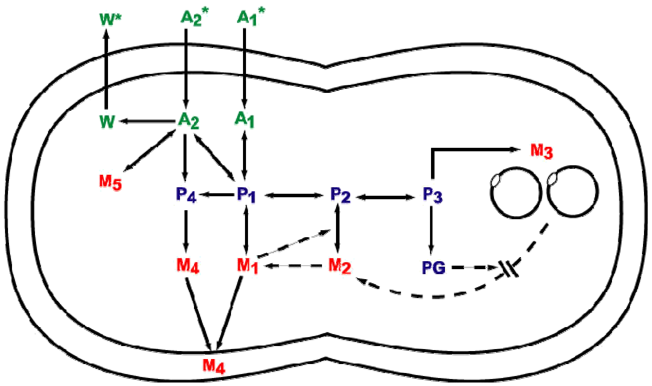
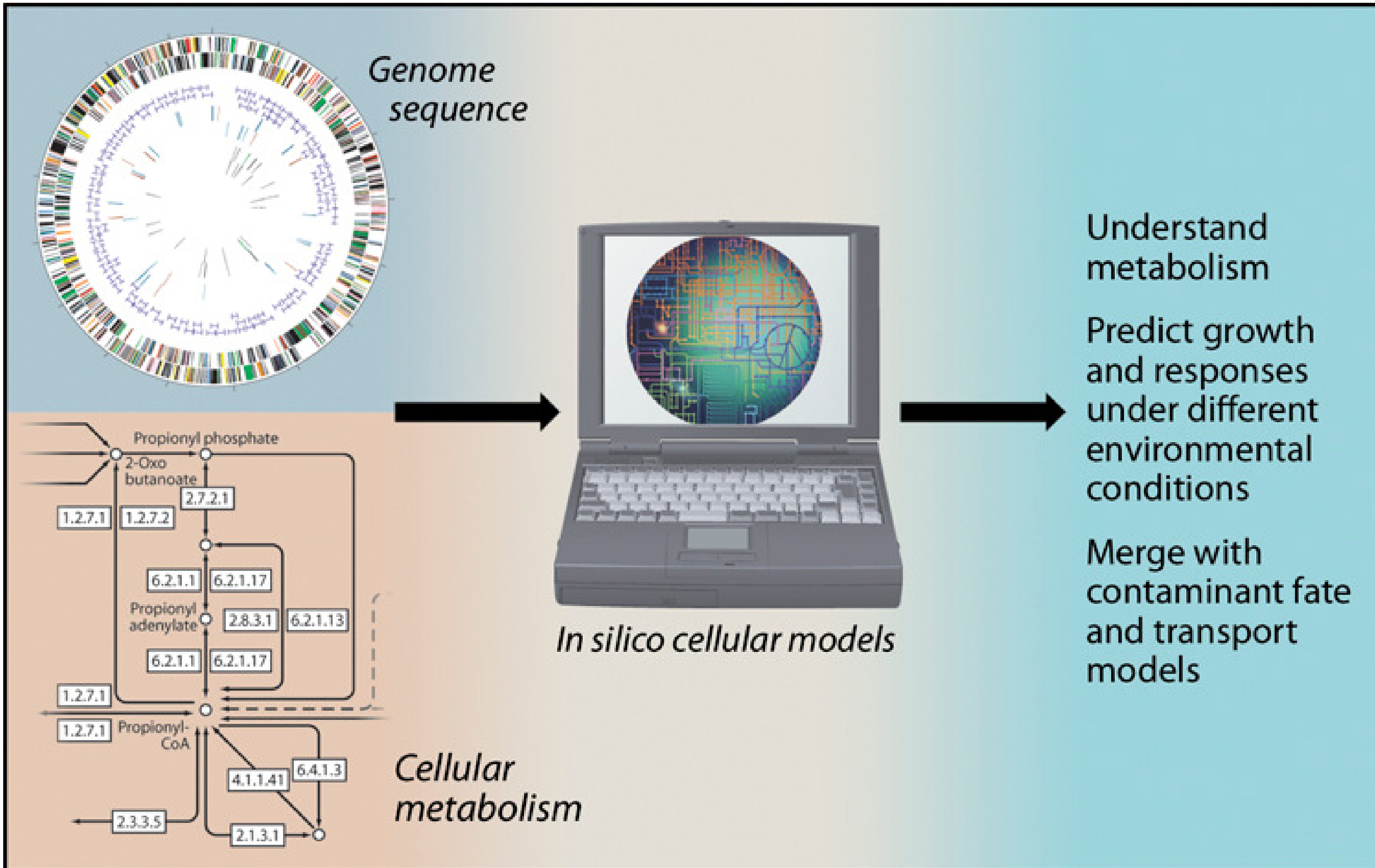
Towards a Genomically Detailed Minimal Cell Model

Jordan C. Atlas, and Michael L. Shuler
CSGF Annual Conference 2009

Shuler Research Group
School of Chemical and Biomolecular Engineering
July 14, 2009

