Research Focus

Inducible systems see the light

William M. Keyes and Alea A. Mills

Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA

Advances in our capacity to design and use novel strategies for achieving inducible gene expression will improve our ability to define gene function. An extremely efficient system designed by nature – that of the regulatable phytochrome system in plants – has provided the basis for developing a novel inducible gene expression system.

The development of regulatable transgene expression systems has had an enormous impact on biomedical, pharmaceutical and agricultural research. These systems have provided the tools for functional genomic analysis, allowed the generation of animal models of human disease and have aided drug discovery [1–3]. The development of more reliable techniques for analyzing gene function is especially timely in this post-sequencing era as knowledge of genome sequence has already enhanced our ability to manipulate the genome.

The ideal system

The ideal inducible gene expression system should have several key characteristics [1,4] – in short, it should be completely controllable. The ideal system would have no background or 'leaky' expression in the absence of inducer, would only be induced within the cell or tissue of choice, gene expression would be readily reversible by the investigator and the inducer would be non-toxic to the organism. Also, the inducer should specifically regulate gene expression to levels high enough to achieve the desired physiological response. At present, there are several inducible systems available, which use various modes of induction and regulation. Each strategy has its own inherent advantages and disadvantages and each fulfills several of the criteria mentioned above. However, many of these regulatable systems use inducing agents that might have adverse physiological effects on the organism under study if not used with due caution and possibly impede their use in gene therapy regimens.

One inducible system that has been successfully used for regulated gene expression is the ecdysone-based system that uses the insect molting hormone ecdysone as an inducer of gene expression in mammalian cells [5]. This system uses a truncated version of the ecdysone receptor, fused to the herpes simplex virus VP16 activation domain (VP16AD). In response to ecdysone agonists, such as ecdysone or ponasterone A, the ecdysone receptor fusion protein dimerizes with ultraspiracle or retinoid X receptor α to form a transcriptionally active complex. Other systems, such as the estrogen- and progesterone-based inducible systems, make use of the yeast GAL4 DNA-binding domain (Gal4DBD) and the VP16AD, coupled to truncated versions of the estrogen- or progesterone nuclear steroid hormone receptors. Endogenous hormones do not bind these truncated receptors. In the presence of the appropriate ligand (estrogen or the progesterone antagonist RU486), dimerization of the specific receptor occurs, which results in the binding of the activation complexes at promoters engineered to contain the Gal4 upstream activation sequence (UAS) and transcription of the target gene occurs [6–8]. Tetracycline-based systems, which use the tetracycline resistance operon from *Escherichia coli*, have also been fused to VP16AD using a similar strategy [9]. Recently, the UAS or GAL4 system was used as a reverse genetic tool that can be used for inducible RNAi-mediated gene silencing (RNAi; RNA interference) [10].

A new light-switchable gene promotor system

Recently, there has been an addition to this field that takes steps towards attaining the ideal inducible system. Shimizu-Sato *et al.* [11] recently described a light-switchable gene promoter system that uses members of the photoreceptor family of phytochromes in plants to induce transcription of a reporter gene upon exposure to visible light (Fig. 1).

In plants, the phytochrome family of sensory photoreceptors (designated phyA to phyE in *Arabidopsis*) can sense light and convert this stimulus into biological processes that alter the expression of genes controlling the growth and development of the plant [12]. These chromoproteins are converted in milliseconds from an inactive (Pr) form to an active (Pfr) form in response to the absorption of photons of red light, a component of normal visible light. This is enabled by a light absorbing chromophore, such as phycocynanobilin (PCB), which is covalently attached to the phytochrome in the natural setting of the plant. This process is rapidly reversible by the absorption of far-red light by the active conformation, thus reverting back to the inactive form. In the presence of chromophore this rapid interconversion between active and inactive forms is a repeatable process that enables a plant to rapidly adjust its physiology in response to environmental light cues.

Intrinsic to this process is phytochrome interacting factor-3 (PIF3), a basic helix-loop-helix containing transcription factor residing in the nucleus. Quail and colleagues demonstrated that PIF-3 is bound by the phytochrome on light-induced activation and is dissociated in response to reconversion of the phytochrome to the inactive state [13]. It is this rapid, repeatable process that made it possible to regulate expression of a reporter gene in response to a light stimulus using a modification of the yeast two-hybrid system.

Corresponding author: Alea A. Mills (mills@cshl.edu).

