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13C Metabolic Flux Analysis of CHO Cells with Parallel Labeling Experiments

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$^{13}$C Metabolic Flux Analysis of CHO Cells with Parallel Labeling Experiments

Maciek Antoniewicz, Woo Suk Ahn
University of Delaware

Metabolic Engineering IX
June 7, 2012
CHO cells

- Most popular cell line to produce biotherapeutics
- CHO metabolism is still poorly understood
- New insights needed for medium optimization and cell line screening
Flux analysis in CHO cells

Metabolism changes in time

- Glucose
- Lactate
- Cells
Flux analysis in CHO cells

Metabolism changes in time

Metabolism is complex

Glucose, Lactate (mM)

Time (day)

VCD (10^5 cells/mL)

Maciek Antoniewicz - mranton@udel.edu
# Metabolic Flux Analysis in CHO cells

TABLE 1. Overview of metabolic flux analysis studies in CHO cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Culture model</th>
<th>Flux analysis method</th>
<th>Major achievements</th>
<th>Year [Ref]</th>
</tr>
</thead>
<tbody>
<tr>
<td>y-CHO</td>
<td>Continuous</td>
<td>MFA</td>
<td>MFA validated the requirement of amino acids derived from peptides in serum-free media.</td>
<td>1996 [101]</td>
</tr>
<tr>
<td>y-CHO</td>
<td>Continuous</td>
<td>MFA</td>
<td>Carbon utilization efficiency was estimated by MFA. IPP by glycolysis was related to TCA cycle flux, not glycolysis.</td>
<td>2002 [102]</td>
</tr>
<tr>
<td>CH-10 TF</td>
<td>Continuous</td>
<td>MFA</td>
<td>The efficiency of carbon utilization was estimated by MFA and linked to reduced production rate of t-PA.</td>
<td>2001 [103]</td>
</tr>
<tr>
<td>CH-10-2UL</td>
<td>Batch</td>
<td>MFA + 13C MFA</td>
<td>Microscopic dynamic modeling approach was linked to a simplified network model for MFA.</td>
<td>2009 [110]</td>
</tr>
<tr>
<td>CH-10-2UL</td>
<td>Batch</td>
<td>MFA + 13C MFA</td>
<td>Dynamic modeling was linked to MFA to estimate fluxes during cell growth, translation and stationary phase.</td>
<td>2008 [112]</td>
</tr>
<tr>
<td>CH-10 TF</td>
<td>Batch</td>
<td>MFA</td>
<td>Co-feeding of glucose and lactate resulted in metabolic shift from lactate production to consumption.</td>
<td>2006 [104]</td>
</tr>
<tr>
<td>Unknown</td>
<td>Perfusion</td>
<td>MFA</td>
<td>Intracellular fluxes were estimated by quasi real-time MFA in perfusion culture.</td>
<td>2005 [107]</td>
</tr>
<tr>
<td>Unknown</td>
<td>Perfusion</td>
<td>MFA</td>
<td>Error propagation from measurements to metabolic fluxes was determined for MFA.</td>
<td>2005 [106]</td>
</tr>
<tr>
<td>Unknown</td>
<td>Perfusion</td>
<td>IC-MFA</td>
<td>MFA and 13C MFA were compared. Fluxes were linked to IC-MFA data.</td>
<td>2011 [114]</td>
</tr>
<tr>
<td>Super-CHO</td>
<td>Batch</td>
<td>MFA</td>
<td>Differences in CHO cell metabolism and hybridoma cell metabolism were identified using MFA.</td>
<td>2010 [109]</td>
</tr>
<tr>
<td>CH-10-2UL</td>
<td>Batch</td>
<td>MFA</td>
<td>To improve flux observability, the number and type of available measurements were optimized for MFA.</td>
<td>2010 [108]</td>
</tr>
<tr>
<td>CS-CHO SF18</td>
<td>Fed-batch</td>
<td>MFA and 13C MFA</td>
<td>Fluxes were estimated by MFA, MFA was used to estimate fluxes in the rest of network model.</td>
<td>2011 [113]</td>
</tr>
<tr>
<td>Unknown</td>
<td>Fed-batch and continuous</td>
<td>MFA</td>
<td>Amino acid composition of culture medium was optimized using MFA. By-product yields were reduced and cell density and antibody production were enhanced.</td>
<td>2011 [105]</td>
</tr>
<tr>
<td>CH-10-K1</td>
<td>Fed-batch</td>
<td>MFA + IC-MFA</td>
<td>MFA was integrated with a kinetic model to simulate metabolic dynamics in fed-batch cultures.</td>
<td>2011 [111]</td>
</tr>
<tr>
<td>hKCHO</td>
<td>Fed-batch</td>
<td>MFA + 13C MFA</td>
<td>Kinetic models for growing and non-growing subpopulations of cells were integrated with a simplified MFA model.</td>
<td>2011 [113]</td>
</tr>
<tr>
<td>CH-10-K1</td>
<td>Fed-batch</td>
<td>TIC MFA and 13C MFA</td>
<td>Metabolic fluxes were determined for growth phase and stationary phase in a fed-batch culture using 13C-based MFA.</td>
<td>2011 [115]</td>
</tr>
</tbody>
</table>

