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Optogenetic control of RNA function and metabolism using engineered light-switchable RNA-binding proteins

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RNA-binding proteins (RBPs) play an essential role in regulating the function of RNAs in a cellular context, but our ability to control RBP activity in time and space is limited. Here, we describe the engineering of LicV, a photoswitchable RBP that binds to a specific RNA sequence in response to blue light irradiation. When fused to various RNA effectors, LicV allows for optogenetic control of RNA localization, splicing, translation and stability in cell culture. Furthermore, LicV-assisted CRISPR–Cas systems allow for efficient and tunable photoswitchable regulation of transcription and genomic locus labeling. These data demonstrate that the photoswitchable RBP LicV can serve as a programmable scaffold for the spatiotemporal control of synthetic RNA effectors.

Recent studies have reshaped prior conceptions about the functions of RNAs, particularly the numerous non-coding RNAs that play important roles in diverse cellular activities[1]. RNAs exhibit complex dynamics and functions at specific times and locations inside cells, and these dynamics include changes in their expression, degradation, translocation, splicing and other chemical modifications[2–4]; thus, techniques capable of the precise and spatiotemporal manipulation of RNA dynamics and functions are highly desirable for understanding the physiological functions of RNA in live cells[5,6]. To this end, light is an ideal trigger because it is easy to obtain, highly tunable, non-toxic and, most importantly, has a high spatiotemporal resolution[7]. Several studies have attempted to control RNA functions through the activation of chemically caged RNAs using UV light; these efforts allowed for the optical control of gene expression[8–12], ribozyme activity[13], CRISPR–Cas function[14] and protein–RNA crosslinking[15,16]. However, the acceptance of these methodologies by biologists has been limited, probably because of the toxicity of UV radiation and the technical complexities associated with the synthesis of caged RNAs[6,17]. Alternatively, RNA functions might be optogenetically controlled by genetically encoded photoswitchable RBPs[6]. Optogenetics is a burgeoning technique in which genetically encoded photoswitchable proteins are used to manipulate biological processes with unsurpassable flexibility and high spatiotemporal precision[7,18]. In eukaryotic cells, numerous proteins are thought to function as RBPs, which govern almost all aspects of RNA metabolism, including transcription, processing, translation, turnover and cellular localization[19,20]. Nevertheless, reports on natural photoswitchable RBPs are extremely rare.

Recently, Weber et al.[21] selected an RNA aptamer that can specifically bind PAL, a natural blue light receptor, in a light-dependent manner. The light-activated PAL–RNA interaction was shown to be capable of translational repression in bacteria and mammalian cells. We hypothesized that synthetic photoswitchable RBPs could also be engineered from natural RBPs and photoreceptors. Previously, we and others developed several photoswitchable DNA-binding proteins (DBPs) and optogenetic transcriptional control systems for the reversible, tunable and spatiotemporal regulation of gene transcription with light[22], and among these, single-component systems under the control of one photoswitchable DBP appear to be favored because of their advantages of simplicity and a large on/off ratio[23–28]. These photoswitchable DBPs are based on straightforward modular designs consisting of a DNA-binding domain and a small light–oxygen–voltage (LOV) domain-containing protein. Following exposure to blue light, the oligomerization states and binding capabilities of the photoswitchable DBPs are altered to those of their cognate DNA sequences, which directly leads to gene transcription activation[24,26–29] or repression[25]. We therefore hypothesized that replacement of the DNA-binding motif in the photoswitchable DBPs with an RNA-binding motif would create photoswitchable RBPs, which might be valuable optogenetic tools for research in RNA biology.

Results

Engineering of a photoswitchable RBP. To identify an RNA-binding motif that is simple and orthogonal to the human transcriptome, we searched sequence databases and identified a transcriptional antiterminator protein from *Bacillus subtilis* termed LicT (Supplementary Fig. 1a and Supplementary Tables 1–7). LicT consists of an N-terminal RNA-binding domain (coantiterminator (CAT)) and two phosphorylatable phosphotransferase system regulation domains. During activation, LicT undergoes conformational changes that stabilize the LicT dimer and specifically binds a ribonucleic antiterminator (RAT) RNA sequence to prevent the

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