Insulin and TSH promote growth in size of PC Cl3 rat thyroid cells, possibly via a pathway different from DNA synthesis: comparison with FRTL-5 cells

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Abstract

In the rat thyroid cell lines PC Cl3, FRTL-5 and WRT, proliferation is mainly regulated by insulin or IGF, and TSH. However, the mechanism regulating cell mass doubling prior to division is still unknown. Our laboratory has shown that in dog thyroid cells insulin promotes growth in size while TSH in the presence of insulin triggers DNA replication. In the absence of insulin, TSH has no effect on cell growth. In this report we investigated insulin action on both cell mass and DNA synthesis and its modulation by TSH and insulin in PC Cl3 and FRTL-5 cells. In PC Cl3 cells, insulin activated not only DNA synthesis but also protein synthesis and accumulation. Although TSH potentiated the stimulation of DNA synthesis induced by insulin, enhancement of protein synthesis by both agents was additive. All TSH effects were reproduced by forskolin. Similar effects were also obtained in FRTL-5 cells. This suggests that insulin and TSH, via cAMP, modulate both growth in size and DNA replication in these cell lines.

Lovastatin, which blocks 3-hydroxy-3-methylglutaryl coenzyme A reductase, decreased the induction of DNA synthesis, but not of protein synthesis induced by insulin or TSH in PC Cl3 cells. In FRTL-5 cells, lovastatin reduced protein and DNA synthesis stimulated by insulin but not TSH-induced protein synthesis. Taking these data together, we propose that insulin and/or TSH both modulate cell mass doubling and DNA synthesis in these cell lines, presumably via different pathways, and that there are at least two pathways which regulate growth in size in FRTL-5 thyroid cells: one triggered by insulin, which is lovastatin sensitive, and the other activated by TSH, which is not sensitive to lovastatin.

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Introduction

Both growth in size and DNA replication are necessary for repetitive cell division to maintain the size that is characteristic of the cell type. This rule does not necessarily apply to any one cell division. Moreover, in some instances, cell mass doubling occurs without DNA synthesis. Thus DNA replication is not necessarily triggered by the doubling of cell size (1). Rat thyroid cell lines, particularly FRTL-5, are widely used to study proliferation. As reported previously this cell line was derived from the thyroid of 3- to 4-week-old rats (2), whereas the other cell line investigated in the current study, PC Cl3, was derived from 18-month-old rat thyroid (3). While numerous reports show that in FRTL-5, and to a lesser extent in PC Cl3 rat thyroid cells, proliferation is regulated mainly by insulin or insulin-like growth factor (IGF) and thyrotropin (TSH) (2–6) the regulatory mechanisms of cell mass doubling are still unclear. Recently our laboratory has clearly shown that in primary cultures of dog thyroid cells, insulin promotes protein accumulation, while TSH, in the presence of insulin, triggers DNA synthesis (7). TSH per se has no effect on cell size. Therefore we have studied the relative roles of insulin and TSH in the regulation of growth in size and DNA synthesis in PC Cl3 and FRTL-5 rat thyroid cells to test whether these cell lines could be used to study the relationship between cell size and DNA synthesis. Moreover, we wanted to establish the repertoire of growth factors acting on PC Cl3 cells.

In rat thyroid cell lines, inhibitors of the Ras pathway (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, or microinjection of Ras dominant negative mutant), decrease IGF-I-stimulated (8) or TSH-stimulated (9, 10) DNA synthesis. An HMG-CoA reductase inhibitor prevents Ras activation; however, it also inhibits other pathways which require mevalonate. The group of Meinkoth has proposed that, in WRT cells, TSH promotes DNA synthesis via both cAMP and a Ras pathway which does not require Raf-1 or MEK (11). Nevertheless, no direct evidence of Ras activation by TSH was shown. On the other hand, in FRTL-5 cells,
TSH, via cAMP, regulates cholesterol biosynthesis which is upstream of Ras farnesylation (12, 13). This could suggest that the TSH stimulation of the mevalonate biosynthetic pathway could be required for the TSH-induced proliferation of these cells. To test this hypothesis, we have investigated the effect of lovastatin, an HMG-CoA reductase inhibitor (14, 15), on protein or DNA synthesis induced by insulin or TSH.

