Communications to the Editor

Light-Activated Gene Expression

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For the study of gene products involved in virtually all cellular processes including cell−cell communication, cell cycle regulation, and development, it is desirable to control gene expression with small-molecule ligands.[1] Commonly used systems are based on modified forms of ligand-dependent repressors,[2,3] chemical inducers of dimerization[4,5] and steroid and nuclear hormone receptors.[6−8] Applications of these methods are potentially limited by the ability to selectively deliver ligands to specific tissues or cells. We present a new straightforward approach to regulating gene expression with light using hormone analogues whose agonist properties are masked by photolabile protecting groups (Figure 1). This new method should provide substantial enhancement of spatial and temporal resolution of existing inducible expression systems.

The estrogen receptor (ER) is a member of the superfamily of nuclear and steroid hormone receptors that act as ligand-dependent transcriptional regulators in eukaryotes. The unliganded ER is bound to HSP90.[9] Upon binding estradiol (E2), the receptor undergoes a conformational change that results in dissociation from HSP90. It then binds to specific eukaryotic promoters and stimulates gene transcription by associating with transcriptional co-activators (Figure 2).[10−12] Recent crystal structures of the liganded forms of the ER ligand binding domain (LBD) and the LBDs of other hormone receptors have provided a basis for the rational molecular design of ER agonists and antagonists.[13,14] This structural information also permits the design of nonagonist/nonantagonists from known ligands as we have done in this report.

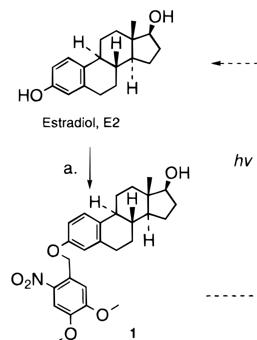

Figure 1. Compound **1** is a nonagonist, photocaged analogue of E2. a. 2-Nitroveratryl-bromide, K_2CO_3, acetone.

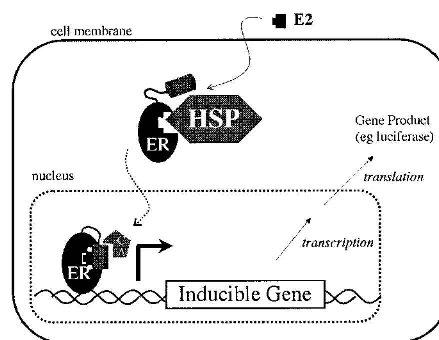

Figure 2. Mechanism of ER action. HSP = heat shock protein 90; CA = transcriptional co-activator.

The interactions of the 1-hydroxyl of E2 with Arg394 and Glu353 have been shown to be critical for ligand binding.[13,14] Thus, compound **1**, a "photocaged" analogue of estradiol was predicted to be a nonligand for ER. However, compound **1** can be rapidly reverted to estradiol upon exposure to UV light. This suggests that **1** may be useful for mediating light-activated gene expression (Figure 1).

We evaluated the ability of **1** to induce luciferase reporter gene expression in HEK293 cells transiently transfected with ER receptor plasmid pSG1-HEO, the luciferase reporter ERE-luc and control plasmid pRLCMV (Promega). Adherent cells growing in standard culture wells were irradiated with a fluorescent UV lamp. Infrared and short wavelength UV light are filtered by a glass plate and the polystyrene lid of the cell culture dish to avoid excessive heating and UV damage.[15] Cells were later lysed and evaluated for luciferase activity using a dual luciferase assay (Promega) (Figure 3).

In the absence of light, **1** (which contains less than 0.1% E2), weakly supresses E2-dependent transactivation (see Supporting Information). Pretreatment of **1** with cell lysates affords no additional activity in cell culture suggesting that **1** is not "uncaged" in cells in the absence of light. Cells grown in the presence of 5 nM **1** and exposed to UV light show as much as 86% of the maximum activity inducible by E2 (Figure 3). Importantly, no significant induction is observed in the absence of ligand. The ability to rapidly introduce active hormones into cells allows us

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(15) HPLC analysis of methanolic solutions of **1** irradiated by this set up show that E2 is liberated at the rate of 3×10^{-2} s^{-1}.

