dependent cell lines in the same fashion that it is regulated by IL-2 in IL-2–dependent T-cell lines. Thus, in both T cells and hematopoietic cells, IL-2 receptor expression is a component of very similar cellular responses to specific growth factor stimulation. These responses also include protein kinase C translocation, $[Ca^{2+}]_i$ flux, and increased expression of c-*fos*, c-*myc*, and c-*myb*. Our results suggest that the IL-2 receptor is important in regulating the growth of hematopoietic cells.

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Induction of the c-*fos* Oncogene by Thyrotropic Hormone in Rat Thyroid Cells in Culture

GIULIA COLLETTA, ANNA MARIA CIRAFICI, GIANCARLO VECCHIO

Rat thyroid cells in culture, rendered quiescent by hormone deprivation, can be stimulated to undergo DNA synthesis in the absence of serum by the addition of purified thyrotropin. The primary effect in response to thyrotropin action in thyroid cells is the induction of the c-*fos* oncogene, followed by c-*myc* expression. This suggests that thyrotropin acts as a competence growth factor.

SEVERAL PROTO-ONCOGENES HAVE been directly implicated in the control of cellular proliferation. Expression of oncogenes coding for nuclear proteins, such as c-*fos* and c-*myc*, is enhanced by stimulation by growth factors and may transduce signals that affect the cellular response to exogenous stimuli.

Moreover, three distinct proto-oncogenes (c-*sis*, c-*erb*B, and c-*fms*) seem to control critical events in the transduction of extracellular growth signals into the cell. Sequence comparisons suggest that c-*sis* codes for platelet-derived growth factor (PDGF) (*1, 2*) and that the c-*erb*B product is partially homologous to the epidermal growth factor (EGF) receptor (*3*). Finally, the mouse c-*fms* product, which is found in high concentrations in macrophages, is closely related to the colony-stimulating factor–1 receptor (*4*).

The c-*myc* oncogene is induced in resting cells of both hematopoietic and fibroblast origin, in response to mitogens and growth factors, and in regenerating liver tissue after partial hepatectomy (*5–7*). The transcription of c-*fos* is also greatly increased within minutes of the administration of purified growth factors to quiescent rat fibroblasts. This is followed by the induction of c-*myc* messenger RNA (mRNA) and the subsequent rapid disappearance of almost all c-*fos* mRNA (*8–10*). This suggests that c-*fos* induction is one of the earliest effects on gene expression triggered by growth factors.

We focused our analysis on the effect of a purified hormone, thyrotropin (TSH), on c-*myc* and c-*fos* expression in epithelial thyroid cells, which are the physiological target for the hormone in vivo. The rat thyroid cell line (FRT-L-5 cell clone) has the following differentiated functions: (i) TSH dependence for growth, (ii) thyroglobulin synthesis and secretion in the culture medium, and (iii) ability to take up iodide. FRT-L-5 cells grow in the presence of calf serum and require a mixture of six hormones (6H), which includes TSH (10 mU/ml), somatostatin (10 μg/ml), insulin (10 μg/ml), glycyl-L-hystidyl-L-lysine acetate (10 μg/ml), transferrin (5 μg/ml), and hydrocortisone ($10^{-8}M$). Under these conditions, the cells have a doubling time of 32 hours and display the fully differentiated phenotype that is typical of the original tissue (*11*). The hormone mixture, and in particular the TSH hormone, is an absolute requirement for FRT-L-5 cell growth. Without the 6H mixture, thyroid cells remain attached to the plate, develop morphological alterations, and do not divide (*12*).

In this system, depriving rat thyroid cells of the hormone mixture can make them quiescent even in the presence of 5% calf serum; only background levels of incorporated [^{3}H]thymidine were observed. However, to exclude any interference by serum proteins in the action of growth factors, we carried out experiments on synchronized thyroid cells in serum-free medium. Treatment of quiescent FRT-L-5 cells with 6H resulted in a 20- to 30-fold increase in the incorporation of [^{3}H]thymidine into trichloroacetic acid (TCA)–precipitable material that reached a peak 19 hours after stimulation (Fig. 1). These data demonstrate a synchronous progression of rat thy-

Dipartimento di Biologia e Patologia Cellulare e Molecolare, Centro di Endocrinologia ed Oncologia Sperimentale del Consiglio Nazionale delle Ricerche, II Facoltà di Medicina e Chirurgia, Via Sergio Pansini 5, Naples, Italy.

roid cells into the S phase of the cell cycle after the addition of hormones (Fig. 1).

