Monitoring Protein Synthesis in living cells with fluorescent labeled tRNA FRET pairs

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Di-Peptide (DIP) Technology

We transfect cells with tRNAs labeled as FRET donors and acceptors. A FRET signal is generated only when a donor labeled tRNA and an acceptor-labeled tRNA come in close contact (< 7 nm), as they do on the ribosome during the elongation cycle. The intensity of the FRET signal correlates with the number of ribosomes engaged in protein synthesis, providing a real-time, live-cell assay for measuring rates of protein synthesis.

Sample results: CHO cells were co-transfected with bulk Cy3- and Rhod-110-labeled yeast tRNAs. At 7h post transfection, cells were treated or not with puromycin or cycloheximide prior to fixation and imaging. [adapted from Barhoom 2011]

Specific DiP: monitoring synthesis of a specific protein with specifically selected pair of tRNAs

In humans there are 48 distinct isoacceptor tRNAs yielding 1176 distinct tRNA pairs. The E-factor of a protein is defined as the maximal ratio of the frequency of appearance of a specific di-tRNA in the synthesis sequence of the protein of interest relative to its appearance in all other proteins in the relevant cell or tissue under the given experimental conditions.

Image of a differentiating neuronal B104 cell showing sites of translation as reported by the occurrence of FRET between tRNAs labeled with the fluorophores Cy3 and Cy5. In this image, FRET efficiency is pseudocolored, with red equaling maximal FRET. Higher FRET signals are seen near the tip of the extending growth cone, indicating that these loci have higher numbers of translating ribosomes. [Image: Vanderklish lab, Scripps Research Institute]

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Length</th>
<th>E-Factor</th>
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</thead>
<tbody>
<tr>
<td>TNFRSF1B</td>
<td>Tumor necrosis factor-binding protein 2</td>
<td>808</td>
<td>46.12</td>
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<tr>
<td>CD40</td>
<td>Tumor necrosis factor receptor superfamily member 5</td>
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<td>60.13</td>
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<tr>
<td>RHOA</td>
<td>Transforming protein RhoA</td>
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<td>60.13</td>
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<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
<td>808</td>
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<td>CD40</td>
<td>Tumor necrosis factor receptor superfamily member 5</td>
<td>381</td>
<td>60.13</td>
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<tr>
<td>PTGFR</td>
<td>Prostaglandin F2-alpha receptor</td>
<td>197</td>
<td>60.13</td>
</tr>
</tbody>
</table>

Table 1: some familiar proteins with their leading di-tRNA and E-Factor

Specific DiP assay distinguishes infected from non-infected cells on 7:1 ratio (single cells)

Sample results: CHO cells were co-transfected with bulk Cy3- and Rhod-110-labeled yeast tRNAs. Arrows point to typical co-localizations, consistent with the formation of ‘viral factories’ [adapted from Barhoom 2011]

Mouse collagen synthesis can be monitored by selecting the appropriate pair of Gly-Pro tRNA out of the 12 possible pairs of Glycine (4 isoacceptors) and Proline (3 isoacceptors). Collagen type I is characterized by high abundance of Pro1:Gly4 di-tRNAs, with E-factors of 72.2 and 82.5 for the Col1a1 and Col1a2 genes, respectively.

Protein synthesis induction in serum-starved mouse fibroblasts treated with or without TGF-β1 (4ng/ml, 24 hours; image: Leask lab, University of Western Ontario)

Synthesis of viral (EHDV-2-IBAV) protein N53 can be monitored using Ile2:Ile2 or Ile2:bulk tRNA FRET. Sheep cells were infected (MOI=1) and transfected 13h post infection with Cy3-ile2-tRNA and yeast bulk Rd110-tRNA for 6h, fixed, immunostained (anti-NS3/Alexa-647) and imaged.