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Air-conditioning for regulated transgene expression

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A recent issue of *Nature Biotechnology* describes an intriguing development in the arena of inducible transgene expression systems, which allows tight regulation of transgene expression by a gaseous agent.

The development of inducible expression systems in recent years conferred the ability to control the timing and level of transgene expression *in vitro* and *in vivo*. This advancement in gene transfer/expression technology opened new avenues for basic research and gene therapy applications, which were utilized by a large number of research groups to investigate a plethora of fundamental biological phenomena and to dissect the mechanisms of natural and pathological processes.

The design of the various inducible expression systems has been aimed mainly at maximizing the ratio between the level of transgene expression at the induced (on) state and the level of background transgene expression with no induction (off state) – and minimizing the systems' effect on the gene expression profile of the host.

These existing systems all comprise three components (Figure 1). One is an inducible promoter, which should ideally exhibit minimal transcription in all potential target cells. A second component is a modulator-controlled transactivator, comprised of three domains: (i) a DNA binding domain (DBD), that can recognize and bind the unique *cis* elements in the inducible promoter, (ii) a transcription activation domain, and (iii) a site for the modulator to bind to. The final component is a modulator, which interacts with the transactivator to alter the ability of the latter to bind and activate the inducible promoter. The modulators are usually small molecules including antibiotics,[1–3] hormone analogs,[4,5] small carbon molecules (ethanol),[6] and metal ions.[7]

How the different modulators affect transactivator binding to the inducible promoter form the characteristics of each inducible system. These include inducing conformational change in the transactivator DBD,[1] facilitating the translocation of the transactivator into the host cell nucleus,[5] or joining the transactivator's DBD with its transcription activation domain.[2,3]

Not surprisingly, in most of the inducible transgene expression systems, the introduction and removal of the modulators from the relevant host cell constitutes the basic signaling gear for activating and silencing transgene expression, respectively.

Currently, in all *in vitro* experiments, the various modulators are added directly to the culture media. In *in vivo* experiments the modulators are administered to experimental animals either by injection or by feeding. Efficient removal of modulators in the setting of a large-scale *in vitro* experiment is labor intensive since it requires washing of a large number of cells and the replacement of large volumes of culture media. Complete clearance of modulators *in vivo* following their withdrawal from the animal diet may last several days. These expensive and laborious methodologies of switching transgene expression on and off do not enable real-time fine-tuning of modulator levels and preclude rapid adjustments in transgene expression level.

Weber's new study was aimed at improving the ability to regulate transgene expression *in vitro* and *in vivo* and describes the development of a gas-inducible transgene expression system in mammalian cells. The system is derived from the filamentous fungus *Aspergillus nidulans* and is based on the enzymatic machinery that regulates the formation of acetyl-CoA from ethanol in a three-step process.[8]

The AlcR transcriptional activator tightly regulates transcription of the *alcA* and the *aldA* genes, which encode the ADH1 and ALDH enzymes, respectively. Importantly, the ability of AlcR to bind its target *cis* elements in the *aldA*, and *alcA* promoters requires interaction with its natural modulator, acetaldehyde. The fact that a boiling point of 21°C renders acetaldehyde gaseous at physiological conditions was the basis on which the new inducible expression system has been established. Similar to the approach taken in earlier studies,[6,9] Weber *et al* developed an inducible AlcR-dependent promoter (P_{AIR}) comprising a minimal CMV promoter and five operators (O_{alcA}) each containing the AlcR binding sequence. Akin to other regulated transactivators the AlcR comprises a DBD, a transcriptional activation domain, and a modulator (acetaldehyde)-binding domain. Although derived from fungus, the activation domain of AlcR is functional in the settings of plant and mammalian cell transcription machinery. Earlier studies employed ethanol to regulate AlcR-dependent transcription in plants;[6,9] however, a recent study by Flipphi *et al*[8] indicated that acetaldehyde is the sole physiological inducer of the ethanol catabolism pathway. Thus, it is reasonable to postulate that ethanol activation of AlcR-dependent transcription (as reported earlier) is a consequence of ethanol catabolism to acetaldehyde.

