Journal of Cellular Biochemistry 84:349–358 (2002)

# Growth Factor-Mediated Altered Expression and Regulation of S-Adenosylmethionine Decarboxylase in a H-Ras Transformed Cell Line Capable of Malignant Progression

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**Abstract** Mammalian S-adenosylmethionine decarboxylase (SAMDC) is a regulatory activity, which is involved in the biosynthesis of polyamines. The polyamines, namely putrescine, spermidine, and spermine, are essential for mammalian cell proliferation. SAMDC expression was examined in a H-ras transformed cell capable of metastasis formation. Serum stimulation of these cells resulted in increased SAMDC mRNA and enzyme activity expression. The effect of several physiologically relevant growth factors on SAMDC expression was also determined. SAMDC mRNA expression was increased in response to epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) stimulation but was unaffected by transforming growth factor beta$_1$ (TGF-beta$_1$) and platelet derived growth factor (PDGF). Increased SAMDC enzyme activity occurred in response to exposure to EGF, bFGF, TGF-beta$_1$, and PDGF. The EGF and bFGF mediated alterations in SAMDC mRNA expression were apparently not due to alterations in the transcriptional apparatus but occurred partly through post-transcriptional mechanisms involving increased SAMDC message stability. EGF and bFGF were able both to cooperate with cycloheximide, an inhibitor of protein synthesis, to augment the expression of SAMDC mRNA. Furthermore, studies with NIH-3T3 fibroblasts transfected with either the normal basic fibroblast growth factor coding sequence that lacks a known secretory signal sequence or a chimeric bFGF sequence that targets the growth factor to the secretory pathway revealed that increased SAMDC expression occurred only in those cells which contained the chimeric bFGF sequence that targets the growth factor to the secretory pathway suggesting that the increase in expression of SAMDC occurs through an autocrine mechanism. Increased ornithine decarboxylase (ODC) expression was found to occur in both types of bFGF transfected cells suggesting that altered ODC expression in response to bFGF stimulation may occur through both autocrine and intracrine mechanisms. In addition, a correlation was found to exist between SAMDC expression and regulation in response to growth factor stimulation and malignant potential. This correlation supports the view that growth factor induced alterations in SAMDC expression, although not sufficient on their own to induce metastasis, are important in the promotion and establishment of events important to the phenotype expressed by H-ras transformed cells capable of malignant progression. J. Cell. Biochem. 84: 349–358, 2002. 

**Key words:** S-adenosylmethionine decarboxylase; H-ras transformation; growth factors; altered expression/regulation

Polyamines are critically important for mammalian cell proliferation [Pegg, 1988]. The two key regulatory and rate-limiting enzymes in the biosynthesis of polyamines are ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC). Mammalian S-adenosylmethionine decarboxylase (SAMDC) is a highly regulated enzyme whose levels can fluctuate depending upon the growth status and the intracellular polyamine levels of the cell [Pegg, 1988]. SAMDC catalyzes the formation of decarboxylated S-adenosylmethionine, which acts as the aminopropyl donor for the biosynthesis of the polyamines, spermidine, and spermine. This decarboxylation reaction is one of the key and rate limiting steps in polyamine biosynthesis. Additionally, this step also acts as a critical point for intracellular S-adenosyl-

Grant sponsor: Natural Sciences and Engineering Research Council of Canada (to R.A.R.H).

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Received 5 January 2001; Accepted 26 June 2001


DOI 10.1002/jcb.1301

methionine metabolism (SAM). SAM is the major methyl donor in cells and its decarboxylation commits it to the polyamine pathway.

The expression of SAMDC and ODC has been well documented to increase in cells which have been stimulated to proliferate [Pegg, 1988]. SAMDC activity can be induced in quiescent mammalian cells by several proliferative stimuli. These include stimuli such as hormones, tumour promoters, and growth factors [Sakai et al., 1980; Nawata et al., 1981; Macaione et al., 1984; Pegg and McCann, 1992; Soininen et al., 1996; Bielecki and Hurta, 2000].

Previously, we have investigated growth factor mediated expression and regulation of ODC in oncogene transformed cells [Hurta et al., 1993; Hurta and Wright, 1994; Hurta et al., 1996; Hurta, 1999]. The effect of a number of growth factors, including transforming growth factor beta$_1$ (TGF-beta$_1$), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and tumor necrosis factor alpha (TNF) on the expression of ODC in H-ras transformed cells has been examined [Hurta et al., 1993; Hurta and Wright, 1994; Hurta et al., 1996; Voskas et al., 1999, unpublished observations]. In order to ascertain the molecular events involved in the growth related regulation of SAMDC expression, this study investigated the effects of growth factors (which influence cell proliferation) on SAMDC expression in a unique H-ras transformed cell capable of malignant progression [Hurta et al., 1996].