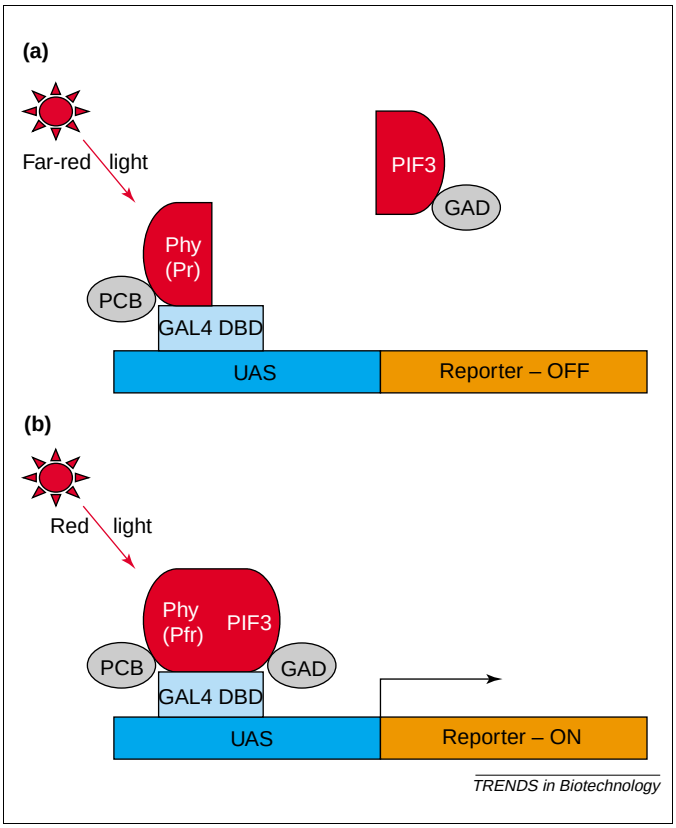

Fig. 1. A schematic diagram of the mechanism of action of the light-switchable expression system (outlined in [11]). (a) In the absence of a light stimulus, or with inactivating far-red light, the phytochrome (Phy), fused to the Gal4 DNA binding-domain (GAL4 DBD), is bound to the upstream activation site (UAS) upstream of the reporter gene. The presence of the chromphore phycocyanobilin (PCB) is necessary for absorption of the photons of light. The inactive conformation (Pr) of the phytochrome prevents binding of the phytochrome-interacting-factor 3 (PIF3)-GAL4-activation domain (GAD) fusion-protein. (b) The absorption of a photon of red-light rapidly alters the conformation of the Phy, to the active state (Pfr), facilitating binding of the PIF3-GAD complex and activating transcription of the downstream reporter gene. The process is then rapidly reversible on re-exposure to far-red light.

How it was made

A bigenic yeast strain was first generated that contained a light-sensing component and an activation component. The light-sensing portion was generated by fusing the phytochrome (either full-length phyA or N-terminal phyB) to the Gal4DBD. The activation component was generated by fusing the PIF3 transcription factor to the GAL4 activation domain (GAD). A yeast strain containing both fusion constructs was generated. Cultures were grown in the presence of the chromophore PCB and pulsed with red light causing the PIF3-GAD fusion protein to bind to the PCB-phytochrome-Gal4DBD complex positioned on the UAS 5′ of the *lacZ* reporter gene (Fig. 1b). Light-induced transcriptional activation was monitored by accumulation of colorimetric product.

Under these conditions, maximal expression of *Lac Z* was obtained after only one minute of exposure to red light. This activation required a pre-incubation period in the dark, presumably to allow for protein synthesis and accumulation of fusion proteins. Activation of the reporter gene was robust and rapid, resulting in a 50-fold increase in gene expression after only 30 minutes, which, when incubated for an extended period of time in the dark (3 hours) resulted in a one thousand-fold increase in gene expression above background levels. This system is tightly regulated with only background levels of gene expression observed in the absence of the stimulus. The chromophore PCB was shown to be essential for activation, facilitating binding of PIF3-GAD. In the case of yeast, it was necessary to supply exogenous PCB to the growth media to achieve inducible gene expression because yeast, in contrast to plants, does not express this chromophore.

One of the key features of this system is that it is readily reversible in response to a stimulus of light of a different wavelength. A single dose of far-red light blocked any further increase in LacZ activity, suggesting that reconversion to the inactive state abolishes activity, presumably by dissociation of the protein complex (Fig. 1a). As was the case for the activation of gene expression, inactivation was also extremely rapid. This differs from many inducible systems that merely rely on removal of the inducer to achieve inactivation. This often results in a slow response because the concentration of the inducer must be lowered below the threshold levels required for gene activation.

