Citations

The In-crowd

RNA interference, which once enjoyed nearly unrivaled popularity in gene therapy circles, has lost some of its cachet. One illustration of this reversal is the growing acceptance that techniques for delivering small interfering RNA (siRNA) into cells and tissues must improve if the therapeutic promise of these molecules is to be attained. Monitoring siRNA entry into cells is usually performed by conjugating a fluorophore to an oligonucleotide terminus. However, adding a bulky fluorescent moiety runs the risk of tampering with the localization and behavior of the labeled siRNA. Of equal concern is that the very same attachment site needed for the fluorophore may be required for conjugating functional groups that enable entry into cells. An alternative approach would be to label the siRNA by incorporating a modified nucleobase with fluorescent properties. One such base analogue, 6-phenylpyrrolocytosine (PhpC) binds faithfully to guanine and is a sufficiently good stand-in for cytosine to substitute for the natural base in enzyme substrates. Based on these promising traits, Wahba et al. decided to test the performance of PhpC-bearing siRNAs for traceable gene silencing, and report their findings in *ACS Chemical Biology*. The authors had several material concerns. An initial worry was that the modified base could disrupt the stability of the siRNA duplex. However, there was little change in melting temperature of siRNA with multiple substitutions of cytosine, PhpC incorporation in both the guide and passenger strands, or placement of the modified deoxynucleotide at the 3' overhangs. When the various

modified siRNAs were evaluated in silencing assays, all knocked-down gene expression though potency diminished with modifications at the 3' end of the guide strand or upon replacement of all the internal cytosines. Modified siRNAs also behaved equivalently to unmodified siRNA in terms of immunostimulation. Although interactions with the innate immune system lead to undesirable off-target effects, the PhpC-containing oligonucleotides are intended as reporters of siRNA behavior, so even this less-desirable trait is important to mimic. Confident that PhpC didn't disrupt any crucial properties of siRNA, Wahba et al. then tested whether the modified siRNAs could be followed in transfection experiments. While siRNA variants with a single modified base required unreasonably long exposure times, oligonucleotides containing 9 PhpC bases were easily monitored after lipofection. With these studies, the authors establish PhpC-modified siRNA, which can be detected using a standard DAPI filter set, as a new reagent for routine monitoring of siRNA uptake in knockdown experiments. More importantly, fluorescence tracking of these siRNAs should give a much-needed boost to understanding and improving siRNA uptake and trafficking.

Wahba et al. Phenylpyrrolocytosine as an unobtrusive base modification for monitoring activity and cellular trafficking of siRNA. *ACS Chem Biol.* [Epub ahead of print, July 21, 2011; doi:10.1021/cb200070k].

In the Loop

Histone modification is a key regulator of chromatin structure and transcriptional activity. While the ways in which acetylation and methylation of particular histone H3 lysine residues correlate with transcribed versus silent chromatin are well known, few studies have succeeded in monitoring these modifications in individual living cells. In an article appearing in *Nucleic Acids Research*, Hayashi-Takanaka et al. introduce the FabLEM (antibody fragment–based live endogenous modification labeling) method for tracking histone modifications. The probes are fluorescently conjugated Fabs prepared from monoclonal antibodies against acetylated or methylated H3 lysines. Once loaded into the cytoplasm of a living cell, the Fabs can diffuse into the nucleus where they bind reversibly with their targets. After testing a variety of monoclonal antibodies, the authors conclude that a dissociation constant in the picomolar range is required for visualizing subnuclear localization of modified histones. Lower-affinity Fabs are suitable for tracking the overall level of histone modification in a given cell, a measure that rests on the ratio of nuclear to cytoplasmic Fab signal. For instance, in cells

Changes in histone acetylation (top) and dimethylation (bottom) visualized by FabLEM monitoring.

treated with the histone deacetylase inhibitor trichostatin A, this ratio rises over a two-hour period for a Fab against acetylated lysine 9 of H3, while concurrently the nuclear fraction of Fabs that recognize dimethylation of this residue (a repressive modification) diminishes. Extended monitoring experiments of this kind are a noteworthy advantage of FabLEM compared with staining of fixed cells. For the most avid Fabs, the technique can also track histone modifications in mouse preimplantation embryos, and neither

Fab injection nor long-term imaging lessens the likelihood of obtaining live pups after embryo transfer. Although FabLEM may not be as sensitive to rapid changes in protein modification as FRET-based measures, the new technique has the advantage of probing endogenous proteins and is also compatible with simultaneous monitoring of more than one modification. For these reasons, FabLEM is an appealing new tool for epigenetics studies in living cells and developing preimplantation embryos.

Hayashi-Takanaka et al. Tracking epigenetic histone modifications in single cells using Fab-based live endogenous modification labeling. *Nucleic Acids Res.* [Epub ahead of print, May 16, 2011; doi:10.1093/nar/gkr343].

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