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Review

Genetically encoded RNA photoswitches as tools for the control of gene expression

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ABSTRACT

An important goal in chemical and synthetic biology is controlling the expression of defined sets of genes by external stimuli, and one of the most attractive stimuli is light. Current approaches to the photocontrol of biological processes utilize photoresponsive proteins. In this article, I will illustrate the prospects of synthetic systems in which the receptor is a photoresponsive nucleic acid, and will review the different tools already in place to develop photoresponsive systems based on RNA. A particular focus is on genetically encoded photoswitches that can be expressed in prokaryotic or eukaryotic cells, and respond to photoisomerizable, cell-permeable small molecules.

1. Introduction

One of the most important goals in chemical and synthetic biology is controlling the expression of defined sets of genes by external stimuli. Such systems allow switching on or off at will the production of proteins of interest, exogenously controlling cell function, or constructing increasingly complex gene networks with unprecedented control. Simple trigger systems involve either chemical (e.g., exogenous small molecules) or physical (e.g., light) signals. Response to these signals requires an appropriate sensor for the stimulus either on the cell surface or inside the cell. Typically, these sensors have a high specificity for the trigger signal, responding only to a certain type of small molecule, or to light of a specific wavelength. Current approaches to the photocontrol of biological processes utilize photoresponsive proteins derived from natural photoreceptors or their domains. In this article, I will examine strengths and limitations of this approach and illustrate the prospects of synthetic systems in which the receptor is not a protein, but a photoresponsive nucleic acid. A particular focus is on genetically encoded photoswitches that represent native, i.e., chemically unmodified, aptameric nucleotide sequences that can be expressed in prokaryotic or eukaryotic cells, and respond to photoisomerizable, cell-permeable small molecules.

The reader should be aware that the ultimate goal, the photocontrol of gene expression at the RNA level, has not been achieved yet. I therefore consider this essay a "concept paper", where I present a personal appraisal of the present status and the potential of the proposed approach. I describe the overall concept as well as the various tools that have been developed by scientists in different fields, and that can now – in my opinion – be combined to develop and establish genetically encoded RNA photoswitches as tools for the control of gene expression.

2. Photoresponsive proteins in Nature

Various processes in Nature use light for vital processes, from photosynthesis to growth control to synthesis of hormones or vision. The utilization of light is achieved by specialized photoreceptors that respond to changes in light intensity, quality, direction, and duration [1]. These light-responsive proteins often bind a small organic cofactor or chromophore that enables them to interact with light. Absorption of light by the chromophore results in photochemical and conformational changes that ultimately lead to activation of the photoreceptor and the initiation of signaling. Currently, six types of natural photoreceptors are distinguished: rhodopsins, xanthopsins, phytochromes, blue-light sensors using flavin cofactors (BLUF), light-oxygen-voltage (LOV) sensors, and chryptochromes [2]. The first three catalyze the *E/Z* isomerization of retinal, phytochromobilin, and *p*-coumaric acid, respectively, while the last three involve different flavin-based photochemistry [3]. Thus, the primary photochemistry of these photoreceptor proteins changes the configuration of the chromophore, which in turn initiates the formation of a signaling state of the receptor to communicate the absorption of a photon to a downstream signal transduction partner.

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3. Photoresponsive proteins in synthetic biology and optogenetics

In addition to providing important information on how organisms detect and respond to light, the obtained knowledge of photoreceptor function has led to advances in synthetic biology, too [4]. The field of "optogenetics" concerns technologies that exploit natural or engineered light-gated proteins (i.e., photoreceptors) to perturb and control biological events in a spatiotemporally exact manner [1,2]. These photoreceptors are genetically encoded, non-invasive, and applicable to intact cells and organisms. In contrast to fluorescent proteins, which should generally not disturb the process under study, photoreceptors are intended to modulate activity. Recently, a number of studies reported the generation of light-induced systems for controlling gene expression in mammalian cells [5–7] (for a comparative discussion, see [8]).

Among the six types of natural photoreceptors, the LOV sensors and the phytochrome receptors are composed of modules, i.e., contiguous compactly folded protein domains. This feature facilitates their application in the construction of photoresponsive fusion proteins. While the photosensor modules mediate light detection and serve as input domains, actuator or effector modules display various biological activities, from enzymatic rate acceleration to DNA or protein binding, transport or channel activity, and serve as output domains. Signal-dependent interactions between sensor and effector modules lead to a change in biological activity in response to the signal. This modularity provides the basis of current design approaches, which are based on (careful) domain fusion [2].