Metabolic Flux Analysis (MFA)

\[ S \mathbf{v} = 0 \]

\[
\begin{pmatrix}
1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 \\
0 & 0 & 0 & 0 & 1 & -1 & 0 & -1
\end{pmatrix}
\begin{pmatrix}
v_1 \\
v_2 \\
v_3 \\
v_4 \\
v_5 \\
v_6 \\
v_7 \\
v_8
\end{pmatrix}
= 
\begin{pmatrix}
0 \\
0 \\
0 \\
0 \\
0 \\
0 \\
0 \\
0
\end{pmatrix}
### Metabolic Flux Analysis (MFA)

The figure illustrates the metabolic fluxes in a metabolic network with the following equations:

\[ S \times v = 0 \]
\[ R \times v = r \]

The table represents the fluxes (\( v \)) in the network:

\[
\begin{align*}
A &= \begin{pmatrix} 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \\
B &= \begin{pmatrix} 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 \end{pmatrix} \\
C &= \begin{pmatrix} 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 \end{pmatrix} \\
D &= \begin{pmatrix} 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 \end{pmatrix} \\
E &= \begin{pmatrix} 0 & 0 & 0 & 0 & 1 & -1 & 0 & -1 \end{pmatrix} \\
S &= \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \\
P &= \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \\
Q &= \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \\
R &= \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix}
\end{align*}
\]

\[
\begin{pmatrix}
v1 \\
v2 \\
v3 \\
v4 \\
v5 \\
v6 \\
v7 \\
v8
\end{pmatrix} \times \begin{pmatrix}
v1 \\
v2 \\
v3 \\
v4 \\
v5 \\
v6 \\
v7 \\
v8
\end{pmatrix} = \begin{pmatrix} 0 \\
0 \\
0 \\
0 \\
r_s \\
r_p \\
r_q \\
r_R \end{pmatrix}
\]
Metabolic flux analysis is only as reliable as your data and modeling assumptions.

- **Observability Problem**
  - If \( \text{rank(data)} = \text{number of fluxes} \) → all fluxes can be determined
  - If \( \text{rank(data)} < \text{number of fluxes} \) → not all fluxes can be uniquely determined
Model of CHO metabolism

Glucose → G6P → 6PG → Ru5P

GL3P → DHAP → GAP

G6P → F6P → FBP

Lipids

Glycogen

Amino Acids (Asp, Asn)

PEPCK → OAA → PEP

Amino Acids (Ser, Cys)

Thr → ThrAl

Gln + CO2

Fatty Acids

MAL → PYR

Amino Acids (Leu, Ile, Phe, Tyr, Lys, Trp)

MAL → OAA → CIT → ICIT

TCA Cycle

FUM → SUC → SuccCoA → AKG

mitochondrion
cytosol

Amino Acids (Gln, Pro, Arg, His)

AcCoA → OAA

C1 + CO2

Ser

Pro

Glu.ex

Amino Acids (Met, Val, Ile, Thr)
Model of CHO metabolism

- **External Measurements**
  - Glucose

```
Glucose
  ↓
G6P
  ↓
F6P
  ↓
FBP
  ↓
DHAP ↔ GAP
  ↓
BPG
  ↓
3PG
  ↓
2PG
  ↓
Pep
  ↓
PYR ————> Lactate
```
Model of CHO metabolism

