

Murine ST2 gene is a member of the primary response gene family induced by growth factors

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Received 27 February 1992

The murine ST2 gene, which encodes a protein remarkably similar to the extracellular portion of murine interleukin 1 receptor types 1 and 2, is expressed in growth-stimulated BALB/c-3T3 cells in the presence of 50 μ g/ml of cycloheximide. The treatment with 1,000 U/ml of purified native murine β -interferon superinduced, rather than suppressed, the ST2 mRNA expression as in the cases of c-myc and JE mRNAs. These results suggested that the murine ST2 gene belongs to the family of primary response genes induced by growth factors. Furthermore, a longer ST2-related mRNA was found in BALB/c-3T3 cells that were stimulated to proliferate in the presence of cycloheximide.

Cell cycle; Primary response gene; Interleukin 1 receptor; Immunoglobulin superfamily; Cell growth

1. INTRODUCTION

Initiation of mammalian cell proliferation is controlled by extracellular growth signals. The signal transduction sequences have been of great interest, and many immediate-early genes have been identified as primary response genes [1]. To elucidate the mechanisms of the Go/S transition, we have chosen to study the late responses and specifically the genes that are activated at 10 h after serum stimulation of resting BALB/c-3T3 cells. We have isolated a battery of growth-specific cDNAs [2], one of which, ST2 cDNA, encodes a protein that could be classified into the immunoglobulin superfamily. This protein is highly similar to the extracellular portion of IL-1R1 and IL-1R2 [3,4]. Furthermore, the St2 locus is very tightly linked to the Il-1R1 and Il-1R2 loci, suggesting the close functional relationship among them [4,5]. Here, we report that ST2 gene expression is a primary response to the growth signal(s), and also report the presence of a longer ST2-related mRNA in growth-stimulated BALB/c-3T3 cells.

2. MATERIALS AND METHODS

The BALB/c-3T3 cells (clone A31) were kindly provided by Dr. C. Stiles (Harvard Medical School). Cycloheximide was purchased from

Abbreviations: IL-1R1, interleukin 1 receptor type 1; IL-1R2, interleukin 1 receptor type 2; β -IFN, β -interferon; CHX, cycloheximide; dCTP, deoxycytidine 5'-triphosphate; DME, Dulbecco's modified Eagle's medium.

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Wako Pure Chemicals. The multiprime DNA labeling system and [α - 32 P]dCTP (specific activity, about 3,000 Ci/mmol) were from Amersham.

The total cytoplasmic RNAs were prepared from BALB/c-3T3 cells as described [6]. Northern blotting was performed using glyoxal and dimethylsulfoxide [7]. Probes for Northern hybridization were labeled with [α - 32 P]dCTP by the multiprime labeling method [8]. Hybridization was carried out at 42°C and the filter (Zeta probe, Bio-Rad) was washed at 50°C in 0.1 \times SSC, 0.1% SDS solution [3]. The natural murine, β -IFN (5 \times 10⁶ IU/mg), was prepared and purified as described [9,10]. The treatment of BALB/c-3T3 cells by β -IFN was carried out as described [10]. In brief, quiescent BALB/c-3T3 cells were incubated for 48 h in the presence or absence of 1,000 IU/ml of β -IFN in DME + 5% platelet-poor plasma. Thereafter, the cells were either stimulated to proliferate by the addition of DME + 10% fetal bovine serum or kept untreated, in the presence or absence of 1,000 IU/ml of β -IFN.

3. RESULTS AND DISCUSSION

BALB/c-3T3 cells were introduced into the Go state as described [10], and then they were stimulated to proliferate by serum addition with or without cycloheximide (CHX). The total cellular RNAs were extracted at 10 h after serum stimulation, because the appearance of ST2 mRNA was maximum at 10 h after the stimulation of quiescent BALB/c-3T3 cells in the absence of CHX [3]. The RNAs were processed for Northern hybridization (Fig. 1). The experiment shown is representative of three similar experiments. Even in the presence of 50 μ g/ml of CHX (Fig. 1B) the ST2 mRNA expression was not suppressed, indicating that the expression is not dependent on protein synthesis. Furthermore, in the presence of 10 μ g/ml of CHX, the ST2 mRNA was apparently superinduced (Fig. 1A). The expression of ST2 by CHX alone was intriguing. Although the precise mechanisms remain unknown, this

