Establishment of an apoptosis-suppressible, cell-cycle arrestable cell line and its application for enhancing protein production of serum-free or -supplemented culture

Yon Hui Kim¹, Atsushi Kitayama², Mareyuki Takahashi¹, Etsuo Niki¹ & Eiji Suzuki^{3*}

¹ Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan

² Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

³ Research Institute of Innovative Technology for the Earth, 9-2, Kizugawadai, Kizu-cho, Soraku-gun, Kyoto, 619-0292, Japan

(* Author for correspondence. E-mail: esuzuki@rite.or.jp)

Received 1 February 1999; accepted 20 September 1999

Key words: antisense, apoptosis, cell cycle, *c-jun*, protein production

Abstract

Expression of *c-jun* gene induces apoptosis of cells cultured in serum-free medium. It also promotes cell-cycling in serum-containing medium, leading cells to die by overgrowth. Previously, we established an apoptosis-suppressible, cell-cycle arrestable cell line, *c-jun* AS, by transfecting Friend murine erythroleukemia (F-MEL) cells with a dexamethasone-inducible antisense *c-jun* gene. Induction of the antisense *c-jun* transcription with dexamethasone suppressed *c-jun* expression. As a result, *c-jun* AS cells survived in serum-free medium containing dexamethasone for a long time, while F-MEL cells died quickly in the presence or absence of dexamethasone. In serum-containing medium, the growth of *c-jun* AS cells was viably blocked by inducing antisense *c-jun* transcription, and the cells survived at the non-growth state avoiding overgrowth. In the present study, protein productivity of *c-jun* AS cells was examined in comparison with that of wild type F-MEL cells. *C-jun* AS and F-MEL cells were further transfected with a vector for expressing alkaline phosphatase as a protein to be produced, and named *c-jun* AS-SEAP and F-MEL-SEAP cells, respectively. In the serum-free medium with dexamethasone, *c-jun* AS-SEAP cells produced the protein for up to 6 days, while F-MEL-SEAP cells stopped production on day 3 due to cell death caused by serum deprivation. In the serum-containing medium with dexamethasone, *c-jun* AS-SEAP cells were viably arrested in the cell cycle, and cell death due to overgrowth was avoided. As the result, they could produce the protein for up to 18 days, while F-MEL-SEAP cells stopped production within 7 days due to cell death caused by overgrowth.

Introduction

Establishment of apoptosis-resistant cell lines is expected to improve the productivity of mammalian cells in culture. For example, in the production of vaccine or viral vectors for genetic therapy, culture of the cells in medium free of serum components is desirable for avoiding biological contamination with virus or mycoplasma in serum. Serum deprivation from culture medium, however, often induces cells to die by ap-

optosis. Therefore, it would be worthwhile to engineer cells to be apoptosis-resistant in serum-free medium.

In non-proliferating viable cells, resources such as amino acids may be utilized more efficiently for production of the protein of interest than in proliferating cells, by being diverted from synthesis of cellular components to production of the protein of interest (Suzuki and Ollis, 1990; Takahashi et al., 1994). However, cells usually continue to grow consuming resources in culture medium until they start dying due to unfavor-

Figure 1. Growth and protein production of the apoptosis-suppressible, cell-cycle controllable *c-jun* AS-SEAP cells cultured under the normal condition. Logarithmically growing *c-jun* AS-SEAP cells and the other cells as controls cultured in the complete medium were collected, resuspended and cultured in each total volume of 10 ml of the fresh complete medium (10%FBS) in the absence of dexamethasone in 25-cm² T flasks. Viable cell count and the amount of produced phosphatase were determined following the procedure in the section 'Materials and methods'. (a) Viable cell count; (b) viability; (c) amount of the produced phosphatase in culture supernatant (shown as luminescence). ▲ wild type F-MEL cells; △ *c-jun* AS, F-MEL cells transfected with antisense *c-jun* gene; ■ F-MEL-SEAP, wild type F-MEL cells transfected with SEAP reporter system 2; □ *c-jun* AS-SEAP, *c-jun* AS cells transfected with SEAP reporter system 2.