## MATERIALS AND METHODS

### Cells and Growth Conditions

The C2 cell line used in this study was derived from 10T½ mouse fibroblasts following cotransfection of the plasmid, pAL8A, which encodes for T-24-H-ras, and the neomycin resistance gene [Egan et al., 1987; Hurta et al., 1996]. C2 cells are morphologically transformed, express high levels of ras and possess malignant characteristics [Egan et al., 1987; Hurta et al., 1996]. Cells were grown on 100-mm Nunc tissue culture plates (Life Technologies) in alpha minimal essential medium (Life Technologies) supplemented with antibiotics and 7% fetal bovine serum (Hyclone) as previously described [Hurta et al., 1996]. In experiments, investigating the effect of growth factors (TGF-beta$_1$, PDGF, bFGF, and EGF) (R&D Systems), a serum free medium which consisted of alpha minimal essential medium supplemented with 10 μg/ml transferrin (Sigma) and 5 μg/ml. insulin (Sigma) was used as previously described [Hurta et al., 1996].

### Northern Blot Analysis

Total cellular RNA was isolated by a rapid RNA isolation method using the TRIZOL reagent (Life Technologies) according to the manufacturer's instructions and then subjected to electrophoresis through 1% formaldehyde-agarose gels followed by transfer to Nytran nylon membranes. Blots were pre-hybridized and hybridized at 65°C using Rapid-Hyb (Amersham) according to manufacturer's instructions. Hybridization occurred in the presence of probe isolated from plasmid clone H2 which encodes for mouse SAMDC (provided by Dr. A.E.I. Pajunen, University of Oulu, Oulu, Finland). Loading of RNA samples was monitored using ethidium bromide stained ribosomal RNA bands prior to transfer of the gels or by monitoring glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression with a GAPDH specific insert which was isolated from the plasmid, pBssK-(provided by Dr. L. Penn, University of Toronto, Toronto, ON), or by monitoring 28S expression with a 28S ribosomal RNA probe isolated from the plasmid $pA_{BB}$ (Gonzalez et al., 1985). Densitometric analysis of appropriately exposed autoradiograms was performed using a GS700 Imaging Densitometer (Bio-Rad Laboratories) and the Molecular Analyst software program (Bio-Rad Laboratories). (Northern blot analysis data presented are representative of observations noted in at least duplicate determinations).

### Determination of S-Adenosylmethionine Decarboxylase and Ornithine Decarboxylase Enzyme Activity

Cell pellets were solubilized in ice-cold 50 mM Tris–HCl, pH 7.5, containing 0.1 mM EDTA, and 5 mM dithiothreitol, then sonicated briefly, and centrifuged at 14,000*g* for 20 min at 4°C to remove the insoluble fraction. The supernatants were assayed for SAMDC activity by measuring the liberation of [$^{14}$C]-$CO_2$ from S-[carboxyl-$^{14}$C] adenosyl-L-methionine essentially as previously described [Wang et al., 1992]. The buffer for the measurement of SAMDC activity contained 50 μM putrescine,

which was added to stabilize the SAMDC. Ornithine decarboxylase enzyme activity was measured as previously described [Hurta et al., 1996]. Radioactivity was determined by liquid scintillation spectroscopy using a LS6500 multi-purpose scintillation counter (Beckman). Enzyme activity was determined and expressed as nmoles $CO_2$/h/mg protein. Protein content was determined using the Biorad reagent (Bio-Rad).

## RESULTS

### Effect of Serum Supplementation on SAMDC Expression

C2 cells were grown to confluence and then placed on a serum free (defined medium) for 48 h. Following this, C2 cells were exposed to medium which contained 30% serum for pre-determined periods of time. SAMDC mRNA expression and SAMDC enzyme activity were then determined. As shown in Figure 1, the stimulation of cell growth in C2 cells was accompanied by changes in the expression of SAMDC mRNA levels. Following growth in medium containing 30% serum for 1, 3, 6, 12, 24, and 48 h, SAMDC mRNA levels increased (taking into consideration loading control levels) about 2.5-, 5.8-, 7.7-, 9.0-, 6.0-, and 3.1-fold, respectively (Fig. 1). Increased SAMDC enzyme activity also occurred in response to serum stimulation of C2 cells. As shown in Table I, SAMDC enzyme activity increased about 4.5-, 5.7-, 16-, 18.5-, 21.1-, and 1.3-fold following treatment of C2 cells with serum for 1, 3, 6, 12, 24, and 48 h, respectively.

### Effect of Serum Growth Factors on SAMDC Expression in C2 Cells

Since serum is known to contain a number of progression and competence determining growth factors, and since serum treatment of C2 cells affected the expression of SAMDC, and since the activity of SAMDC is induced by a number of factors related to cell growth, the effect of specific growth factors, namely, TGF-beta$_1$, PDGF, bFGF, and EGF, on SAMDC expression in C2 cells was determined. C2 cells were grown to confluence and then placed on a serum-free (defined) medium for 48 h. These C2 cells were then treated with TGF-beta$_1$ (10 ng/ml), PDGF (5 ng/ml), bFGF (10 ng/ml), and EGF (20 ng/ml) for 3 h. The expression of SAMDC mRNA levels and enzyme activity was then determined. As shown in Figure 2, EGF and bFGF were able to increase the expression of SAMDC mRNA levels in C2 cells. PDGF and TGF-beta$_1$ did not appreciably affect SAMDC mRNA expression levels. SAMDC mRNA expression levels were elevated by about 7.5- and 4.5-fold in C2 cells in response to EGF and to bFGF treatment, respectively. Interestingly,