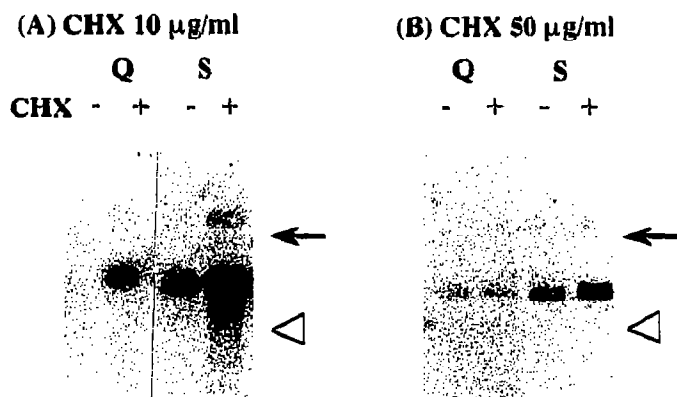


Fig. 1. The effect of cycloheximide on the expression of ST2 mRNA. Total cellular RNAs were prepared from quiescent BALB/c-3T3 cells (Q) and cells at 10 h after serum stimulation (S), incubated without (-) or with (+) 10 μ g/ml (A) or 50 μ g/ml (B) of CHX. RNAs (5 μ g) were loaded on a 1.1% agarose gel for Northern blotting. *Eco*RI fragments of ST2 cDNA [3] were labeled with [α - 32 P]dCTP and used as probe. Hybridization was carried out as described in Materials and Methods. The arrow and the open triangle correspond to the positions of 28 S and 18 S ribosomal RNA, respectively.

phenomenon has been reported as a competence effect of CHX [11,12].

Next, we analyzed the time-course for the appearance of ST2 mRNA in the presence or absence of CHX. As shown in Fig. 2, the appearance of ST2 mRNA was

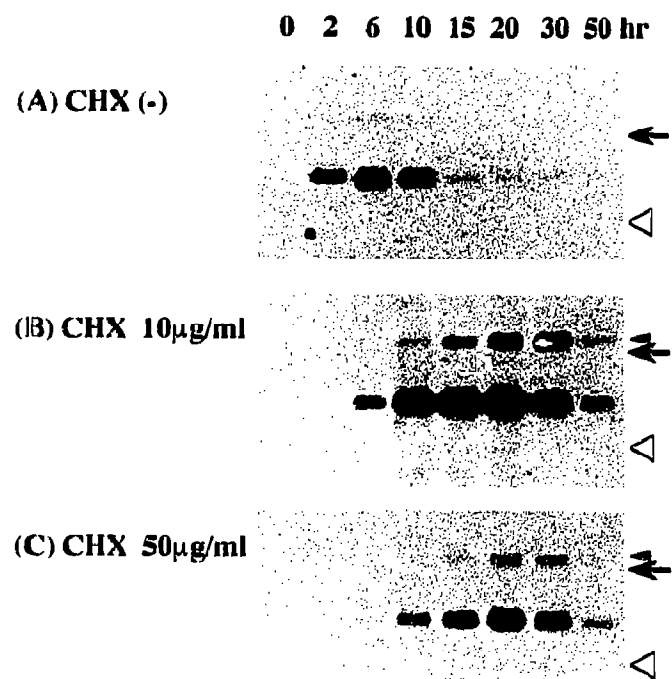


Fig. 2. Time-course of the expression of ST2 mRNA in the presence of cycloheximide. Total cellular RNAs were extracted at the indicated hours after serum stimulation without (A) or with 10 μ g/ml (B) or 50 μ g/ml (C) of CHX. Northern hybridization was performed as described in Fig. 1. The arrow and open triangle correspond to the positions of 28 S and 18 S ribosomal RNA, respectively. The arrowhead indicates the position of the longer ST2-related mRNA.

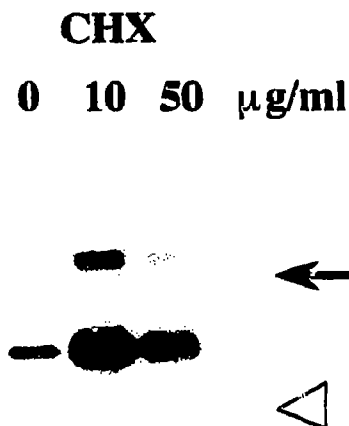


Fig. 3. Comparison of the samples of maximum ST2 expression on the same Northern blot. Total cellular RNA (5 μ g) at 10 h after stimulation from untreated cells or at 20 h after stimulation from CHX-treated cells were loaded on an agarose gel and processed for Northern hybridization as described in Fig. 1. The arrow and open triangle correspond to the positions of 28 S and 18 S ribosomal RNA, respectively.

