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TSH is able to induce cell cycle-related gene expression in rat thyroid cell

Giulia Colletta and Anna Maria Cirafici

Istituto di Patologia Umana e Medicina Sociale, Facoltà di Medicina e Chirurgia, Chieti. Centro di Endocrinologia ed Oncologia Sperimentale del CNR, c/o Dipartimento di Biologia e Patologia Cellulare e Molecolare, II Facolta' di Medicina e Chirurgia, Via S. Pansini 5, 80121 Napoli,Italy

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Rat thyroid cells in culture (FRTL-5 strain) require thyrotropic hormone (TSH) for growth. TSH alone in serum free medium is able to induce DNA synthesis of FRTL-5 cells. DNA synthesis occurs 18-20 hours following TSH stimulation of quiescent cells. Here we demonstrate that two sets of genes, related to the entry of cells in the S phase, are induced by TSH: 1) immediate early genes, such as c-jun and a gene coding for a zinc-finger protein Xrox 20/Egr2, both having a pattern of expression similar to the c-fos oncogene; 2) early delayed genes such as ornithine decarboxylase (ODC), 2F-1, a gene that shows a strong similarity in aminoacid sequence to a mitochondrial ADP/ATP carrier, and the asparagine synthetase gene (TS11). Furthermore, an increased expression of the histone H3 gene, a typical marker of S phase, has been observed in TSH-treated FRTL-5 cells.

Several growth factors that can induce cells to divide have been identified and much effort has been concentrated on identifying the individual genes activated by these factors. Numerous genes are induced upon mitogenic stimulation of quiescent mammalian fibroblasts and cascade of gene activity from early to late times in the G1 phase has been delineated. Some of the late genes in this cascade probably have a role in the induction of DNA synthesis. To understand the biochemical events that control progression through the cell cycle in the system of epithelial thyroid cells, it is important to investigate the mechanisms that regulate the temporal expression of cell cycle regulated genes. Rat thyroid cells in culture can be rendered quiescent when grown in the absence of the thyrotropic hormone (TSH) (1). When quiescent cells are stimulated by TSH in serum free medium, they are able to enter the S phase (2). Two sets of

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genes related to cell growth have been described (3,4,5). The first consists of genes whose induction occurs immediately after the growth factors stimulus. These genes have been named immediate early genes; this set of genes is also defined by the superinduction of their expression in the presence of a growth factor plus a protein synthesis inhibitor. The second set of genes is induced as a secondary consequence of transit through the cell cycle: these genes have been named progression or early delayed genes. The abundance of progression mRNAs fluctuates in cycling cells, and their induction is blocked when growth factors are added together with protein synthesis inhibitors such as cyclohexemide (3,4). Genes from both groups have been analysed in this work, their expression has been monitored after TSH-stimulation of thyroid cells. Although many factors (i.e. insulin, IGF-I, EGF) (6,7) have been shown to modulate thyroid cell growth, we show that TSH alone, in a serum free system, is able to induce expression of both early immediate genes such as c-jun (8) and Xrox 20/Egr2 (9,10) that follow a c-fos like pattern of induction, and progression genes like ODC (11), 2F-1 (12), TS11 (13).

Materials and Methods

Cell culture

The FRTL-5 (1) rat thyroid cells are derived from 3 week old normal Fischer rats, passaged and grown in Coon's modified Ham's F-12 medium (W/O) supplemented with 5% heat inactivated, mycoplasma-free calf serum and a six hormone (6H) mixture containing the following: TSH, 1 x 10^{-10} M; insulin 10µg/ml; hydrocortisone 1 x 10^{-8} M; human transferrin 5 ug/ml; somatostatin 10 ng/ml; and glycyl-L-histidyl-L-lysine acetate 10 ng/ml.

DNA synthesis assay

DNA synthesis was measured by incorporation of ^{3}H-thymidine (1 µCi/ml, 40 Ci/mmol, Amersham, United Kindom) into trichloroacetic acid-insoluble material as described previously (2). Each point was assayed in duplicate. Autoradiography was performed on glass coverslips as described (14). At least 200 cells were counted per point.

RNA extraction and hybridization

RNA was purified from cultured cells by a modification of the guanidine hydrochloride extraction method. Total RNA, 10 ug/lane, was fractionated on a 1% formaldehyde agarose gel , transferred to nytran membranes (Schleicher and Schuell) and hybridized following standard procedure (2). ^{32}P nick-translated gel purified fragments of DNA (2 x 10^{8} cpm/ug) were used for hybridization. The probes used were: the human c-jun

cDNA (P-hcJ-1) (15), the mouse Xrox 20 cDNA (AC16 plasmid) (9,10), the second exon of the rat thyroglobulin gene (PTG1) (16), the human ODC cDNA (pODC934) (17), the human TS11 cDNA (pcD-ts11-5A) (18), the human 2-F1 cDNA (hp2F) (19) and the human H3 (pF0422) (20). The size of transcripts were indicated relative to 18S and 28S markers which were assumed to be 1.8 and 5.4 kilobases, respectively.

