Supplementary information for

Measuring the Dynamics of *E. coli* Ribosome Biogenesis Using Pulse-Labeling and Quantitative Mass Spectrometry

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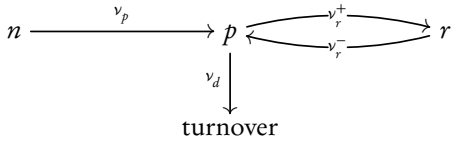
Exchange-plus-turnover model

In the main text, the pulse labeling kinetics of r-proteins S20, S21 and L33 were analyzed using an exchange-plus-turnover model in which the precursor pool p and completed ribosome r are represented by a single aggregate pool r' (see **Results**). Here, we describe the conditions under which labeling differences between the two pools effectively disappear, leading to the model for the turnover of r'.

To achieve labeling that is faster than dilution by growth, $f_{max}(t)$, existing material (which is less labeled than new material) must be removed from completed ribosomes. It can be shown that turnover of r-proteins in p alone will not cause $f_r(t)$ to exceed $f_{max}(t)$ while the degradation of individual ribosome-associated r-proteins or entire ribosomes would give rise to over-labeling. However, it does not seem reasonable to invoke a new biological phenomena in which specific ribosome-incorporated r-proteins are targeted for degradation. It is also unlikely that complete ribosomes are degraded, because the observed labeling kinetics for all other r-proteins are poorly-fit when constrained by a global degradation rate of 8.4% per generation (*i.e.* that of r-protein S21; calculations not shown).

Another mechanism that can explain the over-labeling of a ribosome-associated r-protein is its rapid exchange between the completed ribosome and the free protein pool combined with the turnover of free proteins (*e.g.* due to protein degradation; see **Discussion**). The derivation of this exchange-plus-turnover model, developed below, demonstrates that for reasonable exchange rates the mechanism simplifies to turnover from a single effective pool, r'.

As in the precursor pool model, amino acids in the nutrient pool, n, flow into the r-protein precursor pool, p, with flux ν_p, which in turn flows into the completed ribosome pool, r, with flux ν_r^+. In the exchange-plus-turnover model, r-proteins can also exchange from pool r back to p with flux ν_r^-, and, critically, r-proteins can be turned over from the pool p with flux ν_d (see flow diagram in next column).



Specifically, r-proteins exchange between pools p and r at the rate ϵ, and are degraded from the free pool p at the rate d. The formulas for the various fluxes are given in terms of the rates and pools by the following equations:

$$\nu_p = k\cdot(p+r)+d\cdot p \tag{S1}$$
$$\nu_r^- = \epsilon\cdot r \tag{S2}$$
$$\nu_r^+ = (k+\epsilon)\cdot r \tag{S3}$$
$$\nu_d = d\cdot p \tag{S4}$$

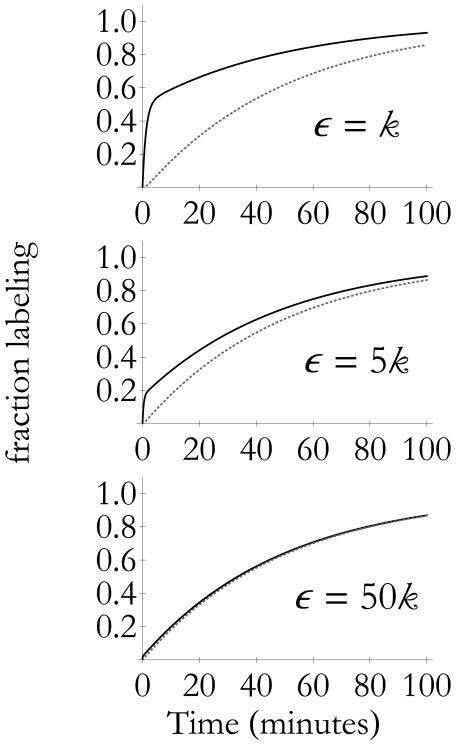
The differential equations describing the fraction labeling of the pools, r^* and p^*, are given by:

$$\frac{dp^*}{dt} = \nu_p + \nu_r^-\cdot f_r - \left(\nu_r^+ + \nu_d\right)\cdot f_p \tag{S5}$$
$$\frac{dr^*}{dt} = \nu_r^+\cdot f_p - \nu_r^-\cdot f_r \tag{S6}$$

