Dissociation of thyrotropin receptor function and thyrotropin dependency in rat thyroid tumour cell lines derived from FRTL-5

CJH van der Kallen¹, JH Coes¹, JP van GraasHorst¹, EMD Schuring², FA Ossendorp², JHH Thijssen¹, MA Blankenstein¹ and TWA de Bruin¹

¹Department of Endocrinology, Utrecht University and Academic Hospital Utrecht, HP GO2.625, PO Box 85300, NL-3508 GA Utrecht, The Netherlands; ²Academic Hospital Leiden, Leiden, The Netherlands.

Summary: Spontaneously transformed somatic thyrocyte mutants, FRTL-5/T and FRTL-5/T, are thyrotropin (TSH) independent for growth and show loss of the thyroid-specific phenotype, with absent thyroglobulin and thyroid peroxidase gene expression. To investigate the role of TSH-receptor (TSH-R) activation in rat thyroid growth and function, binding of TSH and TSH-induced cAMP production were measured in intact cells under identical assay conditions. TSH binding did not differ in terms of affinity and receptor number and presence of 5.6 kb and 3.3 kb mRNA rat TSH-R transcripts was determined in all variants. By contrast, basal cAMP was 11-fold lower in FRTL-5/T and 6-fold lower in FRTL-5/T than in wild-type FRTL-5 (1.1 ± 0.4, P < 0.01). Maximal cAMP production was similar between wild-type and cell variants and stimulation by bovine, rat and recombinant human TSH revealed normal activation patterns. Therefore, a dissociation was present between the loss of TSH control on growth and function, and the presence of a normally functioning TSH-R. Subsequent to TSH incubation FRTL-5/T and FRTL-5/T cell lines showed a different expression pattern of TSH-R and the proto oncoproteins c-myc and fos than FRTL-5 wild-type. The data indicated that the cause of the TSH-independent thyrotropin dependency is located downstream of the cAMP cascade, influencing genes that control the expression of cell cycle-related proto-oncoproteins and thyroid-specific genes.

Keywords: thyroid; thyrotropin; thyrotropin receptor; cAMP; FRTL-5

The glycoprotein hormone thyrotropin (TSH) is the major regulator of thyroid function and growth (Vassart and Dumont, 1992). Actions of TSH are exerted through interaction with the plasma membrane TSH receptor (TSH-R), which has been characterised as a member of the G-protein coupled receptor family (Nagayama et al., 1989; Libert et al., 1989). Following binding of TSH to the TSH-R, G-proteins will be activated, resulting in stimulation of adenylyl cyclase (Dumont et al., 1989) and cAMP production or in stimulation of phosphatase C with subsequent diacylglycerol and inositol-3-phosphate production (Vassart and Dumont, 1992). cAMP is generally assumed to regulate a major part of thyroid function and thyroid growth (Vassart and Dumont, 1992).

Thyroid tumours can be divided into functional and non-functional tumours. With regard to growth and metabolic activity, functional tumours are responsive to TSH like normal thyroid tissue. As a tumour evolves, phenotype-specific metabolic activities decrease or disappear (Christov and Raichev, 1972; Wollman, 1963), as has been described in transgenic models (Ledent et al., 1991). Non-functional tumours show absence of phenotype-specific thyroid functions and, characteristically, TSH is unable to stimulate such functions, or growth. Therefore, loss of TSH responsiveness could result from a TSH-R defect or a defect in a post-receptor element important for signal transduction. For example, absence of high-affinity TSH-R (Abe et al., 1981), a defective coupling between the TSH-R and G-protein (Namba et al., 1993), a TSH-R point mutation (Parma et al., 1993), and a Gα mutation including those found in other endocrine tumours (Lyons et al., 1990; Yoshimoto et al., 1993) have been suggested to explain the observed TSH independence or TSH unresponsiveness. In experimental terms validity of either of the first two possibilities would be supported by findings of abnormal TSH binding or an abnormal pattern of TSH-induced cAMP production. The latter two possibilities are then expected to result in a higher basal cAMP level.