able conditions at the overgrowth state. Depletion of nutrients or accumulation of toxic metabolites in the culture medium induces mainly apoptotic and partly necrotic cell death at the overgrowth state (Glacken et al., 1988; Toyama et al., 1990; Vomastek and Franek, 1993; Mercille and Massie, 1994; Singh et al., 1994; Franek and Chladkova-Sramkova, 1995). In other words, cultured cells usually reach overgrowth inevitably and, as a result, the cells die out. Thus, the arrest of proliferating cells before overgrowth should make it possible to keep the cells viable in the culture for a longer period, and as a result, protein production of the

cells could be continued longer. However, most of the reported methods for arresting proliferating cells, such as addition of growth-inhibitory reagents to medium and deprivation of some components from medium, often induce cell death by apoptosis. Therefore, development of a new method that can block cell growth without inducing apoptosis is desirable.

To establish apoptosis-resistant and cell-cycle controllable cell lines, we applied the antisense *c-jun* gene reported previously (Kim et al., 1998). Proto-oncogene *c-jun*, known as the 'immediate early response' gene, is rapidly induced in response to mi-

Figure 2. Serum-free protein production by the apoptosis suppressible, cell-cycle controllable *c-jun* AS-SEAP cells cultured under antisense *c-jun* inductive condition. Logarithmically growing *c-jun* AS-SEAP cells and the other cells as controls cultured in the complete medium were collected, resuspended and cultured in each total volume of 10 ml of the serum-free medium in the presence of 10^{-6} M dexamethasone in 25-cm^2 T flasks. Viable cell count and the amount of produced phosphatase were determined following the procedure in the section 'Materials and methods'. (a) Viable cell count; (b) viability; (c) amount of the produced phosphatase in culture supernatant (shown as luminescence). ▲ wild type F-MEL cells; △ *c-jun* AS, F-MEL cells transfected with antisense *c-jun* gene; ■ F-MEL-SEAP, wild type F-MEL cells transfected with SEAP reporter system 2; □ *c-jun* AS-SEAP, *c-jun* AS cells transfected with SEAP reporter system 2.

togenic stimuli, and it plays a critical regulatory role in the commitment of the cell to proliferate. Nuclear phosphoprotein c-Jun is a major component of the AP-1 trans-acting transcriptional activator complex that promotes the transcription of various genes required for progression of the cell cycle (Turner and Tjian, 1989). Selective inhibition of *c-jun* expression caused exit from the cell cycle; in other words, it blocked cell growth (Smith and Prochownik, 1992; Soprano et al.,

1992).

C-Jun has been reported to be a potential inducer for natural cell death, apoptosis (Colotta et al., 1992; Estus et al., 1994; Anderson et al., 1995; Rampalli and Zelenka, 1995). Interleukin (IL-6 and IL-2) - dependent mouse cell lines underwent a programmed cell death after growth factor deprivation; in this death process, the *c-jun* gene was rapidly induced, and antisense oligonucleotides directed against *c-jun* reduced

Figure 3a. DNA gel electrophoresis of the cells expressing or not expressing the antisense *c-jun* in serum-free or serum-supplemented culture. DNA was extracted from the cells and gel-electrophoresed following the procedure in the section 'Materials and methods'. (a) DNA ladder pattern of cells sampled on days 9 and 11 from the serum-free culture in the presence of dexamethasone. CS, *c-jun* AS-SEAP cells; FS, F-MEL-SEAP cells, M, molecular size marker. (b) DNA ladder pattern of *c-jun* AS-SEAP cells sampled from the serum-free culture in the absence or presence of dexamethasone. (c) DNA ladder pattern of the F-MEL and F-MEL-SEAP cells sampled from the serum-supplemented culture in the presence of dexamethasone. (d) DNA ladder pattern of the *c-jun* AS and *c-jun* AS-SEAP cells sampled from the serum-supplemented culture in the absence or presence of dexamethasone.

this *c-jun* induction while improving the survival of the cells after growth factor deprivation (Colotta et al., 1992). Antisense oligonucleotides for *c-jun* prevented HL-60 cells from apoptosis induced by ceramide (Sawai et al., 1995).