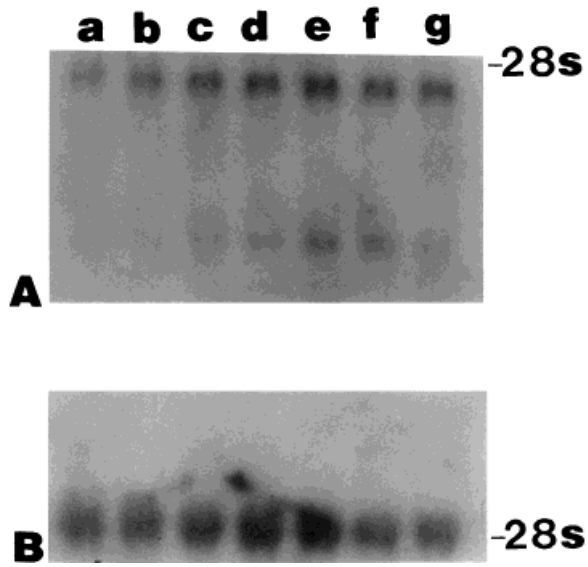


**Fig. 1.** Northern blot analysis of SAMDC expression in C2 cells following exposure to serum. C2 cells were grown to confluence and then placed on a defined medium for 48 h prior to exposure to fresh medium which contained 30% serum. **A:** SAMDC mRNA expression is shown in **(a)** control cells, no serum stimulation, and in cells re-exposed to medium containing 30% serum for 1 **(b)**, 3 **(c)**, 6 **(d)**, 12 **(e)**, 24 **(f)**, and 48 **(g)** h, respectively. **B:** 28S ribosomal RNA expression in shown as a loading control. The autoradiograms shown in A and in B were exposed at −70°C with intensifying screens for 24 h and for 1 h, respectively.

**TABLE I. S-Adenosylmethionine Decarboxylase Enzyme Activity in H-Ras Transformed Malignant C2 Cells Following Serum Stimulation***

| Exposure Time (h) | SAMDC Enzyme Activity[a] |
|---|---|
| C2 cells | |
| 0 | 0.51 ± 0.05 |
| 1 | 2.30 ± 0.12 |
| 3 | 2.91 ± 0.15 |
| 6 | 8.16 ± 0.99 |
| 12 | 9.44 ± 1.11 |
| 24 | 10.77 ± 1.22 |
| 48 | 0.68 ± 0.08 |

*Confluent C2 cells were placed on a defined medium for 48 h prior to exposure to medium supplemented with 30 % serum for pre-determined times.
[a]SAMDC enzyme activity is expressed as nmoles $CO_2$/h/mg protein. The results presented are from duplicate experiments.

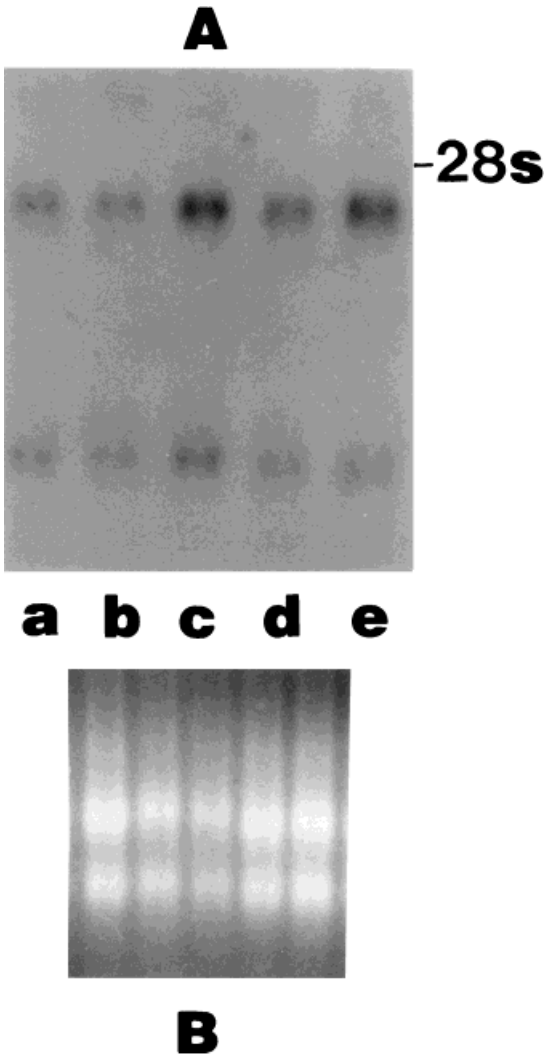


**Fig. 2.** Northern blot analysis of SAMDC mRNA levels in C2 cells. C2 cells were grown to confluence and then placed on a defined medium for 48 h, prior to exposure to growth factors. **A:** SAMDC mRNA levels in C2 cells (control cells) **(a)**, and in C2 cells exposed to TGF-beta$_1$ (10 ng/ml) **(b)**, to EGF (20 ng/ml) **(c)**, to PDGF (5 ng/ml) **(d)**, and to bFGF (10 ng/ml) **(e)**. Exposure time to growth factors was 3-h. **B:** Ethidium bromide stained ribosomal RNA bands are indicated as a loading control. The autoradiogram shown in A was exposed for 24 h at −70°C with intensifying screens.