Results

TSH, Forskolin and TPA are able to stimulate the expression of c-jun and Xrox 20 genes.

Rat thyroid cells (FRTL-5 strain) become quiescent when grown for two days without the TSH containing hormone mixture in the presence of medium supplemented with 0.05% of BSA (bovine serum albumin)(2). Quiescent cells stimulated by 10 mU/ml of TSH can enter the S phase as demonstrated by ^{3}H-thymidine incorporation (fig.2 B), and by nuclear labeling (result not shown). This treatment of quiescent cells is able to increase the levels of expression of the c-jun gene, whose expression is detectable also in untreated quiescent cells, at variance with c-fos or Xrox 20 genes. Both transcripts 2.7 kb and 3.2 kb of the c-jun oncogene are present (Fig.1) (8). TSH stimulation also induces, to a lesser extent, the expression of the mRNA coding for the finger protein Xrox 20, (Fig.1). Both genes are maximally induced 40 minutes after TSH stimulation.

In thyroid cells induction of cell growth is mediated by the increase in cAMP produced by the action of TSH on its own receptor (21). Treatment of cells with 0.5 uM forskolin increases the intracellular levels of cAMP and induces cell growth (22). This treatment induces the expression of c-jun and Xrox 20 genes. Hovewer the highest increase in the expression of these genes is obtained by treating thyroid cells with the tumor promoter, TPA (100ng/ml), that acts through the activation of kinase C. Both TPA and forskolin treatment induce immediate early gene expression, with a kinetic similar to that described for TSH (Fig 1) with the maximal increase 40 minutes after drugs addition.

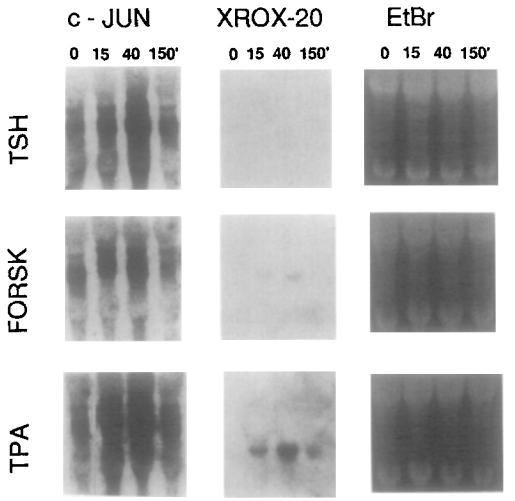

Figure 1. TSH, Forskolin and TPA induce c-jun and Xrox 20.
Total RNA was extracted from quiescent thyroid cells (0),, and from cells treated with 10 mU/ml of TSH, 0.5 μM of Forskolin, 100ng/ml of TPA, at the times indicated after stimulation. Northern blots were hybridized with the two probes c-jun and Xrox 20 as indicated.

TSH-treatment induces the expression of genes related with the cell entry into the S phase.

To follow the thyroid cells entry into the S phase, the expression of three different genes that require ongoing protein synthesis for their response to the growth factor, was analysed by northern blots. Rapid and dramatic induction of ODC activity in response to growth stimuli has been found in all cells and tissues in which it has been studied. Induction of the ODC gene expression has been demonstrated in fibroblasts after serum stimulation as well as in lymphocytes after PHA stimulation (11,23,24). In TSH-stimulated thyroid cells both ODC specific mRNA species were induced (2,7 kb and 2,2 kb), 4-6 hours after stimulation (Fig.2A and 2B). The asparagine synthetase gene, TS11, is also induced by TSH in this cell system. The TS11 gene has been isolated from temperature sensitive cell cycle mutants of a syrian hamster cell line (18). In thyroid cells its induction follows the same pattern shown in serum-stimulated fibroblasts. A clear increase in the mRNA levels (five fold) is demonstrated 4 hours after initiation of the treatment (Fig.2A and 2B). 6 hours after the TSH

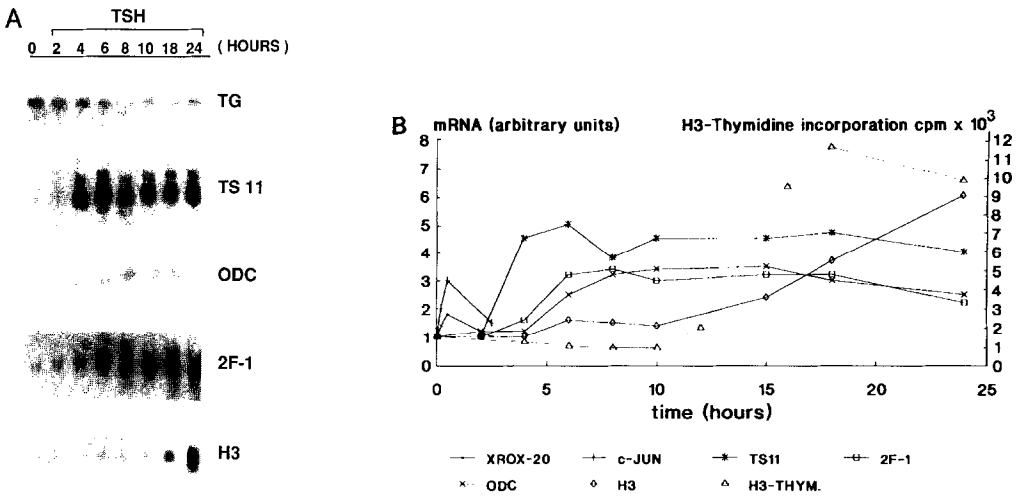

Figure 2 A. TSH induces expression of TG, TS11, ODC, 2F-1 and H3 genes.