Limitations and outlook

As with any novel technology, there might be several inherent limitations with this light-inducible system. A major concern is the feasibility of using this approach for generating inducible gene expression systems in the cells of higher organisms such as mammals. Yeast cells can readily absorb exogenous PCB supplied in the growth media. Whether the cells of higher organisms will also absorb exogenously supplied chromophore remains to be seen. As the authors point out, however, it is likely that a cell or organism could be genetically engineered to synthesize the chromophore, as shown previously with bacteria [14]. Yeast cells have the additional feature of being transparent (or light sensitive), which facilitates the use of photons of light as a switch. Although light possesses the characteristics of an ideal inducer – it is non-toxic, can be regulated by the investigator and is readily available and inexpensive – the use of light as a gene switch could pose some technical difficulties. Without much alteration, the system used in yeast could probably be used in other transparent organisms such as plants, worms, flies and even mammalian cells grown in culture. However, if the system is to be applied to higher mammals, a method of delivery to light-impermeable areas will need to be developed, possibly, by fibre-optics or even laser beams. This could also be circumvented by the limited expression of the gene induction components to areas that are light accessible (e.g. skin and eyes) potentially providing a whole other dimension to gene therapy and drug delivery. The fact that the level of gene expression can be quantitatively regulated by titrating the number of photons of light delivered provides the investigator with even more control in precisely regulating the response.

One obvious technical difficulty with the procedure is the requirement for handling of the organisms in the dark. As shown in yeast, and possibly with some other simple organisms, this might be manageable in the laboratory environment but again poses some challenges with regards to the feasibility of applying this approach to higher organisms. As demonstrated, exposure to light for

>3 hours drastically reduces the level of gene expression. Maximal reporter expression was seen after a single dose of red light, followed by a 3-hour incubation in the dark. However, as dramatic as the increase in reporter expression was (1000-fold over negative controls), it was still only about one-sixth of the level of endogenous GAL4 expression. Whether this translates to gene expression that reaches physiological levels remains to be seen.

Clearly, Shimizu-Sato and colleagues have developed a regulatable system that has the potential to raise the bar on inducible gene expression systems, almost bridging the gap between science fiction and reality. It is with great anticipation that we await the next step in the journey.

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The new role of SAGE in gene discovery

Kenneth R. Boheler and Michael D. Stern

Laboratory of Cardiovascular Science, National Institute on Aging, NIH, 5600 Nathan Shock Drive, Baltimore, MD 21224, USA

The sequencing of the human genome has led to *in silico* predictions of far fewer genes than anticipated. Recent studies using serial analysis of gene expression have cast doubt on this finding. One study predicts the presence of many unidentified low abundance transcripts, whereas two others have mapped unique tags to previously unpredicted exons. Genome and transcriptome complexity is thus greater than predicted and many of the missing genes are probably expressed in low copy numbers or only in early embryonic tissues.

With the near completion of the human genome sequence [1,2], the number of predicted human genes (30 000–40 000) has turned out to be much lower than earlier estimates (45 000–140 000). However, recent data have called these predictions into question [3,4] and the number of functional transcriptional units in the mouse and human genomes remains controversial. Given that the identification of all genes in a mammalian genome is crucial to understanding the function and regulation of transcriptional units, a definitive determination of the correct number of genes depends on an accurate estimate and physical map of all of the genes in a genome.

Expressed sequence tags

Identification of all transcripts and RNA splicing variants in the mouse and human transcriptomes is the ultimate complement to the genome project. This has been accomplished primarily through the analysis of expressed sequence tags (ESTs) with subsequent EST mapping to the corresponding genome. Although data collected from EST projects have contributed significantly to the identification of all the genes, the rate of novel sequence discovery using this method has decreased as a function of time. Although it is possible that most of the unique transcripts have been identified, it is more likely that technical or other limitations to the EST approach have been reached. EST projects on early embryonic cells and stem cells, for example, remain a minor component of the mammalian EST collection (~4 million ESTs from human and ~2 million ESTs from mouse: dbEST [5]) because of technical limits in the production of cDNA or EST libraries from small sample sizes or because of ethical problems associated with obtaining tissues from human embryonic tissues and preimplantation embryos (eggs to blastocysts). It therefore remains probable that many unique transcripts, either from novel genes or in the form of splicing variants, remain to be identified.

Serial analysis of gene expression (SAGE)

Serial analysis of gene expression (SAGE) has the

Corresponding author: Kenneth R. Boheler (bohelerk@grc.nia.nih.gov).