Identify biotin-responsive elements in genes encoding IL-2 in human lymphoid cells

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Introduction:

Immune cells such as T and B cells secrete cytokines in response to stimulation or the immune system. Extracellular cytokines bind to receptors located on the surface of target cells such as T cells and natural killer cells. This receptor binding triggers signaling cascades, leading to cellular growth, proliferation, and differentiation. Numerous studies have provided evidence for an essential role of biotin in immune function. We provide evidence that biotin status affects expression of genes encoding IL-2. Here we test the hypothesis that the regulatory regions encoding this gene contain biotin-responsive elements. Jurkat cells (human T cell line) were used as model. These cells produce IL-2 after appropiate stimulation.

Materials and methods:

Materials

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany). Jurkat cells (clone E6–1) were purchased from American Type Culture Collection (Manassas, VA). Medium for cell culturing contained the following components: RPMI-1640 (Atlanta Biologicals, Norcross, GA) without biotin; Fetal bovine serum without biotin (see below); and 100,000 IU/L penicillin and 100 mg/L streptomycin (final concentrations). Before medium preparation, fetal bovine serum was depleted of biotin using a column packed with immobilized avidin (Immunopure; Pierce, Rockford, IL).

Cells

Cells were cultured (5% CO_2 at 37°C in humidified atmosphere) in the following biotin-defined media for at least 5 wk before sample collection:
25 pM biotin (denoted "deficient"), 250 pM biotin ("physiological"), or 10,000 pM biotin ("pharmacological"); culture medium was replaced with fresh medium every 48 h. Cell viability was monitored at timed intervals using Trypan blue.

Transfection

Plasmids

For transfections, Jurkat cells were cultured in biotin-defined media for 5 wk prior to analyses. The following constructs were generated to model effects of biotin on 5'-flanking regions of genes encoding IL-2.

1) A construct of the regulatory region of the IL-2 gene (spanning 321 bases upstream of the transcription start site) linked to the luciferase gene [denoted p(-321)IL2-Luc] was provided by L. P. Freedman (Memorial Sloan-Kettering Cancer Center, New York, NY; Ref. 1). The regulatory elements of the IL-2 gene are located within 300 bases upstream of the transcription start site.

2) Two constructs of the regulatory region of the IL-2 gene (spanning 1,500, 477 bases, upstream of the transcription start site) linked to the luciferase gene (denoted p(-1,500)IL2-Luc, and p(-477)IL2-Luc

3) A construct of the RSV promoter linked to β-galactosidase gene (denoted RSV β-gal) was used as control for transfection efficiency (provided by B. R. White, University of Nebraska-Lincoln, Lincoln, NE).

4) A positive control plasmid (pGL3-Control) linked to the luciferase gene (Promega), driven by SV40 promoter and enhancer.

Protocol

For transfection standardizations, Jurkat (4×10^5 cells /well) were cultured in 1 mL of fresh medium. Metafectene PRO was complexed with the plasmids as we describe below.
The reagent:DNA ratios were: 1 µL:0.5 µg, 2 µL: 0.5 µg, 3 µL:0.5 µg, 4 µL:0.5 µg; 2 µL:1 µg, 4 µL:1 µg, 8 µL:1 µg, 12 µL:1 µg; 4 µL:1.5 µg, 8 µL:1.5 µg, 12 µL:1.5 µg, 16 µL:1.5 µg. Complexes were prepared by mixing Metafectene PRO with 100µL of sterile PBS followed by the addition of plasmid DNA. The mixture was incubated for 15 minutes at room temperature and added dropwise to the cells and swirling the flask. The cells were incubated at 37°C in a CO_2 incubator for 24 hours.

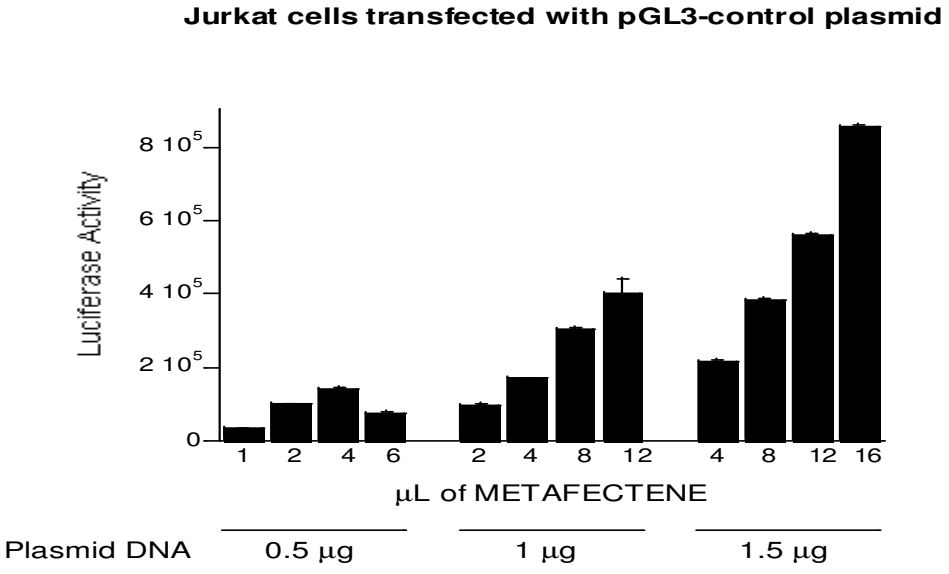
After transfection, cells were stimulated with 50 µg/L of PMA and 2 mg/L of PHA for 6 h to induce expression of reporter genes. Luciferase activity was assayed by LucLite Plus (Packard, Boston, MA), according to the manufacturer's instructions, with a Top Count NXT (Packard). β-Galactosidase activity was assayed with a commercial assay kit (Promega, Madison, WI) and an Emax Microwell Plate Reader (Molecular Devices, Sunnyvale, CA). All data were normalized for transfection efficiency, as judged by β-galactosidase activity in response to transfection with RSV β-gal.

