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Co-regulation of two gene activities by tetracycline via a bidirectional promoter

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Recently, we have described a regulatory system that allows the stringent control of individual gene activities in higher eukaryotic cell lines (1), in plants (2) and in animals (3,4). The essential components of this system are (i) an RNA polymerase II minimal promoter placed downstream of multiple operator sequences (*tetO*) of the *Escherichia coli* Tn*10* tetracycline resistance operon and (ii) a fusion between the Tet repressor (TetR) and the herpes simplex virus protein 16 (VP16), named tTA (1). In the absence of tetracycline (Tc), tTA binds to the *tet* operators to activate transcription from the minimal promoter, whereas in the presence of Tc its association and consequently its transcription activation is prevented.

After the binding of tTA, minimal promoters derived from the cytomegalovirus IE promoter (P_{hCMV}; 5) and fused to seven *tetO* sequences reach the remarkable strength of the parent promoter in HeLa cells when compared in transient expression assays (6). This high activation potential of tTA and the arrangement of its binding sites within $P_{hCMV*-1}$ [(1); see Fig. 1A] suggested the design of a bidirectional promoter which would allow the simultaneous regulation of two transcriptional units from centrally located multiple *tetO* sequences (Fig. 1A). Such a promoter should be useful for a number of experimental approaches. First, it may allow the co-regulation of the synthesis of two gene products in stoichiometric amounts, frequently a prerequisite for the production of heterodimeric (or hetero-oligomeric) proteins. Second, by fusing minimal promoters of differing efficiencies to the centrally located *tetO* sequences, two gene products may be co-regulated at different but defined levels. Third, by integrating an appropriate reporter gene at one side of the bidirectional promoter, the regulation of a not-readily-assayable gene of interest may be monitored via the reporter function. This latter possibility may also facilitate—at the cellular as well as at the organismal level—the screening for properly integrated expression units controlling the gene of interest.

Here, we report the construction of a bidirectional promoter (P_{bi-1}; Fig. 1A) and show that two reporter genes encoding β-galactosidase and luciferase are indeed co-regulated by this promoter in a quantitative manner. Moreover, we describe a family of vectors which readily allows the use of P_{bi-1} for various purposes.

The generalized divergent transcription unit depicted in Figure 1A consists of the bidirectional promoter, of gene x followed by the SV40 late polyadenylation signal (1) as well as of gene y, oriented opposite to gene x and followed by the rabbit β-globin intron/polyadenylation site (7). The bidirectional promoter P_{bi-1} contains seven *tet* operator sequences flanked by two minimal promoters. These minimal promoters span the sequence of P_{hCMV} from position +75 to −53 and −31, respectively. The various elements were retrieved from plasmids pUHG16-1 (8) and

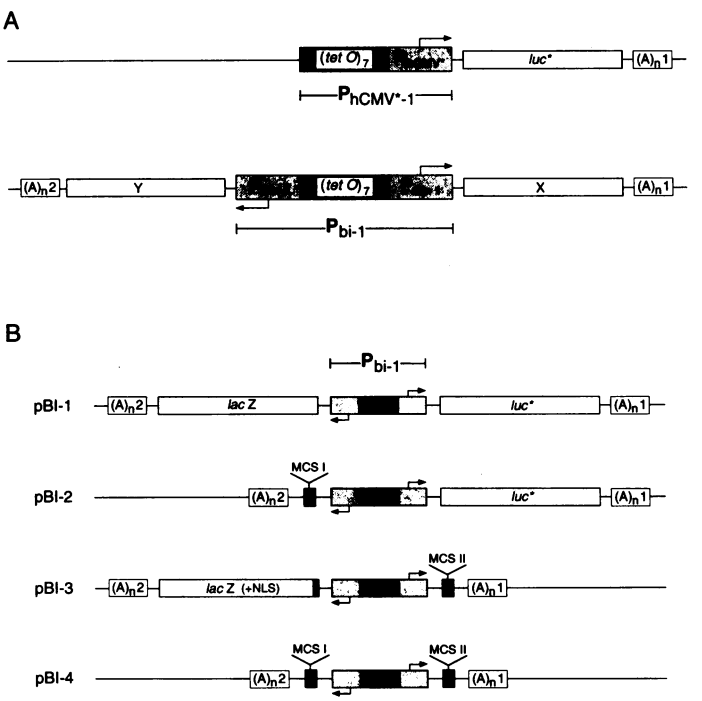

Figure 1. Schematic presentation of the bidirectional transcription unit. (**A**) Plasmid pUHD131-3 (9) containing the promoter $P_{hCMV*-1}$ (1) which consists of seven *tet* operators and a minimal promoter sequence spanning position −53 to +75 of P_{hCMV}IE (5). The principle of a bidirectional promoter in which the *tet* operators are flanked by two minimal promoters (P_{min}) is outlined below. (**B**) Four bidirectional transcription units which all contain promoter P_{bi-1}. The cleavage specificities of the multiple cloning sites are as follows, MCS I: *Pvu*II, *Mlu*I, *Nhe*I, *Eco*RV; MCS II: *Pst*I, *Not*I, *Sal*I. $(A)_n1$ corresponds to the SV40 late polyadenylation site (1) whereas $(A)_n2$ comprises the intron/polyA signal of the rabbit β-globin gene (7).

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pUHD131–3 (9), and combined using standard procedures as described in Sambrook *et al.* (10). In plasmid pBI-1 (Fig. 1B), the luciferase encoding gene as modified by Bonin *et al.* (11) is positioned as gene x and the β-galactosidase encoding sequence as gene y. This vector, pBI-1, was used to examine the functionality of the divergent expression unit and, in particular, of P_{bi-1}.