In the present study we tested these possibilities experimentally using the FRTL-5 rat thyroid cell line and spontaneously transformed somatic cell variants with different degrees of differentiation and sensitivity to TSH (Ossendorp et al., 1990). FRTL-5 cells are an accepted model for normal thyroid function as these cells show responsiveness to TSH for iodide uptake, thyroglobulin (Tg) iodination and secretion, and cell growth. The variant cell lines have been generated by subcutaneous injection of FRTL-5 cells in nude mice. From the tumours that subsequently developed, variant cell lines were derived as described by Ossendorp et al. (1990). The variant cell line FRTL-5/T, isolated from a functionally active tumour, shows normal responsiveness to TSH for most thyroid-specific functions (Ossendorp et al., 1990). This is in contrast to the FRTL-5/T and FRTL-5/T variants, isolated from non-functional tumours, which are insensitive to TSH with respect to iodide uptake, thyroglobulin iodination and secretion, and cell growth, thus fulfilling the criteria of somatic cell mutants with the phenotype of autonomous TSH-independent growth and function (Ossendorp et al., 1990). In the present study we evaluated whether acquisition of autonomy was associated with defects in the binding of the ligand, or with defects in the second messenger system. The production of cAMP in response to TSH was characterised under the same conditions in which TSH binding to its receptor can be measured; binding was evaluated in terms of capacity and affinity. In addition, the response of the proto-oncoproteins c-myc and fos was measured to characterise the effect of TSH at the early steps of the cell cycle.

Materials and methods

Culture of FRTL-5 cells and variant cell lines

FRTL-5, FRTL-5/T and FRTL-5/T cells were cultured in 6H (six-hormone mixture) medium (Ambesi-Impiumbato et al., 1980) consisting of Coon's modified Ham's medium.

Correspondence: CJH van der Kallen
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(Gibco BRL, Breda, The Netherlands) containing 5% fetal calf serum (Gibco) and insulin (10 μg ml⁻¹), hydrocortisone (0.36 ng ml⁻¹), transferrin (5 μg ml⁻¹), somatostatin (10 ng ml⁻¹), glycyl-L-histidyl-L-lysine acetate (2 mg ml⁻¹) (all Sigma, St Louis, MO, USA) and bTSH (bovine TSH) 10 μg ml⁻¹ (Ambinon, Organon, Oss, The Netherlands). As FRTL-5/Ta cells were generated under TSH-free conditions, the cells were cultured in 5H (5 hormone mixture) medium (TSH-free medium, identical to the medium described above but without TSH) as described previously (Ossendorp et al., 1990).

In experiments that measured cAMP production and TSH binding, cells were cultured in six-well plates (Gibco). Five days before the experiments, cell variants were cultured without TSH in such a way that the cells were confluent on the experimental day. One day before the experiments fresh medium was added to the cells.

(125I)TSH binding and competition (whole cell assay)
The iodination of bTSH (kindly supplied by Dr JG Pierce, University of California, CA, USA) was performed as described by Roelen et al. (1992) for human growth hormone. Briefly, 5 μg of bTSH was iodinated in a total volume of 25 μl with 0.3 mg ml⁻¹ chloramine-T (final concentration), the reaction was terminated with 100 μl of 0.96 mg ml⁻¹ Na-meta-bisulphite (final concentration). Free and bound 125I were separated on Sephadex G-25 (PD-10 columns, Pharmacia, Uppsala, Sweden). The incorporation of 125I was 72 ± 0.4% (mean ± s.e.m., n = 9). The (125I)bTSH in the protein fraction obtained from the Sephadex G-25 column was receptor purified by binding to and elution from porcine thyroid plasma membranes (Smith et al., 1977). Eluates were purified on a Sephadex S-200 column with Tris sodium chloride (10–50 mM) containing 0.1% bovine serum albumin (BSA).