Since several growth factors—EGF, PDGF, and fibroblast-derived growth factor—induce oncogene expression in fibroblasts (*8–10*), we have analyzed the expression of c-*fos* and c-*myc* in FRT-L-5 cells after stimulation by the 6H mixture. The level of expression of c-*fos* increased more than 30-fold 15 minutes after the addition of the 6H mixture. This rapid induction was followed by a rapid decrease; c-*fos* levels similar to those expressed by normal proliferating cells were reached within 2 hours (Figs. 1 and 2). The c-*fos* induction preceded the expression of c-*myc*, which resembled the pattern of induction obtained with fibroblasts stimulated by PDGF (*8*). The c-*myc*–specific mRNA appeared after 1 hour and the maximum induction (about tenfold) was observed after 150 minutes (Fig. 1). There was less induction of c-*myc* mRNA than of c-*fos*.

Since TSH seems to be the main factor responsible for the growth-promoting activity in the 6H mixture (*12*), we conducted similar experiments on quiescent rat thyroid cells by adding pure TSH (10 mU/ml), obtained from bovine pituitary glycoproteins (*12, 13*). When quiescent FRT-L-5 cells were treated with pure TSH, they incorporated thymidine at levels comparable to those obtained after stimulation with the total 6H mixture (Fig. 2A). In these conditions TSH seems to act on thyroid cells as a growth factor that makes the cells competent to undergo DNA synthesis, analogous to the effect of PDGF in the rat fibroblast system (*14*). We cannot, however, exclude an autocrine synthesis of progression factors, cooperating with TSH in the induction of the S phase.

We have also measured the levels of c-*fos* and c-*myc* after adding TSH alone. Induction of c-*fos* was observed 5 minutes after addition of pure TSH to thyroid cells (Fig. 2B). The additional RNA band present in the 6H-stimulated cells represents a c-*fos*–related gene r-*fos*; r-*fos* expression has been reported in PDGF-stimulated fibroblasts (*15*). Induction of c-*myc* did not occur until 1 hour after the c-*fos* peak (*16*). Cells treated with the hormone mixture in the absence of TSH (5H) do not replicate (*12*). A very small increase in c-*fos* mRNA has been found in quiescent rat thyroid cells treated with the 5H mixture (Fig. 2B). However, this analysis measured steady-state levels of RNA, so that we cannot exclude the possibility that the increase in c-*fos* is due to an increased half-life of this mRNA.

In contrast, no relevant differences in the level of thyroglobulin (TG) mRNA were observed in synchronized cells after the addition of 6H (Fig. 3B). TG mRNA is abundant in these cells, representing 4% of the total polyadenylated [poly(A)$^+$] RNA. We have analyzed TG mRNA levels in quiescent cells after growth factors are added. Total RNA was extracted at various times after the addition of 6H and hybridized with a rat TG genomic clone containing the first and second exon of the TG gene (*17*). The levels of the rat TG gene in uninduced cells resemble those present in hormone-stimulated ones. No detectable c-*fos* or c-*myc* RNA was present in proliferating cells stimulated over a long term by 6H (Figs. 2 and 3; control).

To determine whether protein synthesis was necessary to promote the c-*fos* or c-*myc* expression, we treated thyroid cells with cycloheximide, which inhibits protein synthesis by 90% (Fig. 3A). Stimulation of quiescent thyroid cells for 30 minutes or for

Fig. 1 (left). Effect of growth factors on DNA synthesis and induction of c-*fos* and c-*myc*. (A) FRT-L-5 cells were grown in Coon's modified Ham's F-12 medium supplemented with 5% calf serum and 6H in 2-cm plates. After 1 day, the medium was changed and cells were made quiescent by incubation in the presence of 1 ml of medium plus 0.25% bovine serum albumin for 3 days. For measurements of DNA synthesis, the cells were exposed to [^{3}H]thymidine (2.0 μCi/ml; Amersham, 40 Ci/mmol) for 2 hours at the times indicated. The stimulation index indicates the ratio of incorporation into TCA-precipitable material (*12*) for the stimulated culture relative to incorporation into unstimulated cells. Duplicate cultures were assayed for each time point. The amounts of c-*fos* and c-*myc* transcripts, expressed as induction relative to that observed in quiescent cells, were determined by quantitative densitometric scanning of autoradiographs (B and C). Total RNA was extracted in the presence of guanidinium hydrochloride as described (*26*) and chromatographed on oligodeoxythymidine cellulose. Polyadenylated RNA, 5 μg per lane, was fractionated on a 1% formaldehyde-agarose gel in morpholinopropanesulfonic acid (MOPS) buffer (200 m*M* MOPS, 50 m*M* sodium acetate, 10 m*M* EDTA) and transferred to nitrocellulose. Bound RNA was pretreated overnight with buffer containing 1% glycine, 50% formamide, 5× standard saline citrate (SSC), 50 m*M* NaH_2PO_4, *p*H 6.5; sonicated salmon sperm DNA (250 μg/ml); and 1× Denhardt's solution. The hybridization was carried out for 12 to 16 hours at 42°C, in the presence of 50% formamide, 2× SSC, 40 m*M* NaH_2PO_4 at *p*H 6.5, salmon sperm DNA (200 μg/ml), 10% dextran sulfate, 10 m*M* EDTA, 0.1% sodium dodecyl sulfate, with 5×10^6 count/min ^{32}P nick-translated purified probes (2×10^8 count/min per microgram of DNA). The oncogene-specific probes used were v-*fos* (*27*) (B) and mouse c-*myc* (*28*) (C). The size of oncogene transcripts were determined relative to 18*S* and 28*S* ribosomal RNA markers, which were assumed to be 1.8 and 4.5 kilobases, respectively.

