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A novel yeast expression system based on a hormone-induced transcriptional cascade

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Background

The yeast *Saccharomyces cerevisiae* is widely utilized in gene expression projects, both as a model eukaryotic organism and as a host for heterologous protein production. Much of this research and biotechnological activity demands the use of highly regulated systems, able to provide accurate control of the expression in gene function analysis, and timely recombinant protein synthesis during fermentative production. Different yeast expression systems have been developed that can be controlled at the transcriptional level. Among these systems, those based on the potent, tightly regulated *GAL1-10* promoter and its cognate transcriptional activator Gal4 are most commonly used [1]. However, induction of the *GAL* system requires the presence of galactose and the absence of glucose in the culture media [2], a major disadvantage when the metabolic changes associated to this switch in carbon source are relevant to the study. In addition, the high cost of the inducer can preclude scaling up production of a commercially valuable protein using this system. A good alternative to regulate transcription driven by *GAL* promoters is the incorporation of the hybrid protein developed by D. Picard [3], which combines features of three different transcriptional activators, the DNA binding domain of Gal4, the hormone response domain of the human estrogen receptor (ER), and the transcription activation domain of herpes-virus VP16. This chimerical protein activates transcription from *GAL1-10* promoters in the presence of estradiol at micromolar concentrations, even in the presence of glucose. However, constitutive expression of this transactivator originates a high basal activity of the *GAL* promoters in the absence of the hormone, therefore diminishing its efficiency as a transcriptional regulator.

Results

In order to improve this expression tool, we have placed the coding sequence of the Gal4-ER-VP16 hybrid activator under the control of a *GAL* promoter. The combination of these regulatory elements results in an amplification feedback loop that is triggered by the hormone and ultimately leads to enhanced expression of recombinant genes fused to similar *GAL* promoters (see Figure 1). The basal expression level of this system is as low as that of *GAL*-driven genes in glucose-containing media. This platform is back compatible with pre-existent *GAL* expression constructs.

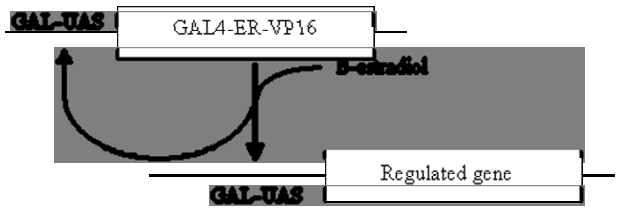

Figure 1
Estradiol-triggered feedback loop.

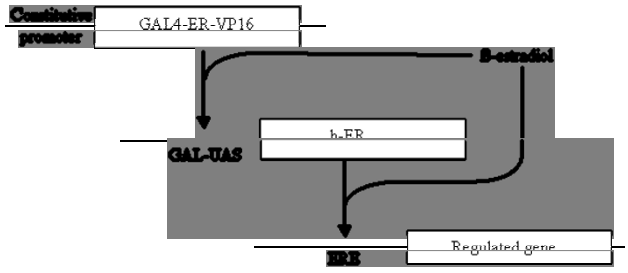

Figure 2
Hormone-dependent transcriptional cascade.

We have further expanded the versatility and capacity of this system, by adding new regulatory elements to those already described. To this end, we have placed the complete human ER coding sequence under the control of a *GAL* promoter, and combined it with another construct, constitutively expressing the Gal4-ER-VP16 hybrid activator. This configuration generated a hormone-dependent transcriptional cascade that allowed accurate regulation of any promoter containing an estrogen-responsive element (ERE) (Fig 2). The basal activity of the ERE-containing promoter was not influenced by expression of Gal4-ER-VP16 in the absence of ligand. The new combined system improved an order of magnitude the expression level windows of the individual regulatory circuits. In addition, we have found that certain mutations affecting *GAL* regulation further increase the level of induction of this system.

Conclusion

We have tested two novel gene/protein expression systems derived from the combination of different eukaryotic transcription elements. One of the systems activated expression of genes under the control of *GAL* promoters in *S. cerevisiae*, uncoupling it from the galactose/glucose signaling and keeping the low basal activity found in *GAL* promoters. The other system consisted of a cascade of estrogen-dependent activators able to stimulate transcription from an ERE-containing promoter. This new CASCADE system could constitute a simple and cost-efficient way to control heterologous protein expression in yeast, especially in projects requiring tightly controlled protein production, including functional genomics and industrial recombinant protein production.

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