**Materials and methods**

**Cell culture and agents**

PC Cl3 cells, developed as previously described (3), and FRTL-5 cells (a gift from Dr R Di Lauro, Stazione Zoologica Anton Dohrn, Napoli, Italy) were cultured in Coon’s modified Ham F12 Medium (Sigma Chemical Co., Milan, Italy) supplemented with 5% calf serum (Gibco Laboratories, Paisley, UK), transferrin (5 μg/ml), insulin (5 μg/ml) and bovine TSH (1 mU/ml) (Sigma) (3H-medium). Culture and subculture techniques were similar to those originally described (16). All cells were maintained in a water-saturated incubator at 37°C in an atmosphere of 5% CO₂, 95% air. Before each experiment, cells from donor culture dishes were harvested by treatment with 0.1% trypsin, 1 mmol/l EDTA solution and plated onto 35 mm plates. The cells were usually subcultured for 3 or 4 days in 3H-medium to reach 2.5 x 10⁴ cells/cm² density, then washed with prewarmed basal medium eagle (BME) medium (Gibco) three times and switched to medium containing transferrin (5 μg/ml) (basal medium) with or without 0.5% calf serum. After 3 days, cells were washed with prewarmed BME medium three times and then treated with test substances. For all the measurements, cells were incubated for 48 h with the test substance, except for protein synthesis experiments in which the incubation lasted 6 h.

DNA synthesis or, more precisely, the fraction of cells entering into DNA synthesis, was estimated by the frequency of [³H]thymidine-labeled nuclei, as estimated by autoradiography. Cells in Petri dishes were incubated for 48 h in the medium containing the indicated agent and, during the last 24 h, were incubated with 3 × 10⁻³ mol/l thymidine, 10⁻⁶ mol/l deoxyctydine (DOC), and 10 μCi/l [³H]thymidine. After removal of the medium, the cells were fixed with methanol and extensively washed (17). Autoradiography was performed as described previously (17), directly in the Petri dishes. The nuclei were stained with toluidine blue, and the proportion of labeled nuclei was determined by counting at least 1000 nuclei in each dish.

For the measurement of cell protein content, the cells were rapidly rinsed four times with prewarmed PBS without Ca²⁺ or Mg²⁺ at pH 7.5. The cells were lysed with 0.4 mol/l NaOH and 5% SDS (final concentrations). Cell protein content was measured using a simplified version of the protein method of Lowry (18).

**Measurement of cell DNA content**

Cells were rapidly rinsed three times with PBS without Ca²⁺ or Mg²⁺ at pH 7.5 prewarmed at 37°C, then treated with pronase for 30 min at 4°C, collected and digested by ribonuclease and pronase. The cell DNA assay was a fluorimetric method using the increase in fluorescence of ethidium bromide when complexed with nucleic acid (19).

**Measurement of [³⁵S]methionine incorporation into proteins**

Cells were incubated for 3 days in basal medium, then washed with prewarmed BME medium three times and [³⁵S]methionine (1000 Ci/mmol, Amersham International, Amersham, Bucks, UK) and test agent were added simultaneously. Methionine (1 mmol/l) was added together with [³⁵S]methionine (20 μCi/ml) to overfill intracellular amino acid pools and avoid artifacts of dilution (20). After 6 h, cells were lysed with 100 μl of 0.4 mol/l NaOH and 5% SDS (final concentrations). Proteins of a 5 μl fraction of the lysates were extracted by the method of Siekevitz (21), allowing the removal of nucleic acid and lipids. The protein precipitate was dissolved in Soluene 100 (Packard, Meriden, CT) and its radioactivity was measured in a liquid scintillation counter (Packard).

**Reagents**

Lovastatin was a kind gift from Merck (Darmstadt, Germany). Hepatocyte growth factor (HGF) was a kind gift from Dr T Nakamura, Osaka University Medical School, Osaka, Japan. Epidermal growth factor (EGF), phorbol myristate ester (TPA), ribonuclease and mevalonic acid lactone were from Sigma. Forskolin and pronase were from Calbiochem (San Diego, CA).

**Statistical analysis and data presentation**

All experiments were performed in duplicate, at least three times. Results are means ± s.e.m of three separate experiments. Determination of the significance of differences between mean values was by ANOVA or paired t-test.