A disadvantage of these nature-derived photosensors is their restriction to a small set of chromophores, and therefore to certain pre-defined wavelength ranges. For broad applicability in synthetic biology, one would like to have multiple switches, each of which responds to a different, narrow wavelength range. This would allow the construction of systems where a certain channel is used for photoswitching, while others are reserved for simultaneous spectroscopic observation, or for the design of orthogonal systems that combine several different switches and function as logic gates, allowing a more sophisticated control of biological processes by light of different wavelengths than simple on/off switches. Furthermore, the development of switches that work in the red or infrared part of the spectrum would be more relevant for triggering inside tissues or life animals due to the superior penetration characteristics of light in this range, while short-wavelength UV-light appears less attractive due to photodamage. Methods for the development of new photoreceptors that involve other chromophores are therefore urgently needed to expand the scope of photoregulation in the context of optogenetics and synthetic biology.

4. Photoresponsive nucleic acids – current status

There are no RNA- or DNA-based photoreceptors known in Nature. Some photochemical processes involve the nucleobases, e.g., thymine dimerization by UV light as a DNA damaging event [9], or the photoreactivity of thiouracil as a minor base in tRNA [10], but no light-dependent reversible structural changes occur in natural systems that could be used to construct artificial receptors. Synthetic photoresponsive nucleic acids, however, have been created and studied quite intensively. The most abundant class is caged oligonucleotides, in which critical molecular positions (e.g., the Watson–Crick face) are chemically modified with photolabile protecting groups [11]. The steric bulk of the protecting group prevents proper folding and/or interaction with partners. The protecting groups can then be removed with high spatial and temporal resolution by irradiation with light of an appropriate wavelength, releasing the native nucleic acid. This approach has been used intensively to initiate folding, binding, or catalysis with high temporal and spatial resolution [12,13]. Applications to miRNAs and siRNAs have been reported [14,15]. Caging is, however, an irreversible event; once uncaged, the process cannot be reverted.

Oligonucleotides have also been covalently endowed with reversibly photoswitchable moieties, mainly with azobenzenes. Upon irradiation, these azobenzenes carry out a reversible *cis/trans* isomerization. The different steric demand of the two isomers can induce structural changes in the attached oligonucleotide, which in turn may have functional consequences [16]. An elegant example is the modification of promoter sequences for T7 RNA polymerase with azobenzenes, thereby bringing transcription of a gene under the control of light [17]. Up to 10-fold modulation of transcription efficiency was achieved.

All these above approaches, however, provide limited utility in synthetic biology, as the covalently modified oligonucleotides must be exogenously delivered and cannot be synthesized by the biosynthetic machinery of the cell, preventing inheritance of this treat.

The ideal nucleic acid photoreceptor for synthetic biology consists of unmodified DNA or RNA so it can be easily incorporated into the genome of a host organism. Like in the protein-based photoreceptors, photosensitivity should be provided by a small organic cofactor or chromophore that will be exogenously delivered. While no such nucleic acid photoreceptors are known to exist in Nature, a few artificial prototypes have been developed in the laboratory already, thereby demonstrating the general feasibility of photoregulation without the help of proteins. These prototypes are RNA aptamers selected in vitro against three different photoisomerizable small molecules (see below). In one case, transmission of the signal from the sensor domain to a catalytic effector domain could be demonstrated [18].

5. Aptamers and riboswitches, SELEX and related techniques

Aptamers are RNA molecules that specifically bind other molecules. They were first isolated by in vitro selection (or SELEX, for Systematic Evolution of Ligands by EXponential enrichment) from huge synthetic combinatorial RNA libraries [19,20]. RNA aptamers for thousands of different targets, from small organic molecules like dyes, amino acids, or nucleotides to large proteins and even cells have been reported. Binding typically involves non-covalent interactions and is governed mostly by hydrogen bonding and π-stacking. Aptamers can be as specific as antibodies and can bind their targets with similar affinity, they are, however, smaller and easier to synthesize. Aptamers have developed into valuable tools in cell biology and diagnostics, and they represent a promising class of drug candidates in different therapeutic areas. Aptamers could also be fused to ribozymes to generate RNA catalysts allosterically regulated by the target molecules (allosteric ribozymes or aptazymes) [21], or to other functional RNAs to generate decoys or logical gates [22].

Later on, such aptamers were discovered in Nature where they were found to be involved in gene regulation by riboswitches [23–25]. These riboswitches are typically part of the 5′-untranslated regions of bacterial mRNAs where they sense the concentration of certain metabolites without any protein cofactors: an aptameric domain binds to the metabolite, thereby changes its structure, and transmits this change to an attached expression platform. If this metabolite binding happens co-transcriptionally, transcription itself may be up- or down-regulated (e.g., by the formation of antitermination or termination signal structures upon refolding), other riboswitches modulate translation (e.g., by sequestering the ribosome binding site).