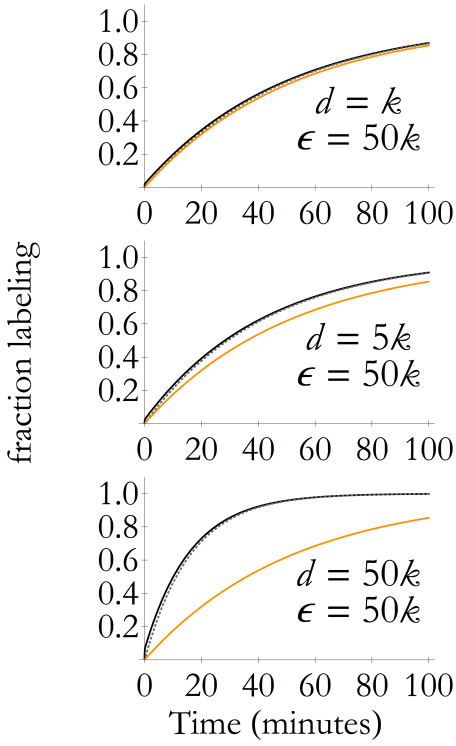
Substituting Equations S1-4 into S5 and S6 and applying the quotient rule gives the following set of equations that describe the time evolution of f_r and f_p:

$$\frac{df_p}{dt} = \left(k+\frac{k}{\mathbf{P}}+d\right)\cdot\left(1-f_p\right)+\frac{\epsilon}{\mathbf{P}}\cdot\left(f_r-f_p\right) \tag{S7}$$
$$\frac{df_r}{dt} = (k+\epsilon)\cdot\left(f_p-f_r\right) \tag{S8}$$

Equation S8 shows that any difference between f_p and f_r acts to eliminate the difference, since if $f_p > f_r$ the correction to f_r is positive, and if $f_p < f_r$ the correction to f_r is negative. For $\epsilon \gg k$, the labeling f_r tracks very closely with f_p.



Plot S1: Fraction labeling in the pools r and p for increasing exchange rate. f_r (dotted line) and f_p (solid line) for $\epsilon \in (k, 5k, 50k)$ with $\mathbf{P} = 5/100$ and $k = \ln(2)/36\ \text{min}^{-1}$.



Plot S2: Fraction labeling in the pools r and p for fast exchange and increasing turnover rate. f_r (dotted line), f_p (solid line) and f_{max} (orange line) for $d \in (k, 5k, 50k)$ with $\mathbf{P} = 5/100$, $k = \ln(2)/36\ \text{min}^{-1}$ and $\epsilon = 50k$.

Plot S1 shows that if the exchange rate between completed ribosomes and the precursor pools, ϵ, is roughly 50 times the cell growth rate, k, then there is no detectable difference between $f_p(t)$ and $f_r(t)$. This holds for all relevant values of the pool size $\mathbf{P}$ and degradation rate d. Thus, when $\epsilon \gg k$, it is reasonable to model the two pools p and r as a single pool, r', from which the turnover process occurs. Degradation from this aggregate pool can explain the observed over-labeling of r-proteins in completed ribosomes, as shown in Plot S2.

Estimation for the exchange rate ϵ

To estimate the average exchange rate ϵ for r-proteins, a typical value of $K_d = k_{off}/k_{on} = \epsilon/k_{on} = 10$ nM for r-proteins[1,2] and a diffusion-limited on rate of $k_{on} = 10^8\ \text{M}^{-1}\text{s}^{-1}$ were assumed. This simple calculation yields an estimated ϵ value of $1\ \text{s}^{-1}$. By comparison, the growth rate k is given by $\ln(2)/36\ \text{min}^{-1} = 3 \times 10^{-4}\ \text{s}^{-1}$. Therefore, ϵ is likely much greater than k, placing the exchange rate safely within the regime in which the labeling of the two pools is indistinguishable.

A note on the turnover rate d

Since the free protein pool is indistinguishable from the ribosome pool by pulse labeling for fast exchange rates, $\mathbf{P}$ is not measurable, and so it is not possible to know the actual fraction of free proteins of a given r-protein that are turned over. However, it can be shown that the reported turnover rate d' is related to the actual turnover rate for the precursor pool as $d' = d \cdot \frac{\mathbf{P}}{1+\mathbf{P}}$. In light of this, we report the conglomerate turnover rate d' for the combined pool r'.

Anomalous labeling kinetics of r-protein S20

In addition to its over-labeling, the labeling kinetics of S20 were observed to be significantly faster in completed 70S ribosomes (Fig. 2) than in dissociated 30S subunits in both S9 over-expression BL21(DE3) Tuner (Fig. S2B) and wild-type MRE600 (data not shown) strains. These anomalous results could be explained by the presence of extra-stoichiometric copies of S20[3,4] reversibly bound to the 50S ribosomal particles in *E. coli*. The presence of this significant precursor pool of on-pathway (and thus highly-labeled) S20 r-protein associated with the 50S sub-

unit could then explain both its over-labeling in the completed 70S ribosome, and its slower labeling kinetics in the dissociated 30S subunit. This potential mechanism is further supported by the observation of higher S20 protein levels in both 50S and 70S ribosomal particles relative to the 30S, as measured by qMS (data not shown).

References

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