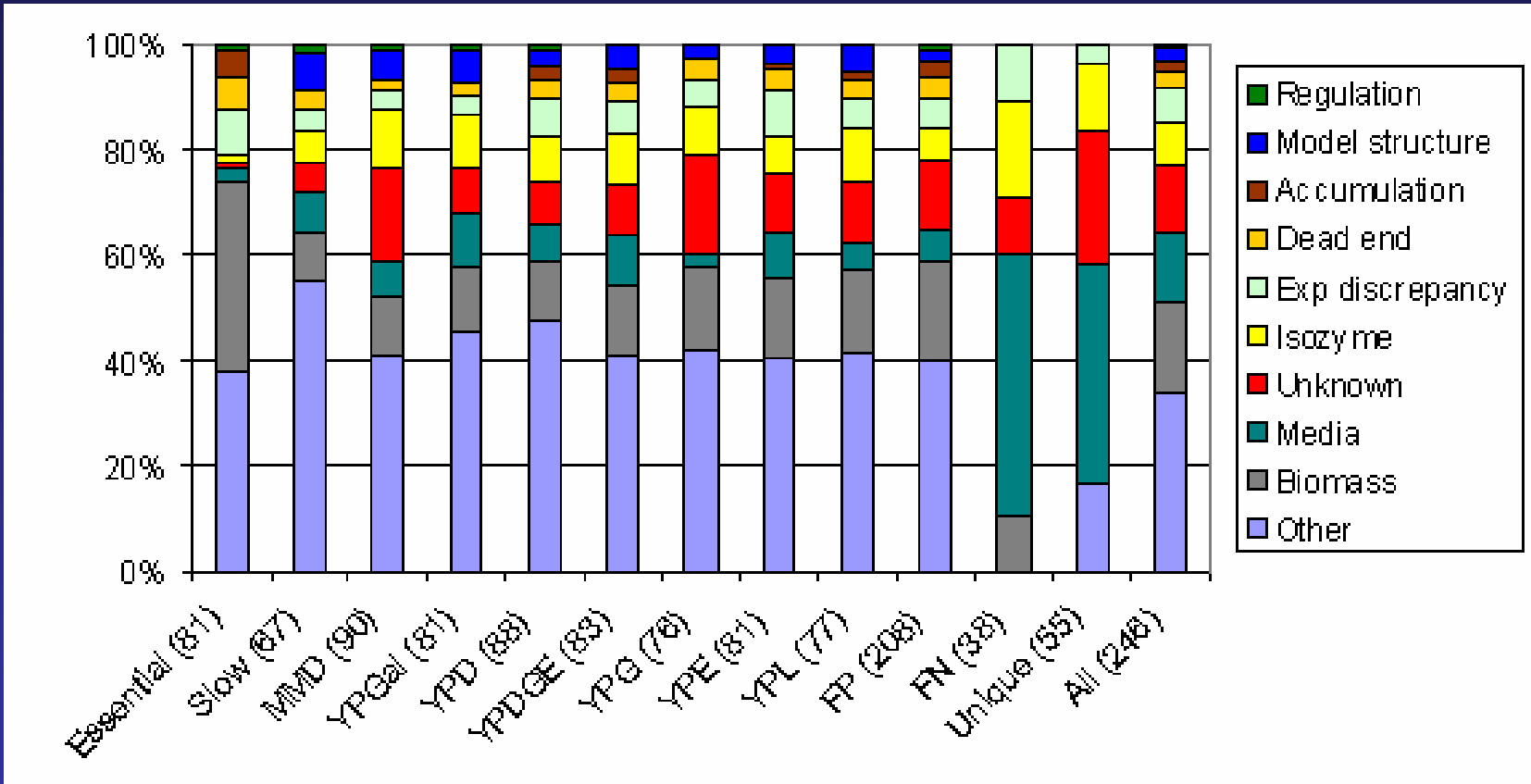
Large-scale Gene Deletion Study

- Compared *in vivo* growth rates for 682 gene deletion strains to *in silico* predictions made by iND750
  - Knockout strains were grown on 7 media conditions, resulting in a total comparison of 4,154 metabolic phenotypes!
  - Results were classified as either false positive, false negative, true positive, or true negative
- iND750 correctly predicted 82.6% growth phenotypes
- Analysis of failure modes can be used to improve model, identify inconsistencies in knowledge base, and highlight areas where further experimental investigation is required

[Steinmetz *et al.*., Nat Genet 2002]
[Giaever *et al.*., Nature 2002]
[Duarte *et al.*., Genome Res 2004]

Breakdown of False Predictions



[Duarte *et al.*., Genome Res 2004]