The above information on the function of c-Jun suggested that the inhibition of *c-jun* expression by itself could arrest cell cycling, while preventing cells from apoptosis. Previously, we established a cell line, *c-jun* AS, by transfecting F-MEL cells with an antisense *c-jun* gene connected downstream of the MMTV promoter. The antisense *c-jun* gene is inducible by dexamethasone (Kim et al., 1998). In Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), *c-jun* AS cells could survive to be quiescent up to 16 days. The non-proliferating

cells showed a high cell viability exceeding 85%, when cultured with 10^{-6} M dexamethasone, while wild type F-MEL cells died out after overgrowth. In serum-free DMEM, *c-jun* AS cells were able to be cultured for longer than 6 days with high cell viability exceeding 90% in the presence of 10^{-6} M dexamethasone, while F-MEL cells started quickly dying within 2 days in the presence or absence of dexamethasone. Thus, *c-jun* AS cell line is apoptosis-suppressive, cell-cycle controllable cell line.

Here, we evaluated the protein productivity of *c-jun* AS cells when they are cultured in medium free of serum components or cultured at the state of cell cycle arrest in the presence of serum. Alkaline phosphatase was chosen as an example of protein of interest because it is secreted from cells into culture medium,

Figure 3, continued..

and hence can be assayed easily by measuring luminescence of culture supernatant. The cells transfected with the antisense *c-jun* gene were further transfected with the alkaline phosphatase gene, and cultured to produce the protein.

Materials and Methods

Cell lines and culture conditions

Friend murine erythroleukemia (F-MEL) cell line was supplied from Riken Cell Bank. The apoptosis-suppressive, cell-cycle controllable cell line, *c-jun* AS was prepared as reported previously (Kim et al., 1998). Other cell lines were prepared as described below. All the cell lines were cultured in a complete medium, that is, DMEM (Nissui, Tokyo) supplemented with 10% (v/v) fetal bovine serum (FBS) (JRH Bioscience), 4 mM glutamine, 100 $\mu\text{g ml}^{-1}$ kanamycin, and 0.2% NaHCO_3 , unless otherwise specified. For serum-free culture, DMEM was supplemented with glutamine, kanamycin, and NaHCO_3 , but not with FBS, at the concentrations described above. The cells were maintained in the logarithmic phase of growth in 25-cm²

plastic T-culture flasks (Sumitomo Bakelite, Tokyo) at 37 °C in a 5% CO_2 -95% air humidified incubator. For induction of the antisense *c-jun* transcripts, cells were cultured in the complete or the serum-free medium supplemented with dexamethasone (Sigma) at a concentration of 1×10^{-6} M. Viable cells were counted by the method of trypan blue dye exclusion employing a hemocytometer.

Transfection and selection

For transfection, 1×10^6 cells of *c-jun* AS and F-MEL cell lines, respectively, were pelleted at 500 g for 10 min, washed twice in phosphate-buffered saline (PBS), and resuspended in 200 μl of DMEM. Twenty micrograms of pSEAP2-control vector (Clontech) plus five micrograms of pZeoSV vector (Invitrogen) were dissolved in a total volume of 50 μl of DMEM. The DNA mixture was mixed with 20 μl of Lipofectamine (GIBCO-BRL) and incubated at room temperature for 40 min. The cells suspended in the serum-free medium were incubated with the complex mixture for 24 h. Then the cells were transferred to the complete medium, and zeocin (Invitrogen) was added to the

Figure 4. Serum-free protein production by *c-jun* AS-SEAP cells cultured without induction of antisense *c-jun*. Logarithmically growing *c-jun* AS-SEAP cells and the other cells as controls cultured in the complete medium were collected, resuspended and cultured in each total volume of 10 ml of the serum-free medium in the absence of dexamethasone in 25-cm² T flasks. Viable cell count and the amount of produced phosphatase were determined following the procedure in the section 'Materials and methods'. (a) Viable cell count; (b) viability; (c) amount of the produced phosphatase in culture supernatant (shown as luminescence). ▲ wild type F-MEL cells; △ *c-jun* AS cells; ■ F-MEL-SEAP cells; □ Tc-*c-jun* AS-SEAP cells.

medium at a final concentration of 1 mg ml^{-1} for selection. Zeocin-resistant clones were detected within 4 to 8 days. Pooled zeocin-resistant clones were then cultured continuously in the complete medium with zeocin.