Northern blots of total RNAs extracted from quiescent thyroid cell (0) and from cells treated with 10 mU/ml of TSH, harvested at the indicated times. The probes used for hybridization are indicated, TG: thyroglobulin probe, used as a control.

Figure 2 B. Kinetic of cell cyle-related gene expression after TSH stimulus.

Quiescent FRTL-5 cells have been stimulated with 10 mU/ml of TSH. Cells were pulse labeled with 1 µCi/ml of ^{3}H-thymidine for 1 hour at the indicated hours after TSH stimulation. The points shown represent the mean of duplicate determinations. Relative amounts of hybridized RNAs were determined by quantitative densitometric scanning of the autoradiograms, and the value expressed as the mean of more than one experiments.

stimulus an increase is observed also in the expression of the 2F-1 gene. The sequence of this gene is related to the ATP/ADP mitochondrial carrier protein and its expression is regulated by growth factors in fibroblasts (12), and increases also after treatment of B lymphocytes with goat-anti-mouse immunoglobulin antibody and citochalasin D (25) (Fig.2A and Fig.2B).

H3 histone gene expression, which is related to the cell entry into the S phase (26), increases, instead, after a longer interval from the TSH stimulus. This result is in agreement with the previous report demonstrating the induction of the H1 histone gene during the S phase, in FRTL-5 cells treated with the six hormone mixture (27). Fig. 2B shows the complete pattern of cell cycle-related genes induction after TSH treatment. The three different sets of genes sequentially induced are evidentiated : immediate early expressed in the early G1, progression genes expressed in late G1 and histone genes, like H3, expressed during S phase.

Discussion

In previous studies we have shown that TSH in a serum-free system is able to induce thyroid cell DNA synthesis, and, 30 minutes after stimulation, the levels of mRNA of two proto-oncogenes related to cell growth: c-fos and, c-myc were also shown to be increased (2,7). In this paper we have enlarged the spectrum of growth-related genes induced by TSH action. TSH alone in serum-free medium is able to induce 60% of the FRTL-5 nuclei to incorporate thymidine 24 hours after treatment. Although we cannot exclude the presence of endogenous growth factor(s) produced by thyroid cells in culture, that can cooperate with the added hormone (28), this treatment is sufficient for the induction of mRNAs coding for transcription factors. TSH induces the expression of genes coding for the classical AP-1 complex (c-fos and c-jun) and for a zinc finger protein, Xrox 20; these nuclear factors are probably involved, not only in the mitogenic stimulus, but also in the cascade of subsequent events regulated by TSH, such as thyroglobulin and thyroid peroxidase synthesis and iodide uptake (29,30). TSH acts on rat thyroid cell through a well characterized receptor, with a high degree of homology with the G-protein coupled receptors (21). This interaction induces high levels of intracytoplasmic cAMP, which mediate the mitogenic signal. Accordingly, forskolin treatment, that mimics TSH action in inducing high levels of cAMP, also increases c-jun, and Xrox 20 mRNAs levels with a pattern of induction similar to that shown by the c-fos oncogene. These results obtained in differentiated epithelial thyroid cells by hormonal stimulus are in agreement with results obtained in other systems where stimulation of proliferation is accompanied by an induction of c-jun, but contrast with data obtained in dog primary thyrocytes where, instead, c-jun induction is inhibited by high level of cytoplasmic cAMP (31).

Activation of protein kinase C pathway by Tumor promoter treatment is less mitogenic in FRTL-5 cells; in spite of that TPA induces both genes. And the levels of induction of the Xrox 20 gene obtained with TPA are

higher with respect to the induction obtained with TSH alone or with forskolin. This is probably due to the strong effect of the TRE elements in the Xrox 20 gene promoter (9).

The TSH mediated entry into the S phase is preceeded by the induction of elevated levels of mRNAs coding for progression genes such as ODC , TS11, and 2F-1. This genes expression increases between 4-6 hours from the stimulus, and remains stable during the S phase. In analogy with other systems where these genes have been studied, we suspect that this induction is at the transcriptional levels. However we cannot exclude at present, that the induction is mediated by different mechanisms.

In conclusion , a sequential gene activation that occurs during cell cycle progression induced by TSH has been shown in the FRTL-5 cells, demonstrating that this is a suitable system for subsequent cell cycle studies.

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