Supporting Information

Kinetics of precursor labeling in SILAC experiments

Tian Zhang[1], John C. Price[3], Eslam Nouri-Nigjeh[2], Jun Li[2], Marc K. Hellerstein[4,5], Jun Qu[2], Sina Ghaemmaghami[1‡]

[1]Department of Biology, University of Rochester, NY, USA

[2]Department of Pharmaceutical Sciences, University at Buffalo, Buffalo, NY, USA

[3]Department of Chemistry and Biochemistry, Brigham Young University, UT, USA

[4]Department of Nutritional Science and Toxicology, University of California, Berkeley, CA, USA

[5] KineMed, Inc., Emeryville, CA, USA

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Supporting Methods

Reagents

Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Dialyzed Fetal Bovine Serum (dialyzed FBS), Phosphate Buffered Saline (PBS), GlutaMAX, Pen-Strep Solution and MEM Vitamin Solution were purchased from Invitrogen. ^{15}N Amino Acid Mix was purchased from Cambridge Isotope Laboratories. Sodium butyrate was purchased from Sigma. Anhydrous calcium chloride, ferric nitrate, magnesium sulfate 7-hydrate, potassium chloride, sodium bicarbonate, sodium chloride, sodium phosphate were purchased from J.T. Baker. Glucose was purchased from USBiological. Vybrant DyeCycle Violet Stain was purchased from Invitrogen. Pyronin Y (PY) was purchased from Santa Cruz Biotechnology.

The molar percentage of each amino acid in the ^{15}N mix is as follows: Aspartic Acid: 9.1%, Glutamic Acid: 11.3%, Asparagine: 6.3%, Serine 4.6%, Glutamine 5.3%, Histidine 0.9%, Glycine 8.3%, Threonine 5.6%, Alanine 11.5%, Arginine 2.6%, Tyrosine 3.0%, Cystine 0.4%, Valine 6.2%, Methionine 1.4%, Tryptophan 2.0%, Phenylalanine 2.9%, Isoleucine 4.8%, Leucine 8.3%, Lysine 3.2% and Proline 2.3%. To make a fully ^{15}N labeled synthetic media, an inorganic salt mix was initially formulated with $CaCl_2$ (1.8 mM), $Fe(NO_3)_3$ (0.25 μM), $MgSO_4$ (0.8 mM), KCl (5.3 mM), $NaHCO_3$ (44 mM), NaCl (110 mM) and NaH_2PO_4 (1.0 mM). 1 g of ^{15}N amino acid mix was directly dissolved in the salt mix. This mix was then supplemented with glucose (4.5 g/L), dialyzed FBS (5%),

Vitamin Solution (1X) and Pen-Strep (1X). All stated concentrations are final concentrations.

FACS analysis and measurement of growth rate

N2a cells were grown to 50% confluency and were either left untreated or treated with 10 mM sodium butyrate for 24 h, 48 h, 64 h and 96 h. Cells were fixed with 70% ethanol for 24 h, suspended in DMEM and centrifuged at 300 xg for 5 min at room temperature. Supernatant was removed and the wash step was repeated. The cells were counted and resuspended in DMEM complete media. The final cell concentration was approximately $1x10^6$ cells/mL. Vybrant DyeCycle Violet Stain (Invitrogen) was added at 1µL per 1 mL to reach the final concentration of 5mM. After mixing gently for 5-10 seconds, cells were incubated at 37 °C for 5 min, 5µL of a 1:10 dilution of the PY stock solution was added. The samples were kept on ice and protected from light for at least 10 min prior sample acquisition on flow cytometer (LSR II BD). At least 20,000 events were collected in each analysis and data were analyzed by Flowjo software. In each sample, single cell populations were selected and the DNA content was analyzed by the distribution of fluorescence intensities. Cell cycle stages were quantified using the Watson cell cycle model within the FlowJo software.

Kinetic model

The kinetic model considered throughout the manuscript, has the following inherent assumptions:

1. Protein synthesis is a zero order process with respect to the protein concentration.

2. Protein degradation occurs at a constant fractional rate that is uniform for the entire protein pool. Thus, protein degradation can be modeled as a first order process with respect to protein concentration.