detected at 6 h after serum stimulation, and the peak in the distribution of this mRNA among the total cellular RNAs was 20 h in the presence of 10 μ g/ml of CHX. Treatment with 50 μ g/ml of CHX further delayed the appearance of mRNA at 10 h after stimulation. Comparison of the intensities of the ST2 signal in the samples (with or without CHX) of maximum expression in this time-course experiment on the same blot (Fig. 3) confirmed that the ST2 expression was not suppressed by the treatment with CHX.

The presence of a longer ST2-related mRNA is of great interest (arrowhead in Fig. 2). The size of this mRNA is comparable to the mRNA of IL-1R [13]. However, it might not be the IL-1R mRNA itself, since IL-1R expression should be suppressed under these conditions [14]. It could be a precursor of ST2 mRNA, but another possibility is that the mRNA is a receptor-type ST2 homologue containing transmembrane and cytoplasmic domains. Cloning of the cDNA corresponding to this longer mRNA is now in progress, and the structural analysis of this cDNA will be described elsewhere.

Superinduction of the mRNA of immediate-early genes, such as JE and c-myc, by serum-stimulation of quiescent BALB/c-3T3 cells in the presence of β -IFN has been reported [10]. Therefore, we examined the effect of purified murine β -IFN on the expression of the ST2 gene (Fig. 4). The addition of 1,000 IU/ml of β -IFN superinduced the ST2 mRNA expression. This result was not a universal effect of β -IFN since the expression of ST1, and also one of the growth-specific mRNAs [2], was not changed by the presence of β -IFN (data not shown).

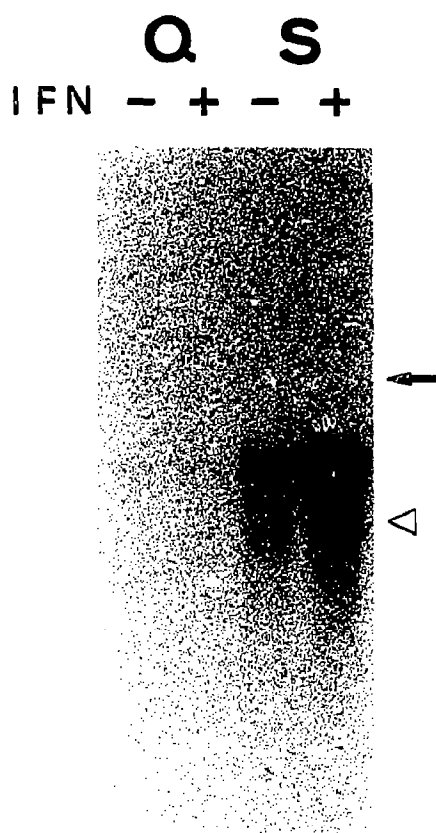


Fig. 4. Superinduction of ST2 mRNA by the treatment with murine β -interferon. Confluent BALB/c-3T3 cells were incubated without (-) or with 1,000 IU/ml of murine β -IFN and then stimulated to proliferate (S) or kept unchanged (Q) as described in the Materials and Methods. After 10 h, the total cellular RNAs were extracted and processed for Northern hybridization as described in Fig. 1. The arrow and open triangle correspond to the positions of 28 S and 18 S ribosomal RNA, respectively.

The fact that the expression of ST2 is not suppressed even in the presence of 50 μ g/ml of CHX suggested that the ST2 gene is a member of the primary response gene family [1]. Furthermore, the superinduction of JE, c-myc and ST2 in two different systems, including 10 μ g/ml of CHX or 1,000 IU/ml of β -IFN, also supports

this concept. The ST2 protein is highly similar to the extracellular portion of IL-1R1 and IL-1R2, and the ST2 locus is tightly linked to the IL-1R1 and IL-1R2 loci on chromosome 1 [4,5]. This suggests a close functional relationship amongst them. However, with respect to gene expression, our results are clearly distinct from the report that IL-1R gene expression is dependent on the expression of immediate-early genes [14].

Acknowledgements: We thank Dr. Tsunao Tetsuka for valuable discussions and Ms. Mayumi Masubuchi and Ms. Reiko Izawa for their excellent technical assistance. This work was supported in part by research grants from the Japanese Ministry of Education, Science and Culture.

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