Confluent cells were washed three times with modified Hanks’ balanced salt solution (HBSS: 5 mM potassium chloride, 1.3 mM calcium chloride, 0.4 mM magnesium sulphate, 0.34 mM disodium hydrogen phosphate, 0.44 mM potassium hydrogen phosphate, 280 mM sucrose and 0.25% BSA) and incubated for 1 h at 37°C in 1 ml of HBSS containing (125I)bTSH (± 10 000 c.p.m. sp. act. 1.2 × 10⁶ d.p.m. fmol⁻¹) and various concentrations of bTSH (1 μM = 2.4 fmol), rTSH (recombinant human TSH, Genzyme, West-Malling, UK; 1 μM = 4.2 fmol) and rTSH (rat TSH, kindly provided by NIDDK, NHPP, Baltimore, MD, USA; 1 μM = 1.0 fmol) (0–2.40, 0–0.42 and 0–0.24 μM respectively). After 1 h the incubation buffer was removed, cells were washed three times with ice-cold HBSS and 1 ml of 1 N sodium hydroxide was added to detach the cells. The bound radioactivity was transferred to tubes and counted in a γ-counter. The dissociation constants (Kd) of bTSH and the number of TSH binding sites (R) were calculated with a linear subtraction method (Van Zoelen, 1989). To compare the affinities of bTSH, rTSH and rhTSH IC₅₀ values (50% competition) were determined from the competition curves.

Effect of TSH on intracellular cAMP response
Confluent cells were washed three times with HBSS. The cells were incubated at 37°C in 1 ml of HBSS containing 0.5 mM 3-isobutyl-1-methylxanthine (Sigma) and various concentrations of bTSH, rTSH, rTSH (0–0.24, 0–0.44 and 0–0.024 μM respectively). After 1 h the incubation was terminated by removing the buffer and addition of 1 ml of 96% ice-cold ethanol. Cells were stored at least overnight at −20°C. Cells were scraped with a rubber policeman, the ethanol was evaporated at 37°C under a stream of nitrogen and 0.5 ml of a Triton X-100 buffer (4% Triton X-100, 5 mM EDTA, 50 mM Tris-HCl pH 7.5) was added. After centrifugation for 10 min at 3600 r.p.m. the supernatants were used to measure cAMP (radioceptor assay TRK 432, Amersham, Little Chalfont, UK). DNA was measured in the pellets (Burton, 1956), using calf thymus DNA (Boehringer Mannheim, Mannheim, Germany) as a standard. To compare the effects of bTSH, rTSH and rhTSH, their concentrations yielding 50% of the maximal cAMP response (EC₅₀) were calculated from the dose–response curves.

Measurements of intracellular cAMP were performed under similar conditions that enabled assay of TSH binding and competition. Control experiments, comparing cells of several passages, revealed similar properties with regard to TSH binding and cAMP response in the course of 4 years with all variants and wild-type FRTL-5.

Detection of TSH-R, c-myc and fos RNA expression
FRTL-5 cells and variants were harvested for RNA isolation after 7 days of TSH depletion (5H medium) or after variable intervals following TSH stimulation (100 μl ml⁻¹). RNA isolation was performed as described previously (Ossendorp et al., 1990). In short, cells were homogenised in 6 μl urea/3 M lithium chloride. After centrifugation (10 000 × g, 45 min, 4°C), the pellet was dissolved in 10 mM Tris-HCl (pH 7.6), 5 mM EDTA, 1% sodium dodecyl sulphate (SDS), deproteinised by two extracts with phenol-chloroform (1:1) and precipitated with ethanol. Samples of RNA were denatured at 60°C for 30 min in a solution containing 50% (v/v) formamide, 2.2 M formaldehyde and RNA running buffer (20 mM) MOPS (pH 7.0), 5 mM sodium acetate, and 1 mM EDTA.