**Results**

**Insulin activated cell proliferation and the effect was potentiated by TSH**

Figure 1 shows that TSH and forskolin by themselves moderately increased the [³H]thymidine labeling index in PC Cl3 cells, as did serum alone. Insulin alone induced a more pronounced effect. EGF as well as HGF had no effect in the absence of insulin; in the presence of insulin an increase of the same magnitude as the effect induced by insulin alone was detected. While TPA per se
increased nuclei labeling, in the presence of insulin the magnitude of the effect appeared slightly less than the theoretical sum of individual effects. By contrast, when insulin was added together with TSH or forskolin, a strong potentiation of nuclear labeling was observed, the magnitude of the effect being comparable to that when cells were incubated in 5% calf serum-containing 3H-medium. This means that co-stimulation by TSH and insulin induced full activation of proliferation. Fig. 2 shows that in PC Cl3 cells insulin enhanced nuclear labeling in a concentration-dependent manner and the effect was potentiated by TSH at and above a concentration of 50 ng/ml insulin.

In FRTL-5 cells, insulin also activated DNA synthesis and the effect was synergistically potentiated by TSH and forskolin (Fig. 3); serum alone (5%) induced an increase of about the same magnitude. TSH and forskolin, EGF and HGF all moderately increased nuclear labeling; these effects were potentiated by insulin. The effects of TPA were comparable to those of insulin. As observed for the PC Cl3 cells, TPA and insulin added together induced an effect slightly lower than would be obtained for the theoretical sum of the individual effects. TPA did not change significantly the TSH-induced increase of labeled nuclei (TPA, 10 ng/ml, percent labeled nuclei: 16.9 ± 0.5; TSH: 14.4 ± 1.2; TPA + TSH: 17.2 ± 0.5).

**Insulin stimulated both protein and DNA accumulation and these effects were modulated by TSH**

As illustrated in Fig. 4, in PC Cl3 cells insulin at concentrations as low as 50 ng/ml already stimulated
protein accumulation, the maximum effect being reached at 250 ng/ml ($t = 5.601; P = 0.0304$). TSH per se also enhanced protein accumulation, but the effect was similar to the effect induced by the lowest concentration of insulin.

Although TSH potentiated insulin-induced protein accumulation, the effect was additive, not synergistic, and reached a plateau at a concentration of 50 ng/ml insulin (Fig. 4A). Insulin per se at concentrations higher than 250 ng/ml, slightly increased DNA accumulation. Although TSH alone did not induce a detectable increase in DNA accumulation after 48 h stimulation, both hormones synergized to induce DNA accumulation (Fig. 4B). Similarly in FRTL-5 cells, insulin per se, but not TSH, could induce a detectable increase of DNA accumulation, but TSH enhanced the effect of insulin (T Kimura, J Golstein & J E Dumont, unpublished observations).

**Insulin increased $[^{15}S]$methionine incorporation into protein**

In PC Cl3 cells insulin increased $[^{15}S]$methionine incorporation into protein in a concentration-dependent fashion and the effect reached a plateau at $1 \mu g/ml$. TSH per se enhanced $[^{15}S]$methionine incorporation and the effect was additive to the insulin action (Fig. 5). TPA (10 $\mu g/ml$) per se also increased $[^{15}S]$methionine incorporation to the same level as insulin (approximately 140% of control) (Fig. 6). This effect was not additive with the insulin effect. By contrast, EGF and HGF as well were without effect. Similar results were obtained in FRTL-5 cells used as control (see below).

**Lovastatin inhibited $[^{3}H]$thymidine labeling of nuclei in both PC Cl3 and FRTL-5 cells; in FRTL-5 cells, it inhibited $[^{15}S]$methionine incorporation induced by insulin but not by TSH**