3. Cell doubling time is constant throughout the entire time-course and cell division is asynchronous within the cell population such that it can also be modeled as a first order process with respect to protein concentration.

4. The total protein concentration of each cell does not change during the experimental time-course (i.e. the system is at steady-state).

Thus, we can devise the following rate equation:

$$\frac{d[Protein]}{dt} = k_{syn} - k_{deg}[Protein] - k_{dil}[Protein] \quad (1)$$

Where k_{syn} is the first order rate constant for protein synthesis, k_{deg} is the first order rate constant for protein degradation and k_{dil} is the rate constant for dilution due to cell division.

With $[Protein](0) = 0$, we solve for $[Protein](t)$:

$$[Protein](t) = \frac{k_{syn}}{k_{dil}+k_{deg}} - \frac{k_{syn}}{k_{dil}+k_{deg}} e^{-(k_{dil}+k_{deg})*t} \quad (2)$$

The protein concentration at steady-state ($\frac{d[Protein]}{dt} = 0$) is:

$$[Protein_{steady-state}] = \frac{k_{syn}}{k_{dil}+k_{deg}} \quad (3)$$

Since our protein labeling measurements are fractional (i.e. normalized with respect to the total steady-state protein level), the observed fractional labeling is derived as:

$$Fraction\ Labeled\ Protein\ (t) = [Protein](t)/[Protein_{steady-state}] = 1 - e^{-(k_{dil}+k_{deg})*t} \quad (4)$$

Thus, the observed first order rate constant for fractional clearance (k_{clr}) is derived as:

$$k_{clr} = k_{dil} + k_{deg} \quad (5)$$

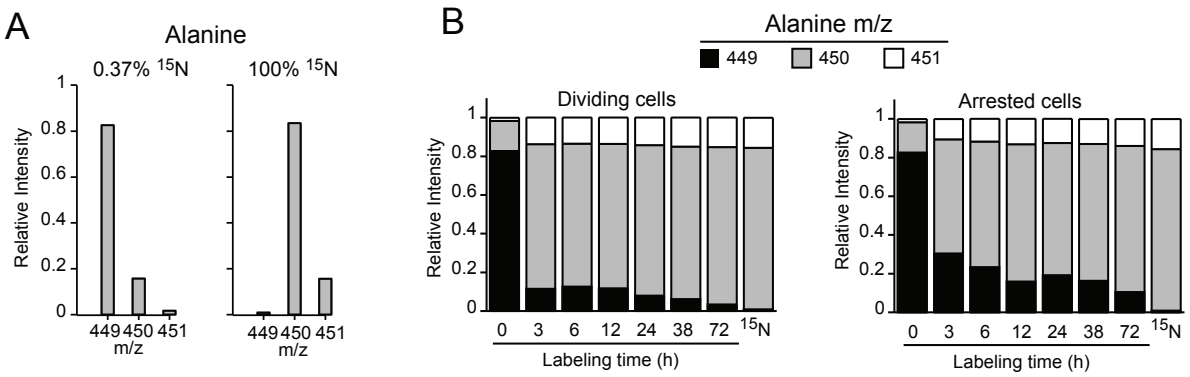

Figure S1. The ^{15}N labeling of the internal free amino acid pool analyzed by GC/MS in dividing N2a cells. (A) The isotopomer distributions of unlabeled and fully labeled alanine (B) The change in the isotopomer distribution of the free internal pool of alanine during the course of isotopic labeling.