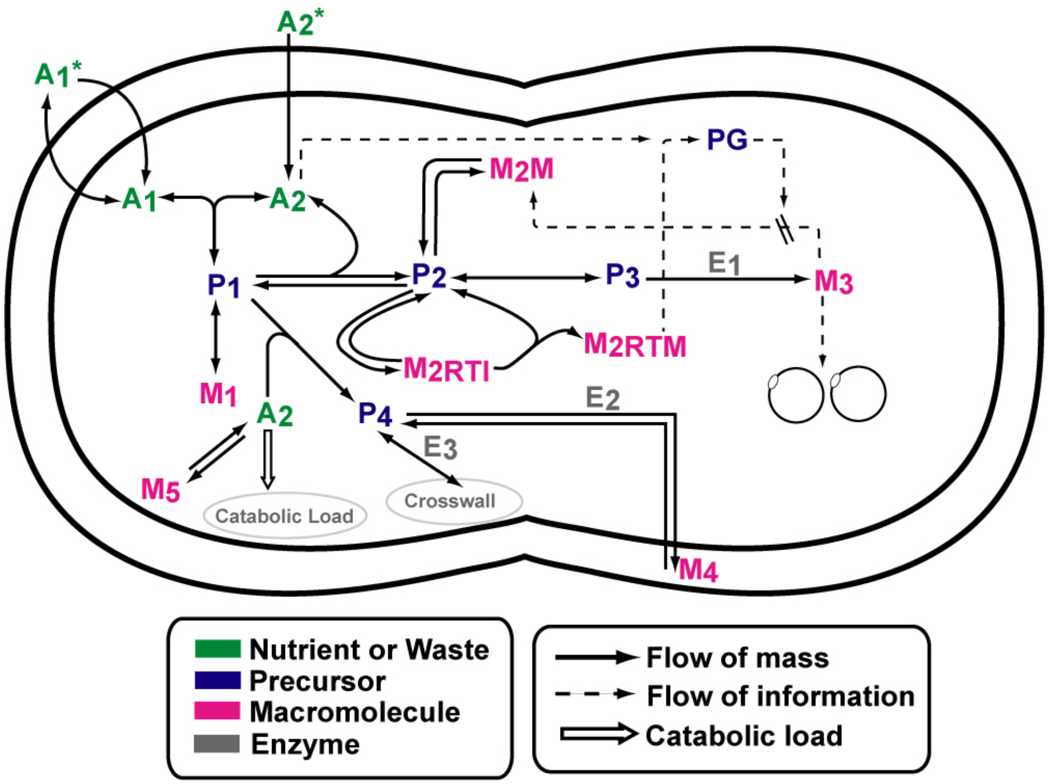


Motivation



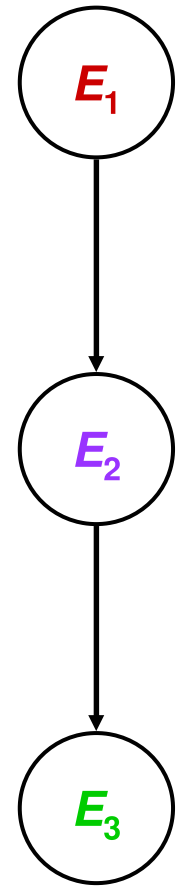
U.S. Department of Energy Genome Programs http://genomics.energy.gov

Coarse-Grained Cell Modeling



- Events - **discrete** changes in cell state
- E_1 – DNA replication initiation
- E_2 – DNA replication termination
- E_3 – cell division

- **Events depend on chemical/genomic detail.**



Nikolaev, Atlas, and Shuler, *J. Phys. Conf. Ser.*, v. 46, 2006.

Coarse-Grained Rate Equations

$$\left(\frac{dP}{dt}\right)_S = v_{S\to P}\underbrace{\left(\frac{K_I}{K_I + \frac{I}{V}}\right)}_{\text{Inhibition Term}}\underbrace{\left(\frac{\frac{A}{V}}{K_A + \frac{A}{V}}\right)}_{\text{Activation Term}}\cdot E$$

