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Effect of drugs on gene expression in mammalian cells: a highly efficient procedure to test large numbers of samples

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Normally, drugs that affect gene expression do not have application in medicine as they are very often tumour promoting agents. However, as more and more diseases are understood at a genetic and molecular level, transcriptionally active drugs must remain a focus of medical research. Since screenings of new drugs that influence gene expression require the analysis of extremely large numbers of samples, the classical protocols to study gene expression are inadequate because of the time, energy and cost involved. We have developed a protocol that is sensitive, simple, quick, cheap, and that allows the processing of several dozens of samples at one time.

First, in the only time-investing step, cell lines carrying the promoter of interest linked to the bacterial β-galactosidase gene (lacZ) are isolated. As a model system, we chose the mouse mammary tumour virus (MMTV) promoter: this a well-studied transcriptional element that depends on the action of the glucocorticoid, progesterone and mineralocorticoid receptors activated by the respective steroid hormones (1–3, and references therein). By co-transfection with an expression vector for the hygromycin resistance gene and subsequent selection in hygromycin B medium (70 μg/ml), we created permanent cell lines of 3Y1 rat fibroblasts harbouring MMTV-lacZ (Figure 1A). We called Kiki-GR those clones that tested positive for β-galactosidase activity (4, 5) in presence of 0.1 μM dexamethasone (a synthetic glucocorticoid).

To simulate a drug screening experiment, the cell lines were plated in 24-wells dishes to 30–40% confluency (medium: DMEM + 10% cadet bovine serum, at 37°C in 5% CO_2). After the cells adhered to the plates, each well was supplied with medium containing increasing concentrations of dexamethasone (final solvent concentration in all wells: 0.1% ethanol) for a two-days incubation. We then performed an *in situ* β-galactosidase assay, that is directly in the wells. In contrast to the classical β-galactosidase assay, this procedure does not require time-consuming harvesting of dozens of different samples. The cells were rinsed once with phosphate-buffered saline (−) (PBS−) and made to swell by overlaying them with hypotonic buffer (25 mM Tris−phosphate (pH 7.8), 2 mM $MgCl_2$) on ice for 10 min. After discarding the hypotonic solution, the cells were disrupted *in situ* by the addition of 50 μl lysis buffer (25 mM Tris−phosphate (pH 7.8), 2 mM DTT, 10% glycerol, 1% Triton-X-100, 0.66 mM PMSF) directly to the wells, followed by brief vortexing of the whole plate and gentle rocking for 15 minutes at room temperature. The 24-wells dishes were then placed on ice, and 250 μl reaction mixture were added to each well (ice cold; 1 mM $MgCl_2$, 45 mM β-mercaptoethanol, 100 mM sodium phosphate (pH 7.8), 0.26 mg/sample 0-nitrophenyl-D-galactopyranoside). Reactions were started contemporaneously by placing the plates in a 37°C incubator for 30 to 60 minutes. The solutions were diluted with 300 μl ice-cold H_2O and placed on ice to stop the reaction.

If the chemical composition of the culture medium allows expression of the lacZ gene, the solutions in the corresponding wells turn bright yellow. This can be easily seen by naked eye and can be quantified, if needed, by measuring the optical density at 420 nM. Distinctly yellow supernatants were observed in all the wells containing the different Kiki-GR clones incubated with dexamethasone (but not in those without it); in contrast, wild type 3Y1 did not yield any β-galactosidase activity. The intensity of the colour (data not shown) and optical density (Figure 1B) were reproducibly proportional to the concentration of dexamethasone, hence to gene expression levels (2). Maximal activity was observed with 10^{-7} M, half-maximal activity with 10^{-8} M dexamethasone (2). The four Kiki-GR clones responded differently to dexamethasone, in correspondence with the number of intact copies of MMTV-lacZ integrated in their genomes (data not shown). Finer modulations of these activities can be also observed (Figure 1C, D): The action of dexamethasone on Kiki-GR # 10 was inhibited by high concentrations of ketokonazole (a low-affinity, non-steroid antagonist of dexamethasone) (6, and references therein), and by moderate concentrations of progesterone. Progesterone probably competes non-specifically for binding to the C-terminus of the glucocorticoid receptor: high doses of progesterone alone (Figure 1D) do not activate β-galactosidase expression because rat fibroblasts do not express progesterone levels at significant levels (2).

This protocol seems to be adequate not only for qualitative but also for semi-quantitative analysis. The choice of the lacZ reporter gene is not casual: microtiter plate-based luciferase assays are more sensitive and compact, but an *in situ* version can be troublesome because they require clean, calcium-free extracts, while β-galactosidase assays can be performed with crude lysates. On the other hand, the bacterial acetyl-transferase (CAT) gene does not allow *in situ* analysis.