Possible Changes to the Model

ORF	Gene	Reason for false prediction	Suggested change and comments
YPL188W	*POS5*	Mod Struct	Change the model so that only Pos5p can provide NADPH in mitochondria.
YMR267W	*PPA2*	Mod Struct	Force the model to utilize Ppa2p instead of the cytoplasmic isoforms by restricting phosphate transport out of the mitochondria.
YMR202W	*ERG2*	Mod Struct	Modify the interconversion between zymosterol and ergosterol biosynthesis to require *ERG2*.
YDR178W	*SDH4*	Isozyme	Make Sdh4p a non-essential part of the succinate dehydrogenase complex.
YML123C	*PHO84*	Isozyme	There are multiple alternative isozymes for the phosphate transporters, but Pho84p should be the dominant one.
YKL067W	*YNK1*	Isozyme	Null mutant retains 10% of nucleoside diphosphate kinase activity. Sources of remaining enzyme activity are unknown. Reaction without gene associations should be added to the model to represent these unidentified enzymes.
YKL148C	*SDH1*	Isozyme	Sdh1p should not be considered to be an essential part of the succinate dehydrogenase complex.
YAL038W	*CDC19*	Regulation	Pyk2p isozyme should only be expressed under conditions of very low glycolytic flux.
YOL086C	*ADH1*	Regulation	This isozyme (out of five) should be the only one active under severely glucose repressed conditions.

[Duarte *et al.*., Genome Res 2004]

Conclusions

- *S. cerevisiae* is a model experimental system because of its technological advantages and similarity to higher eukaryotes.
- We can successfully build multi-compartmental metabolic models of eukaryotic cells.
- Model building is an iterative process, requiring continued updating and testing.
- Other cellular functions may be incorporated to the metabolic model, such as regulation, signal transduction, cell cycle, sporulation and so on.

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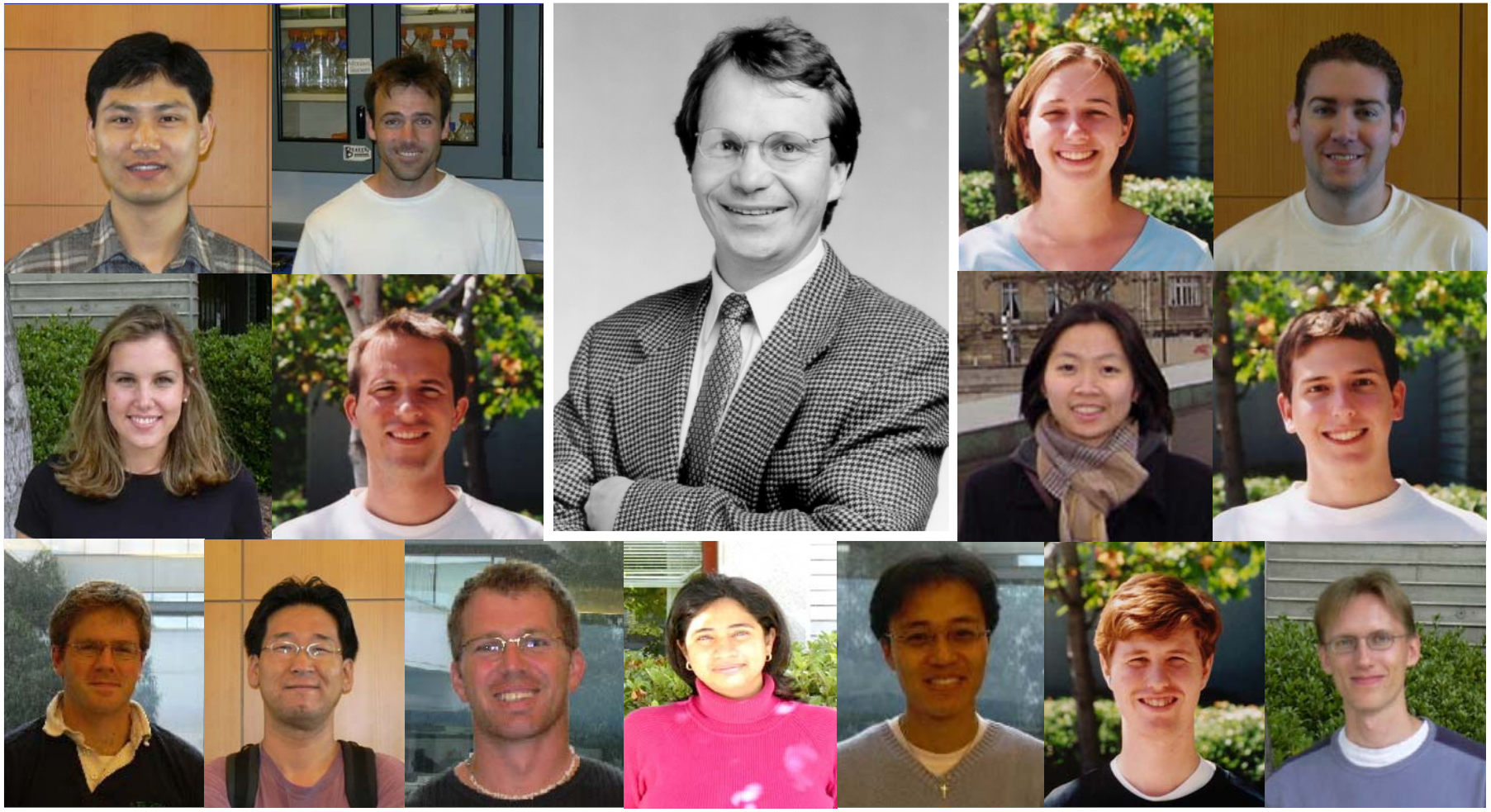
Principal Investigator: Dr. Bernhard Palsson



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