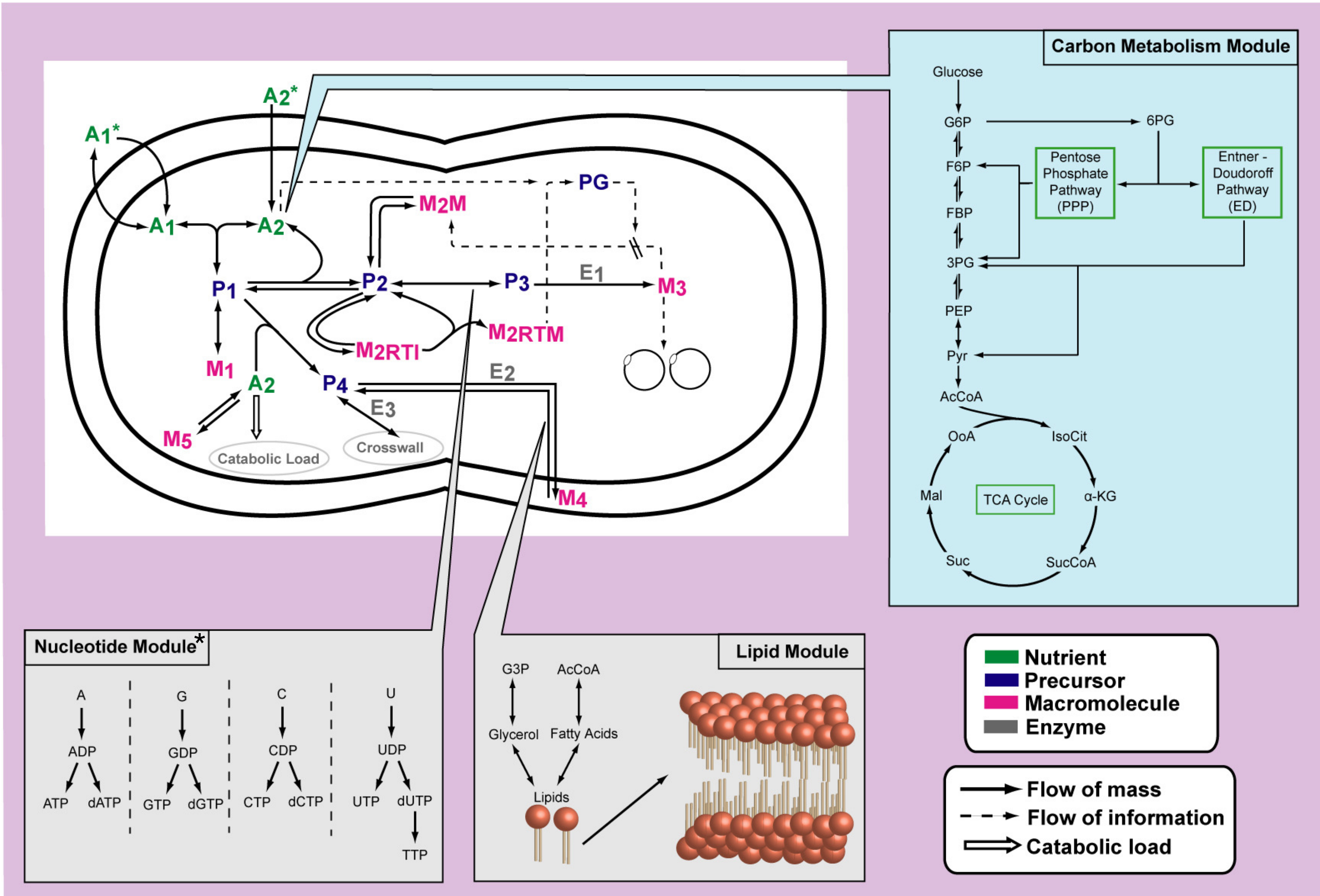
Inhibition Term Activation Term

P – Product (pg)
I – Inhibitor (pg)
A – Reactant (pg)
E – Enzyme (pg)

$v_{S\text{-}P}$ – Rate Constant (pg P/(hr-pg E))
K_I – Inhibition Constant (pg/μm^3)
K_A – Activation Constant (pg/μm^3)
V – Volume (μm^3)

DAE system integrated numerically using SloppyCell

Modular Approach to Adding Chemical/Genomic Detail

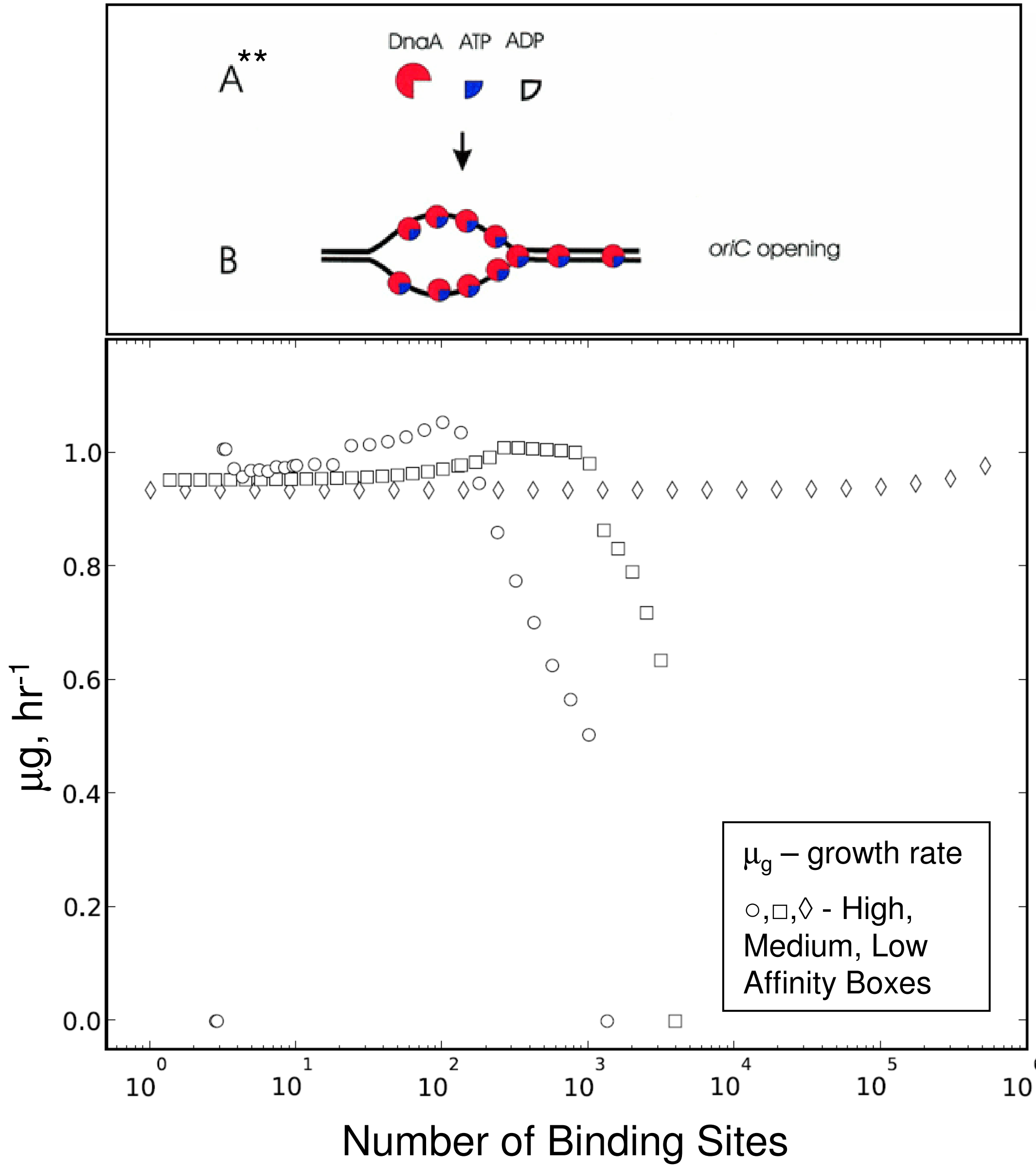


*Castellanos *et al.*, *PNAS,* v. 101(17), 2004.

