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A new system to control the *barnase* expression by a NifA-dependent promoter

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Abstract

Barnase is a potent ribonuclease widely used as a cytotoxic agent, tightly regulated by barstar to maintain cell viability. In this report, we describe a new composite regulatory system to control barnase cytotoxicity and expression, involving *barstar* and *lacI* genes under control of the NifA-, σ^{54}-dependent *Sinorhizobium meliloti nifH* promoter, and the *barnase* gene under control of the LacI-repressible *ptac* promoter. In this system, expression of the*nifH* promoter, activated by constitutively expressed NifA, resulted in constitutive synthesis of the LacI and barstar proteins. LacI, in turn, represses transcription of the *barnase* gene and barstar inhibits any ribonuclease activity. Full expression of the *barnase* gene induced by IPTG led to cell death. Control of barnase synthesis and activity could be achieved by regulating *nifA* expression and NifA protein activity by specific environmental signals.

Keywords: Barnase; Barstar; Nif promoter; LacI; Gene regulation; Biological containment system

1. Introduction

Lethal genes are widely used in biological research and as molecular tools in genetic engineering. Specific examples are: the positive selection of foreign gene expression (Vernet et al., 1985; Deyev et al., 1998; Choi et al., 2002); the enrichment of plant populations transformed with *Agrobacterium* containing only T-DNA sequences (Hanson et al., 1999); biological containment systems of bacterial strains that promote plant growth and which are potentially useful for bioremediation of polluted areas (Szafranski et al., 1997; Molina et al., 1998; Meilan et al., 2000; Ronchel and Ramos, 2001); engineering nuclear male sterility in plants (Block et al., 1997; Meilan et al., 2000; Kuvshinov et al., 2001) and conditional cell ablation in gene therapy of cancer cells (Bi et al., 2001; Leuchtenberger et al., 2001). In all cases, control of the lethal gene expression and of its product activity depend on cytotoxicity and its biotechnological application. Ex-

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pression of lethal genes in vivo, particularly those with highly toxic products, requires tight regulation by a finely adjusted promoter activity to maintain cell viability (Bi et al., 2001; Leuchtenberger et al., 2001). Such regulation depends on either the expression of an inhibitor of the lethal gene product or the strict repression of a regulatory promoter responding to physical or chemical signals, such as heat shock, modulators, such as IPTG and tetracycline, or to environmental pollutants, such as alkyl benzoate. Alternatively, regulation of lethal genes can be obtained by promoters induced at a specific stage of the developmental cycle, e.g. sterile male transgenic plants use a genetic expression switch that allows expression of the lethal function only during anther development (Block et al., 1997; Meilan et al., 2000).

Bacillus amyloliquefaciens barnase is a potent ribonuclease (Hartley, 1989) and highly toxic to cells. It has been widely used to produce sterile male or female transgenic plants to prevent gene transfer to wild plants by cross-pollinating compatible species (Beals and Goldberg, 1997; Block et al., 1997; Kuvshinov et al., 2001), as a potent endotoxin in ablation of mammalian cells (Bi et al., 2001; Leuchtenberger et al., 2001), and to study protein translocation to the cytosol (Prior et al., 1991). The *barstar* gene product (Hartley, 1988), on the other hand, binds specifically to barnase, forming a highly stable equimolar complex that inhibits barnase activity (Hartley, 1989; Schreiber and Fersht, 1995; Deyev et al., 1998). Given the ability of the barstar inhibitor to control barnase activity, the advantage of the barnase:barstar system is that the lethal function can be controlled both at the level of gene expression and product activity.

We have designed a new genetic system to regulate the expression of the *barnase* gene and to control the activity of its product. The scheme for the genetic regulatory circuit is shown in Fig. 1. Cell viability is maintained by repression of a LacI-repressed *ptac* promoter, which regulates the *barnase* gene expression, and by barstar, an inhibitor of barnase, expressed from *pnifH*, a NifA-regulated promoter.

The NifA protein, product of the *nifA* gene, is a positive regulator of *nif* genes of diazotrophic bacteria, and the *Klebsiella pneumoniae* NifA protein (used in this regulatory circuitry) is insensitive to ammonium and oxygen in the absence of the NifL protein, an inhibitor of NifA (Merrick and Edwards, 1995). In an *Escherichia coli* background, which lacks the *nifL* gene, NifA produced by the constitutive expression of the *nifA* gene, activates transcription of the *lacI* and *barstar* genes positioned in tandem, from the *Sinorhizobium meliloti nifH* promoter. The LacI protein, in turn, represses transcription of the *barnase* gene and barstar inhibits remaining ribonuclease activity (Fig. 1).

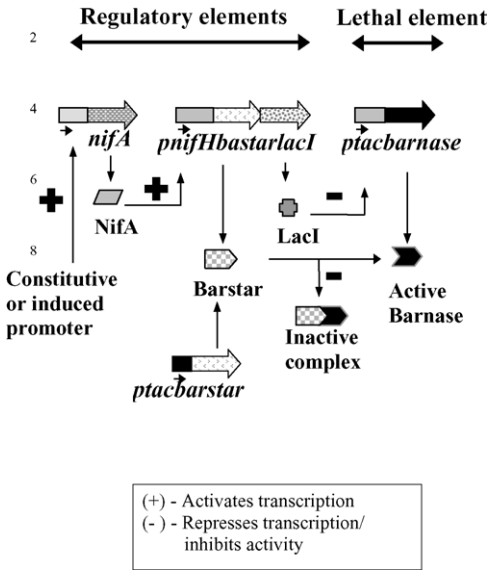

Fig. 1. Scheme of the regulatory circuit designed to control the expression of the *barnase* gene by expression of the *nifA* gene-dependent promoter. (+) Transcription activation; (−) transcription repression or activity inhibition.

2. Materials and methods

2.1. Bacterial strains and media

The bacterial strains used are listed in Table 1. *E. coli* strains were grown on LB or NFDM medium. The growth media were supplemented with antibiotics: ampicillin (250 $\mu g\,mL^{-1}$), spectinomycin (50 $\mu g\,mL^{-1}$), tetracycline (10 $\mu g\,mL^{-1}$), chloramphenicol (30 $\mu g\,mL^{-1}$) or nalidixic acid (10 $\mu g\,mL^{-1}$).

2.2. DNA manipulations

Standard procedures used for plasmid preparations, DNA ligation and agarose gel electrophoresis were as described by Sambrook et al. (1989). Restriction