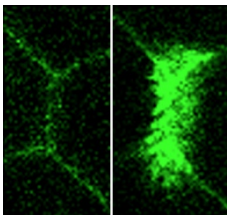
OPTOGENETICS

Follow the PIF

Dev. Cell **36**, 117–126 (2016)



ELSEVIER

Experimental control of protein localization will help decipher protein function and manipulate cell behaviors. The phytochrome optogenetic system exploits the red light–mediated heterodimerization of the phytochrome B protein (PHYB) with its partner phytochrome interaction factor (PIF) in the presence of the chromophore PCB. However, this technique has not yet been applied to multicellular organisms because of the inability of PCB to penetrate deep tissues and the poor expression of PHYB. Buckley *et al.* were able to successfully optimize this system for zebrafish embryos by delivering a newly purified version of PCB into the embryos along with a truncated version of PHYB that optimizes its expression. When a PHYB-CAAX construct is used to drive membrane localization, exposure to 650-nm light rapidly recruits PIF6 to the membrane. Shifting the light to 750 nm reverses membrane binding of PIF6. Subcellular control of localization can be achieved by focusing the 650-nm laser at a point of interest. Given their interest in the regulation of polarity during neurogenesis, the authors generated a Pard3-PIF6 fusion construct and were able to direct the apical polarity protein Pard3 and recruit its binding partner Pard6 to specific membrane locations in the neural progenitors by the spot of the 650-nm laser. Overall, the use of this modified phytochrome system offers the potential to regulate cellular behaviors with greater control in a multicellular organism. *GM*

TARGET IDENTIFICATION

Getting cholesterol out

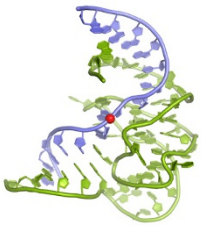
eLife **4**, e12177 (2016)

Cholesterol transport within cells is a well-understood process wherein low-density lipoprotein (LDL) is internalized via receptor-mediated endocytosis and trafficked to lysosomes, where cholesterol is ultimately liberated. Cholesterol is then transported by a lysosome-lumenal protein called NPC2 to the lysosomal membrane–embedded cholesterol efflux transporter NPC1. A deficiency of NPC1, as in the lysosomal storage disease Niemannn-Pick type C, leads to cholesterol accumulation within lysosomes. U18666A is a cationic amphiphile that inhibits the exit of LDL-derived cholesterol from lysosomes, mimicking NPC1 deficiency, though its target has not been formalized. To find the molecular target of U18666A, Lu *et al.* generated a photo-crosslinkable form of the compound, U-X, that also contained an alkyne group useful for a post-crosslinking attachment of functional elements such as fluorophores through click chemistry. Incubation of a CHO cell line with U-X identified a protein that was characterized as NPC1. The threshold for U18666A inhibition increased >100-fold with NPC1 overexpression, whereas NPC1 depletion resulted in a loss of U-X crosslinking. Testing several NPC1 point mutants that cause cholesterol transport deficiencies for their ability to crosslink with U-X led to the conclusion that U18666A binds to the sterol-sensing domain (SSD) of NPC1, not the cholesterol-binding N-terminal domain. These results suggest that the SSD contains a second cholesterol-binding site that may act in a relay to move cholesterol from lysosomes. *MB*

CATALYTIC DNA

Ligating with lambda

Nature **529**, 231–234 (2016)



VLADIMIR PENA

Enzymes comprised solely of DNA have not been observed in nature, but laboratory evolution experiments have identified proficient DNA catalysts. The lack of structural data on deoxyribozymes has limited our understanding of DNA-driven catalysis, but a new study reporting the X-ray crystal structure of the 9DB1 ligase deoxyribozyme, which catalyzes phosphodiester bond formation between the terminal 3′-hydroxyl and the 5′-triphosphate of RNA substrates, changes this landscape. In their 2.8-Å-resolution structure of a 9DB1 DNA construct with a product RNA strand, Ponce-Salvatierra *et al.* revealed a λ-shaped folded complex, which is built from partially stacked short helical domains that are held in place by long-range base-pairing. Nucleotides of the A-G RNA ligation site are organized in a duplex-like conformation at the λ three-way junction by specific base-pairing interactions with the protruding J2/3 region and additional stacking and tertiary interactions, which also enforce the enzyme's 3′,5′-ligation regiochemistry. Unlike with ligase ribozymes, no catalytic metal ions were observed in the active site of 9DB1, but a phosphate oxygen of dA13 is positioned nearby and critical for ligation catalysis. Further functional group mutagenesis of key base and sugar residues validated the 9DB1 structural model, defined how the greater conformational flexibility of DNA nucleotides facilitates catalytic structure formation and informed rational design strategies for engineering 9DB1 for broader substrate specificity. *TLS*

RNA MODIFICATION

Translating for growth

Science **351**, 282–285 (2016)

Hydroxymethylcytosine (5hmC) is a modification that occurs in DNA and RNA and is mediated by Tet methyldioxygenases. Although the biological roles and functions of DNA methylation have been characterized, less is known about the effects of RNA 5hmC modifications. To address this, Delatte *et al.* used *Drosophila* as a system because they do not contain DNA 5hmC modifications and express a single functional Tet gene (*dTet*). Dot blot analysis of *Drosophila* S2 cell extracts using a 5hmC antibody revealed that polyadenylated RNAs were highly modified by 5hmC. On this basis, the authors reasoned that 5hmC modification might regulate translation. Sucrose-gradient fractionation of S2 cell extracts followed by dot blotting showed that mRNAs associated with polysomes had a high level of 5hmC content, while *in vitro* translation analysis of hydroxymethylated RNA templates indicated that 5hmC-modified RNA promoted translation. To determine whether these effects had any biological significance, Delatte *et al.* examined 5hmC and *dTet* levels during *Drosophila* embryogenesis and detected high expression of both in the larval brain. Consistent with the expression patterns, *dTet* mutant larvae, which expressed lower levels of 5hmC, had smaller brain size and decreased neuroblasts. Although these findings reveal new insights regarding 5hmC RNA modifications, further studies are needed to determine whether the 5hmC-mediated regulation of translation is related to the fly brain defects. *GM*

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