L3.3: Protein Half Lives

Prof Jenna Rickus
In this lecture

• Dig deeper into protein half lives
  – The gene product (protein) decay rate constant, $\alpha$
  – From our hill function model of gene expression

• What does the half life of real proteins look like?

• Does this model hold up in real systems?
Return to our basic rate equation for gene expression

\[
\frac{dy}{dt} = \text{generation} - \text{consumption}
\]

\[
\frac{dy}{dt} = \beta f(x) - (\alpha_{\text{dilution}} + \alpha_{\text{degradation}})y
\]

Where
\[y = \text{conc. protein Y inside cell}\]

Consumption

\(\alpha_{\text{degradation}}\) small for a stable protein
\(\alpha_{\text{degradation}}\) large for rapidly degraded protein

\(\alpha_{\text{dilution}}\) large for rapidly dividing cells
\(\alpha_{\text{dilution}}\) large for slowly dividing cells
Rapidly Dividing Cells

\[ \alpha = \alpha_{\text{dilution}} + \alpha_{\text{degradation}} \]

\[ \alpha \approx \alpha_{\text{dilution}} \]

dominated by dilution

Slowly Dividing Cells

\[ \alpha = \alpha_{\text{dilution}} + \alpha_{\text{degradation}} \]

\[ \alpha \approx \alpha_{\text{degradation}} \]

dominated by degradation


\[
\frac{dy}{dt} = \beta f(x) - (\alpha_{\text{dilution}} + \alpha_{\text{degradation}})y
\]

If we could prevent or eliminate production of protein

\[
\frac{dy}{dt} = -\alpha y \quad \alpha = \alpha_{\text{dilution}} + \alpha_{\text{degradation}}
\]

Our model would predict simple exponential decay of protein levels with time

If we measured \( y \) levels over time we should be able to fit our data to a line with slope determined by the overall decay rate

\[\text{testable predictions}\]
Proteome Half-Life Dynamics in Living Human Cells

Eran Eden,*† Naama Geva-Zatorsky,* Irina Issaeva, Ariel Cohen, Erez Dekel, Tamar Danon, Lydia Cohen, Avi Mayo, Uri Alon†

Cells remove proteins by two processes: degradation and dilution due to cell growth. The balance between these basic processes is poorly understood. We addressed this by developing an accurate and noninvasive method for measuring protein half-lives, called “bleach-chase,” that is applicable to fluorescently tagged proteins. Assaying 100 proteins in living human cancer cells showed half-lives that ranged between 45 minutes and 22.5 hours. A variety of stresses that stop cell division showed the same general effect: Long-lived proteins became longer-lived, whereas short-lived proteins remained largely unaffected. This effect is due to the relative strengths of degradation and dilution and suggests a mechanism for differential killing of rapidly growing cells by growth-arresting drugs. This approach opens a way to understand proteome half-life dynamics in living cells.
How do you measure protein half life in living cells?

**Bleach Chase Experiments**

**Fig. 1.** Bleach-chase workflow. (A) Fluorescence of endogenously YFP-tagged proteins is automatically quantified from time-lapse movies (20-min resolution). Average dynamics (black) are means of ~500 individual cells (gray). (B) In bleach-chase, protein fluorescence dynamics is measured in bleached and unbleached cells (P and P$_v$ respectively). The difference between bleached and unbleached cells decays in time, with a slope on a semilogarithmic plot equal to the protein removal rate, \( \alpha \). Half-life is $T_{1/2} = \ln(2)/\alpha$. F.U., fluorescence units.

Eden et al 2011
Expected curves

Protein fluorescence

$\alpha = \text{large}$

$\alpha = \text{medium}$

$\alpha = 0$

$\ln(P(t) - P_v(t))$ (F.U.)

