Citations

Catch as Catch Can

Given their importance in post-transcriptional gene regulation, identifying which miRNAs target particular transcripts is expected to provide important information concerning development, differentiation, and cellular homeostasis. Computational predictions have been used to identify potential miRNA target sequences in mRNAs. However, in silico approaches don't pick up noncanonical sites and cannot account for factors that affect real-world binding, such as mRNA folding, intracellular miRNA concentrations, and the presence of RNA-binding proteins. Circumventing these challenges requires experimental methods, such as co-purification of miRNAs and mRNAs from cells. One such method uses exogenous co-expression of a fusion transcript containing an mRNA bait and the MS2 hairpin sequences along with a fusion of MS2 coat protein and an affinity tag. Although miRNAs can be captured in this system, these pulldowns seem to only pick up the most abundant miRNAs. To get a fuller picture of interacting miRNAs, Braun et al. developed miRNA trapping by RNA in vitro affinity purification (miTRAP), publishing their method in *Nucleic Acids Research*. In

miTRAP, an in vitro transcript containing the 3' untranslated region of the gene of interest plus four MS2 hairpins is immobilized via an MS2 coat protein–MBP fusion to amylose resin. After cell lysate is poured over this bait, co-purifying RNAs are analyzed by quantitative RT-PCR or deep sequencing. Tests with well-characterized miRNA target sequences showed that the method was selective and able to pick up low-abundance miRNAs. In addition to identifying miRNAs that have been shown to regulate the bait mRNA and those that have been predicted by computational methods to interact, miTRAP captured a number of novel miRNA-mRNA interactions. The authors used miRNA-directed decoys and luciferase reporter assays to validate 9 out of 10 candidate miRNAs, suggesting that miTRAP is a reliable method for uncovering new miRNA-mRNA regulatory relationships.

J. Braun et al. 2014. Rapid identification of regulatory microRNAs by miTRAP (miRNA trapping by RNA in vitro affinity purification). *Nucleic Acids Res.* [Epub ahead of print, February 7, 2014; doi:10.1093/nar/gku127]

The Speed of Light

When it comes to speed, nothing beats light-controlled transcriptional activators. This key advantage over small-molecule inducers has led to a blossoming of optogenetics tools. Many take an approach akin to two-hybrid assays and depend upon the recruitment of a transcriptional activator domain to a DNA-binding domain via a light-induced interaction. However, the need for multiple protein components and the possibility of interfering with endogenous protein-DNA interactions are disadvantages of this approach. Other optogenetic tools based on an engineered version of the photosensory LOV (light/oxygen/voltage) domain can bind directly to DNA. However, their weakness is a deactivation time in excess of two hours. In an article in *Nature Chemical Biology*, Motta-Mena et al. describe the development of a light-triggered gene expression system with rapid activation and deactivation kinetics. The basis of their system is the bacterial transcription factor EL222, which consists of an LOV domain and a helix-turn-helix (HTH) DNA-binding domain. Illumination with blue light forms a protein-flavin adduct in LOV, triggering the HTH domain to dimerize and bind DNA. To this small protein, the authors added a VP16 transcriptional activation domain and a nuclear localization signal. This VP-

Light-induced dimerization of VP-EL222 and activation of gene expression. Source: *Nature Chemical Biology*.

EL222 construct, when cotransfected into 293T cells with a luciferase reporter under the control of a minimal promoter with EL222-binding sequences, showed a more than 100-fold increase in luciferase expression (versus background) after exposure to 24 hours' worth of cycling blue light illumination (20 seconds on, 1 minute off). The background signal was due to basal promoter activity of the minimal promoter rather than activation of VP-EL222 in the dark or recruitment of cellular factors by the DNA binding sequence for EL222. In experiments with stably transfected cells, gene expression from a luciferase reporter was shown to be titratable by the length of light exposure. Using these stable lines, Motta-Mena et al. explored the kinetics of gene

expression, and through modeling, calculated VP-EL222 to have an activation time of 3–5 seconds and deactivation (via adduct cleavage) within an impressive 10–40 seconds. With this groundwork in place, the authors tested their construct in zebrafish, showing that microinjection of VP-EL222 led to light-dependent expression with substantially less toxicity than a previously described cryptochrome-based system. The authors concede that the persistent background expression from the minimal promoter may rule out use of VP-EL222 in certain applications and that engineering of the HTH is necessary for the transactivator to target other DNA sequences. However, the simplicity, compatibility with low-intensity light, and quick activation and reset parameters should give VP-EL222 a bright future as an optogenetics tool.

L.B. Motta-Mena et al. 2014. An optogenetic gene expression system with rapid activation and deactivation kinetics. *Nat Chem Biol.* 10:196-202.

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