In PC Cl3 cells, lovastatin (5 $\mu$mol/l), an HMG-CoA reductase inhibitor, strongly decreased the labeling of nuclei induced by insulin, TSH or a combination of insulin and TSH and this effect was reversed by 1 mol/l mevalonate (Fig. 7A). It did not reduce $[^{15}S]$methionine incorporation, either basal or stimulated by insulin or TSH, when the inhibitor was added together with the hormones (Fig. 7B), or when it was added 24 h before them (Fig. 8A).
Comparable experiments were also conducted on FRTL-5 cells. Lovastatin inhibited [3H]thymidine labeling of nuclei; however, with the increased numbers of passages FRTL-5 cells exhibited a progressive decrease in their responsiveness to TSH in [3H]thymidine labeling index experiments and finally became almost unresponsive in this regard; similarly they were becoming unresponsive to forskolin (J Golstein, T Kimura, F Miot & J E Dumont, unpublished observations). Experiments involving [35S]methionine incorporation were performed on such cells. After either TSH or insulin stimulation or both, a significant increase in [35S]methionine incorporation was observed. Contrary to what happens in PC Cl3 cells, lovastatin treatment inhibited [35S]methionine incorporation activated by insulin but not by TSH (Fig. 8B); this effect was reversed by addition of 1 mmol/l mevalonate. Lovastatin induced morphological changes in both cell systems, as reported by Bifulco et al. (22) for FRTL-5 cells. Indeed a marked rounding-up of the cells was observed in treated FRTL-5 cells and in PC Cl3 cells as well (Fig. 9). The morphological changes induced by lovastatin were reversed by 1 mmol/l mevalonate (not shown).

**Discussion**

Despite the fact that FRTL-5 cells have been extensively used for many years, the mechanism regulating their...
growth in size has not yet been clearly defined (T Kimura, J Golstein & J E Dumont, unpublished observations). As far as PC Cl3 cells are concerned, studies on their regulation are scarce (3). In the current study, we first characterized the effects of various mitogenic factors acting through different cascades on \([3H]thymidine incorporation into nuclei of PC Cl3 cells.\) FRTL-5 cells have been used for comparison; the observations related to proliferation experiments obtained in these cells are generally comparable to those widely reported in the literature (2, 5, 23, 24). Some obvious differences between proliferative properties of both cell lines have been found, mainly with regard to EGF and HGF. Contrary to what has been reported by most authors, in FRTL-5 cells EGF, like HGF, induced a slight increase in \([3H]thymidine-labeled nuclei, this effect being potentiated by insulin. This finding corroborates the observations on EGF action made by Asmis and collaborators (23). It should be pointed out that an EGF effect has been described mainly in aged cells; this could indeed be the case in the current investigation, as commented upon below. Serum alone induced an increase of the frequency of labeled nuclei, which could be due to EGF, insulin and/or IGF-I (23). Some obvious differences between proliferative properties of both cell lines have been found, mainly with regard to EGF and HGF. Contrary to what has been reported by most authors, in FRTL-5 cells EGF, like HGF, induced a slight increase in \([3H]thymidine-labeled nuclei, this effect being potentiated by insulin. This finding corroborates the observations on EGF action made by Asmis and collaborators (23). It should be pointed out that an EGF effect has been described mainly in aged cells; this could indeed be the case in the current investigation, as commented upon below. Serum alone induced an increase of the frequency of labeled nuclei, which could be due to EGF, insulin and/or IGF-I (23). FRTL-5 proliferation was enhanced by TPA, as previously shown by some authors (25). By contrast, the PC Cl3 cells did not respond to EGF and serum alone only induced a weak effect. TPA per se appeared almost as potent as insulin.

Contrary to the TPA attenuation of the TSH-induced stimulation of FRTL-5 cell replication observed by Lombardi and coworkers (25), in our experiments on PC Cl3 cells, TPA did not significantly alter the TSH-induced increase of labeled nuclei. Obviously this cell line-related difference needs to be explored in more depth, particularly using a broad range of TPA concentrations. It is interesting to point out that the same authors also showed that TPA markedly inhibits the synergistic interaction between TSH and IGF-I (25).

Bremer-Gati et al. suggested that synergistic stimulation induced by insulin or IGF and TSH in FRTL-5 cells is modulated by 1,2-diacylglycerol/protein kinase C (PKC) (4); however, a PKC activator could not mimic synergistic stimulation. In PC Cl3 cells we did few experiments with IGF-I (an increased frequency of labeled nuclei comparable to that obtained with 1 μg/ml insulin was already observed with 20 ng/ml IGF-I), but both insulin and TPA added together induced a lower response of the labeling index than would have been expected from the theoretical sum of the individual ones, suggestive of a modulation by PKC of the insulin pathway. In this respect, PC Cl3 cells more closely resemble FRTL-5 cells. TPA stimulated both protein and DNA synthesis in PC Cl3 and FRTL-5 cells. Co-stimulation with TPA and TSH or insulin did not show synergistic stimulation of proliferation or protein synthesis in PC Cl3 and FRTL-5 cells. Therefore the TPA-sensitive pathway may modulate, via PKC, both protein and DNA synthesis in PC Cl3 and FRTL-5 rat thyroid cells. In so far as they have been tested, the effects of TPA and insulin are similar, suggesting once more a role for PKC in insulin action.