DNA fragmentation assay by electrophoresis

One million cells were pelleted and lysed by vortexing vigorously in 0.5 ml of TE buffer (10 mM Tris-Tris-HCl, pH 7.4, and 1 mM EDTA) containing 0.2% Triton X-100 (Sigma), and centrifuged for 10 min at 13 000 g at 4 °C. DNA was extracted after addition

of 0.1 ml of 5 M NaCl and 0.7 ml of isopropyl alcohol. The supernatant was vortexed, placed at -20 °C overnight, and then centrifuged at 13 000 g and 4 °C for 10 min. The DNA pellet was dissolved in TE buffer and treated with RNase prior to electrophoresis on a 2% agarose gel (Sigma). For visualizing fragmented DNA bands, the gel was stained with $1 \mu\text{g}$ of ethidium bromide (Sigma) per ml.

Measurement of alkaline phosphatase

For SEAP activity assay, 500 μl culture supernatant was sampled periodically during the culture period and

Figure 5. Protein production in serum-supplemented medium by the apoptosis suppressible, cell-cycle controllable *c-jun* AS-SEAP cells during cell-cycle blocking by the antisense *c-jun* induction. Logarithmically growing *c-jun* AS-SEAP cells and the other cells as controls cultured in the complete medium were collected, resuspended and cultured in each total volume of 10 ml of the complete medium (10% FBS) in the presence of 10^{-6} M dexamethasone in 25-cm² T flasks. Viable cell count and the amount of produced phosphatase were determined following the procedure in the section 'Materials and methods'. (a) Viable cell count; (b) viability; (c) amount of the produced phosphatase in culture supernatant (shown as luminescence). ▲ wild type F-MEL cells; △ *c-jun* AS, F-MEL cells transfected with antisense *c-jun* gene; ■ F-MEL-SEAP, wild type F-MEL cells transfected with SEAP reporter system 2; □ *c-jun* AS-SEAP, *c-jun* AS cells transfected with SEAP reporter system 2.

centrifuged at 12 000 g for 5 min to pellet any cells present in the culture supernatant. Then 450 μ l supernatant was collected and stored at -20°C until assay. Detection of phosphatase activity was performed following the protocol of Chemiluminescent SEAP assay kit (Great EscAPe™) with SEAP reporter system 2 (Clontech) using Luminescencer-JNR, AB-2100 (ATTO). The luminescence count was approximately proportional to the amount of phosphatase.

Results and discussion

Transfection of *c-jun* AS cells with alkaline phosphatase gene

Apoptosis-suppressible, cell-cycle controllable *c-jun* AS cells and wild type F-MEL cells were transfected with a reporter gene, SEAP2-control vector carrying a structural gene of secreted form of alkaline phosphatase as described in the section 'Materials and methods'. The reporter gene encodes a truncated

form of the placental enzyme that lacks a membrane-anchoring domain, thereby allowing the protein to be efficiently secreted from transfected cells. The transfected cells were selected with zeocin and then cultured in the complete medium. The obtained cell lines derived from *c-jun* AS and F-MEL cell lines were named *c-jun* AS-SEAP and F-MEL-SEAP, respectively. For comparison with the untransfected cell lines in a normal condition, the newly obtained cell lines, *c-jun* AS-SEAP and F-MEL-SEAP cells, as well as *c-jun* AS and F-MEL cells were cultured in the complete medium without dexamethasone for 11 days. The viable cell count was determined by the trypan blue exclusion method using a hemocytometer. Figures 1a and b, respectively, show the viable cell count and the viability of cultures of *c-jun* AS-SEAP, F-MEL-SEAP, *c-jun* AS, and F-MEL cells. There was no significant difference in the time course of changes in the viable cell count and viability among the cultures of these four cell lines. The cells grew to overgrowth by day 3, and then started dying. On days 2, 4, 7, 9, and 11 of the cultures of *c-jun* AS-SEAP and F-MEL-SEAP cells, respectively, 500 μ l samples of the culture supernatant were taken. The amount of the extracellularly secreted alkaline phosphatase was determined using a Chemiluminescent SEAP assay kit, (Great EscApe TM/Clontech). Figure 1c shows the time courses of the protein production by *c-jun* AS SEAP and F-MEL-SEAP cells. The transfection with the reporter gene followed by selection resulted in successful expression of the phosphatase. Both cell lines demonstrated a similar productivity of the protein. *C-jun* AS-SEAP and F-MEL-SEAP cells stopped production of the protein at day 7 of the cultures when the viability dropped below 75% owing to overgrowth.