Results and discussion:

The highest expression of luciferase was obtained with 16 µL:1.5 µg DNA when using plasmid pGL3-control (Fig. 1).

Figure 1. Optimization of transfection efficiency in Jurkat cells. The cells were transfected with the indicated amounts of Metafectene PRO and plasmid. The viability of cells was 98–100% when cells were incubated in media containing 25–10,000 pM biotin.

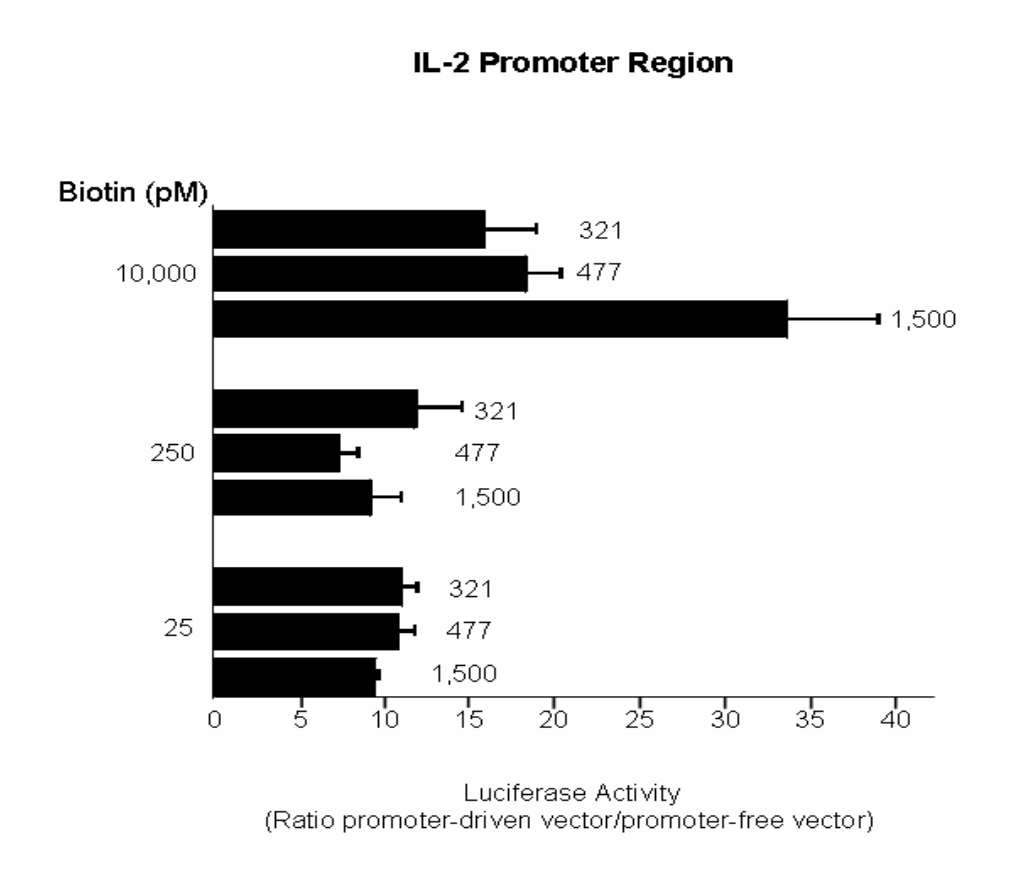
Figure 1



Luciferase activity was greater in p(-1,500)IL2-Luc in biotin-supplemented cells (10,000 pM) compared with physiological (250 pM) and deficient (25 pM) medium (Fig.2). Luciferase activity of constructs p(-477)IL2-Luc and p(-321)IL2-Luc did not depended on biotin. These findings suggest that biotin-responsive elements reside in region -477 to -1,500 from the transcription start site of IL-2.

Figure 2. Luciferase activity in plasmids with IL-2 promoter constructs.The cells were transfected with 16 µL of Metafectene PRO and 1.5 µg of plasmid. The viability of cells was 98–100% when cells were incubated in media containing 25–10,000 pM biotin for all transfections.

Figure 2



Conclusions

1. Jurkat cells were successfully transfected with Metafectene PRO.
2. Cell viabiity was >98%
3. Biotin-responsive elements reside in region -477 to -1,500 in the IL-2 gene