6. In vitro selected aptamers as tools for the control of gene expression

Already before riboswitches were discovered as a natural regulatory principle, in vitro selected aptamers were used to construct artificial regulation systems where the expression of individual genes was put under the control of small molecules [26], for recent reviews see [27,28]. These approaches typically involve the in vitro selection of an aptamer against the small molecule, the incorporation of the aptamer sequence into an untranslated region of the RNA to be regulated, and the subsequent optimization of the linking structure between aptamer and open reading frame, as simple binding of the small molecule not necessarily results in the modulation of gene expression.

RNA-based regulatory elements offer a number of attractive features for use in synthetic biology: their construction from only four building blocks that interact by well-characterized interactions, recent success in structure prediction from primary sequence, and their smaller genetic footprint. Furthermore, RNA molecules do not require a translation process to synthesize the functional element and therefore place a lower energetic and resource load on the host cell. Finally, RNA-based post-transcriptional control strategies are considered to act on faster time scales than transcription-based approaches using regulatory proteins (for a comprehensive review, see [29]).

The two small molecules most commonly used for RNA-based artificial gene regulatory systems are tetracycline and theophylline [27]. For both molecules, aptamers had been isolated many years ago [30,31], and these aptamers were found to bind their targets not only in vitro, but also under in vivo conditions. Most published approaches use a *cis*-acting format for these switches in which the aptamer is covalently joined to the genes under investigation. An interesting alternative format that has been explored to a much lesser extent is that of *trans*-acting switches. Here, the sensor and the open reading frame are not parts of the same molecule. The rationally constructed sensor molecule contains an aptamer against the small molecule, and an antisense domain against some part of the RNA sequence to be regulated. While in the absence of the small molecule the aptamer hybridizes with parts of the antisense domain, blocking it for interactions with its RNA target, aptamer binding refolds the sensor RNA, releasing the antisense domain for target RNA binding. These off-switches have been shown to down-regulate the expression of target genes in eukaryotes, while similarly designed on-switches that sequester the antisense domain upon small-molecule binding up-regulate gene expression [32].

7. Photoisomerizable small molecules

The organic chemist knows a number of molecules that undergo various reversible isomerization reactions upon irradiation. While in some cases, both backward and forward reaction are photochemically driven, requiring light of different wavelengths, in other systems only one of the reactions requires irradiation, while the other is thermally driven. In the context of bioorganic and biomimetic chemistry, the most thoroughly studied system is based on azobenzenes [17]. These show a photochemical *cis*/*trans*-isomerization around an olefinic double bond, with the *cis* isomer preferably being formed under UV irradiation, while irradiation with blue light generates the *trans* isomer. Advantages of this system are the comparatively simple structure and easy synthesis, the considerable steric differences between the two isomers, and the high rate and quantum yield of the reaction. The major disadvantage is the location of the photostationary states: Both isomerization reactions are far from being quantitative; after irradiation typically only 70–90% of the molecules are in the desired state. This non-quantitative switching behavior naturally limits the usefulness of these switches, as – even if one isomer can bind effectively to a receptor and the other cannot – the maximal achievable switching factor is less than 10. While such small effects have successfully been exploited in amplified signaling systems like G-protein coupled receptors where binding of one effector molecule to one receptor molecule can trigger changes in thousands of target molecules downstream in the signaling cascade [33], in non-amplified systems larger switching factors are required to achieve biological effects.

Other classes of photoswitchable molecules with potential utility are spiropyranes, dihydropyrenes, and fulgides. In a biological context, all of these are, however, less explored than the azobenzenes.

Diarylethenes have been investigated for many years, originally in the context of optical information storage materials. Hundreds of derivatives have been synthesized and their switching behavior studied [34]. Upon irradiation, these molecules undergo a reversible electrocyclic ring closure/ring opening reaction. Some of them show excellent reversibility, high fatigue resistance, and a very favorable location of the photostationary states with near-quantitative yields of both isomerization reactions. Importantly, by attaching different substituents to the diarylethene ring system, the switching wavelengths can be tuned to range from the UV through the whole visible spectrum to IR frequencies. Surprisingly, despite their good photochromic performance, interesting spectral properties, and useful structural features, diarylethenes are still largely unexplored in a biochemical context.

