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

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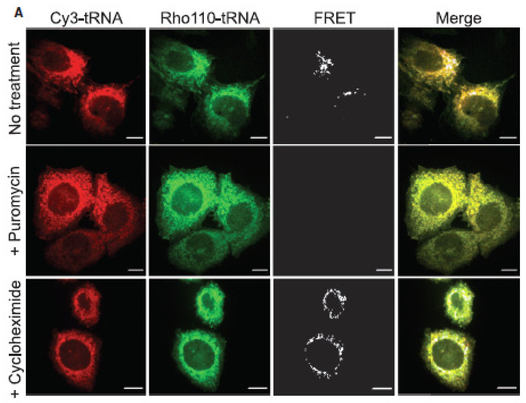
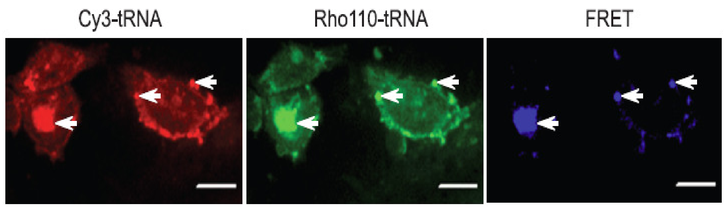
Di-Peptide (DiP) Technology

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 thev 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 Rho110-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]



Overall DiP: monitoring synthesis of overall protein with bulk tRNA



CHO cells, infected with EHDV2-IBAV were co-transfected with Cy3 and Rho110 fl-tRNA. Arrows point to typical triple co-localizations, consist-ent with the formation of 'viral factories' (adapted from Barhoom 2011]

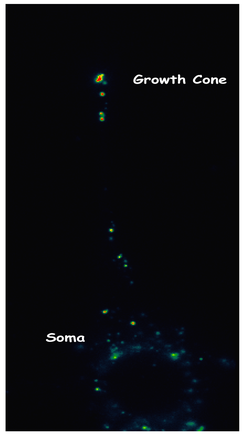
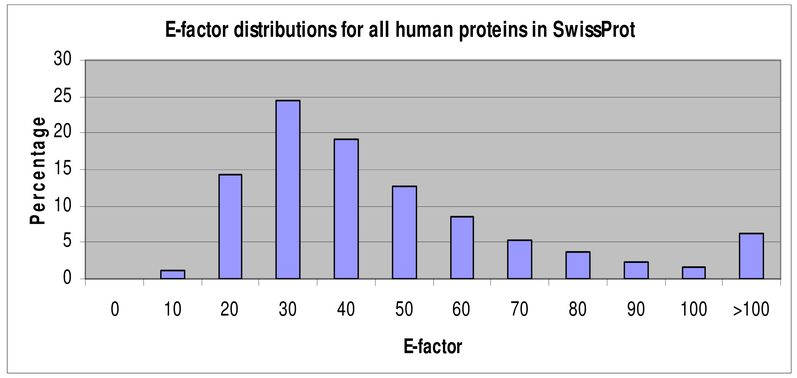


Image of a differentiating neuronal B104 cell showing sites of translation as reported by the occurrence of FRET between tRNAs labeled with the fluorophores Cy3 and Cy5. In this image, FRET efficiency is pseudocolored, with red equaling maximal FRET. Higher FRET signals are seen near the tip of the extending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

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

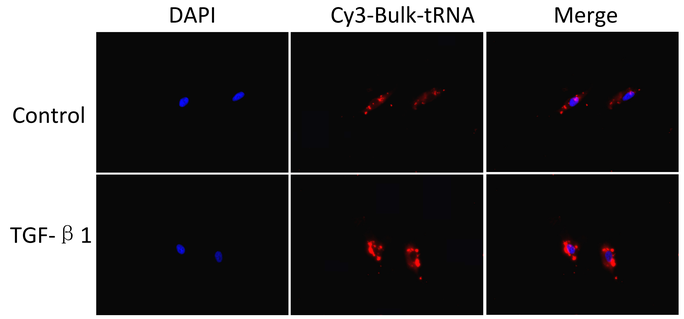
In humans there are 48 distinct isoacceptor tRNAs yielding 1176 distinct tRNA pairs. The E-factor of a protein is defined as the maximal ratio of the frequency of appearance of a specific di-tRNA in the synthesis sequence of the protein of interest relative to its appearance in all other proteins in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