For Northern analysis, RNA samples were separated by electrophoresis in 1.0% agarose gels, followed by transfer to nitrocellulose filters and fixed by heating (80°C for 4 h) as described in detail (Ossendorp et al., 1990). Membranes were hybridised with 32P-labelled probes (Ossendorp et al., 1990). The following probes were used: cDNA clones of the c-myc and fos genes (Tramontano et al., 1986) and a prepared cDNA rat TSH-R insert (1039–1190) probe. The TSH-R cDNA probe (1039–1190) was prepared by polymerase chain reaction (PCR) of the TBAFβ clone (courteously donated by Dr Leonard Kohn, NIH, Bethesda, MD, USA) containing the full-length rat cDNA coding sequence (~ 54 to 2780 bp). For reprobing the filters were stripped by washing the filter twice with 0.05 x SSC, 0.01 M EDTA (hot) and 0.1% SDS for 15 min and once briefly with 0.01 x SSC at room temperature.

Ethidium bromide staining of whole RNA in the agarose gel revealed the 28S (4.8 kb) and 18S (2.0 kb) ribosomal RNA markers respectively, which were used as a control for application of equal amounts of RNA. Quantification of intensity was performed by densitometer.

Statistical analysis
Results are given as means ± s.e.m. (n). Significances of the observed differences were calculated with the non-parametric Mann–Whitney U-test. P-values ≤ 0.05 were considered to reflect statistical significance.

Results

([125]I)TSH binding and competition (whole cell assay) (Figure 1 and 2, Table 1)

Total binding of [125]IbTSH to FRTL-5 cells was 8.3 ± 1.4% of the amount added (n = 8), with a non-specific binding of 0.31 ± 0.12%. These values did not differ significantly between the different cell line variants. The combined data of the competition curves and Scatchard plot analysis of FRTL-5 cells (n = 7–8, separate experiments) are shown in Figures 1a and 2. The analysis was performed both for one and two classes of binding sites. Analysis according to a two-class model yielded a high-affinity, low-capacity binding site with properties similar to the results of the analysis for one binding site. In addition, a binding site with extremely low affinity and high capacity was detectable. As this binding site was considered to represent non-specific binding, further
analysis is only reported for the one class model (Table I). Neither the $K_D$ (1.0–2.4 nM) nor the receptor number (R) differed significantly among the different cell lines. Competition by TSH from several species (rat, bovine and recombinant human) showed a similar pattern between cell variants (Figure 1). Differences between TSH species were noted, i.e. the IC$_{50}$ value of rhTSH was approximately one order of magnitude higher than the IC$_{50}$ value of bTSH and rTSH, by contrast the IC$_{50}$ value of rTSH did not differ from IC$_{50}$ found with bTSH (Table I).

Measurement of cAMP response to TSH (Figure 1, Table II)

The basal intracellular cAMP level was 11-fold lower in FRTL-5/TA cells and 5.8-fold lower in FRTL-5/TP cells than in wild-type FRTL-5 (1.1 ± 0.35 pmol CAMP $\mu$g$^{-1}$ DNA). In contrast a cAMP response was elicited by TSH to the same maximal level in each cell variant (7.9–11.1 pmol CAMP $\mu$g$^{-1}$ DNA) by 240 nm. Thus, the induction of cAMP production by TSH is different between wild-type (10-fold induction) and the TSH-independent growing cell lines FRTL-5/TP and FRTL-5/TA (64- to 79-fold induction). Similar EC$_{50}$ values were obtained with rTSH and bTSH. The EC$_{50}$ value of rhTSH was approximately one order of magnitude higher than the EC$_{50}$ value of bTSH and rTSH. The significant differences between rhTSH IC$_{50}$ and EC$_{50}$ values, observed in the variant cell lines, was probably caused by the use of two different batches of rhTSH. Different batches of rhTSH have different degrees of sialylation and sulphation, causing different TSH bioactivity (Szkudlinski et al., 1993).