A common diarylethene subclass incorporates two heterocyclic thiophene rings, connected via a cyclopentene system (Fig. 1). Our lab recently described the expansion of this switching principle to nucleoside systems in which one of the thiophenes was substituted by an adenosine derivative [35]. These compounds resemble nucleosides, exhibit normal Watson–Crick base-pairing, and are therefore promising candidates for interaction with nucleic acid–based receptors like aptamers. Fig. 2a shows three compounds belonging to this substance class, differing in the substituent attached to the thiophene rings. The open-ring form is – in all cases – colorless, but upon short-wavelength irradiation, intensive color develops (Fig. 2b). The different compounds have different optical properties, as indicated by the varying colors of the solutions in the

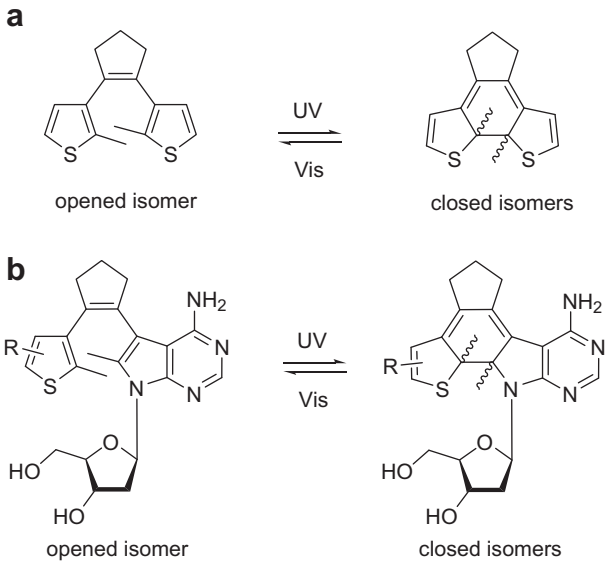

Fig. 1. Diarylethene photoswitches and the isomerization reaction. (a) Thiophene-based diarylethenes and (b) deaza-adenosine-derived diarylethene photoswitches.

