Reprod Nutr Dev (1990) 30, 455-458

Research article

Regulation of *c-fos* expression in primary culture of guinea pig glandular epithelial cells stimulated by growth factors and estradiol

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(15th meeting of the INRA development group, Paris, 24–26 May 1989)

Summary — The *c-fos* expression was investigated in primary culture of guinea pig glandular epithelial cells. These cells were made quiescent by serum deprivation and stimulated with fetal calf serum (FCS, 15%), 17β-estradiol (E_2, 10^{-8} mol/l) alone or in combination with epidermal growth factor (EGF, 100 ng/ml) and insulin (I, 10 μg/ml). Low levels of *c-fos* mRNA were detectable in quiescent cells and were not increased in cells stimulated with either E_2, EGF, I, or EGF plus I. On the contrary, the *c-fos* mRNA were early and transiently increased by FCS or E_2 plus EGF plus I (4.5 and 9.5 fold induction, respectively). This effect was independent of *de novo* protein synthesis since it was not abolished in the presence of cycloheximide.

It appears that E_2 acts in a multiple step process including the stimulation by EGF plus insulin.

uterus / epithelial cell / proto-oncogene / growth factor / estradiol

Résumé — Expression de *c-fos* dans des cellules épithéliales glandulaires d'endomètre en culture primaire : rôle de l'œstradiol et des facteurs de croissance. *Nous avons recherché l'expression de* c-fos *dans des cultures primaires de cellules épithéliales glandulaires d'endomètre rendues quiescentes puis soumises à différents stimuli : sérum de veau fœtal (SVF 15%), 17ß-estradiol (E_2, 10^{-8} mol/l) seul ou en association avec le facteur épidermique de croissance (EGF, 100 ng/ml) et l'insuline (I, 10 μg/ml).*

Les ARN c-fos *étaient à peine détectables dans les cellules quiescentes et non par E_2, ou EGF, ou I ou EGF + I. Ils étaient augmentés précocément et transitoirement par SVF et par E_2 + EGF + I (induction 4,5 et 9,5 fois respectivement). Cette réponse était indépendante de la synthèse* de novo *de protéines puisqu'elle survenait même en présence de cycloheximide.*

*L'estradiol régule donc l'expression de c-*fos *quand il est inclus dans un processus multi-étapes faisant intervenir la stimulation par des facteurs de croissance.*

utérus / cellules épithéliale / proto-oncogène / facteur de croissance / estradiol

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INTRODUCTION

Proto-oncogenes are normal cellular genes whose alteration has been implicated in cancerous proliferation and transformation (Bishop, 1987). Recent evidence supports the hypothesis that these genes play key roles in the biochemical pathways controlling normal cell proliferation. One class of proto-oncogenes, including *c-myc* and *c-fos*, encodes nuclear proteins whose precise function is unknown. Numerous reports have established that the expression of these genes may be regulated by protein hormones and growth factors (Adamson, 1987). *In vivo* studies for hormone responsive cells have associated c-*fos* and c-*myc* expression with the response to growth factor or estrogen stimulation (Loose-Mitchell *et al,* 1988; Weisz and Bresciani, 1988). In the present study, we investigated the expression of *c-fos* gene in primary culture of uterine epithelial cells (from guinea pig) stimulated to grow.

MATERIAL AND METHODS

Cells and culture conditions

Epithelial cells were isolated and cultured as previously described (Chaminadas *et al,* 1986). In brief, after the appropriate growth time, the subconfluent cultures were made quiescent by serum deprivation for 60 h. Then, the G_0/G_1 synchronized cells were stimulated either with fresh fetal calf serum (FCS 15%) or with 1% DCC-FCS (dextran charcoal-treated fetal calf serum) plus epidermal growth factor (EGF, 100 ng/ml) and/or insulin (I, 10 μg/ml) and/or estradiol (E_2, 10^{-8} mol/l), with or without cycloheximide (chx, 10 μg/ml). The control cells were either submitted to 1% FCS (*vs* FCS 15% stimulated cells) or to 1% DCC-FCS (*vs* cells stimulated by 1% DCC-FCS plus a combination of E_2 and growth factors).

Preparation of RNA

Total RNA was isolated from stimulated and unstimulated control cells at different times, using the AGPC method (Chomczynski and Sacchi, 1987). Samples of total RNA (15 μg) from control and treated cells were denatured and slotted onto nylon filters. After baking (80 °C, 2 h), the filters were probed with *v-fos* obtained from Oncor (Gaithersburg, MD, USA) and labelled with ^{32}P-dCTP by nick translation.

Hybridization analysis

The filters were prehybridized and hybridized at 42 °C in the presence of 50% (v/v) formamide. After stringent washes, the filters were exposed to X-ray films. Autoradiographies were quantified by scanning densitometry.