• External Measurements
  – Glucose
  – Lactate
Model of CHO metabolism

- **External Measurements**
  - Glucose
  - Lactate
  - Glutamine
Model of CHO metabolism

- External Measurements
  - Glucose
  - Lactate
  - Glutamine
  - Growth rate
Model of CHO metabolism

- **External Measurements**
  - Glucose
  - Lactate
  - Glutamine
  - Growth rate
  - Amino acids
Model of CHO metabolism

- **External Measurements**
  - Glucose
  - Lactate
  - Glutamine
  - Growth rate
  - Amino acids

- **$^{13}$C-Labeling GC-MS**
  - Typical 200+ mass isotopomers measured
• **CHO cell fed-batch culture**
  - CHO-K1 cells (ATCC CCL-61) in T-25 flasks
  - Humidified incubator, 5% CO$_2$, 37 deg.C

• **Medium composition**
  - DMEM + 10% FBS + 1% PS
  - 6.7 mM [1,2-$^{13}$C]glucose

• **Measurements**
  - Glucose, lactate (YSI)
  - Amino acid concentrations (GC-MS)
  - $^{13}$C-Labeling of intracellular metabolites (GC-MS)
CHO Fed-batch culture

Ahn WS & Antoniewicz MR, Metab Eng, 2011
Shift in Lactate Metabolism

\[ \frac{\Delta \text{ (Lact)}}{\Delta \text{ (Gluc)}} = 1.48 \]

Ahn WS & Antoniewicz MR, Metab Eng, 2011
CHO Fed-batch culture

![Graph showing glucose, lactate, cell count, and viable cell density over time.](https://example.com/graph.png)

Ahn WS & Antoniewicz MR, Metab Eng, 2011
CHO Fed-batch culture

![Graph showing glucose and lactate levels over time in CHO Fed-batch culture]

- Exponential Phase
- Stationary Phase

Glucose and Lactate (mM) vs. Time (day)

[1,2-13C]glucose addition

Metabolism quenching and GC-MS analysis

Ahn WS & Antoniewicz MR, Metab Eng, 2011
Extracellular metabolite profiling

Ahn WS & Antoniewicz MR, Metab Eng, 2011
## Biomass specific fluxes

### Biomass specific uptake and excretion rates of extracellular metabolites (nmol/10^6 cells/hr)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Exponential Phase</th>
<th>Stationary Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>-201</td>
<td>-48.8</td>
</tr>
<tr>
<td>Lactate</td>
<td>299</td>
<td>-2.5</td>
</tr>
<tr>
<td>NH₃</td>
<td>24.5</td>
<td>5.2</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Valine</td>
<td>-5.2</td>
<td>-0.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>-7.0</td>
<td>-0.3</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>-5.6</td>
<td>-0.3</td>
</tr>
<tr>
<td>Proline</td>
<td>-0.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>-1.9</td>
<td>-0.1</td>
</tr>
<tr>
<td>Serine</td>
<td>-8.1</td>
<td>-0.9</td>
</tr>
<tr>
<td>Threonine</td>
<td>-4.4</td>
<td>-0.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>-2.7</td>
<td>-0.2</td>
</tr>
<tr>
<td>Aspartate</td>
<td>-0.0</td>
<td>-0.3</td>
</tr>
<tr>
<td>Glutamate</td>
<td>5.4</td>
<td>-0.4</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>-0.9</td>
<td>-0.1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>-35.0</td>
<td>-5.7</td>
</tr>
</tbody>
</table>

Ahn WS & Antoniewicz MR, Metab Eng, 2011
**Intracellular Metabolite Analysis**

**Tracer experiments with CHO cell culture in T25 flasks**

- Removal of media
  - 1.5 mL cold methanol
  - 5 min in ice

- Cold methanol

- Chloroform/water
  - 1 v CHCl₃
  - 1 v H₂O
  - pH 7–8, overnight in cold chamber, centrif.