all these growth factors were able to increase SAMDC enzyme activity levels in C2 cells. As shown in Table II, TGF-beta$_1$, PDGF, EGF, and bFGF increased SAMDC enzyme activity in C2 cells by about 5.6-, 5.5-, 20.6-, and 12.1-fold, respectively.

In order to further investigate the effect of growth factor-mediated effects on SAMDC expression, the expression of SAMDC (and ODC) was determined in NIH-3T3 fibroblasts transfected with either the normal basic fibroblast growth factor coding sequence that lacks a known secretory signal sequence (BNM46 cells) or a chimeric bFGF sequence fused to an immunoglobulin signal sequence that targets the growth factor to the secretory pathway (Ig60 cells) [Egan et al., 1990; Taylor et al., 1993]. BNM46 cells are non-metastatic whereas Ig60 cells are metastatic [Taylor et al., 1993]. As shown in Figure 3, SAMDC mRNA expression levels in Ig60 cells were increased about 9.0-fold over those found in A1 cells. (A1 cells are NIH-3T3 cells that contained vector only) [Taylor et al., 1993]. The level of SAMDC mRNA expression found in BNM46 cells was very similar to that found in A1 cells. Interestingly, ODC mRNA expression levels were elevated in both BNM46 and in Ig60 cells. As shown in Figure 3, ODC mRNA levels were increased about 3.3- and 25-fold in BNM 46 and in Ig60 cells, respectively (relative to the level of expression noted in A1 cells). Increased SAMDC enzyme activity was evident in Ig60 cells and increased ODC enzyme activity was noted in both BNM46 and in Ig60 cells (Table III). SAMDC enzyme activity was increased about 8.5-fold in Ig60 cells. ODC enzyme activity was increased about 2.6- and 11.3-fold in BNM46 and Ig60 cells, respectively.

**TABLE II. Effect of Growth Factors on S-Adenosylmethionine Decarboxylase Enzyme Activity in H-Ras Transformed C2 Cells***

| | SAMDC Enzyme Activity[a] |
|---|---|
| Control | $0.55 \pm 0.07$ |
| TGF-beta$_1$ | $3.08 \pm 0.09$ |
| PDGF | $3.00 \pm 0.15$ |
| EGF | $11.32 \pm 1.88$ |
| bFGF | $6.66 \pm 0.33$ |

*Confluent C2 cells were placed on a defined medium for 48 h prior to exposure to growth factors. The concentration of growth factors used was: TGF-beta$_1$ (10 ng/ml), PDGF (5 ng/ml), EGF (20 ng/ml), and bFGF (10 ng/ml). Exposure time was 3 h. Results presented are from duplicate experiments.
[a]SAMDC enzyme activity was determined and is expressed as nmoles $CO_2$/h/mg protein.

### Mechanisms Responsible for the EGF- and the bFGF Mediated Alterations in SAMDC Expression in C2 Cells

In order to acquire a better understanding of how EGF and bFGF were affecting SAMDC expression in C2 cells, studies were performed to determine aspects of the temporal mechanisms involved. The possibility that the elevations in SAMDC message expression observed

