Basics of metabolite profiling and metabolic flux analysis

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Cells respond to environment

- Cold
- Heat
- Various external messages
- Environmental conditions
- Nutrients
The Pyramid of Life

Signals (light, O₂, food, etc.)

Metabolome

Proteome

Genome

External stimuli

Response

Metabolome

Proteome

Genome

Time
Metabolites are central in cell physiology.
Metabolites can directly control gene expression

Regulation of chromatin accessibility → Gene expression

Metabolomics connects Proteome and Genome to Phenotype

- Metabolomics data provides insights into underlying biology
- Metabolomics data provides information behind the mechanisms by which genes function
- Multiple omics data pointing to the same biological pathways builds scientific hypotheses and bring us closer to translational science
Quantitative Metabolomics: Measurement of metabolite levels at one instant $t$
Know your textbook
Literature recommended

Metabolomics and Isotope Tracing

Cholsoon Jang, Li Chen, and Joshua D. Rabinowitz

1Lewis Sigler Institute for Integrative Genomics and Department of Chemistry, Princeton University, Washington Rd, Princeton, NJ 08544, USA

Published in final edited form as:

A roadmap for interpreting $^{13}$C metabolite labeling patterns from cells

Nutrient and the response of metabolism

The quantitative inflows and effluxes from each metabolite must be balanced.
The origin of correlations in metabolomics data

Diogo Camacho, Alberto de la Fuente, and Pedro Mendes*

Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, MC 0477, Washington St., Blacksburg, VA, 24061, USA

Received 19 August 2004; accepted 15 September 2004
Glucose removal decreases flux through glycolysis but some glycolytic intermediates increase (e.g., PEP)

Adapted from Jang C, et al., Cell 2018
Metabolic flux

$^{13}$C-Glucose $\rightarrow$ $^{13}$C-lactate

$^{13}$C-F 1,6-BP $\rightarrow$ $^{13}$C-pyruvate
Isotopic tracer

Glucose

$^{13}$C$_6$-Glucose

U-$^{13}$C-Glucose

- Heavy isotope
- Non radioactive
- Similar physical properties
Because the labeled atom has the same number of protons, it will behave in almost exactly the same way as its unlabeled counterpart and, will not interfere with the reaction under investigation.
Isotopic tracing to study the activity of metabolic pathways in cells

**U-^{13}C-Glucose**

1. **G6-P**
2. **Nucleotides (IMP, AMP, GMP)**
3. **lactate**
4. **pyruvate**

**13C-enrichment in metabolites**

- **Steady-state labeling**
- **Dynamic labeling**

**13C-Glucose**

- **Metabolite A** (e.g., G6P, lactate)
- **Metabolite B** (e.g., IMP, AMP, GMP)

**13C-glucose/serine**

- **Steady-state labeling**
- **Dynamic labeling**

**glucose**

- **G6-P**
- **Nucleotides (IMP, AMP, GMP)**
- **lactate**
- **pyruvate**

**13C-glycine or 13C-serine**

- **Steady-state labeling**
- **Dynamic labeling**

**glycolysis**

- ATP
- ADP
- Fructose 1,6-BP
- Glyceraldehyde 3-P
- ATP
- ADP
- ADP

**U-13C-Glucose**

- **Intensity of labeling**

**Nucleotides**

- IMP
- AMP
- GMP

**Ribose**

**ATP**

- glycolysis

**U-13C-Glucose**

- **Intensity of labeling**

**Nucleotides**

- IMP
- AMP
- GMP

**Ribose**

**ATP**

- glycolysis

**U-13C-Glucose**

- **Intensity of labeling**

**Nucleotides**

- IMP
- AMP
- GMP

**Ribose**

**ATP**

- glycolysis
Isotopic tracing: Consider the size of the unlabeled pool

Dynamic labeling data

$^{13}C$ enrichment over time

-glutamate (condition A)

-glutamate (condition B)

$^{13}C_5$-glutamine

metabolite levels
Isotopic tracing to study the activity of metabolic pathways

Recommendation for tracing experiments:
- Consider tracer uptake (e.g., glucose uptake) before performing the tracing
- Perform the flux in tracer-free medium (e.g., glucose-free medium)
- Timing of labeling matters:

For $^{13}$C-glucose:
- ~15-30 min of labeling enables to label glycolytic intermediates at the steady-state level
- 2-4h of labeling is required to label the TCA cycle
- 6-15h of labeling is required to label nucleotides
Representation of the metabolic tracing diagram

13C6-glucose → pyruvate → Acetyl-CoA → TCA cycle
- Citrate
- OAA
- α-KG
- Glutamate
- Lactate
- Asp

PC, PDH

n rounds (n≥2)

12C

13C

n rounds (n≥2)
Isotopic tracing: Impact on scientific research

$^{13}C_5$-glutamine $\rightarrow ^{13}C_5$-$\alpha$-ketoglutarate ($\alpha$-KG)

Adapted from Buescher JM et al., *Curr Opin Biotechnol* 2015
Isotopic tracing: Impact on scientific research

\[ \text{Fractional abundance } \alpha\text{-KG (M+5)} = \frac{5615000}{6800500} \approx 0.82 \]

Adapted from Buescher JM et al., *Curr Opin Biotechnol* 2015
Isotopic tracing: Ben-Sahra lab

**Tracer:** $^{13}$C$_2$-$^{15}$N-glycine

**Glycine**
- **PRPP**
  - **PRA**
    - **GAR**
      - **FGAR**
      - **FGAM**
        - **AIR**
          - **CAIR**
            - **SAICAR**
              - **AICAR**
                - **FAICAR**
                  - **IMP**

**Formyl-THF**
- **THF**
- **Q**
- **E**
- **PPAT**
- **PFAS**
- **PAICS (E1)**
- **PAICS (E2)**
- **ATIC (E1)**
- **ATIC (E2)**
- **ADSL**
- **GART (E1)**
- **GART (E2)**
- **GART (E3)**

**De novo purine synthesis**

**SK-MEL-28**

**Ali ES, Sahu U et al., Molecular Cell in press**
Presentation of the tracing data

Chart

Untreated

IL-4

Histogram

$^{13}$C metabolic flux analysis (MFA) is a mathematical approach for quantifying intracellular metabolic fluxes in cancer cells.

Antoniewicz MR et al., Exp & Mol Med 2018
The software INCA can be used to perform MFA calculations.
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