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Light-inducible activation of target mRNA translation in mammalian cells†

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A genetically encoded optogenetic system was constructed that activates mRNA translation in mammalian cells in response to light. Blue light induces the reconstitution of an RNA binding domain and a translation initiation domain, thereby activating target mRNA translation downstream of the binding sites.

In recent years, there has been an increased interest in controlling cellular function using visible light.[1–9] Light offers several advantages over traditional ligands such as small molecules or proteins: it is an orthogonal signal that in most cells does not interfere with endogenous ligands; most mammalian cells do not ordinarily respond to light; it is easy to control the concentration of this signal, *i.e.*, light intensity; the intensity is tunable over a broad range and is not susceptible to the limitations of solubility in culture medium; one can exert spatiotemporal control over this signal; and multiplexing is possible by varying the wavelength of light. Most of the prior reports have focused on optogenetic systems and tools to regulate the activity of targeted proteins at the post-translational level or to control gene expression at the transcriptional level. Indeed, while light-inducible control of gene expression by transcriptional activation has been successfully demonstrated in bacteria,[3,10] yeast,[6,11] and mammalian cells,[5,12] optogenetic tools to regulate other processes that control protein levels, such as mRNA translation, have remained elusive.

Previous fundamental studies have shown that tethering the eukaryotic initiation factor 4G (eIF4G)[13] or eIF4E[14] alone to target mRNA is sufficient to initiate mRNA translation. Translation initiation in eukaryotic cells involves the recruitment of the 40S small ribosomal subunit to the 5′cap of an mRNA, and the cap-binding complex eIF4F (consisting of subunits eIF4A, eIF4E and eIF4G) is responsible for establishing this interaction between the target mRNA and the small ribosomal subunit.[15] In greater detail, the eIF4E can bind to the 5′cap structure of mRNA, and poly(A)-binding protein PABP binds to the poly(A) tail. Endogenous eIF4G is then recruited to bridge the eIF4E and PABP and thereby circularize the mRNA, and the resulting complex recruits the small ribosomal subunit resulting in translational initiation. Interestingly, when a fusion protein of eIF4E and an RNA binding domain is tethered to an aptamer upstream of an open reading frame (ORF), translation can be driven downstream of the aptamer in a 5′cap independent manner.[14,16]

Here, we report a modular platform to control various post-transcriptional processes in response to light. We reasoned that the ability to bring together an RNA tether – a protein domain that binds to a specific RNA motif – and a translation activator such as eIF4E in response to light would enable light-inducible control of mRNA translation (Fig. 1). To achieve this objective, we made use of

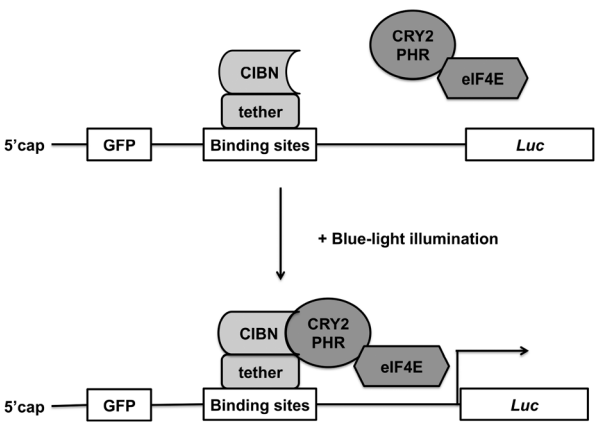

Fig. 1 Strategy for initiating mRNA translation in mammalian cells in response to light. In the cytoplasm, CIBN is tethered to the binding sites on the target mRNA *via* fusion to an RNA binding domain (tether). Upon exposure to blue light, the CRY2PHR–CIBN interaction helps recruit eIF4E to the target mRNA, thereby initiating the translation of luciferase.

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the photosensitivity of the *Arabidopsis thaliana* Cryptochrome 2 (CRY2) protein, which binds to its partner protein Cryptochrome-interacting basic-helix–loop–helix 1 (CIB1) upon light illumination.[17] CRY2 transits into the photoexcited state in the presence of blue light and interacts with the protein CIB1, and the protein subsequently reverts to the ground state and dissociates from CIB1 within minutes after light is turned off. Since mRNA translation occurs in the cytoplasm, we chose the N-terminal photolyase homology region (PHR) (1–498) of CRY2, which lacks a nuclear localization signal (NLS), and an NLS-deficient truncated version of the CIB1 protein (CIBN) (1–170)[6] for this study. We reasoned that CRY2PHR would bind to CIBN upon blue light illumination, and a reconstituted complex also containing a tether and translational activation domain would then help initiate translation downstream of the tether binding sites on the target mRNA (Fig. 1).