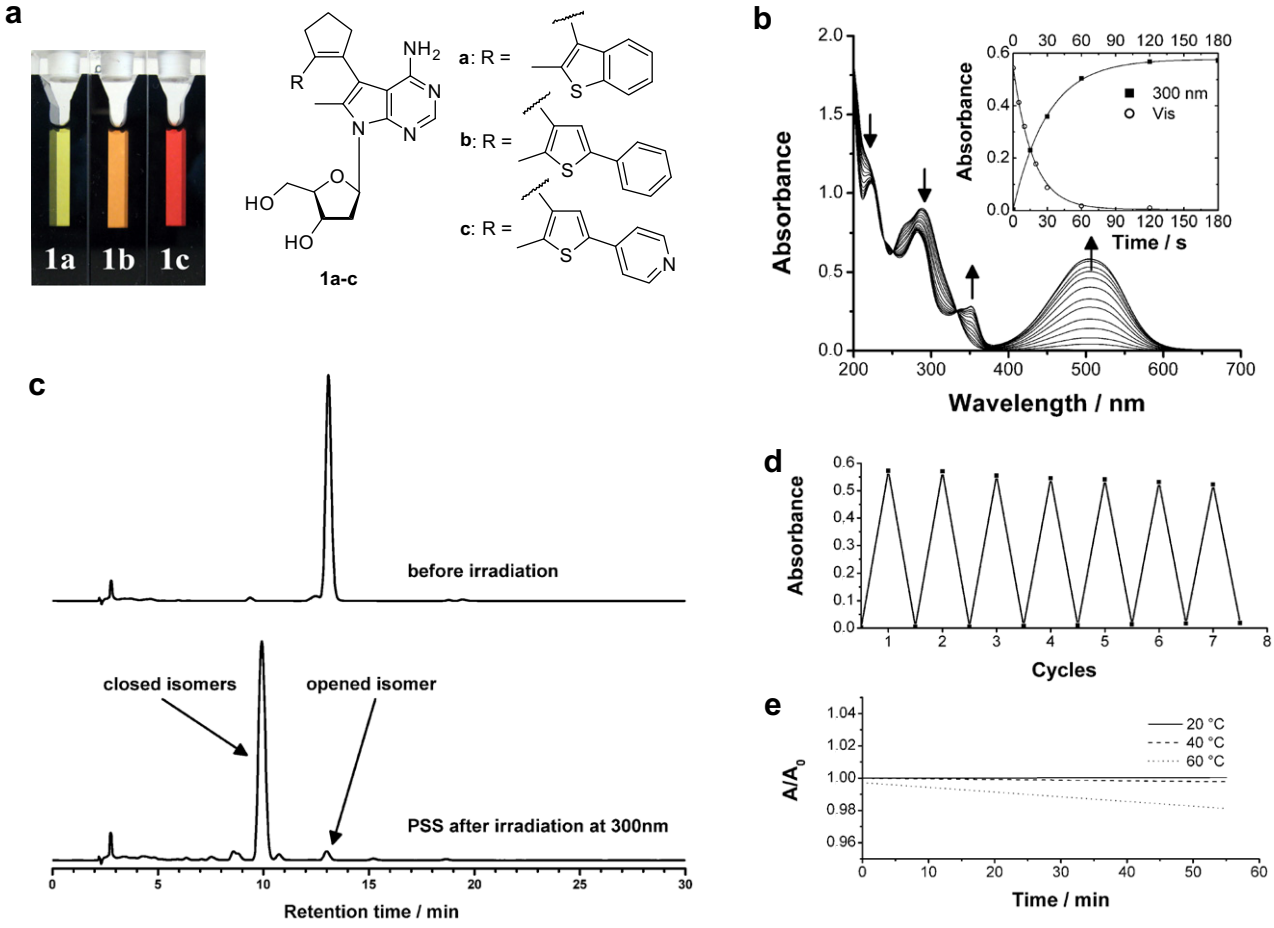

Fig. 2. Characterization of adenosine-based photoswitches (see text for details). Figure modified from Ref. [35].

cuvettes. Compound **1c** is particularly interesting, as its switching behavior is almost ideal: after short-wavelength irradiation, ~97% of the compound are in the closed-ring form, while after subsequent long-wavelength exposure > 99% have isomerized back into the open-ring form (Fig. 2c). Switching is highly reversible, and only very little bleaching and "fatigue" can be observed. Over seven cycles of switching only minor decomposition is observed (Fig. 2d). Thermally, this system is very stable and does not isomerize (Fig. 2e).

As a proof of concept for orthogonal switching systems, we mixed compounds **1a** and **1c** in aqueous buffer and subjected them to light at different wavelengths: At the start of the experiment, both compounds were present in the open-ring form. By using different illumination regimes, both compounds could either be switches concurrently or selectively, one after the other (for details, see [35]). This experiment demonstrates that different switches can be used as mixtures, and the individual species can be selectively controlled by the wavelength of the light used for illumination.

8. Aptamers and ribozymes that respond to photoisomerizable small molecules

Three published studies [18,36,37] investigate the possibility to generate aptamers or ribozymes that respond to photoswitchable small molecules. Young and Deiters selected RNA aptamers against an immobilized spiropyran, and eluted binders by photoswitching, i.e., by irradiation of the matrix with light of the appropriate wavelength. Surprisingly few aptamers were found to distinguish between the two isomeric forms, and the best candidate showed a ~14-fold discrimination and a micromolar binding dissociation constant [36]. Hayashi et al. immobilized a tetrapeptide KRAzR containing an internal azobenzene moiety (Az) on agarose beads and carried out SELEX with an RNA library and competitive elution, yielding aptamers with K_d values in the high nanomolar to low micromolar range. The effect of photoswitching the azobenzene on the strength of RNA binding was studied by surface plasmon spectroscopy, and discrimination between the two isomeric forms was found to be roughly 10-fold [37]. In both studies, only the isolated aptamer (i.e., sensor) domain was studied, and no attempt was made to connect it to and investigate the effects on an output domain. Lee et al. attached a dihydropyrene switch to acrylamide beads, and eluted the selected RNA libraries by photoswitching with light of the appropriate wavelength. Against the immobilized photoswitch, their best aptamer showed a ~35-fold discrimination in binding and a low micromolar K_d. Importantly, after studying the aptamer these authors decided to connect this sensor to a hammerhead ribozyme as actuator domain by screening a number of published "communication modules". Using the so-called "UG module", they obtained an allosteric ribozyme that was almost as active as an unmodified hammerhead ribozyme in the presence of the "closed-ring" form of their dihydropyrene, while it was almost completely inactive with the "open-ring" form. Analysis of the initial rates of hammerhead ribozyme cleavage revealed an impressive switching factor of 900, thereby demonstrating that in this system, an RNA receptor was able to transmit the sensing of a photon to a significant change in (ribo)enzymatic activity [18].

It should be noted that all three photoswitchable systems are far from ideal for applications inside biological systems. Most notably, the switching wavelengths (365 vs. >490 nm for the spiropyranes, 360 vs. 430 nm for azobenzenes, 280–375 vs. >400 nm for the dihydropyrene) are quite short and in a range where many cellular constituents absorb strongly. Converting these photoswitchable molecules into drug-like cell-permeable compounds will also not be a trivial task. Therefore, an expansion to other substance classes, like the diarylethene switches introduced above is urgently needed.