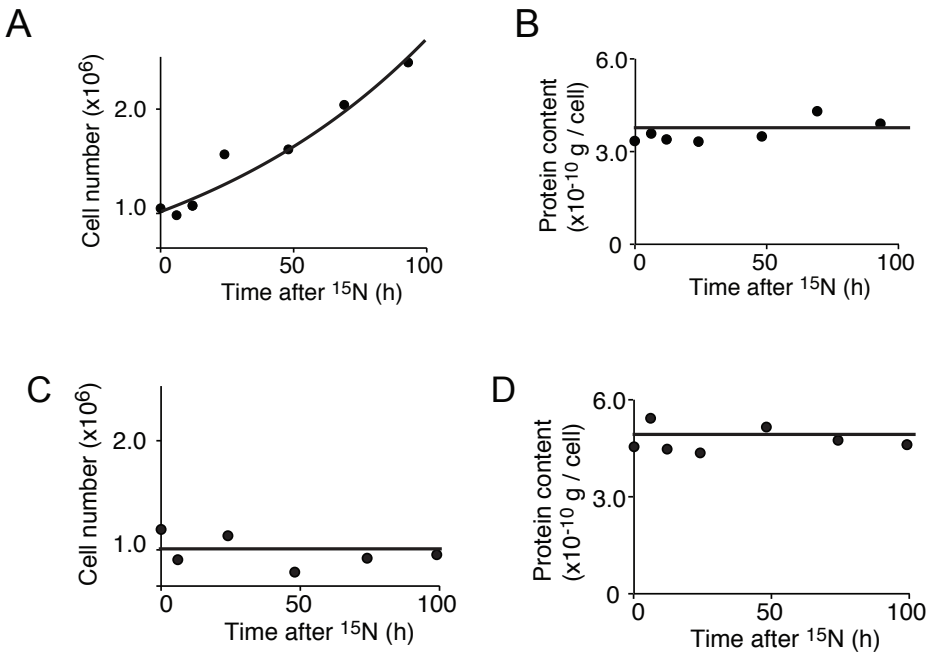

Figure S2. Growth curve and protein content after the initiation of ^{15}N labeling in dividing (A,B) and division arrested cells (C,D). Protein content was determined by BCA assay and normalized by cell number.

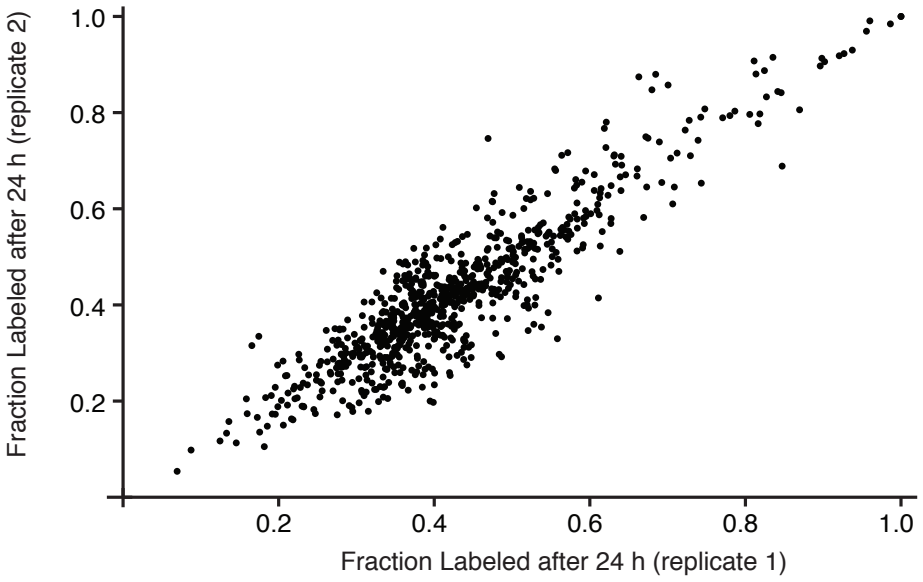

Figure S3. Biological replicate measurements of fraction labeled after 24 h in dividing N2a cells. The replicate measurements have a Pearson correlation coefficient of 0.91.