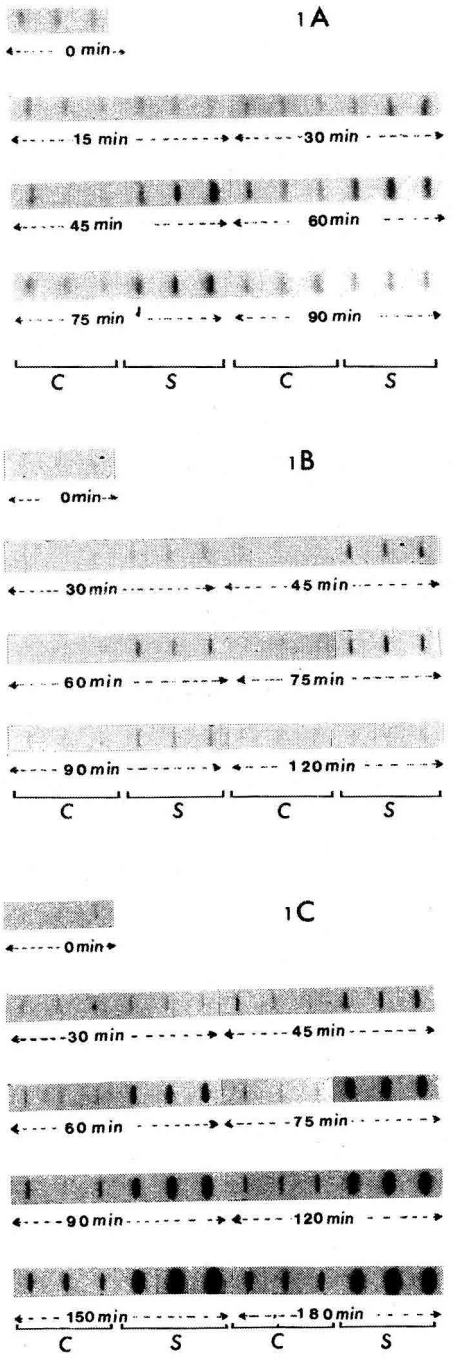
RESULTS

Effect of fetal calf serum on c-fos *mRNA concentration*

Quiescent synchronized epithelial cells were stimulated unspecifically by 15% FCS. To monitor *c-fos* mRNA levels, slot blot analysis was performed on RNAs isolated at various times after serum addition. The results obtained with *v-fos* DNA probe are shown in figure 1A as specific hybridization signals. Low levels of *c-fos* RNA were detectable in quiescent cells. A 4.5-fold increase in *c-fos* mRNA levels was achieved by the addition of 15% FCS. The *c-fos* mRNA levels increased within 30 min, and the expression returned to the basal level within 90 min. Such an induction could not be obtained with 1% FCS (control cells).

Effect of estradiol on c-fos *mRNA concentration*

We investigated the possibility that the increase of *c-fos* mRNA represents an



early response to the hormone stimulation, as well as to unspecific serum stimulation. Quiescent cells were treated with either 10^{-8} mol/l, E_2 1% DCC-FCS, 4 mmol/l L-glutamine and 20 mmol/l Hepes buffer E_2 or with 1% DCC-FCS, 4 mmol/l L-glutamine and 20 mmol/l Hepes buffer (control cells). The levels of *c-fos* mRNA were assessed immediately (time 0) and at different times after treatment.

Throughout 3 h, E_2 did not affect the *c-fos* mRNA level, compared to the level in the control cells (data not shown).

Effect of growth factors on c-fos *mRNA concentration*

No transient induction of *c-fos* gene was observed when EGF or I (in basal medium: 1% DCC-FCS, 4 mmol/l L-glutamine and 20 mmol/l Hepes buffer) was added to quiescent epithelial cells. Treatment by both growth factors did not have any further effect. In these 3 cases, the *c-fos* expression at different times (0-180 min) was the same as that observed for control cells in the presence of basal medium alone (data shown).

Fig 1. Gene expression during transition of epithelial cultured cells from quiescence. Hybridization signals between ^{32}P-*v-fos* DNA and total RNA (15 µg) extracted at indicated times following addition of: 1A) 1% FCS in basal medium plus insulin (1 µg/ml) for C (control cells); 15% FCS in basal medium plus insulin (1 µg/ml) for S (stimulated cells); 1B) 1% DCC-FCS in basal medium for C (control cells); 1% DCC-FCS in basal medium plus E_2 plus EGF plus I for S (stimulated cells); 1C) 1% DCC-FCS in basal medium plus Chx (10 µg/ml) for C (control cells); 1% DCC-FCS in basal medium plus Chx (10 µg/ml) plus E_2 plus EGF plus I for S (stimulated cells). Basal medium consisted of: phenol-red free RPMI 1640, 4 mmol/l L-glutamine and 20 mmol/l Hepes buffer.

Effect of growth factors and estradiol

The results are shown in figure 1B. The level of *c-fos* was significantly increased by EGF plus I plus E_2. The mRNA concentration peaked at 75 min and returned to the basal level within 1 h. Compared to the control, a 9.5-fold increase in *c-fos* mRNA levels was achieved by addition of growth factors plus E_2. As shown in figure 1C, when the stimulation (EGF plus I plus E_2) was performed in the presence of chx, the *c-fos* mRNA level was further increased and did not return to the basal level during the experimental period. The ratio to the control reached a maximum value of 23.8 at 120 min and decreased in the following hour. This fall in the ratio was due to the slight increase of the mRNA level in the control.

DISCUSSION

The type of RNA analysis used here measures steady-state level. Therefore, we are unable to distinguish whether the increase in the level of *c-fos* mRNA and its rapid disappearance are the results of a modulated rate of transcription only, or whether it also reflects modulation of *c-fos* mRNA stability. However, our results demonstrate that:

— *fos* mRNA level is increased in quiescent epithelial cells stimulated by FCS or growth factors plus E_2;

– this increase appears to be independent of *de novo* protein synthesis since it is not prevented by chx treatment;

– it cannot be observed in the presence of E_2 alone or EGF plus I.

Other results obtained in our laboratory suggest that the expression of *fos* gene in uterine epithelial cultured cells is not correlated to DNA synthesis, but correlated to cell proliferation.

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