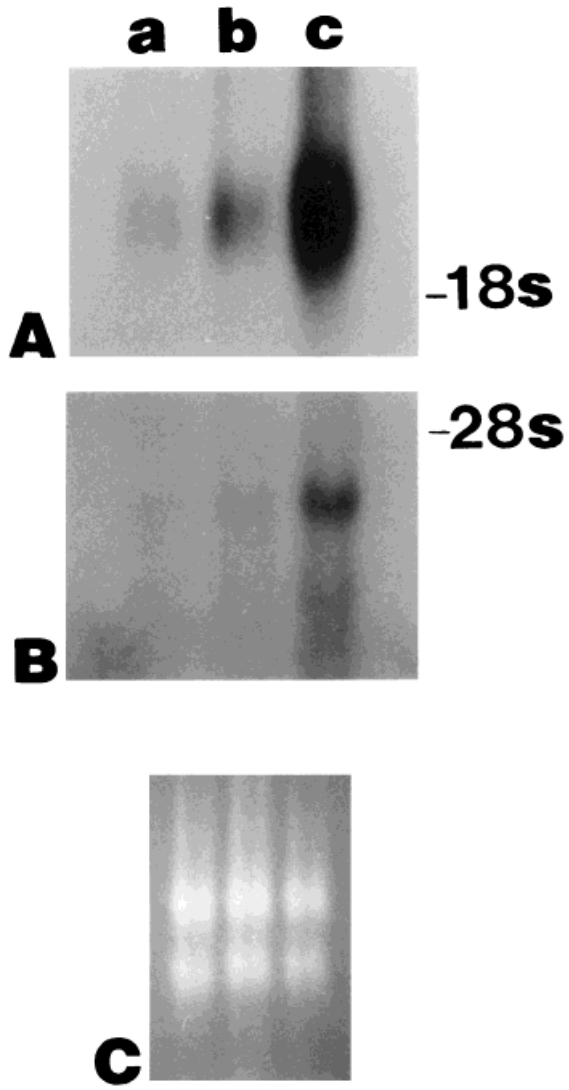


**Fig. 3.** ODC and SAMDC mRNA expression levels in bFGF over-expressing cells. **A:** Northern blot analysis of ODC mRNA levels in **(a)** A1 cells, **(b)** BNM 46 cells, and in **(c)** Ig60 cells. **B:** Northern blot analysis of SAMDC mRNA in cells as described in (A). **C:** Ethidium bromide stained ribosomal RNA bands are shown as a loading control. The autoradiograms shown in (A) and in (B) were exposed at −70°C for 24 and for 48 h, respectively.

to occur in malignant C2 cells following exposure to EGF and to bFGF were due, in part, to changes in the transcriptional apparatus was evaluated. This possibility was examined by pre-treating C2 cells with actinomycin D (5 μg/ml) [Phillips and Crothers, 1986; Hurta et al., 1996] prior to either EGF or to bFGF treatment. As shown in Figure 4, actinomycin D pre-treatment of C2 cells did not affect the ability of either EGF or bFGF to increase the levels of SAMDC mRNA expression in C2 cells.

**TABLE III. Ornithine Decarboxylase and S-Adenosylmethionine Decarboxylase Enzyme Activities in bFGF Over-Expressing Cells***

| Cell Line | ODC Enzyme Activity | SAMDC Enzyme Activity |
|---|---|---|
| A1 | 0.11 ± 0.05 | 0.13 ± 0.07 |
| BNM 46 | 0.28 ± 0.05 | 0.15 ± 0.04 |
| Ig60 | 1.24 ± 0.11 | 1.10 ± 0.09 |

*ODC and SAMDC enzyme activities were determined in exponentially growing control and bFGF over-expressing cells. Results are from duplicate experiments. ODC and SAMDC enzyme activity is expressed as nmoles $CO_2$/h/mg protein.

In the absence of actinomycin D, about 5- and 4.2-fold increases in SAMDC mRNA levels were noted in response to exposure to EGF and to bFGF, respectively. In the presence of this transcription blocker, similar increases in SAMDC mRNA levels in response to EGF and to bFGF were still evident (Fig. 4). Increases of about 5.2- and 4.5-fold in SAMDC mRNA levels were noted following exposure to EGF and to bFGF, respectively (Fig. 4). Inhibitors are commonly used to investigate questions regarding gene expression control at the level of transcription [Phillips and Crothers, 1986, Hurta et al., 1996 ] and although this approach is somewhat indirect, the findings obtained in this study would imply that neither the effects of EGF nor the effects of bFGF on SAMDC

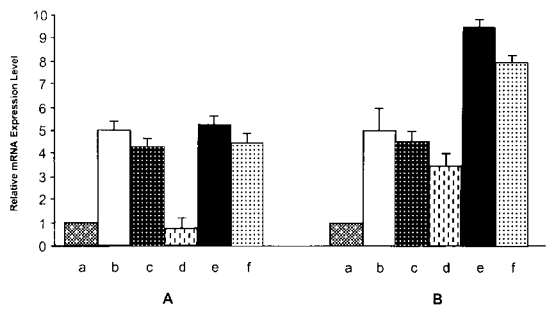


**Fig. 4.** SAMDC mRNA levels in C2 cells. **A:** The relative levels of SAMDC mRNA expression are indicated in C2 cells (control cells) **(a)**, in cells exposed to EGF (20 ng/ml) for 3 h **(b)**, in cells exposed to bFGF (10 ng/ml) for 3 h **(c)**, and in C2 cells exposed to actinomycin D (5 μg/ml) for l h **(d)**, in C2 cells exposed to actinomycin D for 1 h prior to exposure to EGF (20 ng/ml) for 3 h, **(e)** and in C2 cells exposed to actinomycin D for l h prior to exposure to bFGF (10 ng/ml) for 3 h **(f)**. **B:** The relative levels of SAMDC mRNA expression are indicated in C2 cells (control cells) **(a)**, and in C2 cells exposed to EGF (20 ng/ml) for 3 h **(b)**, and in C2 cells exposed to bFGF (10 ng/ml) for 3 h **(c)**, and in C2 cells exposed to cycloheximide (10 μg/ml) for 1 h **(d)**, and in C2 cells exposed to cycloheximide for 1 h prior to exposure to EGF (20 ng/ml) for 3 h **(e)**, and in C2 cells exposed to cycloheximide for 1 h prior to exposure to bFGF (10 ng/ml) for 3 h **(f)**.

mRNA expression involve changes in the transcription process.