An RNA tether – the N-terminal 22 amino acids of a λ phage antiterminator protein (λN), which binds to a 19-nucleotide RNA hairpin (boxB) with a dissociation constant of 1.3 nM[13,18] – was fused to CIBN, and the translational activator eIF4E was fused to CRY2PHR (Fig. 2a). The reporter plasmid contained 6 boxB aptamers between the green fluorescent protein GFP (upstream) and the firefly luciferase (Luc) reporter (downstream) (Fig. 2a). We chose 6 copies of boxB since we observed that reporter plasmids containing 6 boxB aptamers showed a significantly higher level of translational activation upon the expression of a λ-eIF4E fusion protein than those containing 0, 1, or 2 boxB aptamers (see Fig. S1 in ESI†). The boxB aptamers were placed 80 nt downstream of the stop codon of GFP and 75 nt upstream of the start codon of luciferase[13] (Fig. 2a). The coding sequence for GFP was under the control of 5′cap-dependent translation and GFP therefore served as a visual reporter for cell transfection. The stop codon of the *gfp* gene also prevented 5′cap dependent translation of the downstream *luc* gene.

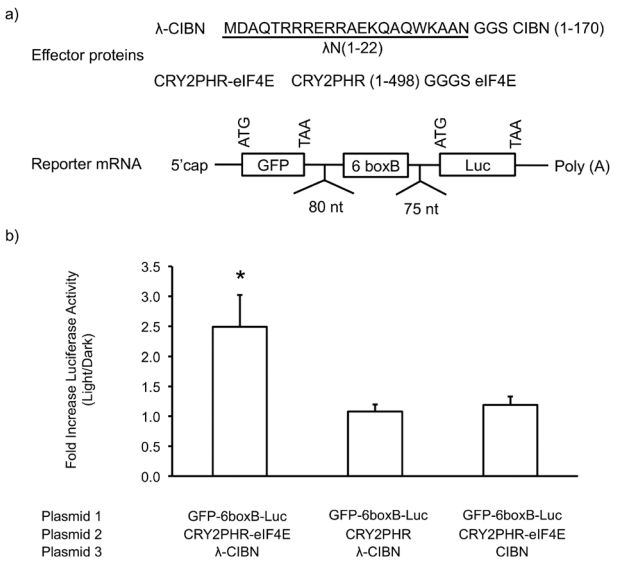

Fig. 2 Light-inducible activation of mRNA translation. (a) Schematic representation of the effectors and reporter. (b) Influence of blue light on the luciferase activity of HEK 293T cells transfected with 3 plasmids: (i) reporter (GFP–6boxB–Luc) containing 6 boxB aptamers; (ii) CRY2PHR–eIF4E or CRY2PHR; and (iii) λ-CIBN or CIBN (*$p < 0.005$ *vs.* dark; $n = 9$).

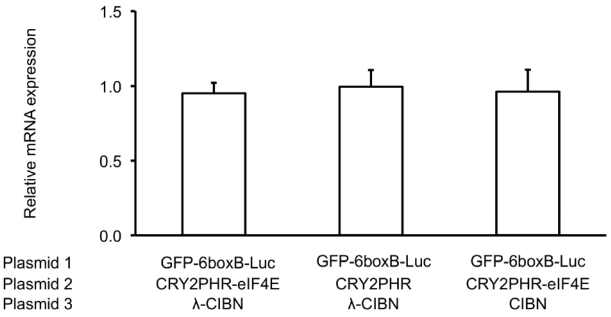

Fig. 3 Quantitative real-time PCR assay shows that the reporter mRNA amounts of HEK 293T cells transfected with CRY2PHR–eIF4E, λ-CIBN and a luciferase reporter containing 6 boxB aptamers are similar in the dark and after exposure to blue light. The mRNA amount of cells in the dark was set at 1.

To test the ability to activate the translation of luciferase in a light-sensitive manner, human embryonic kidney 293T (HEK 293T) cells were plated one day before transfection and co-transfected with plasmids encoding CRY2PHR–eIF4E, λ-CIBN, and the bicistronic reporter. One day after transfection, the cells from each plate were harvested and equally distributed to two plates: one plate was placed in the dark and the other was exposed to an uninterrupted series of 30 second blue light pulses (with a 30 second gap between successive pulses) delivered using a customized LED array for a period of 48 hours. After 48 hours, the luciferase activity was measured in both the plates. We observed a 2.5-fold increase in luciferase activity in the cells exposed to blue light compared to cells in the dark (Fig. 2b). Very little change in luciferase levels was observed in control experiments where CRY2PHR–eIF4E was replaced by CRY2PHR, or where λ-CIBN was replaced by CIBN. Quantitative real-time polymerase chain reaction (QRT-PCR) results revealed that the amount of luciferase mRNA in transfected cells remained essentially unchanged under dark and light conditions (Fig. 3). These results confirm that the light-induced increase in luciferase activity was not due to an increase in levels of reporter mRNA and are consistent with an increase in translational activation in response to light. It may be possible to achieve even greater translational activation by optimizing the position of the aptamer in the intercistronic region.[13]