Northern blotting (Figure 3)

The rat TSH-R-specific probe hybridised with a 5.6 and 3.3 kb mRNA signal respectively, following Northern blotting. These sizes of mRNA transcripts have been identified in FRTL-5 cells as transcripts of the rat TSH-R (Akamizu et al., 1990). In all variants normal sized TSH-R mRNA transcripts were observed (Figure 3), although the intensity differed. This was confirmed by densitometry. Culture of FRTL-5 cells with TSH (6H) down-regulated TSH-R mRNA and this effect was also demonstrated in

**Table I** Capacity (R) and affinity ($K_D$) of bTSH binding to FRTL-5 wild type and variant cell lines and IC$_{50}$-values for the competition of $[^{125}]$bTSH by radioinert competitors

| Parameter | FRTL-5 | FRTL-5/T | FRTL-5/TP | FRTL-5/TA |
|-----------|--------|----------|-----------|-----------|
| (R)       | 134 ± 20 (8) | 259 ± 93 (5) | 127 ± 28 (5) | 102 ± 29 (7) |
| $K_D$ bTSH | 1.0 ± 0.4 (8) | 2.4 ± 0.5 (5) | 1.8 ± 0.3 (5) | 2.3 ± 0.5 (8) |
| IC$_{50}$ bTSH | 2.7 ± 0.6* (8) | 2.7 ± 0.7* (5) | 3.6 ± 1.1* (5) | 3.7 ± 0.7* (7) |
| IC$_{50}$ rTSH | 2.0 ± 0.9* (6) | 4.3 ± 1.4 (4) | 1.9 ± 1.3* (3) | 1.9 ± 0.5* (4) |
| IC$_{50}$ rhTSH | 56 ± 24 (3) | 35 ± 19 (3) | 127 ± 22* (3) | 103 ± 34 (3) |

*K$_D$ and IC$_{50}$ are given in nm, (R) in fmol/well. $K_D$ and R are calculated based on the presence of one binding site. Values represent means ± s.e.m. (n). *Significantly different (P<0.05) vs rhTSH.

Significantly different (P<0.05) vs FRTL-5/T.
TSH-dependent FRTL-5/T cells. In FRTL-5/TP and FRTL-5/TA (TSH-independent variants) the intensity of the TSH-R transcript was substantially lower in the absence of TSH (5H) and stimulation by TSH did not lead to a decreased intensity.

Discussion

In the present study we characterised functional TSH binding, cAMP response, TSH-R gene expression, and c-myc/fos proto-oncogene response in rat thyrocytes. As a model we used stable rat FRTL-5/T, FRTL-5/TP and FRTL-5/TA thyroid cell lines that spontaneously originated from wild-type FRTL-5 cells transplanted into nude mice and that were characterised by TSH-independent growth in combination with dedifferentiation (Ossendorp et al., 1990). The functional binding of radiolabelled TSH, characterised by KD and number of binding sites, as well as IC50 values of rat TSH and bovine TSH, were similar between TSH-dependent (FRTL-5 and T cells) and TSH-independent (TP and TA) cells. Because of the combination of low-affinity (KD 101.5 nM) and an exceptionally high number of binding sites per cells (± 1.1 x 10⁶), we concluded that this class of low-affinity binding sites represents non-specific binding. This is in agreement with other reports (Chazengek et al., 1990; Nagayama and Rapoport, 1992), which concluded that this class of binding sites is an artefact because both untransfected and TSH-R transfected CHO cells show the same low-affinity site. The FRTL-5/TP and FRTL-5/TA variants showed normal maximal cAMP response, but 6- to 11-fold lower basal intracellular cAMP concentrations than wild-type FRTL-5. The EC50 values obtained with rat and bovine TSH were similar between all variants and wild-type FRTL-5. Assays of TSH binding and TSH-induced cAMP production were carried out under identical conditions of cell culture, excluding artefacts which might be caused by low-salt and high-salt culture conditions (Tramontano and Ingbarg, 1986). The present findings therefore formally demonstrate the dissociation between presence of a functionally intact TSH-R, able to activate the cAMP cascade and TSH control on the growth and differentiation in FRTL-5/TP and FRTL-5/TA cells.