To determine whether or not the EGF- and the bFGF-mediated elevations in SAMDC mRNA levels determined to occur in C2 cells required de novo protein synthesis, the effects of the protein synthesis inhibitor, cycloheximide [Edwards and Mahadevan, 1992] on the EGF- and on the bFGF-mediated alterations of SAMDC mRNA expression were determined. Figure 4 shows that cycloheximide (10 μg/ml) treatment alone markedly elevated SAMDC mRNA levels in C2 cells. A 3.5-fold increase in SAMDC message levels was found in C2 cells treated with cycloheximide. Figure 4 also illustrates that the elevation of SAMDC mRNA levels observed in the presence of cycloheximide can be further increased when C2 cells are exposed to cycloheximide and either EGF or bFGF together. Following exposure of C2 cells to cycloheximide and EGF together and to cycloheximide and bFGF together, a 9.6-fold elevation and a 8-fold elevation in SAMDC mRNA levels was found (Fig. 4). In the absence of cycloheximide treatment, a 5- and a 4.5-fold elevation in SAMDC mRNA levels were noted in C2 cells following exposure to EGF and to bFGF, respectively. These observations suggest that the expression of SAMDC message levels in the ras-transformed, highly malignant C2 cells is controlled in a positive manner by protein synthesis inhibition.

### Stability of S-Adenosylmethionine Decarboxylase Message in Malignant C2 Cells Following Exposure to either EGF or to bFGF

Since, neither EGF nor bFGF are able to influence SAMDC message through increased gene transcription, the possibility that the EGF- and bFGF-mediated elevations of SAMDC mRNA levels may occur via post-transcriptional mechanisms through alterations in message stability was investigated. Therefore, the rate of decay of the mature SAMDC message was evaluated in "untreated" (vehicle only) and "treated" (either EGF or bFGF) C2 cells after exposure to an inhibitor of transcriptional initiation, 5,6-dichloro-1-beta-D-ribofuranosyl-benzimidazole (DRB) [Mukherjee and Molloy, 1987, Hurta et al., 1996]. As shown in Figure 5, SAMDC mRNA in C2 cells is markedly more stable following exposure to either EGF or to bFGF. Assuming that the decay of SAMDC mRNA after blocking RNA synthesis follows

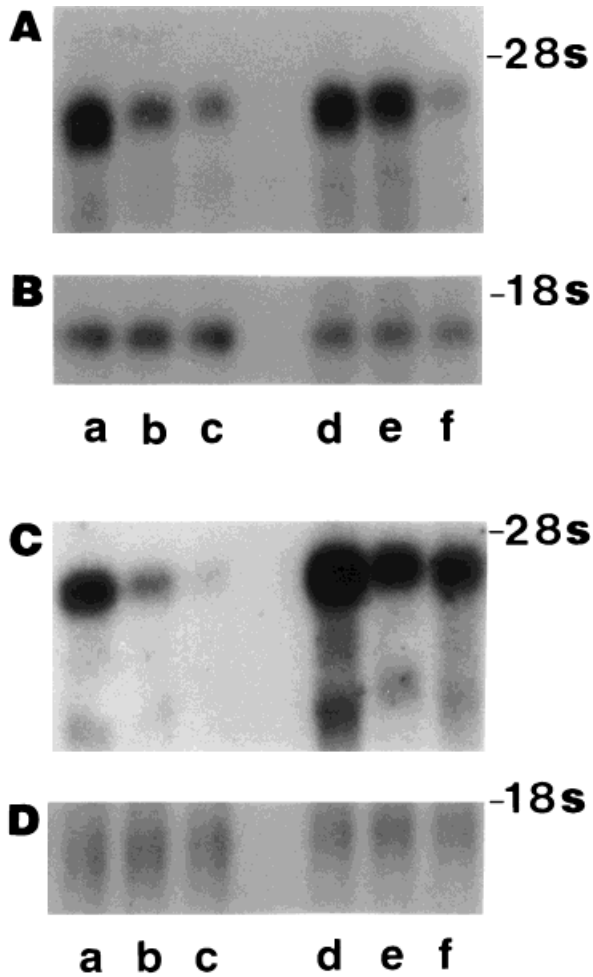


**Fig. 5.** Stability of the SAMDC mRNA transcript in C2 cells in response to bFGF and to EGF treatment. **A:** SAMDC mRNA levels in C2 cells in the absence **(a–c)** ("untreated cells") and in the presence of bFGF (10 ng/ml) **(d–f)** ("treated cells"). Exposure time to bFGF was 3 h. "Untreated cells" were exposed to vehicle alone for 3 h. After the 3 h of treatment, transcription was blocked by the addition of DRB (80 μM). Total RNA was isolated following exposure to DRB for 2 h (b, e) and 5 h (c, f), respectively, and subjected to Northern blot analysis as described and SAMDC mRNA expression levels examined. SAMDC mRNA levels in the absence of DRB and in the absence of bFGF are shown in (a) and in the absence of DRB and in the presence of bFGF for 3 h are shown in (d). **B:** GAPDH mRNA expression levels are shown as a loading control. **C:** SAMDC mRNA levels in C2 cells as described above but only in the presence of EGF (20 ng/ml). **D:** GAPDH mRNA expression levels are indicated as a loading control. The autoradiograms shown in A, B, C, and D were exposed for 24 h at −70°C with intensifying screens.