Protein production in serum-free medium

Depriving a cell culture of serum components is a typical apoptosis-inducing condition. On the other hand, protein production in medium free of serum components is often desirable for medical use. Previously (Kim et al., 1998), we confirmed that *c-jun* AS cells were able to survive better than wild type F-MEL cells in serum-free culture. Therefore, we examined whether *c-jun* AS-SEAP cells can produce more protein in serum-free culture than F-MEL-SEAP cells when apoptosis is suppressed by inducing the antisense *c-jun* transcription.

Logarithmically growing *c-jun* AS and *c-jun* AS-SEAP cells in the complete medium were collected and washed three times in PBS, respectively. The cells

were then placed in the serum-free medium, and cultured in the presence of dexamethasone. F-MEL and F-MEL-SEAP cells were also subjected to the same procedure. The viable cell count and viability are shown in Figures 2a and b, respectively. In the serum-free medium, wild type F-MEL and F-MEL-SEAP cells started dying within 2 days, and the viability decreased to 25% within 4 days. By contrast, *c-jun* AS and *c-jun* AS-SEAP cells survived well in the serum-free medium, and the viability was maintained above 80% for 8 days in the presence of dexamethasone. In the serum-free medium, production of phosphatase by *c-jun* AS-SEAP cells continued up to 6 days in the presence of dexamethasone while that by F-MEL-SEAP cells stopped by day 3 of culture, when the viability dropped below 75% (Figure 2c). Thus surviving longer, *C-jun* AS-SEAP cells cultured with dexamethasone could produce about two-fold amount of the protein in the serum-free medium in comparison with F-MEL-SEAP cells.

We examined the emergence of the ladder pattern, a landmark of apoptotic cells, on electrophoresis of the DNA extracted from the cells of the above experiments. *C-jun* AS-SEAP and F-MEL-SEAP cells were collected from the cultures of the above experiments on days 2, 9, and 11. The DNA samples extracted from the cells were treated as described in the section 'Materials and methods' for detecting DNA fragmentation. On days 9 and 11 (Figure 3a), the ladder pattern is faint for *c-jun* AS-SEAP cells, while it is distinctive for F-MEL-SEAP cells. On day 2, the ladder pattern was undetectable for *c-jun* AS-SEAP cells, but detectable though faint for F-MEL-SEAP cells (data not shown). The ladder pattern indicated that *c-jun* AS-SEAP cells were prevented from apoptosis caused by serum deprivation, and as a result, they produced more protein than F-MEL-SEAP cells, whose apoptosis started within 2 days.

The protein production rate of *c-jun*-AS-SEAP cells became slower after day 3 as shown in Figure 2c, even though the cells were protected from cell death. A possible explanation of the slow protein production rate follows. A cell line closely related to F-MEL, from which the *c-jun* AS-SEAP cell line was derived, was arrested in a G0 like state by the antisense *c-jun* expression (Smith and Prochownik, 1992). In addition, cells arrested in the G0 phase produce protein at a rate slower than cycling cells. For example, 3Y1 cells produced an exogenous protein at a lower rate in the G0 phase, where they were arrested by contact inhibition, than in the logarithmic growth phase. The protein

production rate per 3Y1 cell gradually decreased to 20 and 10% of that in the logarithmic growth phase in 6 and 20 days, respectively, after the onset of the growth arrest (Takahashi, 1996). Therefore, we presumed that *c-jun* AS-SEAP cells were arrested in the G0 state, consequently decreasing the protein production rate.

We repeated the serum-free culture experiment described above, but in the absence of dexamethasone. The viable cell density and viability are shown in Figures 4a and b, respectively. In the absence of dexamethasone, *c-jun* AS-SEAP cells did not survive in the serum-free culture. Hence *c-jun* AS-SEAP cells as well as F-MEL-SEAP cells stopped producing phosphatase within 3 days of culture (Figure 4c). The DNA ladder pattern is detectable on day 3 of culture of *c-jun* AS-SEAP cells in the absence of dexamethasone while it is undetectable on day 4 in the presence of dexamethasone (Figure 3b).