The exact role of TSH in the control of thyroid growth is still unresolved because thyroid mitogenic and anti-mitogenic effects have been reported in different species (Maenhaut et al., 1990; Vassart and Dummont, 1992). TSH is a proven growth factor in FRTL-5 cells (Jin et al., 1986; Coletta et al., 1986), dog thyrocytes (Maenhaut et al., 1990) and human thyrocytes (Dummont et al., 1992). TSH is able to activate

### Table II. cAMP response to TSH in FRTL-5 wild type and sublines and EC50 values of different species of TSH

| Parameter     | FRTL-5  | FRTL-5/T | FRTL-5/TP | FRTL-5/TA |
|---------------|---------|----------|-----------|-----------|
| Basal cAMP    | 1.1±0.4 (7) | 0.38±0.18* (5) | 0.19±0.08* (5) | 0.10±0.03* (6) |
| Max cAMP      | 11.1±2.0 (7) | 11.5±3.8 (5) | 12.2±3.4 (5) | 7.9±2.3 (6) |
| EC50 TSH      | 0.22±0.05* (7) | 0.22±0.05* (7) | 0.26±0.08* (5) | 0.29±0.08* (6) |
| EC50 TSH      | 0.28±0.11* (4) | 0.44±0.09* (4) | 0.90±0.80* (3) | 0.44±0.16* (3) |
| EC50 rhTSH    | 5.1±0.6* (3) | 10.1±5.9 (3) | 5.8±1.2* (4) | 14.3±1.8 (3) |

Max cAMP reflects cAMP production at TSH 240 nM, cAMP values are given in pmol mg⁻¹ DNA, EC50 values are given in nM, values represent mean± S.E.M. (n). *Significantly different (P<0.05) vs FRTL-5/TA. **Significantly different (P<0.01) vs FRTL-5. **Significantly different (P<0.05) vs rhTSH.

**Figure 2.** Scatchard analysis of the competition curve (Figure 1a) of FRTL-5 cells (n=7-8).

**Figure 3.** Northern blot analysis of TSH-R, fos and c-myc mRNA in FRTL-5 and variant cells and effect of the presence of TSH (100 µU mL⁻¹). 5H, culture without TSH; 6H, culture in the continuous presence of TSH, 40 min and 24 h reflect times following addition of TSH. The experimental protocol used is described in detail in the Materials and methods.
both the cAMP cascade and phospholipase C pathway independently by binding to its receptor (Maenhaut et al., 1990; Fujimoto and Brenner-Gatti, 1992). The current theory is that enhancement of the cAMP cascade relates to stimulation and control of functional characteristics in human and FRTL-5 (rat) thyrocytes, including iodide uptake and expression of thyroid-specific proteins thyroglobulin and thyroid peroxidase (Milligan et al., 1995). In agreement with this theory is the recent demonstration that gain-of-function mutations in the TSH-R cause hyperfunctioning thyroid adenomas (Parma et al., 1993) and congenital hyperthyroidism (Kopp et al., 1995), which are characterised by higher basal intracellular cAMP concentrations in agreement with the original report by Kasagi et al. (1980) in a collection of human adenoma tissues. Dedifferentiating tumours may then be caused by activation of growth factor and phorbol ester cascades (Maenhaut et al., 1990; Mockel et al., 1994), and not through activation of cAMP cascade. The finding of reduced basal cAMP concentrations in dedifferentiated FRTL-5/TP and FRTL-5/TA cells, a factor 6 to 11 lower than FRTL-5 cells, therefore represents novel evidence to support this theory, and possibly indicates a mechanism of inverse agonism, causing a reduction in the basal regulation of the adenylate cyclase (Milligan et al., 1995). FRTL-5/TA and FRTL-5/TP cells have lost most of the thyroid-specific phenotype of wild-type FRTL-5 cells as demonstrated by virtual absence of iodide uptake, and absence of thyroglobulin and TPO mRNA (Ossendorp et al., 1990). In addition, FRTL-5/TP and FRTL-5/TA cells demonstrate a dissociation between intact TSH-R function in the cAMP cascade and loss of TSH control over thyroid growth. Thus, acquisition of dominant signal transduction pathways other than the cAMP cascade, or in theory further downstream in the cAMP cascade, is relevant and advantageous for thyrocytes to develop autonomous growth and dedifferentiation.