DnaA Binding Boxes Can Interfere With Growth

- **Initiation and control of DNA replication** by DnaA-ATP molecules*.

- **Real genomic sequence data** for distribution of DnaA binding sites and *dnaA* genes used.



*Atlas *et al.*, *IET Sys. Bio.*, v. 2(5), 2008.
**Boye *et al.*, *EMBO Reports,* v. 1(6), 2000.

Conclusions from Coarse-Grained Cell Models

- Applied to measure general growth trends and physiological parameters
- Mechanistic base to capture discrete physiological events in cell cycle
- Modularity demonstrated with lipids, nucleotides, and DNA replication initiation
- Can incorporate genomic information

What Genes are Necessary to Support Life?

- These genes define a minimal cell

- Hypothetical organism that contains the fewest genes required for 'life'
  - Metabolic homeostasis
  - Reproduction

- Optimally supportive environment (constant pH, all necessary nutrients, waste products dilute)

Mathematical Model of a Minimal Cell

- Basic design rules of life – minimal gene set and regulatory structure
- Guide experiments to construct such a cell (e.g. impact of initial conditions on success)
- Basis for a biotechnological platform cell and evaluation of alternative design strategies

1. Obtain minimal gene set.
2. Integrate with coarse-grained modeling framework

Minimal Gene Set

Approaches Employed

- Transposon mutagenesis, single gene knockouts
- Computational comparison

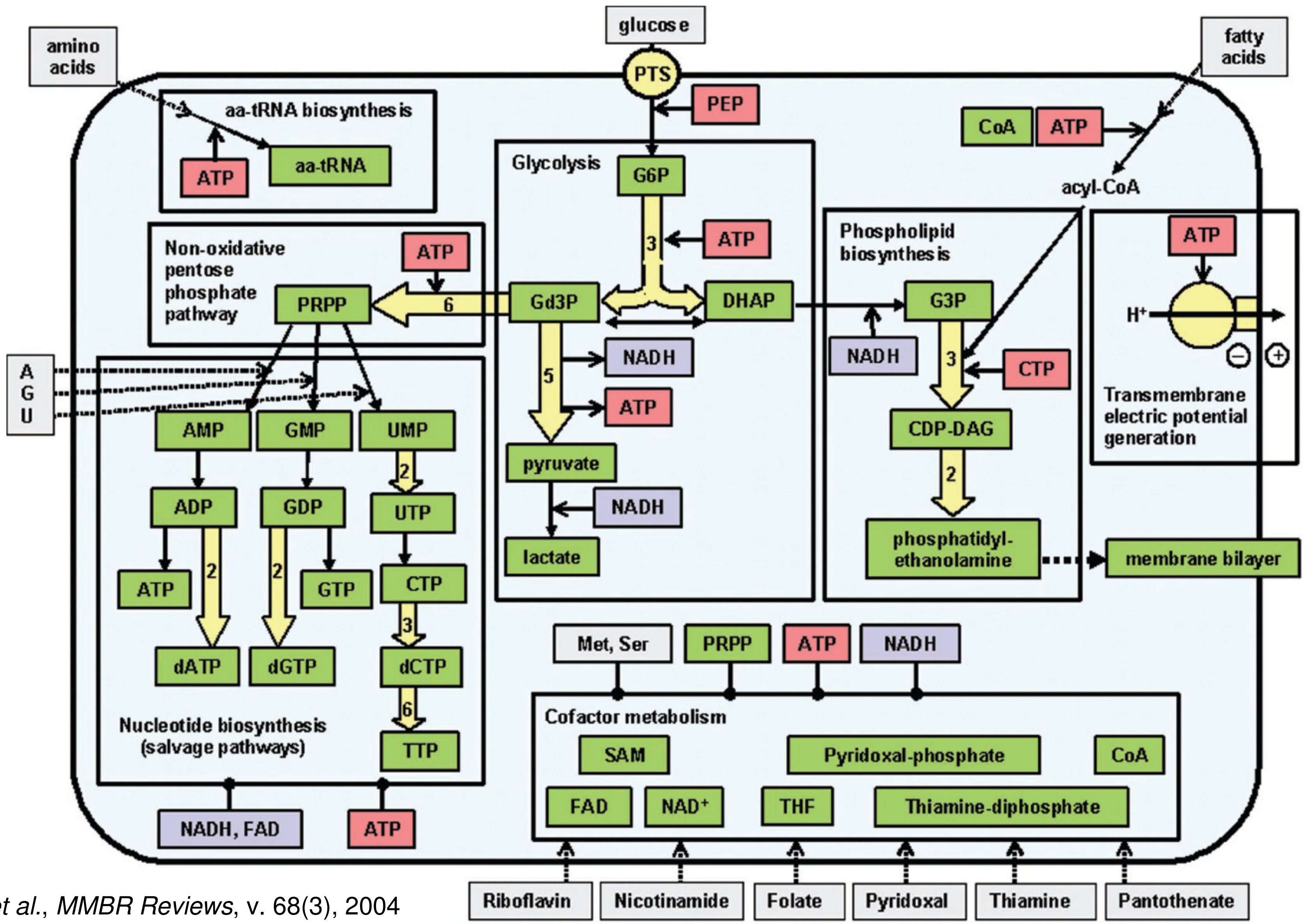
Approach of Gil *et al.*[*,]**

- Present an "enhanced review" of previous work as well as comparison to five endosymbionts.
- Proposed minimal gene set with **206 protein coding genes** (no tRNAs, minimal transport)