Unlike what has been generally observed in thyroid cells, in PC Cl3 cells HGF had no effect (26). Both EGF and HGF had no effect on protein synthesis or accumulation either. In PC Cl3 cells, insulin and TSH activate both growth in size and DNA replication.
However, while in PC Cl3 cells TSH and forskolin per se had a well-defined effect, additive with the effect of insulin, in FRTL-5 cells the proliferative effects of TSH and forskolin were weak or non-existent, but TSH enhanced the insulin effect. The results obtained in PC Cl3 cells are different from the results in the dog thyroid cell system. Indeed, in dog thyroid cells, dissociation of the stimuli for cell mass doubling and DNA synthesis is quite clear. Insulin induces protein accumulation while TSH triggers DNA replication; both protein and DNA accumulation clearly respond to distinct stimuli (7). Therefore, in these cells the protein/DNA ratio constitutes a good marker to demonstrate a clear contrast between insulin and TSH action. Contrary to that in the dog system, in rat thyroid cell lines, the protein/DNA ratio did not show a significant difference because of the double effects of insulin and TSH on protein and DNA accumulation. In these cell lines there is a tight coupling between the two processes; it is therefore difficult to study them separately. In PC Cl3 cells a concentration of 50 ng/ml insulin promoted a just detectable induction of DNA synthesis but greatly potentiated the effects of TSH. Thus very low concentrations of insulin would allow a study of the nature of the permissive effect of insulin on TSH-induced DNA synthesis. In PC Cl3 cells the effects of TSH and insulin on protein accumulation and synthesis were parallel, suggesting that protein accumulation primarily results from an increase in protein synthesis, rather than from an inhibition of protein degradation.

For both cell lines we observed that lovastatin, an HMG-CoA reductase inhibitor, inhibited the synthesis of DNA induced by insulin, TSH or a combination of both hormones, and that this effect was reversed by mevalonate, suggesting that the lovastatin effect was reversible and not toxic. It is well documented in different types of cells that the Ras pathway participates in insulin- or IGF-induced DNA synthesis (8, 14, 15, 27). Activation of Ras needs its prior farnesylation, which requires the first part of the cholesterol biosynthetic
Pravastatin, another HMG-CoA reductase inhibitor, decreased IGF-induced proliferation in FRTL-5 cells (8). Lovastatin inhibits Ras activity through inhibition of HMG-CoA reductase and therefore of farnesyl synthesis and coupling to proteins (14, 15). However, it inhibits other pathways requiring mevalonate. These reports and our results indicate that insulin-stimulated proliferation requires the activity of a lovastatin-sensitive pathway in rat thyroid cells, presumably the Ras pathway. This conclusion would be compatible with the results from studies on other cell types, in which the existence of a positive feedback between insulin activation of the Ras–Map kinase pathway and augmentation of the cellular pool of farnesylated p21 Ras available for activation by growth factors have been reported (27).

Lovastatin also inhibited TSH-induced proliferation in PC Cl3 and FRTL-5 cells. TSH activates proliferation through the adenyl cyclase/cAMP cascade in rat thyroid cell lines (2–6). In addition, TSH, via cAMP, regulates mRNA expression of HMG-CoA reductase and an active cholesterol biosynthetic pathway is required for DNA replication in FRTL-5 cells (12, 13). The group of Meinkoth has shown that Ras inhibition decreases the TSH effect on proliferation in WRT cells (10) and that TSH-stimulated proliferation needs both cAMP-dependent kinase and Ras activity, which is independent of Raf-1 or MEK (11). Although their study using inhibitors suggests that the Ras pathway is required for TSH action, there is no direct proof that TSH stimulates Ras. Dremier et al. showed in dog thyrocytes that activation of PKA is not sufficient and another complementary mechanism could exist in the cAMP-dependent DNA synthesis induced in dog thyroid cells (28). Our results suggest that TSH-stimulated DNA synthesis via cAMP also requires a lovastatin-sensitive cholesterol biosynthetic pathway in PC Cl3 and FRTL-5 cells.