The present findings do not support the claim by Berlingeri et al. (1990) that the loss of TSH control on growth correlates with the complete loss of rat TSH-R gene expression. Using the same rat TSH receptor cDNA probe, these authors could not detect TSH-R mRNA in oncogene transformed FRTL-5 cells; functional TSH binding was not measured. We detected TSH-R mRNA in TSH-independent FRTL-5/TP and FRTL-5/TA variants, although the gene expression was lower than the corresponding transcript signal in FRTL-5 cells. Interestingly, this reduction in TSH-R gene expression did not affect the binding characteristics of radiolabeled TSH. The present data indicated that TSH-R mRNA does not correspond to the number of functioning receptors, suggesting: (1) that degradation or stability of mRNA species (5.6 and 3.3 kb) is different between cell variants; (2) TSH-R protein may have a larger residence time on the cell surface; and (3) a functional assay of TSH binding needs to be combined with measurement mRNA TSH-R levels. In the literature, species-specific responses of TSH-R mRNA expression to TSH exposure have been reported: down-regulation in rat thyrocytes (Akamizu et al., 1990; and the present findings), a slight down-regulation in dog thyrocytes and no marked down-regulation in human thyrocytes (Maenhaut et al., 1992). Significantly reduced TSH-R gene expression has also been found in neoplastic human thyroid tissues in conjunction with reduced thyroglobulin gene expression (Ohta et al., 1990; Elisei et al., 1994). However, several laboratories have shown that functional TSH binding is, in general, not different between normal and neoplastic human thyroid tissues (Karlsson and Dahlberg, 1979; Matsuo et al., 1993). Therefore, a similar discrepancy as found in FRTL-5/TA cells may exist between reduced TSH-R gene expression and intact TSH binding can exist in human thyroid neoplasms.

Coletta et al. (1986) showed that TSH increased fos and c-myc gene expression in FRTL-5 cells under controlled conditions of thyrocyte growth. Our findings on fos and c-myc gene expression in FRTL-5 cells are similar to those by Coletta et al. (1986), and to the findings in dog thyrocytes (Reuse et al., 1990); fos and c-myc gene expression were transiently stimulated after the addition of exogenous TSH to TSH-deprived FRTL-5 cells or T variant cells. Interestingly, in TSH-independent FRTL-5/TP and FRTL-5/TA variants addition of exogenous TSH was again associated with an increase in fos and c-myc gene expression, although the latter transcript signal was reduced compared with wild-type FRTL-5. Our data unequivocally show that the TSH-induced gene expression of fos and c-myc can be dissociated from the phenomenon of TSH-dependent growth and are in agreement with and extend the conclusion by Heldin and Westmark (1988) and Wylie et al. (1989). Other possible mechanisms, known to activate the thyroid-specific genes Tg, TPO and TSH-R, are Pax-8 and thyroid transcription factor 1 and 2 (Zannini et al., 1992; Civitareale et al., 1993; Shimura et al., 1994). Interestingly, transformation of FRTL-5 cells with viral oncogenes (Ki-ras, Ha-ras and mos) leads to undetectable expression of Tg and TPO genes (as in the FRTL-5/TP and FRTL-5/TA variants), in combination with absence of Pax-8 expression (Francis-Lang et al., 1992).

Further studies are planned to characterise the dominant signal transduction pathways in FRTL-5/TP and FRTL-5/TA cells and their effect on genes known to be involved in thyroid growth and function. In conclusion, a functionally normal TSH-R was found in FRTL-5 cells and its TSH-independent variants. Mutations in the TSH-R or Gαq are therefore unlikely explanations for the TSH independence in FRTL-5/TP and FRTL-5/TA cells. In these cells the functionally intact TSH-R is dissociated from the TSH control on growth and differentiation. Supported by the reduced basal levels of cAMP in FRTL-5/TP (6-fold) and FRTL-5/TA (11-fold), we conclude that a different mechanism, down-stream of the cAMP cascade, is responsible for a different activation pattern of fos, c-myc and TSH-R mRNA and the autonomous growth of FRTL-5/TP and FRTL-5/TA cells.

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