Hypusine pathway gene expression examined by single molecule RNA fluorescence in situ hybridization (smRNA-FISH).

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Abstract

The fate of any given mRNA has remained difficult to experimentally follow and has left mechanisms of unexpected gene expression phenomena in the dark. Examples are differential stability of mRNA expression from different promoters, and inhibition of expression in trans from co-transfected plasmids encoding N1-spermidine/spermine, acetyltransferase and a second unrelated gene. Recent advances in single molecule fluorescence in situ hybridization (smRNA FISH) now bring such central aspects of cellular mRNA metabolism into view. Streamlining both the design and synthesis of probe sets of multiple tiled singly labeled 20-mer oligonucleotides with balanced GC content have made most mRNAs targetable. In contrast to traditional cell-disruptive RNA analyses - northern blotting, QPCR, and microarrays - that yield average and relative information on the content of the probed mRNA, smRNA FISH affords discrete and cell-specific copy numbers. That added spatial dimension can be applied to provide cell-to-cell variability and the actual range of mRNA numbers in a given cell within the field of focus, or to examine defined subsets of cells.
The hypusine pathway genes (EIF5A1, EIF5A2, DHPS, DOHH, and SAT1) are essential with deep evolutionary conservation. The gene products are tightly linked to the growth of the cell with aberrant expression in cells that have lost proliferation control, and in several cancers. SAT1, encoding N1-spermidine/spermine, acetyl-transferase, in particular plays a pivotal function in regulating polyamine levels and in turn determining the cells' proliferative state. Recently, vectors episomally expressing SAT1 were found to inhibit the expression from co-transfected plasmids. Inhibition only occurred with enzymatically active SAT1. To gain a better understanding of how the expression of the hypusine pathway genes may contribute to the transformed phenotype, the fate of these mRNAs and those of unrelated reporter genes was examined by smRNA FISH. Single cell expression data for the hypusine pathway genes is presented and compared with previous results from northern analyses. Data is also presented on the further use of the probe sets to examine changes in the numerical and spatial expression in cells after manipulation of the hypusine status. Lastly, data on the expression - mRNA and protein - in cells co-transfected with vectors for SAT1 and unrelated reporter genes is presented, distinguishing between post-transcriptional events that may cause the inhibition. In summary, we show smRNA FISH as a suitable tool to disseminate post-transcriptional events for genes relevant to polyamine homeostasis and regulated protein synthesis in normal and aberrant cells.

Hypusine pathway

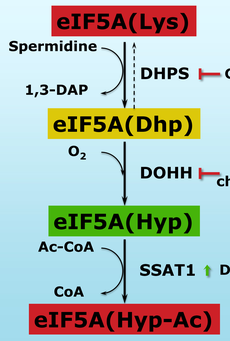

Figure 1. Both isoforms of eukaryotic translation initiation factor 5A (eIF5A-1, and eIF5A-2) are activated by deoxyhypusine synthase (DHPS) and deoxyhypusine hydroxylase (DOHH) in subsequent reactions to yield hypusine (Hyp). DHPS is inhibited by *N1*-guanyl-*N1,N7*-diamino-heptane (GC7), and DOHH by iron chelators. Hypusine is a target for Spd/Spm-N1-Ac-transferase 1 (SSAT1). Di-ethyl-nor-Spermine (DENSpm) strongly induces SSAT1 expression, but also serves as a suicide substrate, allowing cells to survive. *In vivo* evidence for SSAT1-mediated acetylation of eIF5A(Hyp) has not yet been possible to obtain.

Probe designs

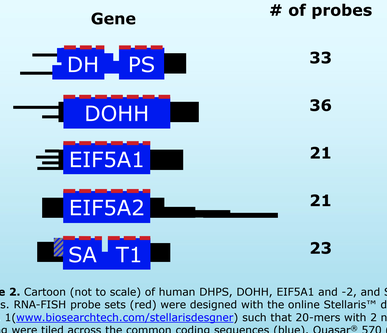

Figure 2. Cartoon (not to scale) of human DHPS, DOHH, EIF5A1 and -2, and SAT1 mRNAs. RNA-FISH probe sets (red) were designed with the online Stellaris™ design tool V. 1(www.biosearchtech.com/stellarisdesgner) such that 20-mers with 2 nt spacing were tiled across the common coding sequences (blue). Quasar® 570 (Q570; Cy3 alternative) was chosen for DHPS, DOHH, and EIF5A1, -2, and Quasar® 670 (Q670; Cy5 alternative) for SAT1 and and for GAPDH (control not shown)