**Non-polar metabolites**
- Lipids in CHCl₃
- Drying

**Polar metabolites**
- Amino acids/organic acids
- Drying; 37°C, 9 hr

**SPE separation**
- fractionation

**Triglyceride/FFA**
- Derivatization
- Lipid esters, TMS der. of FFA

**Organic/Amino acids**
- MOX/TBDMS der. of metabolites

**GC-MS Analysis**
- Derivatization
- MOX: 37°C, 90 min
- TBDMS; 60°C, 30 min

**Mass isotopomer distribution quantification**
## GC-MS Measurements

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Base mass</th>
<th>Carbon atoms</th>
<th>Fragment formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>174</td>
<td>1-2-3</td>
<td>C₆H₁₂O₇NS1</td>
</tr>
<tr>
<td>Lactate</td>
<td>233</td>
<td>2-3</td>
<td>C₁₀H₁₇O₄S1</td>
</tr>
<tr>
<td>Lactate</td>
<td>261</td>
<td>1-2-3</td>
<td>C₁₁H₂₅O₇Si₂</td>
</tr>
<tr>
<td>Succinate</td>
<td>289</td>
<td>1-2-3-4</td>
<td>C₁₂H₂₅O₇Si₂</td>
</tr>
<tr>
<td>Fumarate</td>
<td>287</td>
<td>1-2-3-4</td>
<td>C₁₂H₂₅O₇Si₂</td>
</tr>
<tr>
<td>AKG</td>
<td>346</td>
<td>1-2-3-4-5</td>
<td>C₁₀H₂₅O₇NS₁</td>
</tr>
<tr>
<td>Malate</td>
<td>419</td>
<td>1-2-3-4</td>
<td>C₁₂H₂₅O₇Si₂</td>
</tr>
<tr>
<td>PEP</td>
<td>453</td>
<td>1-2-3</td>
<td>C₁₂H₂₅O₇Si₃P</td>
</tr>
<tr>
<td>GAP</td>
<td>484</td>
<td>1-2-3</td>
<td>C₁₂H₂₅O₇NS₁₃P</td>
</tr>
<tr>
<td>Glyc3P</td>
<td>571</td>
<td>1-2-3</td>
<td>C₂₁H₉O₆Si₄P</td>
</tr>
<tr>
<td>Citrate</td>
<td>459</td>
<td>1-2-3-4-5-6</td>
<td>C₂₀H₂₉O₆Si₃</td>
</tr>
<tr>
<td>3PG</td>
<td>585</td>
<td>1-2-3</td>
<td>C₂₀H₂₉O₆Si₄P</td>
</tr>
</tbody>
</table>

### Amino acids
<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Base mass</th>
<th>Carbon atoms</th>
<th>Fragment formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>232</td>
<td>2-3</td>
<td>C₁₆H₇O₆NS₁₂</td>
</tr>
<tr>
<td>Alanine</td>
<td>260</td>
<td>1-2-3</td>
<td>C₁₁H₂₉O₂NS₁₂</td>
</tr>
<tr>
<td>Glycine</td>
<td>246</td>
<td>1-2</td>
<td>C₁₀H₄O₂NS₁₂</td>
</tr>
<tr>
<td>Valine</td>
<td>260</td>
<td>2-3-4-5</td>
<td>C₁₂H₂₀O₇ NS₁₂</td>
</tr>
<tr>
<td>Leucine</td>
<td>274</td>
<td>2-3-4-5-6</td>
<td>C₁₃H₂₅O₇NS₁₂</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>274</td>
<td>2-3-4-5-6</td>
<td>C₁₃H₂₅O₇NS₁₂</td>
</tr>
<tr>
<td>Proline</td>
<td>286</td>
<td>1-2-3-4-5</td>
<td>C₁₂H₂₀O₇NS₁₂</td>
</tr>
<tr>
<td>Methionine</td>
<td>320</td>
<td>1-2-3-4</td>
<td>C₁₂H₂₀O₂NS₁₂S</td>
</tr>
<tr>
<td>Serine</td>
<td>390</td>
<td>1-2-3</td>
<td>C₁₇H₄O₃NS₁₃</td>
</tr>
<tr>
<td>Threonine</td>
<td>404</td>
<td>1-2-3-4</td>
<td>C₁₃H₂₉O₂NS₁₃</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>302</td>
<td>1-2</td>
<td>C₁₄H₂₂O₂NS₁₂</td>
</tr>
<tr>
<td>Aspartate</td>
<td>302</td>
<td>1-2</td>
<td>C₁₄H₂₂O₂NS₁₂</td>
</tr>
<tr>
<td>Aspartate</td>
<td>390</td>
<td>2-3-4</td>
<td>C₁₇H₂₀O₇ NS₁₃</td>
</tr>
<tr>
<td>Aspartate</td>
<td>418</td>
<td>1-2-3-4</td>
<td>C₁₈H₂₄O₄NS₁₃</td>
</tr>
<tr>
<td>Glutamate</td>
<td>330</td>
<td>2-3-4-5</td>
<td>C₁₆H₃₂O₂NS₁₂</td>
</tr>
<tr>
<td>Glutamate</td>
<td>432</td>
<td>1-2-3-4-5</td>
<td>C₁₉H₂₄O₂NS₁₃</td>
</tr>
<tr>
<td>Glutamine</td>
<td>431</td>
<td>1-2-3-4-5</td>
<td>C₁₉H₂₄O₂NS₁₃</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>302</td>
<td>1-2</td>
<td>C₁₄H₂₂O₂NS₁₂</td>
</tr>
</tbody>
</table>

