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The effects of glucocorticoid hormone on the expression of c-jun

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The effects of glucocorticoid hormone on the expression of c-jun in the fibroblasts were studied. The expression of c-jun was repressed by dexamethasone in the NIH3T3 cells, but not in the transformed B104-1 or EJ-Ras cells. The repression was not relieved by the addition of cycloheximide.

Glucocorticoid; Oncogene; Jun

1. INTRODUCTION

The oncogene jun was first identified as the transforming component of avian sarcoma virus 17 which causes fibrosarcomas in chickens [1]. Nucleotide sequence analysis revealed that the C-terminus of v-jun shares more than 40% homology with the C-terminus of yeast transcriptional activator GCN4, which is the DNA binding domain of GCN4 [2,3]. This finding leads to the suggestion that the v-jun oncogene product acts as a transcriptional factor to influence the cellular gene expression pattern. Later studies have shown that the human AP-1 (activator protein 1) gene is the cellular homologue of the transforming v-jun gene [4,5]. The AP-1 complexes consist of several distinct proteins including those encoded by the proto-oncogenes c-jun and c-fos, and other members in these two gene families [6–10]. At least two other members, jun-B and jun-D, have been identified in the jun family. Jun-B was shown to inhibit the *trans*-activating activities of c-jun when both were co-transfected into F9 cells [11–14].

Collagenase gene is one of the many genes known to be activated by Jun [15]. The AP-1 site in the promoter of the collagenase gene mediates biological responses induced by phorbol esters, growth factors and steroid hormones [16–21]. Direct interaction between the glucocorticoid hormone receptor and the AP-1 complex on the AP-1 site has recently been demonstrated, and was shown to be responsible for the inhibition of collagenase expression by glucocorticoid hormone in CV-1 cells and other cells [18–21]. As the expression of c-jun is regulated by itself, the amount of c-jun mRNA should be affected by glucocorticoid hormone according to the model proposed by several groups [18–21]. In this report, we investigated the effects of glucocorticoid hormone on the expression of c-jun in the fibroblasts. The results indicated that the expression of c-jun is indeed inhibited by dexamethasone in NIH3T3 fibroblasts.

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2. MATERIALS AND METHODS

2.1. *Cell culture*

NIH3T3, B104-1 and EJ-Ras cells were cultured in DMEM supplemented with 10% fetal calf serum in a humidified atmosphere containing 5% CO_2, 95% air on 10-cm plastic dishes.

2.2. *RNA isolation and analysis*

Total RNA was isolated from cells according to the single step method published by Chomczynski and Sacchi [22], and subjected to agarose gel electrophoresis and Northern blot analysis as described [23]. The plasmid pSV-jun containing cDNA of c-jun was used to prepare labeled probes for hybridization. pCJ125 probe containing a ribosomal RNA gene was used as an internal control.

3. RESULTS AND DISCUSSION

3.1. *The effects of dexamethasone on the expression of c-jun*

10^{-6} M dexamethasone was added at time zero to the confluent NIH3T3 cells or B104-1 cells. Cells were harvested at different time intervals and 20 μg total RNA was applied each lane. After 12 h, the expression of c-jun decreased significantly in NIH3T3 cells (lane 1 and lane 3, Fig. 1A), and remained low for at least another 24 h (lane 4, Fig. 1A). While the expression of c-jun stayed constant or increased a little in the neu-transformed fibroblasts cells, B104-1 cells, as shown in Fig. 1B (the relative level has been determined by densitometry scanning). Similar results were observed in

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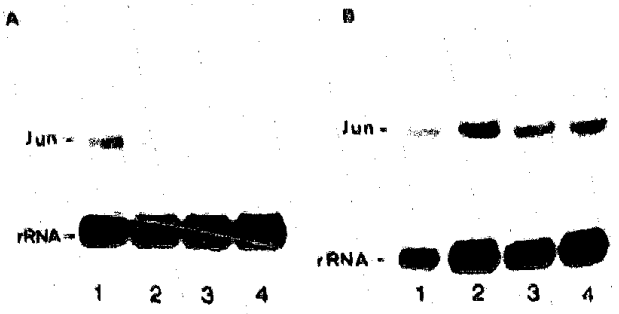

Fig. 1. The effects of dexamethasone on the expression of c-jun in NIH3T3 cells (A) and B104-1 cells (B). Cells were harvested at 0 h (lane 1), 4 h (lane 2), 12 h (lane 3), and 36 h (lane 4) after treatment with dexamethasone. Total RNA isolation and Northern blot analysis were carried out as described in section 2.

EJ-Ras cells which are NIH3T3 cells transformed with oncogene ras (data not shown).

3.2. *Cycloheximide cannot relieve the repression caused by dexamethasone*

The effects of glucocorticoid hormone on the expression of several other genes require ongoing protein synthesis. We wished to study the role of de novo protein synthesis during the repression of c-jun by dexamethasone. Cycloheximide has been shown to increase the steady-state level of the c-jun mRNA concentration by inhibiting the synthesis of a hypothetic labile protein which can destabilize the c-jun mRNA. A similar effect was observed in our experiments (lane 1 and 4, Fig. 2). Comparing the mRNA levels of c-jun in the presence of dexamethasone and that in the absence of dexamethasone when cycloheximide was added (lane 3 and 4, Fig. 2), the repression of the expression of c-jun was clearly not affected by the addition of cycloheximide.

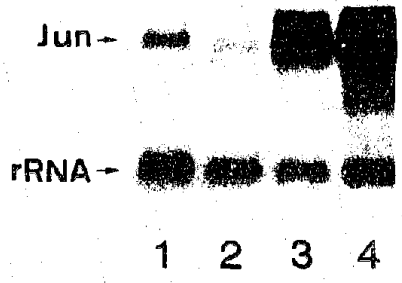

Fig. 2. The effect of cycloheximide on the repression of c-jun by dexamethasone. NIH3T3 cells were treated with dexamethasone or cycloheximide or both for 12 h, and total RNA was isolated, electrophoresesed, blotted and hybridized as described in section 2. In the cells treated with both dexamethasone and cycloheximide, cycloheximide was added 1 h prior to the addition of dexamethasone. RNA from cells untreated (lane 1), cells treated with dexamethasone (lane 2), cells treated with dexamethasone and cycloheximide (lane 3), and cells treated with cycloheximide (lane 4).

In our report, we demonstrated that the expression of c-jun is down-regulated by the glucocorticoid hormone directly in normal fibroblasts but not in transformed fibroblasts (i.e. B104-1 cells). The repression observed in NIH3T3 cells is probably mediated through the interaction between the glucocorticoid hormone receptor and the AP-1 complex on the AP-1 site in the promoter of c-jun [24]. The difference of response to dexamethasone between transformed cells and normal fibroblast cells possibly reflects the ratio of functional Jun and Fos in each cell line according to the model proposed by Yamamoto [18]. However, other explanations, such as decreasing number of glucocorticoid hormone receptor in B104-1 cells, cannot be excluded.

Our results and a recent report by Herrlich et al. [19] indicated that the expression of c-jun can be repressed, induced, or unaffected by the treatment of glucocorticoid hormone in different cell types. Therefore, the interaction between the AP-1 complex and the glucocorticoid hormone receptor on the AP-1 site not only affects the expression of other genes but also affects the expression of c-jun itself.

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