

Development of FRET probes for imaging beta-actin mRNA in live cells

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Introduction:

The ability to accurately and repeatably track mRNA in mammalian cells would positively impact a broad range of biomedical studies. Much research is being done to understand the complicated cellular machinery controlling the localization of mRNA and protein translation, but little is known with certainty so far. β -actin is an excellent model system because it is well-studied, and its localization is known. In addition, it is commonly utilized as a standard RNA with which to compare expression level experimental results. There are a variety of tools currently used to study intracellular mRNA, including molecular beacons and fluorescently labeled oligonucleotide probes. Both of these tools will be employed to conduct fixed-cell, fluorescent in-situ hybridization (FISH) assays in primary human dermal fibroblasts (HDF) in order to more fully understand the localization of β -actin mRNA. Once a set of probes has been shown to work well in fixed cells, live cell testing will follow.

Methods & Materials:

Probes

Two sets of probes were tested; a pair of biotinylated DNA probes approximately 50 bp in length developed from the work of Kislaukis et. al¹ and a FRET pair of dye-labeled 2'-O-Methyl RNA molecular beacons derived from Abe and Kool² (see sequences in Table 1 in Appendix).

FISH

Fluorescent In-Situ Hybridization was used to detect β -actin mRNA in fixed samples. Samples were fixed using 4% ultra-pure paraformaldehyde for 10 minutes at 37°C and permeabilized in 0.1% Triton for 2 minutes at 37°C. Samples were allowed to hybridize for 30 minutes at 37°C in a blocking and hybridization buffer (200 nM final concentration of probe) as described by Singer³.

Results & Discussion:

Fluorescent imaging of hybridized cells shows both disperse and granular deposits of mRNA in the peripheral cytoplasmic regions when using the donor RNA molecular beacon. Currently, only the donor was tested; future experiments will include

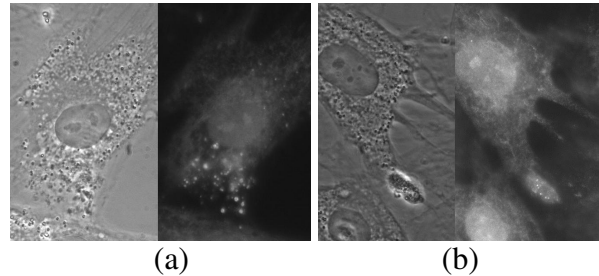


Figure 1: White-light and fluorescent images showing localization of β -actin mRNA deposits; fluorescence is from a CY3 labeled 2'OMe molecular beacon. Panel (a) shows the granular morphology, while panel (b) shows a diffuse region of elevated signal

the use of both the donor and acceptor once the hybridization conditions are optimized. DNA probes can be imaged by conjugating Alexa-488-labeled streptavidin to the 5' biotin before introducing the probes to the fixed cells; attempting to introduce streptavidin after hybridization results in almost exclusive binding of streptavidin to endogenous mitochondrial biotin. This effect may also be mitigated by using lower concentrations of the dye labeled streptavidin. Further experiments must be done to improve the specificity of binding and signal, as well as to perform control experiments using siRNA to lower the actin mRNA expression level.

These results agree in principle with what has been seen in the literature and with what would be expected.^{4,5} Future experiments will utilize both donor and acceptor molecular beacons to achieve higher specificity and sensitivity in both fixed and live cells. Also, a variety of techniques for altering the cells localization of β -actin mRNA will be used to verify that the imaging probes developed accurately describe β -actin mRNA localization and their dynamics.

Conclusion:

There is much future work to be done, but the research done thus far, supported by relevant literature, is an excellent step towards reliable techniques for studying the localization of mRNA in live cells.

References:

1. Kislauskis EH, Zhu X, Singer RH. beta-Actin messenger RNA localization and protein synthesis augment cell motility. *J Cell Biol.* 1997 Mar 24;136(6):1263-70.
2. Abe H, Kool ET. Flow cytometric detection of specific RNAs in native human cells with quenched autologating FRET probes. *Proc Natl Acad Sci U S A.* 2006 Jan 10;103(2):263-8.
3. Singer RH. In situ hybridization of mammalian cells (RNA and oligonucleotide probes)
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4. Hill MA, Schedlich L, Gunning P. Serum-induced signal transduction determines the peripheral location of beta-actin mRNA within the cell. *J Cell Biol.* 1994 Sep;126(5):1221-9.
5. Latham VM Jr, Kislauskis EH, Singer RH, Ross AF. Beta-actin mRNA localization is regulated by signal transduction mechanisms. *J Cell Biol.* 1994 Sep;126(5):1211-9.

Appendix:

DNA Probe 1:

5'—biotin—CACCAACTGGGACGACATGGAGAAAATCTGGCACCACACCTTCTACAATGAGCT-3'

DNA Probe 2:

5'—biotin—GGCATCCACGAAACTACCTTCAACTCCATCATGAAGTGTGACGTGGACATC—3'

RNA Probe FRET DONOR

5'-Cy3-ATAGCACAGCCTGGATAT-BHQ2-3'

RNA Probe FRET ACCEPTOR

5'-BHQ2-TACGGCCAGAGGCGTAC-Cy5-3'

Table 1: Sequences for the two sets of probes used in FISH assays. For DNA probes, location of Biotin is marked at the 5' end. For RNA probes, the BHQ2 represents the quencher molecule.