9. Concept: Control of gene expression by using aptamers against photoisomerizable small molecules

All three studies reviewed above stop at the in vitro characterization of the selected RNA molecules and do not investigate the modules in an in vivo context. However, in author's opinion, the different technologies described in this essay can now be combined to generate artificial photoresponsive systems based on aptamers against photoisomerizable small molecules, and these could be implemented to control prokaryotic or eukaryotic gene networks (Fig. 3). It should be emphasized that this goal has not been reached yet and the topic is subject of current work in author's laboratory. The route to RNA-based photoreceptors for use in synthetic biology is outlined below:

First, various photoisomerizable small molecules are synthesized, sharing a common scaffold but differing in the substitution pattern. Thereby, the switching wavelengths can be tuned over a wide spectral range. High switching efficiency, low bleaching, high cellular permeability and low cytotoxicity are goals to be achieved by systematic variation of substituents already at this stage. Candidates that fulfill the above criteria serve as targets for the generation of aptamers that specifically bind to one isomer while rejecting the other. This can be achieved by standard SELEX methodology, either using immobilized target molecules as described above, or by allosteric selections [21,38]. For allosteric selections, a random nucleotide domain can be fused to a functional RNA, e.g., a hammerhead ribozyme, and the library screened for molecules that are (catalytically) active only in the presence of one isomeric form of the target compound, using light of appropriate wavelengths to switch between the isomers. Allosteric selection has the advantage that the winners have been selected for switching a functional process (including the communication of the sensing event to an actuator domain), while SELEX against immobilized targets selects strictly for binding (i.e., sensing only). Converting the aptamers into artificial riboswitches might therefore be easier with allosterically selected aptamers, as typically only a very small fraction of the aptamers isolated using in vitro selection techniques are active under in vivo conditions. While in vitro selection enriches for high binding affinity and specific recognition of the cognate ligands, these characteristics are not sufficient to obtain aptamers that can be turned into engineered riboswitches that function in vivo. There are examples for strictly rational engineering of artificial riboswitches, but combinatorial selections offer an attractive alternative for obtaining in vivo applicable aptamers [reviewed in [27]]. Weigand et al. cloned an in vitro selected aptamer library directly upstream of a *gfp* reporter gene, and transformed the resulting plasmid library into yeast cells. Yeast colonies were then screened for ligand-dependent changes in GFP expression. Using this approach, numerous new aptamers were identified that control gene expression in vivo [39]. Lynch et al. used a high-throughput screen to optimize the connecting sequence between aptamer and ribosome binding site to markedly increase activity in vivo [40].

By adaptation of the selection methodology, the specificity of the aptamers can be tweaked, and different types of binders can be generated [30]: those that specifically bind to only one type of small-molecule effector, and those binding a whole group with different substituents and spectral characteristics. The first type would have benefits for use in multiply orthogonal switching systems, while the latter one would act as a master key, where – depending on the task, the tissue and other variables – the same switching process could be initiated with different light sources.

The most common format for the aptameric regulators described here will be *cis*-acting riboswitches, in which the aptamer is a part of the untranslated region associated with the gene to be regulated. Alternatively, *trans*-acting riboswitches can be constructed that respond to photoswitchable molecules. These combine the aptameric domain with an antisense or miRNA domain that is either unveiled or sequestered upon binding the small-molecule target. This may allow photoswitching of whole families of genes; e.g., those having the same miRNA target sequence.

10. Outlook

Several recent studies utilize aptamers, ribozymes, and aptazymes for the design of increasing complex synthetic biological devices [29,41]. These approaches utilize small molecules as external stimuli in combination with aptamers and host-specific gene regulatory components. The introduction of photoresponsive small molecules may allow expanding the scope of RNA-based synthetic devices, as a change can be triggered with higher spatial and temporal resolution than achievable with small molecules. Different chromophores targeted to different aptamers will furthermore