first order kinetics, the half-life of SAMDC mRNA in EGF-treated and in bFGF-treated C2 cells, estimated by extrapolation, was increased approximately 5.6- and 4-fold in EGF-treated and in bFGF-treated C2 cells over that found in C2 cells not treated with either

growth factor. The half-life of SAMDC message was estimated to about 2.5 h in "untreated" C2 cells (Fig. 5), whereas the half-life of SAMDC message was altered and increased to about 14 h and to about 10 h in EGF-treated and in bFGF-treated C2 cells, respectively (Fig. 5). These results suggest that both EGF and bFGF have the capacity to regulate SAMDC mRNA levels through mechanisms which involve post-transcriptional stabilization.

## DISCUSSION

Polyamines are believed to be essential for mammalian cell proliferation. Alterations in the expression and regulation of SAMDC, one of the key activities which is responsible for the regulation of cellular polyamine levels, is therefore of utmost importance in influencing cellular proliferation and cellular growth related events. SAMDC expression may be subject to a number of different regulatory phenomena, including transcriptional, post-transcriptional, translational, and post-translational events [Shirahata and Pegg, 1986; Kameji and Pegg, 1987; Pegg et al., 1987; Pajunen et al., 1988; Persson et al., 1989; Soininen et al., 1996]. Serum stimulation of C2 cells resulted in a marked increase in SAMDC mRNA expression and a marked accompanying increase in SAMDC enzyme activity levels. Increased mRNA expression was noted as early as 1 h post-stimulation with serum with maximal stimulation occurring at 6–12 h post-stimulation with serum. These observations are very similar to those seen with serum starved SV-3T3 cells which were then induced to grow by the addition of serum [Shantz et al., 1992]. Unlike what occurred in SV-3T3 cells, the levels of SAMDC mRNA expression in C2 cells were still elevated at 24 and 48 h post-stimulation with serum. Increased SAMDC enzyme activity also occurred in C2 cells in response to serum stimulation with increases in enzyme activity seen as earlier as 1 h post-stimulation with serum. Interestingly, this increased enzyme activity was maintained until 24 h later. The SAMDC enzyme activity levels seen at 48 h post-stimulation had almost returned to those levels seen in unstimulated cells. These results are also similar to those reported with SV-3T3 cells, wherein serum stimulation increased SAMDC enzyme activity 7- to 8-fold with a peak occurring 6 h after induction [Shantz et al., 1992]. In the SV-3T3 cells, the increases in SAMDC enzyme activity were not evident 24 h following induction and the SAMDC activity had almost returned to levels found in uninduced cells [Shantz et al., 1992]. Unlike what occurred in SV-3T3 cells, SAMDC enzyme activity in C2 cells remained elevated for a longer period of time and did not return to the approximate level seen in uninduced cells until 48 h. These differences may be due, in part, to the distinct cellular phenotypes associated with these cells.

The present study provides evidence of a link between alterations in growth factor responses during malignant conversion and the expression of SAMDC. This appears to be especially so in regards to the effects of bFGF and EGF on the regulation of SAMDC expression. Increased levels of SAMDC message were observed in malignant H-ras transformed C2 cells following exposure to bFGF and to EGF. These increases in SAMDC message levels in response to these growth factors occurred through changes at the post-transcriptional level. These observations suggest that both bFGF and EGF are capable of regulating SAMDC mRNA levels through mechanisms of post-transcriptional stabilization of the SAMDC message. These observations are similar to those previously observed in the bFGF-mediated regulation of ODC expression in malignant H-ras transformed cells [Hurta et al., 1996]. The post-transcriptional stabilization of both ODC and SAMDC in response to bFGF represents another aspect of an altered growth regulatory program associated with H-ras mediated cellular transformation, which has culminated in the formation of a malignant phenotype.