Gene	Description	Length	di-tRNA	E-factor
AVPR1A	Vasopressin V1a receptor	418	Arg1Ser2	39.02
TNFRSF1B	Tumor necrosis factor-binding protein 2	461	Leu3Leu3	33.13
CD40	Tumor necrosis factor receptor superfamily member 5	277	Ile3Leu3	40.23
RHOA	Transforming protein RhoA	193	Thr2Arg5	60.09
TTN	Titin	34350	Leu5Leu4	306.35
CD1A	T-cell surface glycoprotein CD1a	327	Ile3Arg1	55.22
PRM1	Sperm protamine P1	51	Pro2Arg3	354.07
SORD	Sorbitol dehydrogenase	357	Val3Ser2	59.82
RB1	Retinoblastoma-associated protein	928	Ile3Ser2	35.92
REN	Renin	406	Gln2Ser2	62.08
RAC1	Ras-related C3 botulinum toxin substrate 1	192	Pro2Tyr1	106.64
PSMB5	Proteasome subunit beta type-5	263	Pro2Leu3	135.94
PTGFR	Prostaglandin F2-alpha receptor	359	Ile2Val3	82.06

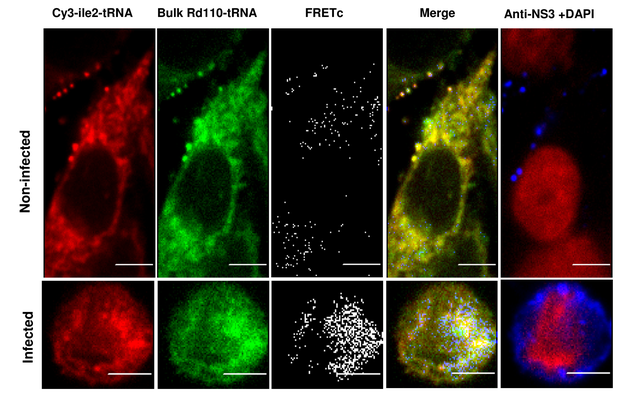
Table 1: some familiar proteins with their leading di-tRNA and E-factors

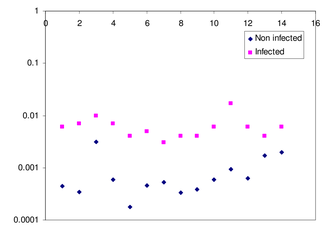
Mouse collagen synthesis can be monitored by selecting the appropriate pair of Gly-Pro tRNA out of the 12 possible pairs of Glycine (4 isoacceptors) and Proline (3 isoacceptors). Collagen type 1 is characterized by high abundance of Pro1:Gly4 di-tRNAs, with E-factors of 72.2 and 82.5 for the Col1a1 and 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 NS3 can be monitored using Ile2:Ile2 or Ile2:bulk tRNA FRET. Sheep cells were infected (MOI=1) and transfected 13 h post infection with Cy3-ile2-tRNA and yeast bulk Rd110-tRNA for 6 h, fixed, immunostained (anti-NS3/Alexa-647) and imaged. Courtesy Elroy-Stein and Ehrlich, Tel Aviv University.





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

Reference: Barhoom S, Kaur J, Cooperman BS, Smorodinsky NI, Smilansky Z, Ehrlich M, Elroy Stein O. Quantitative single cell detection of protein synthesis at subcellular resolution using fluorescently labeled tRNA. Nucleic Acids Research, 39(19):e129. 2011