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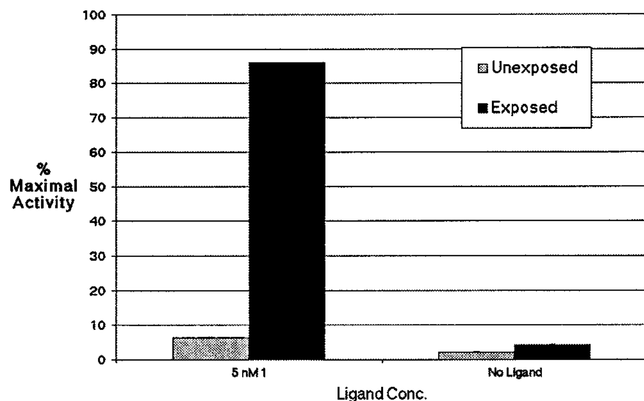

Figure 3. Gene product (luciferase) activity measured 24 h after a 3 min exposure.

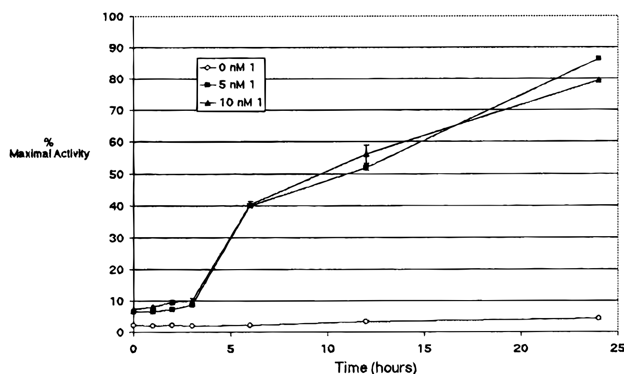

Figure 4. Time dependence of gene product formation after photorelease of E2.

to rapidly trigger hormone receptor-mediated transcription/translation (Figure 4).[16]

These results show that light can activate ER responsive genes in cells growing in media containing **1**. However, these results do not necessarily demonstrate that the uncaging occurs within the cell. To rule out the possibility that the ligand is being uncaged only in the media which then diffuses into the cells, cells were incubated with **1** or E2 for 12 h at which time the media was removed, the cells were washed once with PBS buffer and then returned to ligand-free media just prior to exposure. Cells preincubated with **1** show a temporary burst in gene expression, which reaches a maximum approximately 4 h after exposure and then decreases as a result of the ligand diffusing out of the cell (Figure 5).

Cells preincubated with E2 show maximal luciferase activity, which starts to decrease after 4 h and approaches the same final level of expression as cells preincubated with **1**. These results clearly illustrate that **1** is being deprotected intracellularly and

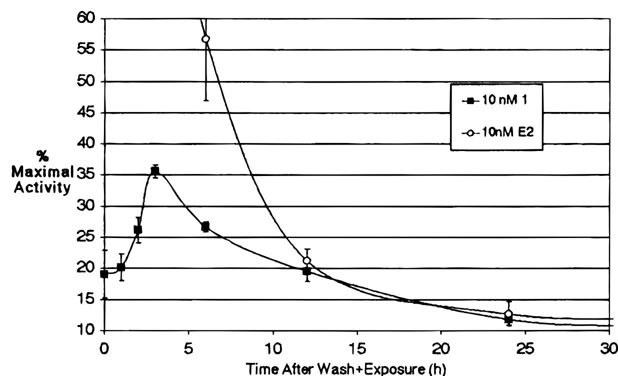

Figure 5. Time dependence of gene product formation after exposure. Cells incubated in 10 nM **1** or E2 are washed prior to a 12 min exposure.

that light activation of **1** can be used to control gene expression within a defined window of time and space.

Others have already demonstrated that hormone receptors can be modified to target different and unique promoter sequences.[17,18] Taken together this suggests that light activated gene expression mediated by **1** could in principle be targeted to virtually any gene of interest.

Light-activated gene expression mediated by photocaged hormones offers a unique tool for regulating genes in culture and possibly in vivo. The potential use of focused light sources, such as lasers, to uncage hormones using 1- or 2-photon methods,[19] may enable one to regulate gene expression in highly localized regions of tissue, potentially at the level of single cells. By placing genes under the spatial and temporal control of light, this approach to gene regulation is uniquely independent of the pharmicokinetic phenomena inherent to using small molecule regulators in living systems and should provide a powerful new tool for studying gene function.

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Supporting Information Available: Experimental procedures for the synthesis of **1** and spectra, experimental procedure and specifications for photo-uncaging of **1** in culture, exposure dependence of transcription mediated by **1** and behavior of **1** in competition with E2 in culture (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) The luciferase activity shows a delayed response as a result of the inherently slower rate of transcription/translation.

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