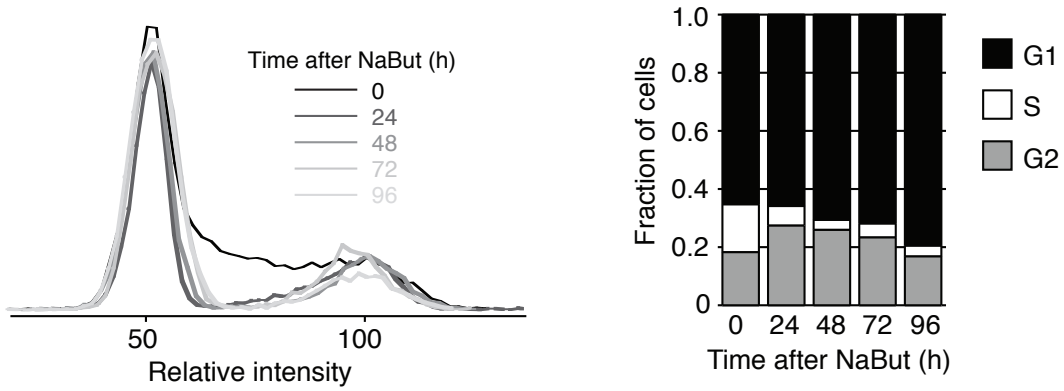

Figure S4. FACS analysis of cell cycle distribution after sodium butyrate (NaBut) arrest. The relative population of each cell cycle stage was quantified by fitting the observed distribution to the sum of three Gaussian distributions as described in Experimental Procedures.

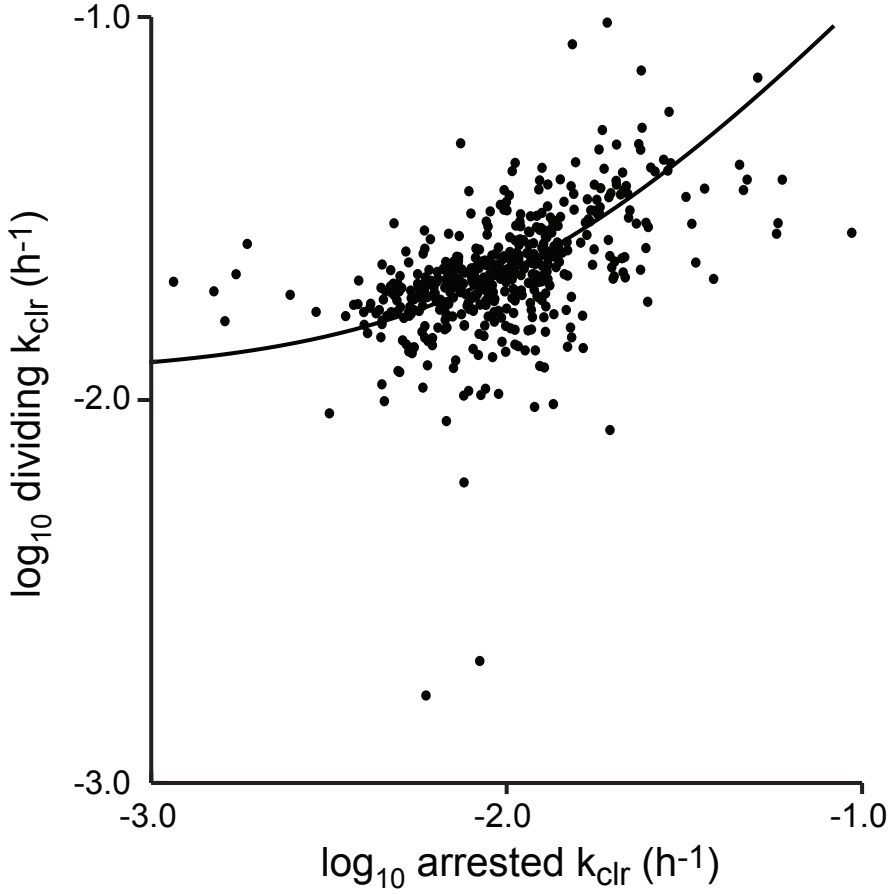

Figure S5. Pairwise comparison of clearance rates between dividing and arrested cells. Note that proteins with faster rates of turnover have similar k_{clr} values in the two states, whereas proteins with slower rates of turnover have faster k_{clr} values in dividing cells where they are primarily cleared through the dilution effects of cell division.