Prolonged protein production by cell-cycle-arrested cells in serum-supplemented medium

Previously (Kim et al., 1998), we found that arrest of proliferating *c-jun* AS cells by inducing the antisense *c-jun* transcription with dexamethasone let the cells survive longer in culture. Here, we examined whether this blocking of cell growth can improve the protein productivity of cultured cells.

C-jun AS, *c-jun* AS-SEAP, F-MEL, and FMEL-SEAP cells in the logarithmic growth phase in the complete medium were collected and resuspended in 10 ml of the fresh complete medium in each of 25-cm² plastic T-flasks at day 0. Dexamethasone was added to the cultures at a concentration of 10⁻⁶ M on day 0 for inducing transcription of the antisense *c-jun*. Then the cultures were incubated in the normal condition. The viable cell count and viability are shown in Figures 5a and b, respectively. The growth of *c-jun* AS and *c-jun* AS-SEAP cells was almost blocked within two days after dexamethasone addition. Thereafter, the viable cell count was kept almost unchanged up to day 9. Viability of above 80% was maintained for 16 days when *c-jun* AS and *c-jun* AS-SEAP cells were cultured with dexamethasone (data after day 9 not shown). In the absence of dexamethasone, *c-jun* AS and *c-jun* AS-SEAP cells reached overgrowth on day 4, and the viability dropped to below 50% on day 9 as shown in Figures 1a and b. The *c-jun* expression level in *c-jun* AS cells cultured in the serum-containing medium was 48% lower in the presence of 10⁻⁶ M dexamethasone than in its absence, and 73% lower in its presence

than that in F-MEL cells in its absence when estimated from the relative density of *c-Jun* protein band on western blotting of the cell extract. The growth of F-MEL and F-MEL-SEAP cells could not be blocked with dexamethasone because they did not carry the dexamethasone-inducible antisense *c-jun* gene. Therefore, the cultures reached overgrowth by day 2 and the viability dropped below 10% within 9 days in the presence of dexamethasone (Figures 5a and b) and in the absence of dexamethasone as well (data not shown).

The secreted alkaline phosphatase in the supernatant of the cultures was assayed as described in the section 'Materials and methods'. Figure 5c shows the time courses of the phosphatase production, which is represented by luminescence count, by the *c-jun* AS-SEAP and F-MEL-SEAP cell cultures. *C-jun* AS-SEAP cells in viable non-growth phase could produce the functional secreted alkaline phosphatase continuously until day 16. F-MEL-SEAP cells stopped production on day 9 because of cell death due to overgrowth. The protein production rate decreased gradually during the non-growth viable phase, although the decrease was less remarkable than in the serum-free culture. Since blocking of growth of *c-jun* AS cells by the antisense *c-jun* was reversible (Kim et al., 1998), this decrease in protein production rate might have been avoided by replacing the culture medium with dexamethasone-free fresh medium intermittently for reactivating the cells.

We examined the ladder pattern on electrophoresis of DNA extracted from the cells in the above experiments. F-MEL and F-MEL-SEAP cells were collected on days 7 and 9, and *c-jun* AS and *c-jun* AS-SEAP cells on days 16 and 18. DNA samples extracted from the cells were treated as described in the Section 'Materials and Methods' for detecting DNA fragmentation. The obtained electrophoresis data are shown in Figures 3c and d. F-MEL and F-MEL-SEAP cells sampled on days 7 and 9 showed a clear ladder pattern. *C-jun* AS and *c-jun* AS-SEAP cells sampled on day 16 did not show a distinct ladder pattern, but those sampled on day 18 did. This indicated that in the presence of dexamethasone *c-jun* AS-SEAP cells were prevented from apoptosis caused by overgrowth until day 16.

Conclusions

Transfection with the antisense *c-jun* gene was a successful method for establishing apoptosis-

suppressible, cell-cycle-arrestable cell line, when it was applied to a Friend murine erythroleukemia cell line. The obtained apoptosis-suppressible, cell-cycle-arrestable cells are useful to produce protein free of serum components. Also they make possible prolonged protein production avoiding cell death due to overgrowth. Whether this method using the antisense *c-jun* gene can be applied to other cell lines awaits further studies.

