Nascent Peptide SILAC: A Proteomic Approach to Studying Translational Repression

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Outline

Introduction to SILAC

How SILAC has been used in the miRNA field

How I intend to use SILAC
Mass Spectrometry is not Quantitative

Traditional Mass Spectrometry is inherently not quantitative
proteins protealize differently
differ in solubilization
differ in ability to be ionized

Quantification is necessary to measure changes in protein levels within cells or organelles

SILAC allows relative quantitation of peptides
SILAC:
Stable Isotope Labeling of Amino Acids in Cell Culture

control

“light”

^{12}\text{C}^{14}\text{N} \text{ Lys, Arg}

\downarrow

pool 1:1

\downarrow

experimental

“heavy”

^{13}\text{C}^{15}\text{N} \text{ Lys, Arg}

\downarrow

In labeled media ~5 doublings

\downarrow

LC-MS/MS

SILAC: Stable Isotope Labeling of Amino Acids in Cell Culture

Depends on protein half life and doubling rate of the cells

SILAC: For more than one condition

- Control
  - $^{12}\text{C}_6\ ^{14}\text{N}_4$-Arg (Arg 0)

- Experimental 1
  - $^{13}\text{C}_6\ ^{14}\text{N}_4$-Arg (Arg 6)

- Experimental 2
  - $^{13}\text{C}_6\ ^{15}\text{N}_4$-Arg (Arg 10)

Red bar: $\Delta$ 10 Da
Blue bar: $\Delta$ 6 Da
SILAC
Enriched for subsets of proteins

Mix cell lysates

Phospho-tyrosine

IP

10% SDS-PAGE

In-gel trypsin digestion

LC-MS/MS

Reduce complexity

Nucleolar proteome dynamics

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Control (time 0)  Treatment (time 1)  Treatment (time 2)

GFP-p68  GFP-p68, +act-D  GFP-p68, +act-D

Arg 0
\(^{12}\text{C}_6^{14}\text{N}_4\text{Arg}\)

Arg 6
\(^{13}\text{C}_6^{14}\text{N}_4\text{Arg}\)

Arg 10
\(^{13}\text{C}_6^{15}\text{N}_4\text{Arg}\)

Combine cells  \(\Rightarrow\) purify organelle  \(\Rightarrow\) 1D-PAGE of proteins

\(\Rightarrow\) in-gel digest with trypsin  \(\Rightarrow\) LC-MS\(^2\)  \(\Rightarrow\) quantitation

Relative intensity  Relative intensity

Arg 0  Arg 6  Arg 10

m/z  Time

Proteins (1–489)

NATURE | VOL 433 | 6 JANUARY 2005 | www.nature.com/nature
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MicroRNAs Target mRNAs Resulting in Diminished Protein Levels

- Translation inhibition
- mRNA degradation

Less protein production and subsequent mRNA destruction

Less protein production
A Correlation Between the Change in mRNA and Protein Abundance

Modes of miRNA-Induced Translational Repression

Peptide SILAC

Normal Translation

Density

Post-initiation block

Initiation block

Peptide SILAC

miRNP

PABP

PABP

AAAAAA

miRNP

PABP

PABP

AAAAAA

miRNP

PABP

PABP

AAAAAA

miRNP

PABP

PABP

AAAAAA

miRNP

PABP

PABP

AAAAAA
Modes of miRNA-Induced Translational Repression

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Polysome Profiling: Measuring Ribosome Density and Occupancy

Polysome Profiling

Velocity Sedimentation

Sucrose Gradient Fractions

JWM, 2007
Modes of miRNA-Induced Translational Repression

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Nascent Peptide SILAC

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purify nascent peptides

tryptsinize → LC-MS/MS
Use Puromycin to Pull Out Nascent Peptides

Tyrosyl tRNA

Puromycin
Conjugation of Tagged Puromycin to Peptides

Using Click Chemistry to Purify Alkyne-Tagged Puromycin + Peptide

Click Chemistry

\[ R^1_-N-N\equiv N + R^2-\equiv H \rightarrow R^1_-N-N\equiv N \\text{syn} + R^1_-N-N\equiv N \\text{anti} \]

Azide + alkyne

resin

Azide resin + Alkyne-puromycin
Alkyne-Puromycin Derivatives are “Clickable”

Alkyne Puromycin reacts with Fluorescent Azide

Currently optimizing incorporation into peptides
Mapping Peptides

Elute with Trypsin digestion

Analysis of N-terminal tryptic peptides should allow quantitation of peptide production
We can compare nascent peptide production to ribosome density (and mRNA expression).

One Possible Scenario

*Example of possible dataset - not real data
Getting a Handle on Translation

mRNA expression (microarray)

Ribosome occupancy (density gradient) ↔ New protein synthesis (peptide SILAC)
Getting a Handle on Translation

- Short timescale
- Sequence of events
  (mRNA turnover vs translation inhibition)
- Localization of translation
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