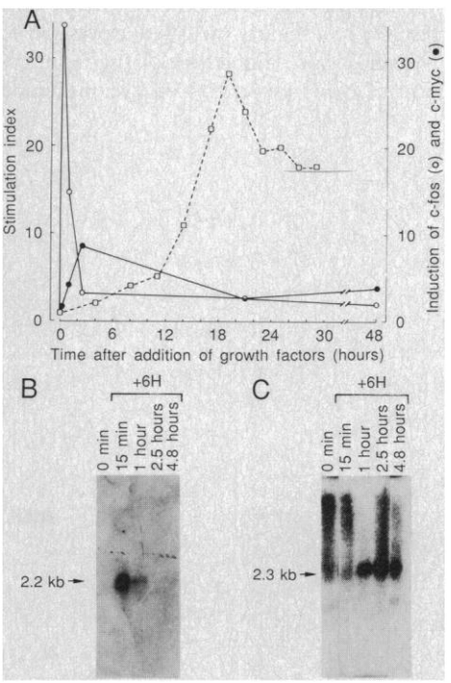
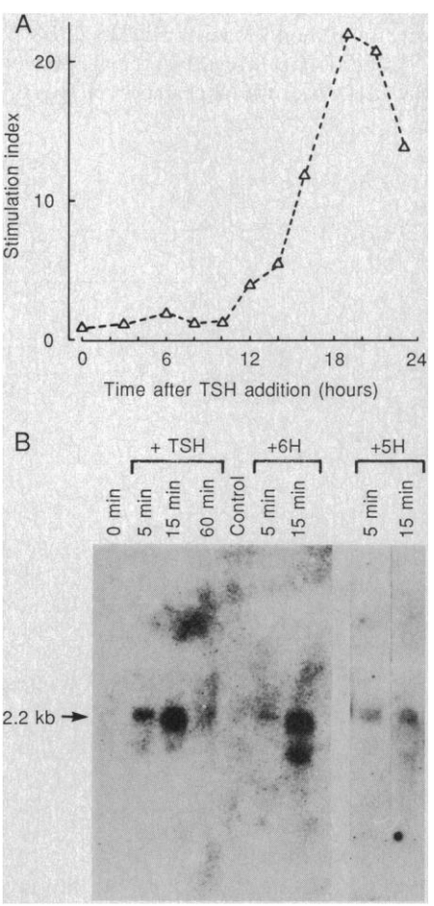


Fig. 2 (right). (A) Incorporation of [^{3}H]thymidine in quiescent FRT-L-5 cells treated with TSH (10 mU/ml). (B) Transcription of c-*fos* in rat thyroid cells at various times after the addition of pure TSH (10 mU/ml), 6H, and 6H without TSH (5H). Total RNA (20 μg per lane) was fractionated on a 1% formaldehyde-agarose gel, transferred to nitrocellulose, and hybridized to the oncogene-specific probes. The control shows RNA from cells that were actively proliferating in the continuous presence of 6H.



2 hours in the presence of cycloheximide led to the accumulation of c-*fos* and c-*myc* RNA to levels that were two to three times as high as the maximum observed in the absence of cycloheximide, as measured by densitometric scans of different blots (Fig. 3, C and D). The maximum increase in c-*fos* mRNA was observed in cells treated with cycloheximide and used 30 minutes after 6H was added. In contrast, no differences were observed in TG mRNA levels (Fig. 3B).

The differentiated functions of the thyroid gland are regulated in large measure by the action of TSH. This action consists of the regulation of the levels of thyroid hormones present in the bloodstream by promotion of thyroglobulin reabsorption from the thyroid follicle, the subsequent proteolytic degradation of TG, and the secretion of free thyroxine and triiodothyronine (*18*).

In addition, TSH can increase the number of dividing cells in thyroid tissue and can induce thymidine uptake in primary cultures of thyroid cells or in isolated thyroid follicles (*19*). For rat thyroid cells in culture, we have shown that TSH is necessary for normal growth and is able to stimulate quiescent cells to enter the S phase. TSH may act on a specific cell-surface receptor that has been partially characterized in FRT-L-5 cells by the use of monoclonal antibodies (*20*).