- All five probe sets are inclusive and recognize >1 mRNA from each gene.
- Even short CDSs can be targeted with > 20 probes

Tissue specific expression

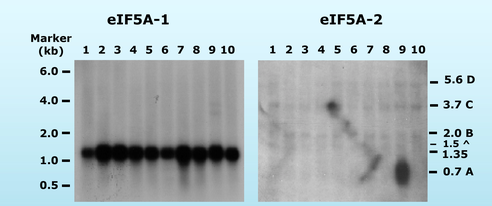

Figure 3 Multiple tissue northern blot (Ambion) of human poly A RNA probed with fragments of the CDS (A) 393 bp (exons 2-5) and (B) 425 bp (exons 2-4). The RNAs were from the following adult tissues: 1: Brain, 2: liver, 3: placenta, 4: small intestine, 5: colon, 6: pancreas, 7: spleen, 8: prostate, 9: testes, and 10: ovary. RNA size markers (kb) are on the left.

- EIF5A1 is highly expressed in all tissues. Northern does not distinguish between the isoforms.
- Five full length (and one partially spliced) mRNAs for EIF5A2. Longer forms predominant in brain and short transcript in testis.

Probe verification

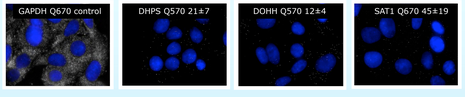

Figure 4. Fixed SW480 cells probed with sets in Fig. 2. From left: GAPDH (Q670), DHPS (Q570), DOHH (Q570), and SAT1 (Q670) as in Raj and Tyagi (2010), and Orjalo *et al.* (2011). Discrete spots identified and counted with RNA QUANT software.

SAT1 expression

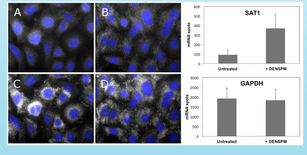

Figure 5 Induction of SAT1 expression. SW480 cells either mock treated (A and C), or with 10 µM DENSpm for 24 h (B and D) and simultaneously probed for SAT1 (A and B; Q670) and control GAPDH (C and D; Q570).

- Even the probe sets with <25 probes produce clearly visible spots of uniform intensity.
- In some cells, active transcription sites are visible for GAPDH and SAT1.

smRNA-FISH vs. northern

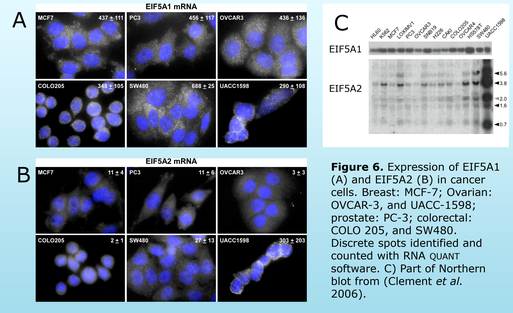

Figure 6. Expression of EIF5A1 (A) and EIF5A2 (B) in cancer cells. Breast: MCF-7; Ovarian: OVCAR-3, and UACC-1598; prostate: PC-3; colorectal: COLO 205, and SW480. Discrete spots identified and counted with RNA QUANT software. C) Part of Northern blot from (Clement *et al.* 2006).

- EIF5A1 and EIF5A2 expression consistent between smRNA FISH and northern analysis.
- smRNA FISH suitable for various cell typed with different morphology and thickness.

Conclusions

- Expression of EIF5A1 is ubiquitous in tissue, whereas that of EIF5A2 is high in testis, and barely detectable in brain.
- Expression of the normally repressed EIF5A2 is activated in select cancer cells
- Data on the expression levels of hypusine pathway mRNAs from northern analysis and smRNA-FISH correlate well with.
- There is little variation in the numbers of mRNAs from each of the tested genes in any of the tested cell populations.
- After induction of SAT1 the mRNAs are by smRNA-FISH found localized to the cytoplasm, indicating rapid post-transcriptional processing and transport.
- smRNA-FISH probes should prove invaluable in distinguishing cells not only with gene duplications, but also for reporting on the activity of such genes.
- smRNA-FISH probes also hold promise for more precise expression profiles, such that non-expressing, low-expressing and high-expressing cells can be identified.
- Probe sets with fewer than 25 probes can be used to faithfully detect short mRNAs in cultured cells.

References

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Acknowledgments and disclaimers

- We thank Sally Coassin for expert technical assistance.
- Biosearch Technologies, Inc. markets Stellaris™ RNA FISH probes.