Weber *et al* demonstrated the ability to utilize directly gaseous acetaldehyde as a means to regulate tightly the expression level of human secreted placental alkaline phosphatase (SEAP) and human interferon-β from the inducible P_{AIR}. The inducibility of the new system and the correlation between transgene expression level and gaseous acetaldehyde concentration was comparable in different cell lines including BHK-21, CHO-K1, and HeLa cells. Furthermore, the acetaldehyde-inducible cassette was successfully incorporated into simple retroviral and lentiviral vector systems, and proved efficient at controlling transgene expression in microencapsulated CHO-K1 cells implanted into mouse peritoneum. However, the increase in

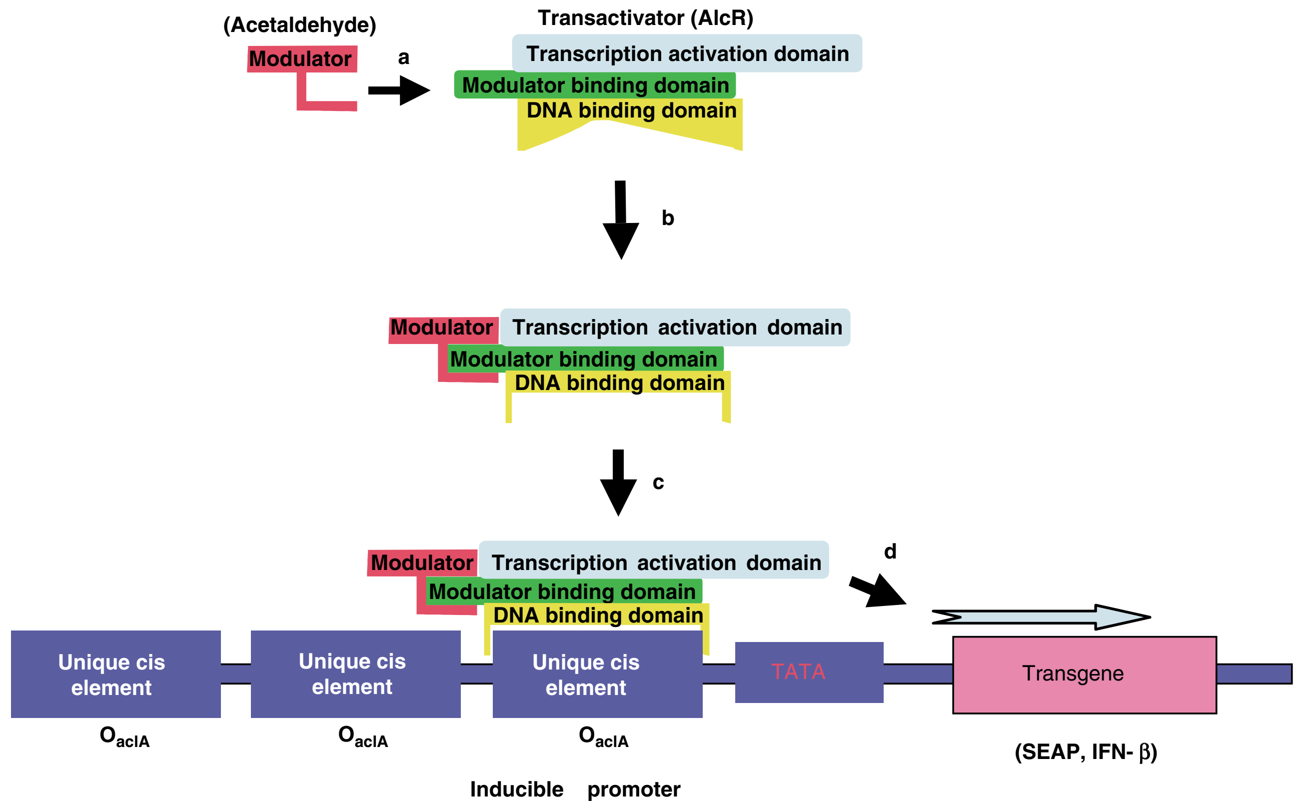

Figure 1 *Three elements comprise a typical inducible transgene expression system: (1) an inducible promoter made of a minimal promoter (TATA box) and a series of unique cis elements (O_{aclA}), (2) a modulator-controlled transactivator (AlcR) made of three domains: (i) a transcription activation domain, (ii) a modulator binding domain, and (iii) a DNA binding domain, and (3) a modulator (acetaldehyde). (a) Modulator binding to the transactivator. (b) Conformational change in the modulator DBD. (c) Transactivator binding to the minimal promoter. (d) Activation of the minimal promoter results in a high level of transgene expression.*

transgene (SEAP) expression levels *in vivo* did not exhibit linear correlation with the increase in acetaldehyde concentrations to which the animals were exposed, and at the maximal acetaldehyde concentration (1000 p.p.m.) the level of serum SEAP activity in the treated animal did not reach its plateau, thus indicating that higher concentrations of acetaldehyde are required for optimal induction *in vivo*. Based on earlier studies with different inducible expression systems, one may consider replacing the parental transcriptional activation domain of AlcR with a mammalian-derived activation domain to improve the potency of AlcR in mammalian cells.[10]

Other considerations regarding the prospects of employing this newly developed system in animal models include the possibility that endogenously generated acetaldehyde will affect transgene expression *in vivo*, and that the ability to employ the fungus-derived-inducible system *in vivo* may be restricted by the development of an AlcR-directed immune response.