Ahn WS & Antoniewicz MR, Metab Eng, 2011
13C-Labeling in Metabolites

Isotopic steady state

Stationary 13C-MFA

Wiechert et al., Met. Eng. 1999

Isotopic non-steady state

Non-stationary 13C-MFA

Young et al., Biotech. Bioeng. 2008
Labeling dynamics of intracellular metabolites

Ahn WS & Antoniewicz MR, Metab Eng, 2011
Labeling dynamics of intracellular metabolites

Ahn WS & Antoniewicz MR, Metab Eng, 2011
Isotopic non-stationary $^{13}$C-MFA

Ahn WS & Antoniewicz MR, Metab Eng, 2011
Parallel Labeling Experiments

Glucose and Lactate (mM)
- Lact: 0, 5, 10, 15, 20
- Glucose: 0, 5, 10, 15, 20

Viable Cell Density (cells/mL)
- 10⁷, 10⁶, 10⁵, 10⁴, 10³

Glutamine (mM)
- NH₃: 0, 1, 2, 3, 4, 5
- Gln: 0, 1, 2, 3, 4

Time (day)
- 0, 2, 4, 6

[1,2-¹³C]Glucose
[U⁻¹³C]Glutamine

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
Isotopic steady state $< 3$ hr

Labeling by $[1,2^{13}C]glucose$ for glycolysis

- **Growth phase**
  - DHAP
  - 3PG
  - PEP

- **Non-growth phase**
  - DHAP
  - 3PG
  - PEP

Labeling by $[U^{13}C]glutamine$ for TCA cycle

- **Gln**
- **Glu**
- **AKG**
- **Cit**

Time (h)
Intracellular Mass Isotopomers

[1,2-\textsuperscript{13}C]Glucose

- Exponential Phase
- Stationary Phase

- DHAP
- 3PG
- PEP
- GLP

[Fractional abundance]

[U-\textsuperscript{13}C]Glutamine

- Exponential Phase
- Stationary Phase

- Gln
- Glu5
- AKG
- Glu4
- Suc
- Fum
- Mal
- Asp
- Cit6
- Cit5

[Fractional abundance]

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
Metabolic Fluxes Growth vs. Stationary

Exponential Phase

Stationary Phase

Flux results from [1,2,13C]glucose tracer data and external rates

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
Metabolic Fluxes Growth vs. Stationary

Flux results from [U-13C]glutamine tracer data and external rates.

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
Metabolic Fluxes Growth vs. Stationary

Exponential Phase

Stationary Phase

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
Combined $^{13}$C-MFA of parallel experiments

Exponential Phase (day 2)

- Glucose → G6P (204)
- G6P → F6P (201)
- F6P → X5P (200 FBP)
- X5P → Ru5P → NTP
- GAP → E4P (200 FBP)
- E4P → F6P
- GLP ← DHAP ← GAP ← 3PG (397)
- Lipid (1)
- OAC (16) → PEP (0)
- Mal (12) → Pyr (42)
- Pyr (42) → Lact (48)
- Growth (0)
- Other (24)
- Lipid (0)

Stationary Phase (day 5)