This study investigated the effects of a number of physiologically relevant growth factors on the expression of SAMDC. Unlike what occurred at the mRNA level, TGF-beta$_1$, PDGF, EGF, and bFGF were all able to increase the level of SAMDC enzyme activity. The extent of stimulation of SAMDC enzyme activity varied with the specific growth factor investigated. It is important to note that growth factor treatment of C2 cells results in the altered expression of a number of growth-related events including an enhancement of growth rate. An enhancement of growth rate in response to growth factor treatment would imply that increased SAMDC expression in response to growth factor stimulation is of biological

relevance. These observations with SAMDC are also in keeping with studies which indicate that TGF-beta$_1$, bFGF, EGF, and PDGF can all increase the expression of ODC by increasing ODC enzyme activity [Hurta et al., 1993; Hurta et al., 1996; Voskas et al., 1999; Hurta et al., 2001 unpublished observations]. These observations are also consistent with studies, which show a co-ordinate regulation of both ODC and SAMDC expression in malignant H-ras transformed cells in response to tumour promoters [Voskas et al., 2001]. The observation that PDGF and TGF beta$_1$ were both able to increase SAMDC enzyme activity in the absence of any readily apparent or substantial alterations in SAMDC message levels is intriguing. A possible explanation for this disparity is that treatment of these cells by PDGF and TGF-beta$_1$ results in enhanced SAMDC enzyme activity via translational or post-translational mechanisms. This may involve the synthesis of specific factors which are potentially involved in the stabilization of SAMDC enzyme protein, whose expression is initiated in response to treatment of these cells with either TGF-beta$_1$ or PDGF. Whether this does occur remains to be determined.

Fibroblast growth factors have many physiological roles in mammalian cellular biology. The contribution of fibroblast growth factors to tumour formation and malignancy may also depend on an ability to stimulate cellular proliferation, and in so doing, to modulate the expression and regulation of SAMDC (and ODC) expression. Therefore, this study also examined the expression of SAMDC and ODC in cell lines transfected with bFGF sequences. Studies have shown that such cells transfected with bFGF sequences exhibit a number of altered characteristics including alterations in cellular locomotion and cellular invasion rates and alterations in protease expression [Taylor et al., 1993; unpublished observations]. This study indicates that, in addition to these changes, alterations in the expression of both SAMDC and ODC occur. This study showed that increased SAMDC expression occurred in cells transfected with bFGF which contained a heterologous signal sequence for secretion. Interestingly, this study also indicated that cell lines transfected with bFGF sequences exhibited altered levels of expression of ODC and that these alterations were not dependent upon the presence of a heterologous signal sequence for secretion. These observations are consistent with previous studies which have shown that elevations in intracellular bFGF levels can modify the properties of cells [Huang and Huang, 1988; Bejcek et al., 1989; Taylor et al., 1993]. Therefore, this study supports models of cellular regulation wherein stimulation of cellular growth-related behaviours, in this case, polyamine biosynthesis, can occur through both autocrine (SAMDC and ODC) and intracrine (ODC) pathways [Taylor et al., 1993]. Additionally, these observations are consistent with other studies of growth stimulating factors which are known to be secreted from the cell [Huang and Huang, 1988; Bejcek et al., 1989]. These studies suggest that a part of the mechanisms being employed by cells may involve an interaction with intracellular components [Huang and Huang, 1988; Bejcek et al., 1989]. The intracrine pathways of fibroblast growth factor stimulation are not well understood. The mechanism of the intracrine stimulation of ODC is unknown and is subject to further investigation.

The stimulation of SAMDC expression in C2 cells in response to EGF is consistent with studies which have shown an enhancement of S-adenosylmethionine decarboxylase activity and the utilization of exogenous putrescine by colon cancer cells stimulated to grow by EGF [Milovic et al., 1998]. The response of cells to EGF appears to be very cell-type specific. In this regard, in studies with rat hepatoma cells, EGF did not significantly change the level of either SAMDC mRNA or SAMDC enzyme activity [Soininen et al., 1996].

The cycloheximide induced SAMDC mRNA expression observed in this study indicate that the SAMDC gene in malignant C2 cells can also be controlled in a positive manner by an inhibitor of protein synthesis. This observation is consistent with studies which have shown that S-adenosylmethionine decarboxylase expression in rat hepatoma cells can be regulated by protein synthesis inhibition [Soininen et al., 1996]. Interactions between bFGF and EGF and cycloheximide resulted in a further marked elevation of SAMDC mRNA expression. bFGF, in conjunction with cycloheximide, resulted in a marked elevation in the level of SAMDC mRNA expression which occurred in an approximately additive manner. This also occurred with EGF. This suggests that cycloheximide and either bFGF or EGF increase SAMDC message by two

separate although interactive mechanisms. These results also indicate the presence of a cycloheximide sensitive regulator of SAMDC mRNA expression in the highly malignant H-ras transformed C2 cells. These results are in agreement with previous studies which have shown that ODC expression is also regulated in response to protein synthesis inhibition alone and through cycloheximide and growth factor interactions [Olsen and Spizz, 1986; Hurta et al., 1993; Hurta and Wright, 1994; Hurta et al., 1996]. The exact nature of this regulation of SAMDC mRNA expression in malignant H-ras transformed C2 cells (and the nature of the specific factor(s) involved) remains to be further investigated. However, a part of the cycloheximide mediated regulation of SAMDC (and ODC) mRNA in these C2 cells involves mechanisms of post-transcriptional stabilization [Hurta, 2000].

In summary, this study has demonstrated the existence of a novel relationship between growth factor signaling and SAMDC expression in H-ras transformed cells capable of malignant progression.

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