To establish the generality of the system, we applied it to another tether–aptamer pair. We chose the bacteriophage MS2 coat protein (MS2) as a second tether because it is known to bind with high affinity to a 21-nt RNA hairpin.[19] HEK 293T cells expressing CRY2PHR–eIF4E, CIBN–MS2, and the reporter containing 6 MS2-binding aptamers showed light-inducible luciferase expression similar to that seen previously for a reporter containing 6 boxB aptamers (see Fig. S2 in ESI†). To investigate whether the two tethers recognize their own aptamer specifically, we transfected HEK 293T cells with one of the reporters (GFP–6boxB–Luc or GFP–6MS2–Luc), CRY2PHR–eIF4E, and either λ-CIBN or CIBN–MS2. Only cells transfected with plasmids encoding matched aptamer–tether pairs showed a significant light-induced increase in luciferase activity, while those transfected with plasmids encoding mismatched aptamer–tether pairs did not (see Fig. S3 in ESI†). We have thus demonstrated the specificity of this approach. We note that RNA aptamers that recognize new molecular targets specifically can

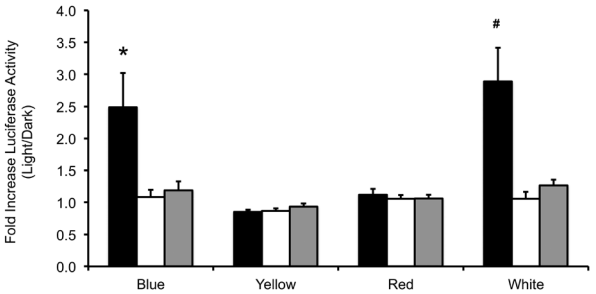

Fig. 4 Luciferase activity of HEK 293T cells expressing CRY2PHR–eIF4E, λ-CIBN and a luciferase reporter containing 6 boxB aptamers significantly increases in the presence of white light but not yellow or red light (*$p < 0.005$ *vs.* dark, #$p < 0.01$ *vs.* dark; $n \geq 4$). Black bar: GFP–6boxB–Luc + CRY2PHR–eIF4E + λ-CIBN; white bar: GFP–6boxB–Luc + CRY2PHR + λ-CIBN; grey bar: GFP–6boxB–Luc + CRY2PHR–eIF4E + CIBN.

be obtained efficiently by SELEX (Systematic Evolution of Ligands by Exponential Enrichment)[20,21] and the light-inducible activation of mRNA translation could therefore be extended to a large number of orthogonal aptamer–tether pairs.

We next investigated the response of this system to different wavelengths of light. CRY2 protein has an absorption peak at 450 nm and absorbs little above 510 nm.[22] HEK 293T cells expressing the reporter, CRY2PHR–eIF4E and λ-CIBN were placed in the dark or exposed to yellow (595 nm), red (630 nm), or white light from an LED array. We found that for transfected cells exposed to yellow and red light, the luciferase activity was similar to that of cells placed in the dark. In contrast, the luciferase activity of the cells exposed to white light increased significantly compared with that of cells kept in the dark (Fig. 4). This result indicates that the light-inducible translational activation system is specific for blue light and that translation can be prevented when not desired by carrying out experiments under yellow or red light. The experiment also confirms that white LED light could be used to activate the translation of the reporter.

The light-inducible translational activation system, in combination with other developed optogenetic systems and tools, further expands the capability to control fundamental cellular processes using light. We note that translation can also be induced in mammalian cells using small molecules. Boutonnet *et al.*[23] reported a 2-fold activation of translation upon addition of a farnesyl transferase inhibitor in mammalian cells transiently co-transfected with inducer and reporter plasmids. However, in contrast to small molecules, some of which may have undesired pharmacological activity, light (at the levels used here) is non-toxic and inert towards cellular targets in most mammalian cells. Moreover, all the components involved in our system are genetically encoded and do not require addition of an exogenous cofactor as is required for some light-sensitive proteins.[24]

In summary, we have developed a light-inducible translational activation system in mammalian cells based on the light-inducible reconstitution of a RNA binding domain and a translation initiation domain. To our knowledge, this is the first optogenetic system applied at the translational level. In addition to controlling mRNA translation, the system can be modified and potentially extended to control mRNA splicing, repression, stability, and translocation by tethering different effector domains to target mRNA in response to light.[25–27] Therefore, the combination of light-inducible protein heterodimerization and RNA tether function technology would greatly extend the optogenetic control of cellular behaviors and of the fate of mRNA at the post-transcriptional level.