Thus, HeLa cells were transiently transfected with pBI-1 and the tTA expression vector pUHD15–1 (1), and high levels of luciferase and β-galactosidase activity were monitored in cell lysates. These enzyme activities were sensitive to Tc (data not shown). Plasmids pBI-1 and pHMR272 (12) encoding hygromycin resistance were then used to stably transfect HtTA-1 cells previously shown to express tTA (1). Out of 21 clones resistant to hygromycin, 16 were positive when assayed for luciferase activity. Fifteen of those clones were also positive for β-galactosidase and the activities of both enzymes were sensitive to Tc in the medium.

Quantitative analysis revealed that nine clones could be regulated for both enzyme activities over at least three orders of magnitude, and that in the presence of 100 ng/ml Tc in the medium, luciferase as well as β-galactosidase activities were indistinguishable from the background as exemplified by the *luc*$^+$/*lacZ*$^+$ HtTA-1 cell line HtBI-1/205. A dose-response analysis of the HtBI-1/205 cell line at different Tc concentrations showed that both luciferase and β-galactosidase activities follow the same slopes, demonstrating that both reporter genes are coregulated (Fig. 2). The difference in the apparent regulation factors of 10^4 versus 10^3 for luciferase and β-galactosidase, respectively, can be explained by the largely differing sensitivities of the two enzymatic assays which allow the monitoring of luciferase activity at much lower enzyme concentrations than β-galactosidase. Expression of the reporter genes in several clones was further analyzed by double immunofluorescence staining with antibodies directed against luciferase and β-galactosidase. The results proved that both reporter genes are co-expressed at the single cell level (data not shown).

Based on pBI-1, a family of plasmids was developed (Fig. 1B). By exchanging the sequences encoding β-galactosidase or luciferase for multiple cloning sites (MCS), both pBI-2 and pBI-3 were obtained. Finally replacing both reporter genes by MCSs, pBI-4 was created for the co-regulation of two genes of interest. All plasmids described have been successfully used, particularly for the screening of stably integrated expression units in cell lines as well as in transgenic animals.

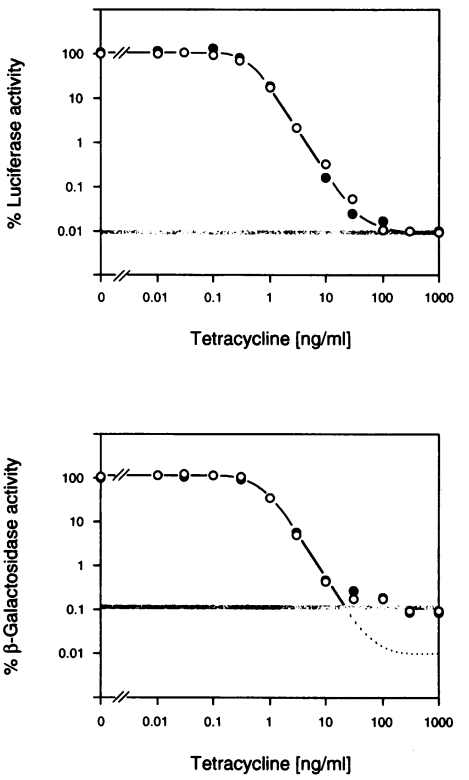

Figure 2. Co-regulation of luciferase and β-galactosidase by P_{bi-1}. HeLa cell line HtBI-1/205 constitutively expressing tTA and stably transfected with pBI-1 was seeded at a density of ~10 000 cells/35 mm dish and incubated with medium containing tetracycline in the concentrations indicated. After 5 days, cells were harvested and assayed for luciferase and β-galactosidase activity. Data shown are from two independently grown dishes. Determination of β-galactosidase activity was performed according to (13). Cell culture, luciferase assays and protein determination were carried out as described previously (1). The graphs illustrate the decrease of luciferase or β-galactosidase activity, respectively, at increasing concentrations of Tc in the medium. For better comparison relative enzyme activities are plotted versus Tc concentration. The grey lines indicate the background values of non-transfected HeLa cells. We anticipate that the insensitivity of the ONPG assay for β-galactosidase obscures the full range of regulation of the β-galactosidase.

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REFERENCES

1 Gossen, M. and Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5547–5551.
2 Weinmann, P., Gossen, M., Hillen, W., Bujard, H. and Gatz, C. (1994) *Plant J.* **5**, 559–569.
3 Furth, P.A., Onge, L. St., Böger, H., Gruss, P., Gossen, M., Kistner, A., Bujard, H. and Hennighausen, L. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 9302–9306.
4 Kistner, A., in preparation.
5 Boshart, M., Weber, F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B. and Schaffner, W.(1985) *Cell* **41**, 521–530.
6 M. Gossen, Ph.D. thesis, Universität Heidelberg.
7 Seveme, Y., Wieland, S., Schaffner, W. and Rusconi, S. (1987) *EMBO J.* **7**, 2505–2508.
8 Resnitzky, D., Gossen, M., Bujard, H. and Reed, S.I. (1994) *Mol. Cell. Biol.* **14**, 1669–1679.
9 A.L. Bonin, unpublished.
10 Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd edn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
11 Bonin, A.L., Gossen, M. and Bujard, H. (1994) *Gene* **141**, 75–77.
12 Bernard, H.-U., Krämmer, G. and Röwekamp, W. G. (1985) *Exp. Cell Res.* **158**, 237–243.
13 Miller, J.H. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.