*Gil, *et al.*, *MMBR Reviews*, v. 68(3), 2004

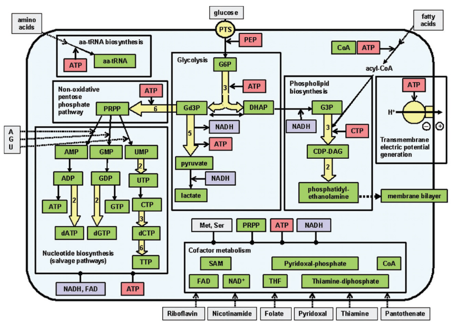
**Gabaldón, *et al.*, *Phil Trans Roy Soc B*, v. 362, 2007

Metabolic Features of the Minimal Gene Set



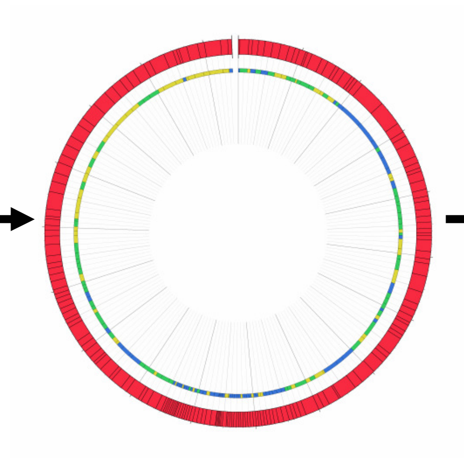
Gil, *et al.*, *MMBR Reviews*, v. 68(3), 2004

How Might We Create a Minimal Cell Model?



Gil, *et al.* gene set

(206 protein coding genes)



Computational Chromosome

Includes protein and RNA encoding genes

(109 single genes, 20 gene clusters)

Proposed Reaction Rates

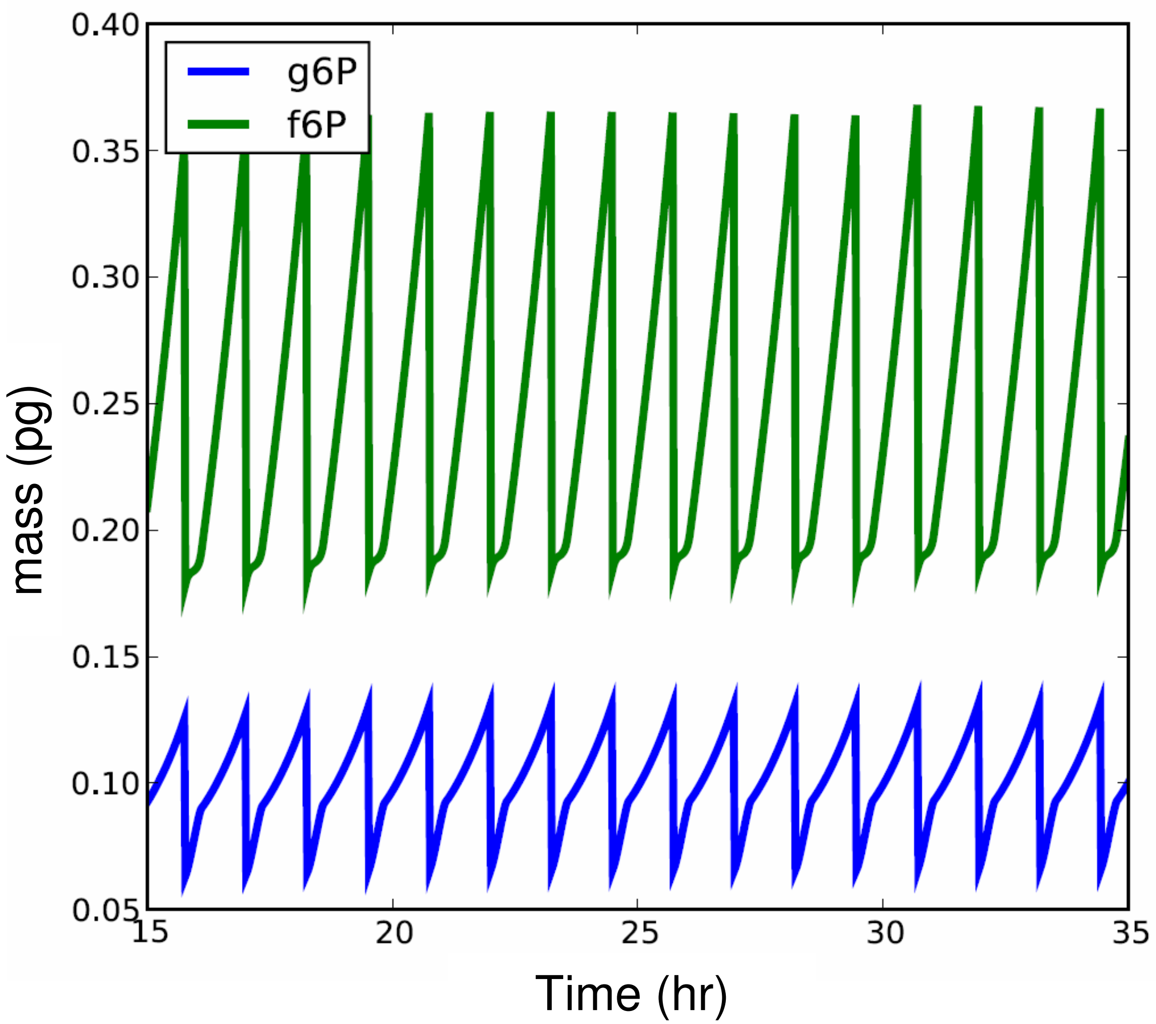
$$\left(\frac{dS_i}{dt}\right)_j = f\left(S, E_j\right)$$