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Notes and references

1 K. Deisseroth, *Nat. Methods*, 2011, **8**, 26–29.
2 E. S. Boyden, F. Zhang, E. Bamberg, G. Nagel and K. Deisseroth, *Nat. Neurosci.*, 2005, **8**, 1263–1268.
3 A. Levskaya, A. A. Chevalier, J. J. Tabor, Z. B. Simpson, L. A. Lavery, M. Levy, E. A. Davidson, A. Scouras, A. D. Ellington, E. M. Marcotte and C. A. Voigt, *Nature*, 2005, **438**, 441–442.
4 Y. I. Wu, D. Frey, O. I. Lungu, A. Jaehrig, I. Schlichting, B. Kuhlman and K. M. Hahn, *Nature*, 2009, **461**, 104–108.
5 M. Yazawa, A. M. Sadaghiani, B. Hsueh and R. E. Dolmetsch, *Nat. Biotechnol.*, 2009, **27**, 941–945.
6 M. J. Kennedy, R. M. Hughes, L. A. Peteya, J. W. Schwartz, M. D. Ehlers and C. L. Tucker, *Nat. Methods*, 2010, **7**, 12–16.
7 H. Ye, M. Daoud-El Baba, R. W. Peng and M. Fussenegger, *Science*, 2011, **332**, 1565–1568.
8 M. Ui, Y. Tanaka, Y. Araki, T. Wada, T. Takei, K. Tsumoto and K. Kinbara, *Chem. Commun.*, 2012, **48**, 4737–4739.
9 L. J. Bugaj, A. T. Choksi, C. K. Mesuda, R. S. Kane and D. V. Schaffer, *Nat. Methods*, 2013, **10**, 249–252.
10 R. Ohlendorf, R. R. Vidavski, A. Eldar, K. Moffat and A. Möglich, *J. Mol. Biol.*, 2012, **416**, 534–542.
11 S. Shimizu-Sato, E. Huq, J. M. Tepperman and P. H. Quail, *Nat. Biotechnol.*, 2002, **20**, 1041–1044.
12 L. R. Polstein and C. A. Gersbach, *J. Am. Chem. Soc.*, 2012, **134**, 16480–16483.
13 E. De Gregorio, T. Preiss and M. W. Hentze, *EMBO J.*, 1999, **18**, 4865–4874.
14 E. De Gregorio, J. Baron, T. Preiss and M. W. Hentze, *RNA*, 2001, **7**, 106–113.
15 N. Sonenberg and A. G. Hinnebusch, *Cell*, 2009, **136**, 731–745.
16 R. S. Pillai, S. N. Bhattacharyya, C. G. Artus, T. Zoller, N. Cougot, E. Basyuk, E. Bertrand and W. Filipowicz, *Science*, 2005, **309**, 1573–1576.
17 H. Liu, X. Yu, K. Li, J. Klejnot, H. Yang, D. Lisiero and C. Lin, *Science*, 2008, **322**, 1535–1539.
18 J. Baron-Benhamou, N. H. Gehring, A. E. Kulozik and M. W. Hentze, *Methods Mol. Biol.*, 2004, **257**, 135–154.
19 J. Carey and O. C. Uhlenbeck, *Biochemistry*, 1983, **22**, 2610–2615.
20 C. Tuerk and L. Gold, *Science*, 1990, **249**, 505–510.
21 A. D. Ellington and J. W. Szostak, *Nature*, 1990, **346**, 818–822.
22 R. Banerjee, E. Schleicher, S. Meier, R. M. Viana, R. Pokorny, M. Ahmad, R. Bittl and A. Batschauer, *J. Biol. Chem.*, 2007, **282**, 14916–14922.
23 C. Boutonnet, O. Boijoux, S. Bernat, A. Kharrat, G. Favre, J. Faye and S. Vagner, *EMBO Rep.*, 2004, **5**, 721–727.
24 R. Khanna, E. Huq, E. A. Kikis, B. Al-Sady, C. Lanzatella and P. H. Quail, *Plant Cell*, 2004, **16**, 3033–3044.
25 R. S. Pillai, C. G. Artus and W. Filipowicz, *RNA*, 2004, **10**, 1518–1525.
26 C. Chou, A. Mulky, S. Maitra, W. Lin, R. Gherzi, J. Kappes and C. Chen, *Mol. Cell. Biol.*, 2006, **26**, 3695–3706.
27 Y. Wang, C. G. Cheong, T. M. T. Hall and Z. Wang, *Nat. Methods*, 2009, **6**, 825–830.