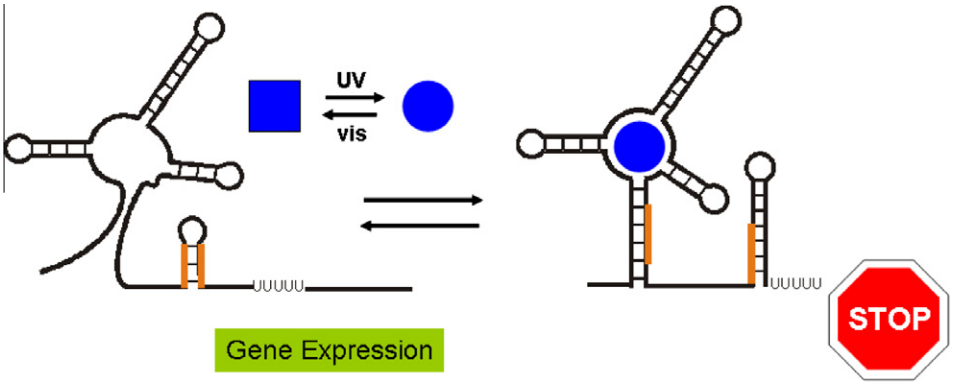

Fig. 3. Aptamers against photoswitchable small molecules as genetic control elements. In the present case, the aptamer recognizes only one isomer of the photoswitchable target, represented as the blue circle.

provide the opportunity to address several switches is parallel by using light of different wavelengths, and thereby enable complex switching decisions.