S_i = species i

S = all species

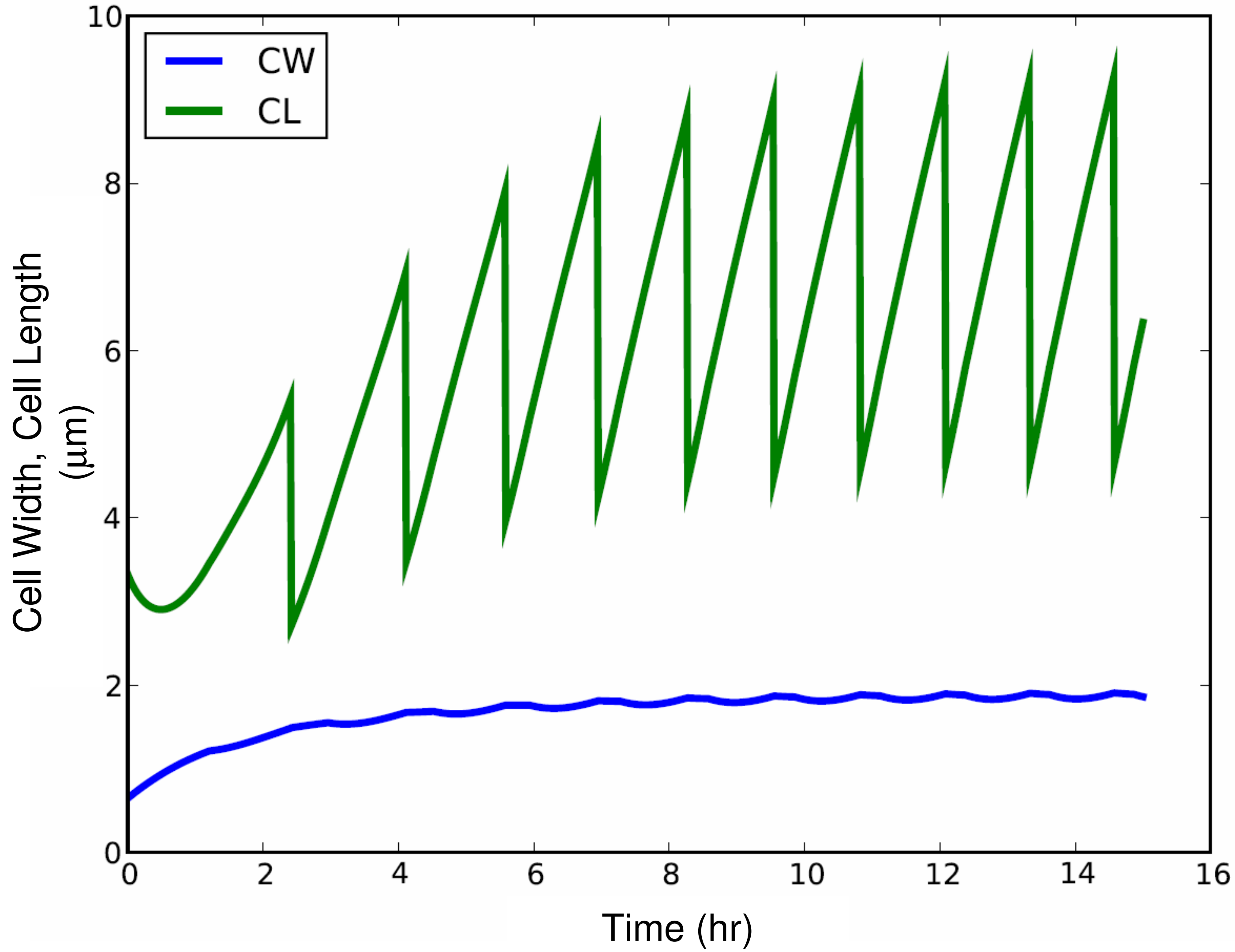
E_j = enzyme j

Stable Cell Cycle



g6P – glucose 6-phosphate, f6P – fructose 6-phosphate

Physiological State - Shape and Size



Venter Institute Synthetic Cell

3 part strategy:

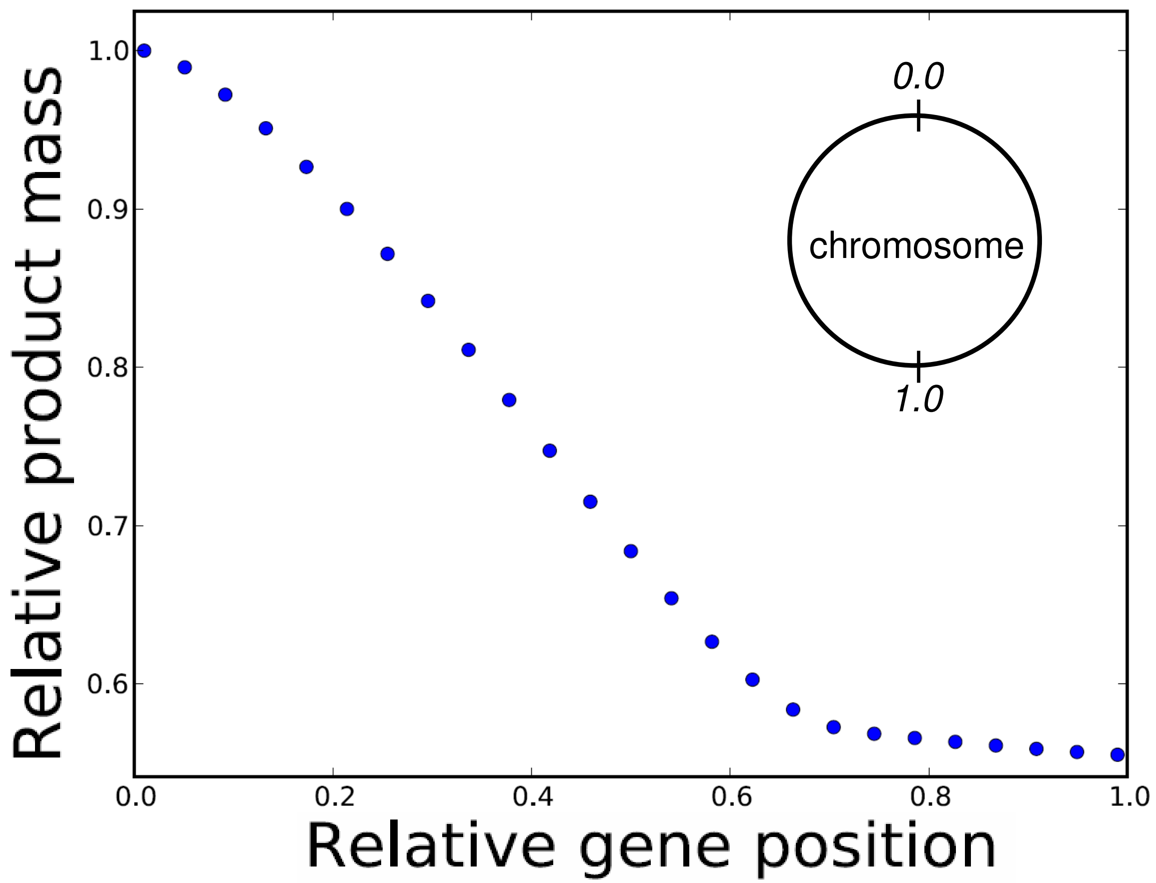
1) **Genome transplantation**: DNA from *Mycoplasma mycoides* into *Mycoplasma capricolum*[*]

2) **Construction of synthetic *Mycoplasma genitalium* genome**[**]

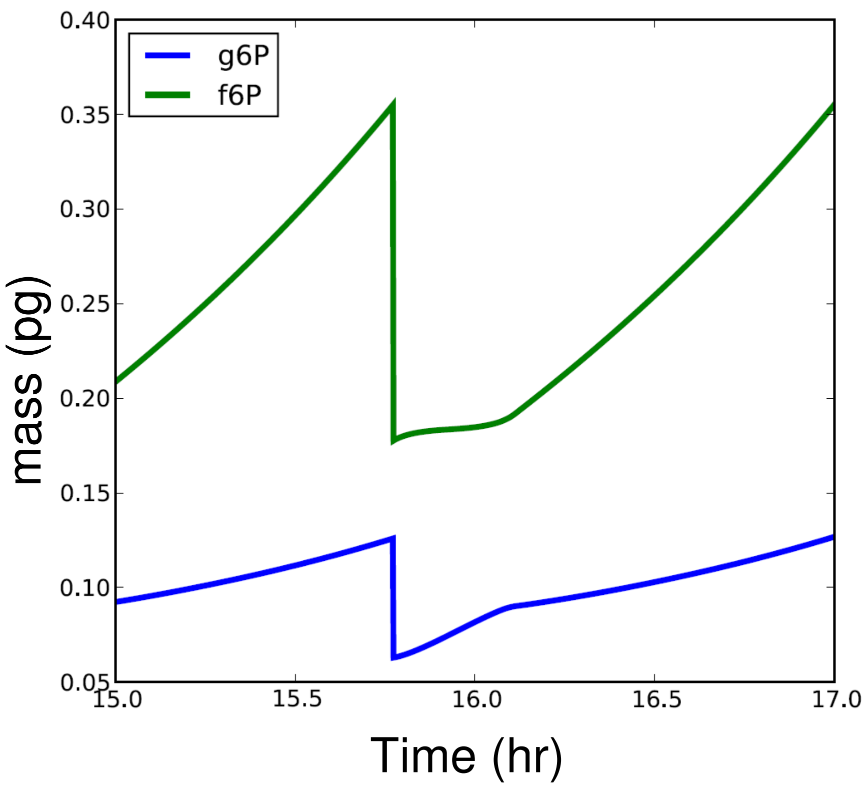
3) **Transplant synthetic genome** into bacterium to make first synthetic bacterium—No Report!

[*] Science 317: 632-638, 2007
[**] Science 319: 1215-20, 2008

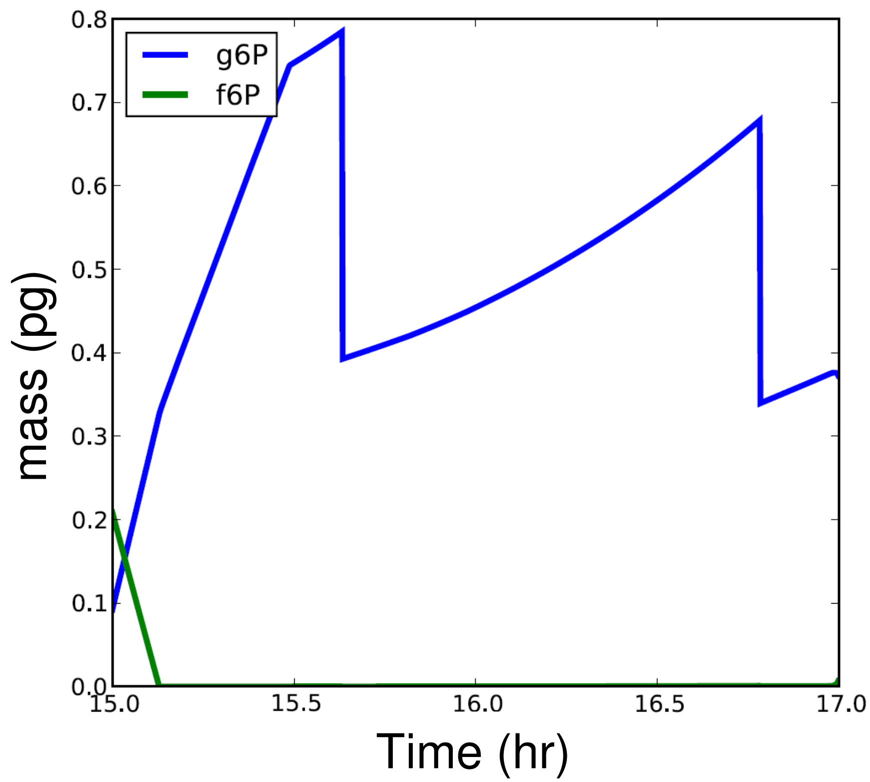
Gene Position Influences Product Production