- Glucose (42)
- G6P → Ru5P → NTP (13)
- F5P → X5P → Ru5P (13)
- GAP → E4P (38 FBP)
- E4P → F6P
- GLP ← DHAP ← GAP ← 3PG (80)
- Lipid (0)
- OAC (17) → PEP (0)
- Mal (2) → Pyr (24)
- Pyr (24) → Lact (66)
- Growth (0)
- Other (17)
- Lipid (0)

nmol/10^6 cells/h:
- 400
- 200
- 100
- 50
- 25
- 10
- 5
- < 2

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
Combined $^{13}$C-MFA of parallel experiments

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
Validating loss of metabolites in glycolysis

Aldonitrile pentaacetate derivatization of media metabolites

#1 peak (M0, 189 m/z)

#2 peak (M0, 345 m/z)

#3 peak (M0, 345 m/z)

Methyloxime trimethylsilylation derivatization of media metabolites

#1 peak (M0, 217 m/z)

#2 peak (M0, 217 m/z)

Methyloxime tert-butyldimethylsilylation derivatization of media metabolites

#1 peak (M0, 315 m/z)

#2 peak (M0, 344 m/z)
13C-Labeling in Lipids

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
De novo lipid biosynthesis

Fraction of newly synthesized palmitate, g(t)

Exp. phase

3h 6h 9h 12h

Stat. phase

3h 6h 9h 12h

Predicted from growth rate

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
Validating Pentose Phosphate Pathway

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
Estimating oxPPP flux using $^{13}\text{C}-\text{MFA}$

- $[1,2-^{13}\text{C}]$glucose tracer
  - Glycolysis produces M2
  - oxPPP produces M1
  - non-oxPPP produces M3

- **Exponential growth phase**
  - Low oxPPP (1% of glucose)

- **Stationary phase**
  - Increased oxPPP (20% of glucose)

Ahn WS & Antoniewicz MR, Metab Eng (Submitted)
Parallel Labeling Experiments for PPP

[1] + [4,5,6]Gluc

Glucose → G6P → F6P → GAP → M1M3 → 3PG/PEP

High oxPPP

M1/M3 < 1

[2] + [4,5,6]Gluc

Glucose → G6P → F6P → GAP → M1M3 → 3PG/PEP

Control exp.

M1/M3 = 1

[3] + [4,5,6]Gluc

Glucose → G6P → F6P → GAP → M1M3 → 3PG/PEP

Control exp.

M1/M3 = 1

Ahn WS & Antoniewicz MR, J Biol Chem (Submitted)
Mass Isotopomer Distributions

Ahn WS & Antoniewicz MR, J Biol Chem (Submitted)
oxPPP estimated with different tracers

Ahn WS & Antoniewicz MR, J Biol Chem (Submitted)
Mass isotopomer analysis

![Graph showing isotopomer analysis results](image-url)
Mass isotopomer analysis

(Oxidative PPP)
Mass isotopomer analysis

(M1 + M2 x 2) / M3

[1]+[4,5,6]Gluc
[2]+[4,5,6]Gluc
[3]+[4,5,6]Gluc

Lost C1-C3 ?
Oxidative PPP

Ahn WS & Antoniewicz MR, J Biol Chem (Submitted)
Loss of carbon atoms in non-oxPPP

Ahn WS & Antoniewicz MR, J Biol Chem (Submitted)
Validated oxPPP flux

Ahn WS & Antoniewicz MR, J Biol Chem (Submitted)
### Reversible non-oxPPP fluxes

<table>
<thead>
<tr>
<th>Glucose Combination</th>
<th>Original PPP model</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1,2]Gluc</td>
<td>Not observable</td>
</tr>
<tr>
<td>Combined analysis</td>
<td>N/A (fit not accepted)</td>
</tr>
</tbody>
</table>

**TA exch, ν15 (nmol/10⁶ cells/h)**
Reversible non-oxPPP fluxes

Extended PPP model with F6P data

- [1,2]Gluc
- Combined analysis

TA exch, $v15$ (nmol/10$^6$ cells/h)
Conclusions CHO cells

• Flux analysis is only as reliable as the model
• $^{13}$C-MFA for model validation

• In the transition from growth phase to stationary phase, CHO cells dramatically changed metabolism

• New insights into CHO metabolism:
  – Oxidative Pentose Phosphate
  – Lipid metabolism
  – Loss of carbon atoms in glycolysis
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