Importantly, Weber *et al* were able to utilize efficiently the acetaldehyde-regulated promoter to control SEAP and IFN-β production in the setting of a stirred tank bioreactor. Of particular significance was the fact that tight regulation of transgene production in this setting was obtained by simply adjusting acetaldehyde inflow, which did not necessitate replacement of culture media. Clearly, the ability to incorporate the acetaldehyde-inducible expression cassette into a large-scale protein production system points at its potential for biopharmaceutical applications as one of the most promising aspects of this intriguing inducible expression system. ■

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1 Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci USA* 1992; **89**: 5547–5551.
2 Rivera VM *et al*. Long-term regulated expression of growth hormone in mice after intramuscular gene transfer. *Proc Natl Acad Sci USA* 1999; **96**: 8657–8662.
3 Amara JF *et al*. A versatile synthetic dimerizer for the regulation of protein–protein interactions. *Proc Natl Acad Sci USA* 1997; **94**: 10618–10623.
4 No D, Yao TP, Evans RM. Ecdysone-inducible gene expression in mammalian cells and transgenic mice. *Proc Natl Acad Sci USA* 1996; **93**: 3346–3351.
5 Wang Y, O'Malley Jr BW, Tsai SY, O'Malley BW. A regulatory system for use in gene transfer. *Proc Natl Acad Sci USA* 1994; **91**: 8180–8184.
6 Caddick MX *et al*. An ethanol inducible gene switch for plants used to manipulate carbon metabolism. *Nat Biotechnol* 1998; **16**: 177–180.
7 Mett VL, Lochhead LP, Reynolds PH. Copper-controllable gene expression system for whole plants. *Proc Natl Acad Sci USA* 1993; **90**: 4567–4571.

8 Flipphi M *et al*. Regulation of the aldehyde dehydrogenase gene (aldA) and its role in the control of the coinducer level necessary for induction of the ethanol utilization pathway in *Aspergillus nidulans*. *J Biol Chem* 2001; **276**: 6950–6958.
9 Felenbok B. The ethanol utilization regulon of *Aspergillus nidulans*: the alcA–alcR system as a tool for the expression of recombinant proteins. *J Biotechnol* 1991; **17**: 11–17.
10 Haack K *et al*. Transactivator and structurally optimized inducible lentiviral vectors. *Mol Ther* 2004; **10**: 585–596.