

A novel rapid and sensitive on/off switch regulated by coumarin for gene expression in mammalian cells

SUMMARY

Regulated expression of transgenes in mammalian cells is a valuable tool for both functional genomic studies and clinical applications. Attempts to control gene activity in eukaryotes with inducible expression systems have been made but often resulted in modest induction levels or in pleiotropic effects. A regulated mammalian transgene expression system was designed based on coumarin-switched dimerization of a chimeric transactivator. This rapid and precise on/off switch can be turned on by coumermycin or promptly switched off by addition of novobiocin.

APPLICATIONS

- Tightly controlled regulation of gene expression in mammalian cells for functional analysis of cellular proteins and drug discovery applications.
- Transient production of highly toxic proteins or proteins interfering with host cell functions.
- Rapid and precise regulation of gene expression *in vivo* using low antibiotics concentration for gene therapy applications.

CONCEPT

Inducible mammalian expression systems that feature low levels of basal activity and rapid induction of gene expression in response to safe and reliable inducers are needed to control protein expression. A novel on/off switch that relies on the rapid and sensitive modulation of a chimeric transactivator by coumarins was developed to provide tightly controlled gene expression in mammalian cells. The transactivator comprises a repressor protein DNA binding domain (λ RA), a transcription activation domain (AD) and the bacterial DNA gyrase B subunit (GyrB). The interaction of coumermycin with GyrB induces

homodimerization of the transactivator maintained at a low basal level by a relatively weak hybrid SV40-CMV promoter. This homodimer triggers transactivator self-expression through a positive regulatory feedback loop designed by insertion of operator sequences (4x λ Op). In turn, transgene expression is triggered by the increased number of homodimers available for binding to the operator sequences (12x λ Op) situated upstream of the modified CMV minipromoter that regulates it. Conversely, the expression system can be promptly and effectively switched off by addition of novobiocin, a competitive monomer of coumermycin that readily abolishes transactivator dimerization (see Figure).

FEATURES AND BENEFITS

High level of inductibility

Inducibility was improved through reduced basal expression and transactivator auto-amplification. The λ R moiety of the transactivator was mutated to reduce its intrinsic dimerization activity and consequently increase the coumermycin-induced/basal-level ratio of gene expression. As well, a positive regulatory feedback loop was used to increase the activation potency of the transactivator controlled by the relatively weak SV40 early promoter.

Readily transferable expression cassette

The chimeric transactivator and the coumarin-responsive transgene expression cassettes, included on one or two separate plasmids, can be moved to any commonly used vector for *in vitro* or *in vivo* delivery into cells or to a pharmaceutically suitable carrier for human administration.

Rapid and sensitive on/off switch

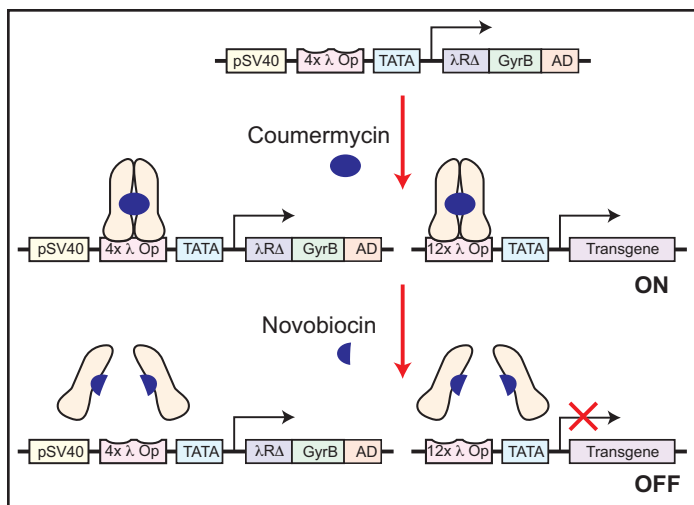
The coumarin-regulated expression system is induced by coumermycin concentrations in the nM range; this compares favorably to rapamycin and tetracycline-regulated systems. Conversely, novobiocin which also binds gyrB with high affinity effectively promotes dimer dissociation and shuts down the system in only a few hours. The very short half life of both compounds also contributes to the highly dynamic nature of the coumarin-regulated expression system.

Safety for gene therapy applications

The coumarin-based on/off switch is well adapted for the safe *in vivo* regulation of gene expression in mammalian cells. Both coumermycin and novobiocin have excellent pharmacokinetic properties, display no overt sign of toxicity, and have no known endogenous binding targets in mammalian cells.

PROTECTION STATUS

A coumermycin/novobiocin-regulated gene expression system (NRC no. 11444).



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