In fibroblasts arrested in the G_0 phase of the cell cycle, the appearance of the c-*fos* peak after the addition of PDGF has been correlated with the passage from G_0/G_1 to the S phase. However, EGF (which has been classified as a progression factor) can induce high levels of c-*fos* and c-*myc* oncogenes in A431 cells; the stimulation is independent of the growth state of the cells (*21*). In the rat thyroid cell system, the expression of c-*fos* and c-*myc* is closely related to the degree of cellular proliferation. Activation of these oncogenes may also be related to the availability of specific TSH receptors on thyroid cell membranes (*20*).

In addition to the proposed role of the c-*fos* oncogene in the control of cell proliferation, expression of this oncogene has also been reported to increase in pheochromocytoma cells and macrophages upon differentiation (*22–25*). In differentiated rat thyroid cells there was no correlation between the expression of the major product of these cells (TG), as measured by RNA levels, and the TSH induction of c-*fos* or c-*myc* oncogenes. No change of TG gene expression was observed after stimulation by the 6H mixture in synchronized cells. Moreover, the inhibition of protein synthesis by cycloheximide did not alter the high level of TG mRNA expression.

Cycloheximide treatment of thyroid cells resulted in a slightly increased expression of c-*fos* and c-*myc*. Higher levels of superinduction of c-*myc* expression by cycloheximide have been observed in lymphocytes and in fibroblasts after the addition of mitogens and in regenerating liver (*5–7*). Superinduction of c-*fos* has been demonstrated in fibroblasts after serum addition (*10*). Hence, although these two oncogenes could be modulated or repressed by an unstable protein in thyroid cells as well, the effect of blocking protein synthesis was less dramatic than has been observed in lymphocytes and fibroblasts.

In rat thyroid cells in culture the TSH hormone seems to behave as a competence growth factor. It stimulates the quiescent cells to undergo DNA synthesis and, by analogy to the PDGF on fibroblasts, induces c-*fos* and c-*myc* mRNA's.

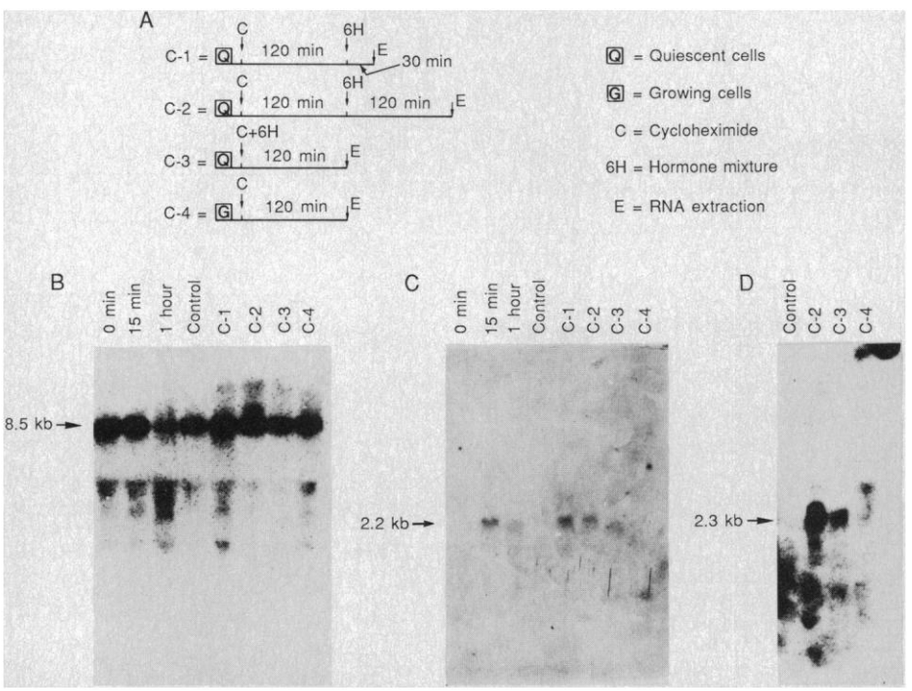


Fig. 3. (A) Cycloheximide treatment. Effects of cycloheximide on transcription of (B) TG gene, (C) c-*fos*, and (D) c-*myc*. Rat thyroid cells were treated with cycloheximide (10 μg/ml), as described in (A). Total RNA (20 μg per lane) was extracted at the indicated times after the addition of the 6H mixture and separated on a 1% agarose gel. Hybridization with the TG (*17*), v-*fos* (*27*), and c-*myc* (*28*) probes were performed as described in the legend to Fig. 1. The control is the same as described for Fig. 2.

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