References

[1] Christie, J.M., Gawthorne, J., Young, G., Fraser, N.J. and Roe, A.J. (2012) LOV to BLUF: flavoprotein contributions to the optogenetic toolkit. Mol. Plant 5, 533–544.
[2] Moglich, A. and Moffat, K. (2010) Engineered photoreceptors as novel optogenetic tools. Photochem. Photobiol. Sci. 9, 1286–1300.
[3] Van der Horst, M.A. and Hellingwerf, K.J. (2004) Photoreceptor proteins, "star actors of modern times": a review of the functional dynamics in the structure of representative members of six different photoreceptor families. Acc. Chem. Res. 37, 13–20.
[4] Fenno, L., Yizhar, O. and Deisseroth, K. (2011) The development and application of optogenetics. Annu. Rev. Neurosci. 34, 389–412.
[5] Wu, Y.I., Frey, D., Lungu, O.I., Jaehrig, A., Schlichting, I., Kuhlman, B. and Hahn, K.M. (2009) A genetically encoded photoactivatable Rac controls the motility of living cells. Nature 461, 104–108.
[6] Levskaya, A., Weiner, O.D., Lim, W.A. and Voigt, C.A. (2009) Spatiotemporal control of cell signalling using a light-switchable protein interaction. Nature 461, 997–1001.
[7] Yazawa, M., Sadaghiani, A.M., Hsueh, B. and Dolmetsch, R.E. (2009) Induction of protein–protein interactions in live cells using light. Nat. Biotechnol. 27, 941–945.
[8] Weber, W. and Fussenegger, M. (2010) Synthetic gene networks in mammalian cells. Curr. Opin. Biotechnol. 21, 690–696.
[9] Heil, K., Pearson, D. and Carell, T. (2011) Chemical investigation of light induced DNA bipyrimidine damage and repair. Chem. Soc. Rev. 40, 4271–4278.
[10] Milder, S.J. and Kliger, K. (1985) Spectroscopy and photochemistry of thiouracils: implications for the mechanism of photocrosslinking in tRNA. J. Am. Chem. Soc. 107, 7365–7373.
[11] Mayer, G. and Heckel, A. (2006) Biologically active molecules with a "light switch". Angew. Chem. Int. Ed. 45, 4900–4921.
[12] Chaulk, S.G. and MacMillan, A.M. (1998) Caged RNA: photo-control of a ribozyme reaction. Nucleic Acids Res. 26, 3173–3178.
[13] Nierth, A., Singer, M. and Jäschke, A. (2010) Efficient photoactivation of a Diels–Alderase ribozyme. Chem. Commun. 46, 7975–7977.
[14] Mikat, V. and Heckel, A. (2007) Light-dependent RNA interference with nucleobase-caged siRNAs. RNA 13, 2341–2347.
[15] Zheng, G., Cochella, L., Liu, J., Hobert, O. and Li, W.H. (2011) Temporal and spatial regulation of microRNA activity with photoactivatable cantimirs. ACS Chem. Biol. 6, 1332–1338.
[16] Keiper, S. and Vyle, J.S. (2006) Reversible photocontrol of deoxyribozyme-catalyzed RNA cleavage under multiple-turnover conditions. Angew. Chem. Int. Ed. 45, 3306–3309.
[17] Liang, X.G., Wakuda, R., Fujioka, K. and Asanuma, H. (2010) Photoregulation of DNA transcription by using photoresponsive T7 promoters and clarification of its mechanism. FEBS J. 277, 1551–1561.
[18] Lee, H.W., Robinson, S.G., Bandyopadhyay, S., Mitchel, R.H. and Sen, D. (2007) Reversible photo-regulation of a hammerhead ribozyme using a diffusible effector. J. Mol. Biol. 371, 1163–1173.
[19] Tuerk, C. and Gold, L. (1990) Systematic evolution of ligands by exponential enrichment – RNA ligands to bacteriophage-t4 DNA-polymerase. Science 249, 505–510.
[20] Ellington, A.D. and Szostak, J.W. (1990) Invitro selection of RNA molecules that bind specific ligands. Nature 346, 818–822.
[21] Koizumi, M., Soukup, G.A., Kerr, J.N.Q. and Breaker, R.R. (1999) Allosteric selection of ribozymes that respond to the second messengers cGMP and cAMP. Nat. Struct. Biol. 6, 1062–1071.
[22] Vinkenborg, J.L., Karnowski, N. and Famulok, M. (2011) Aptamers for allosteric regulation. Nat. Chem. Biol. 7, 519–527.
[23] Winkler, W., Nahvi, A. and Breaker, R.R. (2002) Thiamine derivatives bind messenger RNAs directly to regulate bacterial gene expression. Nature 419, 952–956.
[24] Epshtein, V., Mironov, A.S. and Nudler, E. (2003) The riboswitch-mediated control of sulfur metabolism in bacteria. Proc. Natl. Acad. Sci. USA 100, 5052–5056.
[25] Fuchs, R.T., Grundy, F.J. and Henkin, T.M. (2006) The S(MK) box is a new SAM-binding RNA for translational regulation of SAM synthetase. Nat. Struct. Mol. Biol. 13, 226–233.
[26] Werstuck, G. and Green, M.R. (1998) Controlling gene expression in living cells through small molecule–RNA interactions. Science 282, 296–298.
[27] Suess, B. and Weigand, J.E. (2008) Engineered riboswitches – overview, problems and trends. RNA Biol. 5, 24–29.
[28] Wieland, M. and Hartig, J.S. (2008) Artificial riboswitches: synthetic mRNA-based regulators of gene expression. ChemBioChem 9, 1873–1878.
[29] Liang, J.C., Bloom, R.J. and Smolke, C.D. (2011) Engineering biological systems with synthetic RNA molecules. Mol. Cell 43, 915–926.
[30] Jenison, R.D., Gill, S.C., Pardi, A. and Polisky, B. (1994) High-resolution molecular discrimination by RNA. Science 263, 1425–1429.
[31] Berens, C., Thain, A. and Schroeder, R. (2001) A tetracycline-binding RNA aptamer. Bioorg. Med. Chem. 9, 2549–2556.
[32] Bayer, T.S. and Smolke, C.D. (2005) Programmable ligand-controlled riboregulators of eukaryotic gene expression. Nat. Biotechnol. 23, 337–343.
[33] Volgraf, M., Gorostiza, P., Numano, R., Kramer, R.H., Isacoff, E.Y. and Trauner, D. (2006) Allosteric control of an ionotropic glutamate receptor with an optical switch. Nat. Chem. Biol. 2, 47–52.
[34] Irie, M. (2000) Diarylethenes for memories and switches. Chem. Rev. 100, 1685–1716.
[35] Singer, M. and Jäschke, A. (2010) Reversibly photoswitchable nucleosides: synthesis and photochromic properties of diarylethene-functionalized 7-deazaadenosine derivatives. J. Am. Chem. Soc. 132, 8372–8377.
[36] Young, D.D. and Deiters, A. (2008) Light-regulated RNA–small molecule interactions. ChemBioChem 9, 1225–1228.
[37] Hayashi, G., Hagihara, M. and Nakatani, K. (2009) RNA aptamers that reversibly bind photoresponsive azobenzene-containing peptides. Chem. Eur. J. 15, 424–432.
[38] Helm, M., Petermeier, M., Ge, B., Fiammengo, R. and Jäschke, A. (2005) Allosterically activated Diels-Alder catalysis by a ribozyme. J. Am. Chem. Soc. 127, 10492–10493.
[39] Weigand, J.E., Sanchez, M., Gunnesch, E.B., Zeiher, S., Schroeder, R. and Suess, B. (2008) Screening for engineered neomycin riboswitches that control translation initiation. RNA 14, 89–97.
[40] Lynch, S.A., Desai, S.K., Sajja, H.K. and Gallivan, J.P. (2007) A high-throughput screen for synthetic riboswitches reveals mechanistic insights into their function. Chem. Biol. 14, 173–184.
[41] Carothers, J.M., Goler, J.A., Juminaga, D. and Keasling, J.D. (2011) Model-driven engineering of RNA devices to quantitatively program gene expression. Science 334, 1716–1719.