As observed in different cell types with various HMG-CoA inhibitors, lovastatin caused cell rounding in both PC Cl3 and FRTL-5 cells, an effect which was reversed by mevalonate (22). We did not investigate further these alterations, as Bibfulco and collaborators (22) previously studied cytoskeleton alterations in FRTL-5 cells. They postulated that these inhibitors induce cell rounding by prevention of isoprenylation of proteins important for cytoskeletal organization. Nevertheless, as rounding-up of the cells could lead to reduced cellular adherence representing the precursory events of anoikis (i.e. acquisition of anchorage independence resulting in an increase in the number of dead cells (29)), we determined whether lovastatin, at 5 μmol/l working concentration, could induce some apoptotic or necrotic effect. Only a very small effect was observed (using a method previously described, 3% of fragmented DNA and 4% necrosis respectively were detected after 48 h incubation) (30); it has not been taken into consideration. This confirms that the toxicity of the drug was negligible.

Unexpectedly, TSH per se promoted protein synthesis and accumulation in both rat thyroid cell lines. TSH-induced protein synthesis was reproduced by forskolin, an adenylyl cyclase activator. This suggests that TSH activates protein synthesis via the adenylyl cyclase/cAMP pathway and that TSH stimulates protein accumulation by protein synthesis, not by prevention of degradation. Lovastatin did not inhibit TSH- or forskolin-stimulated protein synthesis. TSH-stimulated
HMG-CoA reductase activity could not cause an increased protein biosynthesis because it is strongly increased only 10 h after stimulation (12), while we could show that TSH activates protein synthesis already during the first 6 h of the incubation. From these results we could suggest that in the rat thyroid cells TSH-induced DNA replication via cAMP requires an active cholesterol biosynthetic pathway, while the TSH hypertrophic action does not.

Lovastatin decreased insulin-stimulated protein synthesis in FRTL-5 cells, but not in PC Cl3 cells. Relief of the inhibitory effect of lovastatin by mevalonate again suggests that the effect of lovastatin was specific, not toxic. This suggests that insulin-induced protein synthesis is regulated via a different pathway than DNA synthesis and likely not via the Ras signaling cascade in PC Cl3 cells. On the contrary, insulin activates both protein and DNA synthesis, at least in part through Ras or a lovastatin-sensitive pathway in FRTL-5 cells.

Co-stimulation with insulin and TSH did not synergistically stimulate protein synthesis in either cell line, while it did synergistically stimulate DNA synthesis. This also suggests a dissociation of the regulation of protein synthesis and DNA replication.

In FRTL-5 cells, we observed that the TSH-induced response to proliferation decreased with the number of passages of the cells (it remained between 20 and 40 in the course of the study). This characteristic was accompanied by morphological changes consisting mainly of rounding-up of the cells and, to a minor extent, in a loss of cell contact. Such an observation has already been reported by others (31, 32). Additional modifications, such as a loss of susceptibility to drug-induced apoptosis, developed together with these alterations (J Golstein, T Kimura, F Miot & J E Dumont, unpublished observations). It should be pointed out that these cells were still responsive to TSH in experiments involving [35S]methionine incorporation. This discrimination in TSH responsiveness to proliferation on the one hand and protein synthesis on the other constitutes additional support for the existence of different pathways regulating both phenomena. Comparison of young and aged cells might constitute a useful tool in the elucidation of the steps responsible for the divergence in TSH responsiveness, which might in turn bring some additional data to elucidate the physiological aging process. As far as PC Cl3 cells are concerned, they were most stable throughout the study, despite the fact that they also have been repeatedly passaged. This difference in the stability between both cell lines can be related to the difference in the acquisition of some malignancy properties in the corresponding murine retrovirus-bearing oncogene-infected cells; indeed it has been established that virus-infected PC Cl3 cells (KiMSV, AF-1, HaMSV bearing v-ras for example) did not grow in agar and did not give rise to tumors in vivo, while virus-infected FRTL-5 cells achieved complete transformation (3). FRTL-5 cells need only one event to become tumoral while PC Cl3 cells require at least two.

Thus due to their stability and the fact that low concentrations of insulin are mitogenically poorly active, but are permissive for TSH action as in dog thyroid cells (33), PC Cl3 cells appear to constitute the best cell line model to study the mitogenic action of TSH and cAMP.

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