Acknowledgements

We thank Dr. Edward V. Prochownik for the generous gift of the antisense *c-jun* gene.

References

- Anderson AJ, Pike CJ & Cotman CW (1995) Differential induction of immediate early gene proteins in cultured neurons by beta-amyloid (A beta): association of c-Jun with A beta-induced apoptosis. *J Neurochem* 65: 1487–1498.
- Colotta F, Polentarutti N, Sironi M, & Mantovani A (1992) Expression and involvement of c fos and c-jun protooncogenes in programmed cell death induced by growth factor deprivation in lymphoid cell lines. *J Biol Chem* 267: 18278–18283.
- Estus S, Zaks WJ, Freeman RS, Gruda M, Bravo R & Johnson Jr EM (1994) Altered gene expression in neurons during programmed cell death: identification of c-jun as necessary for neuronal apoptosis. *J Cell Biol* 127: 1717–1727.
- Franek F & Chladkova-Sramkova K (1995) Apoptosis and nutrition: Involvement of amino acid transport system in repression of hybridoma cell death. *Cytotechnology* 18: 113–117.
- Glacken MW, Adema E & Sinskey AJ (1988) Mathematical descriptions of hybridoma culture kinetics: I. initial metabolic rates. *Biotechnol Bioeng* 32: 491–506.
- Kim YH, Iida T, Fujita T, Terada S, Kitayama A, Ueda H, Prochownik EV & Suzuki E (1998) Establishment of an apoptosis-resistant and growth-controllable cell line by transfecting with inducible antisense *c-Jun* gene. *Biotechnol Bioeng* 58: 65–72.
- Mercille S & Massie B (1994) Induction of apoptosis in nutrient-deprived cultures of hybridoma and myeloma cells. *Biotechnol Bioeng* 44: 1140–1154.
- Rampalli AM & Zelenka PS (1995) Insulin regulates expression of c-fos and c-jun and suppresses apoptosis of lens epithelial cells. *Cell Growth Differ* 6: 945–953.
- Sawai H, Okazaki T, Yamamoto H, Okano H, Takeda Y, Tashima M, Sawada H, Okuma M, Ishikura H, Umehara H & Domae N (1995) Requirement of AP-1 for ceramide induced apoptosis in human leukemia HL-60 cells. *J Biol Chem* 270: 27326–27331.
- Singh RP, Al-Rubeai M, Gregory CD & Emery AN (1994) Cell death in bioreactors: role for apoptosis. *Biotechnol Bioeng* 44: 720–726.
- Smith MJ & Prochownik EV (1992) Inhibition of c-jun causes reversible proliferative arrest and withdrawal from the cell cycle. *Blood* 79: 2107–2115.
- Soprano KJ, Cosenza SC, Yumet G & Soprano DR (1992) Use of antisense oligomers to study the role of c-jun in G1 progression. *Ann N Y Acad Sci* 660: 231–239.
- Suzuki E & Ollis DF (1990) Enhanced antibody production at slowed growth rates: experimental demonstration and a simple structured model. *Biotechnol Prog* 6: 231–236.
- Takahashi K, Terada S, Ueda H, Makishima F & Suzuki E (1994) Growth rate suppression of cultured mammalian cells enhances protein productivity. *Cytotechnology* 15: 54–64.
- Takahashi K (1996) Methods of controlling growth rate of animal cells cultured for protein production. Ph.D. thesis, University of Tokyo, Tokyo, Japan.
- Tohyama N, Karasuyama H & Tada T (1990) Growth autonomy and tumorigenicity of interleukin-6-dependent B cells transfected with interleukin-6 cDNA. *J Exp Med* 171: 389–400.
- Turner R & Tijl R (1989) Leucine repeats and an adjacent DNA binding domain mediate the formation of functional cFos-cJun heterodimers. *Science* 243: 1689–1694.
- Vomastek T & Franek F (1993) Kinetics of development of spontaneous apoptosis in B cell hybridoma cultures. *Immunol Lett* 35: 19–24.