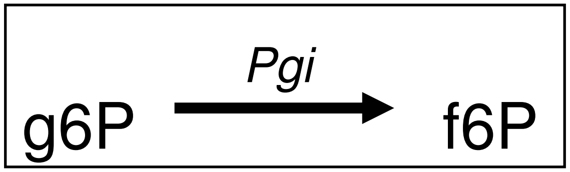
Initial Conditions Affect Cell Viability



Default Pgi (glucose 6-P isomerase) Mass



Pgi Reduced by 50% - Results in Cell Death

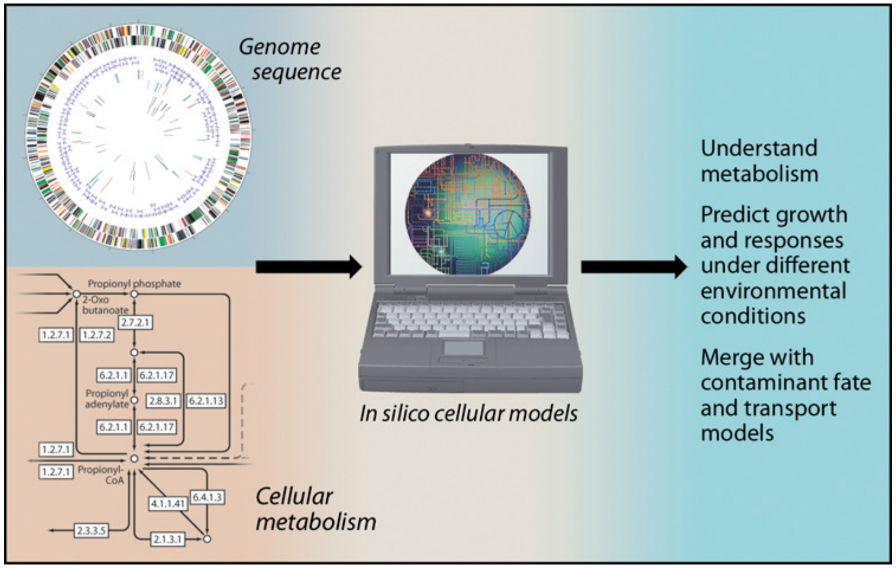


Minimal Cell Conclusions

- Dynamic models can translate genomic detail into physiological predictions
- Outputs:
  - Concentrations of all chemical species
  - Physiological state, including size and dimensions of cell
- Cell can sustain replication indefinitely
- Synthetic cell more difficult than predicted
- Mechanistic details can (and should) be added

What Has Been Accomplished?

Shown for the first time that it is possible to build a genomically and chemically complete cell model capable of indefinite growth and replication



Acknowledgements

- Professor Michael Shuler
- Dr. Evgeni Nikolaev
- Professor Jim Sethna
- Dr. Ryan Gutenkunst
- Dr. Joshua Waterfall

- Funding

Questions?

References

- Atlas, J.C., Nikolaev, E.N., Shuler, M.L., *Incorporating Genome-Wide DNA Sequence Information into a Dynamic Whole-Cell Model of Escherichia coli: Application to DNA Replication*. IET Sys. Biol.,2008. **2**(5): p. 369-382.

- Brown, K.S., Sethna, J.P., *Statistical Mechanical Approaches to Models with Many Poorly Known Parameters*. Phys. Rev. E., 2003. **68**(2).

- Browning, S.T., Shuler, M.L., *Towards the Development of a Minimal Cell Model by Generalization of a Model of Escherichia Coli: Use of Dimensionless Rate Parameters*. Biotech. and Bioeng., 2001. **76**(1): p. 25-30.

- Browning, S.T., Castellanos, M., Shuler, M.L., *Robust Control of Initiation of Prokaryotic Chromosome Replication: Essential Considerations for a Minimal Cell*. Biotech. and Bioeng., 2004. **88**(5): p. 575-584.

- Castellanos, M., Wilson, D.B., Shuler, M.L., *A Modular Minimal Cell Model: Purine and Pyrimdine Transport and Metabolism*. PNAS, 2004. **101**(17): p. 6681-6686.

- Castellanos, M., Wilson, D.B., Shuler, M.L., *A Genomically/Chemically Complete Module for Synthesis of Membrane in a Minimal Cell (in press)*, 2006.

- Domach, M.M., et al., *Computer Model for Glucose-Limited Growth of a Single Cell of Escherichia Coli B/R-a.* Biotech. and Bioeng., 2000. **67**(6): p. 827-840.

- Nikolaev, E.V., Atlas, J.C., Shuler, M.L., *Computer Models of Bacterial Cells: From Generalized Course-Grained to Genome Specific Modular Models*. J. Phys. Conf. Ser., 2006. **46**: p. 322-326.