$\alpha = \text{large}$

$\alpha = \text{medium}$

$\alpha = 0$

Time (hours)

C

Eden et al. 2011
½ life of proteins in lung cancer cells

$\alpha = \text{protein removal rate}$
units = [time$^{-1}$]

$\alpha = \alpha_{\text{deg}} + \alpha_{\text{dil}}$

$\alpha_{\text{dil}} = 0.03 \pm 0.004 \text{ hr}^{-1}$
(based on cell cycle time)

$\alpha$ range: 0.03 hr$^{-1}$ - 0.82 hr$^{-1}$
ave. $\alpha = 0.1 \text{hr}^{-1}$

$T_{1/2} = \frac{\ln 2}{\alpha}$

- Wide range of $T_{1/2}$
- 45 min - 22.5 hours
- Mean = 9.0 hrs

Eden et al 2011
What if you add drug? Model Predictions

$\alpha^*, T_{1/2}^*$ are altered $\alpha, T_{1/2}$ after drug treatment

**Model I:**
Effect of reduced degradation

\[ \alpha^* = k_1 \cdot \alpha_{\text{deg}} + \alpha_{\text{dil}} \]

\[ T_{1/2}^* = \frac{T_{1/2}}{k_1 + (1-k_1) \frac{T_{1/2}}{T_{cc}}} \]

**Model II:**
Effect of reduced dilution

\[ \alpha^* = \alpha_{\text{deg}} + k_2 \cdot \alpha_{\text{dil}} \]

\[ T_{1/2}^* = \frac{T_{1/2}}{1 + (k_2 - 1) \frac{T_{1/2}}{T_{cc}}} \]

$k_1 = 10\%$

$k_2 = 10\%$

Eden et al 2011
Apply anti-cancer drug, CPT, which arrests cell growth

**Observed:**
See global increase in protein half life (decrease in $\alpha$, slope of bottom plot)
Is this dilution or degradation? CPT does change cell division rate.

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**Data:**
2 example proteins of the 100, DDX5 and LMNA

![Graphs showing protein half-life data](image)

**Equation:**
\[
\alpha = 0.12 \pm 0.01 \\
\alpha = 0.06 \pm 0.02 \\
\alpha = 0.05 \pm 0.01 \\
\alpha = 0.02 \pm 0.01
\]

**Table:**

<table>
<thead>
<tr>
<th>E</th>
<th>Degradation dominance</th>
<th>Mixed</th>
<th>Dilution dominance</th>
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<tbody>
<tr>
<td></td>
<td>48%</td>
<td>42%</td>
<td>10%</td>
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Eden et al 2011
Observed: CPT arrests cell growth, so $k_2 = 0$

Good Fit of Experimental Data to Model

Expected increase in $T_{1/2}$ due to complete stop in dilution ($k_2=0\%$)

Eden et al 2011
holds up quantitatively not just qualitatively

apply drugs that alter cell doubling time to varying degree, there by affecting $k_2$ to varying degrees

good fit of experimental to model

Design Curves: Can predict effects of altering cell cycle time on protein half lives

Eq. (2) $\alpha^* = k_1 \cdot \alpha_{deg} + \alpha_{dil}$

Eq. (3) $T_{1/2}^* = \frac{T_{1/2}}{k_1 + (1 - k_1) \frac{T_{1/2}}{T_{ee}}}$

Eq. (4) $\alpha^* = \alpha_{deg} + k_2 \cdot \alpha_{dil}$

Eq. (5) $T_{1/2}^* = \frac{T_{1/2}}{1 + (k_2 - 1) \frac{T_{1/2}}{T_{ee}}}$

Eden et al 2011
Design Curves

Functional consequence:
- Proteome of more rapidly dividing cells will get more out of whack when growth is arrested
- Long half-life proteins are more affected by growth arrest than short $T_{1/2}$ proteins

Eden et al. 2011
other implications of model

Degradation dominates short half life proteins

Dilution dominates long half life proteins

Proteins within a common functional cluster tend to have similar half lives.

Eden et al 2011
Coming up …

• Gene Circuit Motifs
  – Autoregulation

• Design features that are enabled
  – E.g. robustness