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REFERENCES

1. Beato,M. (1989) *Cell* **56**, 335–344.
2. Cato,A.B.C., Mikisicek,R., Schlitz,G., Arnemann,J. and Beato,M. (1986) *EMBO J.* **5**, 2237–2240.
3. Ham,J., Thomson,A., Needham,M., Webb,P. and Parker,M. (1988) *Nucleic Acids Res.* **16**, 5263–5276.
4. β-galactosidase assay, protocol from manual of Promega Corp.
5. Sanes,J.R., Rubenstein,J.L.R. and Nicolas,J.-F. (1986) *EMBO J.* **5**, 3133–3142.
6. Rusconi,S. and Yamamoto,K.R. (1989) *EMBO J.* **6**, 1309–1315.

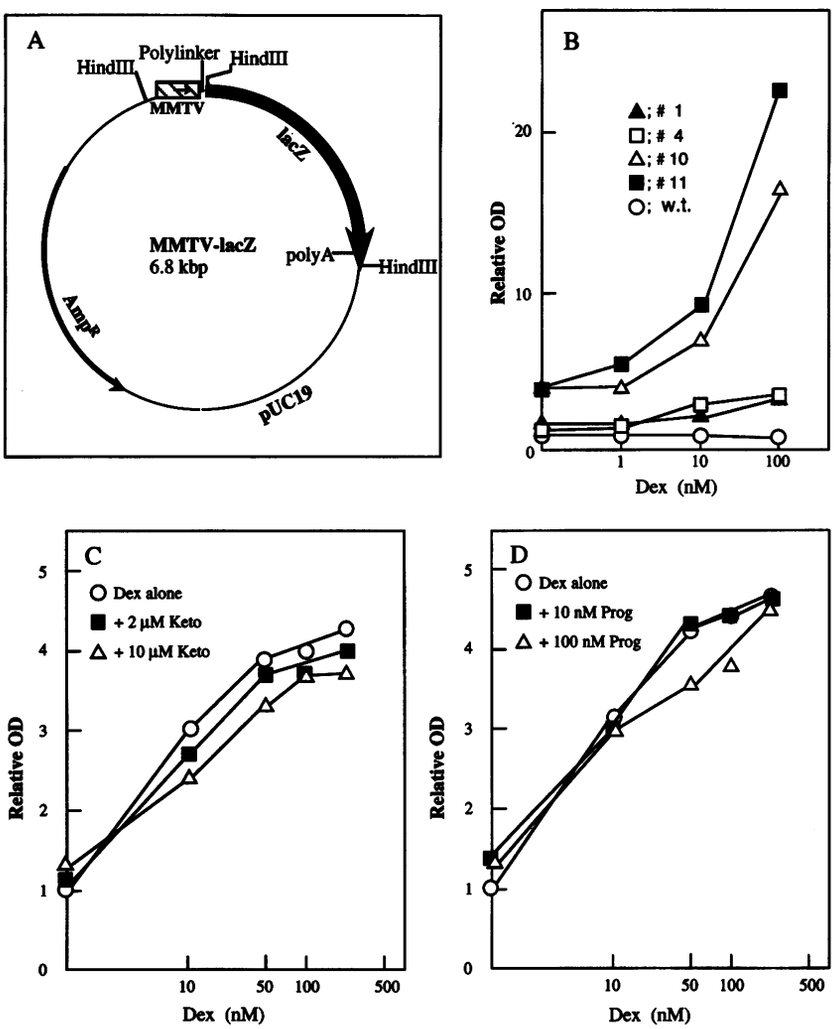

Figure 1. β-galactosidase activities of the cells harbouring MMTV-lacZ. **A.** Plasmid map of MMTV-lacZ. **B.** Kiki-GR (#1, 4, 10 and 11) and original 3Y1 (w.t.) cells were treated with various concentrations of dexamethasone (Dex) for 48 hrs, and their β-galactosidase activities were measured *in situ*. Relative activities to that of wild type 3Y1 cells incubated without dexamethasone (value 1) are shown. **C.** Competition of dexamethasone action with ketokonazole. Kiki-GR #10 cells were treated with various concentrations of dexamethasone plus 2 or 10 μM ketokonazole (Keto). Relative β-galactosidase activities to that of Kiki-GR #10 in absence of dexamethasone are shown. **D.** Competition of dexamethasone action with progesterone. Kiki-GR #10 cells were treated with various concentrations of dexamethasone plus 10 or 100 nM progesterone (Prog). Relative β-galactosidase activities to that of Kiki-GR #10 in absence of dexamethasone are shown.