The Tissue-specific Pathways Regulating Cell Proliferation Are Inherited Independently in Somatic Hybrid between Thyroid and Liver Cells

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Abstract. Thyroid stimulating hormone (TSH) and insulin-like growth factors type 1 (IGF-I) regulate the proliferation and differentiation of cultured thyroid cells but not of cultured liver cells. We have examined the influence of TSH and IGF-I on the metabolic functions and proliferation of somatic hybrids obtained by fusing rat thyroid cells (FRTL5) with rat liver cells (BRL).

While IGF-I is able to stimulate the proliferation of the hybrid cells (TxL) TSH fails to induce their growth. However, the hybrid TxL cells have surface TSH receptors with normal ligand characteristics. The addition of TSH to TxL cells led to typical enhancement of cAMP production and depolymerization of actin filaments. Yet, TSH failed to stimulate iodine uptake in the hybrid cells. Interestingly, iodine inhibited TxL proliferation induced by IGF-I but not by serum.

It is concluded that the hybrid TxL cells inherited from the parental thyroid cells several important differentiated traits including mitogenic pathways induced and used by IGF-I, functional TSH receptors, and sensitivity to the inhibitory action of iodine.

In the thyroid gland the control of cell proliferation depends upon the balance between stimulatory and inhibitory signals. At least two mechanisms are involved in the stimulation of thyroid cell proliferation, one cAMP dependent, triggered by thyroid stimulating hormone (TSH), and one cAMP independent, triggered by insulin-like growth factors (IGFs), insulin, or EGF (Bacharach et al., 1985; Dere and Rapoport, 1986; Ingbar et al., 1987; Jin et al., 1986; Roger and Dumont, 1982, 1987; Roger et al., 1983, 1988; Smith et al., 1986; Tramontano et al., 1986*b*, 1988; Westermak and Westermak, 1982; Westermak et al., 1983). In the FRTL5 cells, a line of normal rat thyrocytes in culture, it has been demonstrated that TSH interacts synergistically with IGFs to modulate mitogenesis (Tramontano et al., 1986*b*), suggesting that only the concomitant activation of the two pathways allows the full expression of the proliferative response of the thyroid follicular cells.

More importantly, recent data indicate that the interaction between TSH and IGFs observed in cells in culture may have a physiological role since it has been shown that IGF-like peptides are produced by the thyrocytes of human, porcine, rat, and ovine origin in vivo and in vitro (Brown et al., 1986; Clemmons and Van Vyk, 1985; D'Ercole et al., 1980, 1984; Errick et al., 1986; Han et al., 1987; Maciel et al., 1988; Mak et al., 1985; Stiles et al., 1985).

Iodine is one of the most important regulators of the thyroid gland functions and it is well known that iodine inhibits proliferation of thyroid follicular cells both in vivo and in vitro (Bray, 1968; Chapman, 1941; Gartner et al., 1985; Halmi, 1971; Ingbar, 1972; Nagataki and Ingbar, 1986). In the FRTL5 cells iodine inhibits growth induced through both the cAMP dependent and the cAMP independent pathways (Becks et al., 1988; Tramontano et al., 1989).

This complex network of interaction regulating thyroid cell proliferation represents a distinctive "trait" of thyrocytes and it can be analyzed as a differentiated function. Thus, we have investigated how the various pathways regulating thyroid cell growth are inherited by hybrid cells, TxL (Ambesi-Impiombato et al., 1985), obtained by fusing FRTL5 cells (Ambesi-Impiombato et al., 1982; Ambesi-Impiombato and Villone, 1987), a line of normal rat thyroid follicular cells whose growth is dependent upon and regulated by TSH and IGF (Tramontano et al., 1986b, 1987; Tramontano and Ingbar, 1986), and BRL-30E cells (BRL), a line of normal rat liver cells, whose growth is regulated by serum (Nissley et al., 1977).

We have investigated the effect of serum, TSH, IGFs, and iodine on the proliferation and differentiated functions of the TxL cells. The results presented here provide evidence that the hybrid TxL clones have inherited the mechanisms con-

1. *Abbreviations used in this paper*: BRL, Buffalo rat liver; bTSH, bovine thyroid stimulating hormone; IGF-I, insulin-like growth factors type 1; KRB, Krebs-Ringer bicarbonate buffer; TSH, thyroid stimulating hormone.

trolling growth from both liver and thyroid cells, in that, they respond to serum as the BRL cells, and to IGF-I as the FRTL5 cells. In addition, similarly to the thyroid parental cells, growth of TxL cells is inhibited by iodine. On the other hand, the growth pathway dependent upon TSH is lost in the TxL cells, in spite of the presence of biologically active TSH receptors on the plasma membrane of TxL cells.

Materials and Methods

Materials

Materials were purchased from the following sources: Coon's modified Ham F-12 tissue culture medium from Hazleton Research Products, Inc. (Denver, CO); calf serum and L-glutamine from Gibco Laboratories (Grand Island, NY); DME from Flow Laboratories, Inc. (Irvine, Scotland); BSA from Reheis Chemical Co (Phoenix, AZ); biosynthetic IGF-I (Thr^{59}-IGF-I) from AMgen Biologicals (Thousand Oak, CA); tissue culture dishes (100 × 20 and 60 × 15-mm style) and centrifuge tubes (15 ml) from Falcon Labware, Becton, Dickinson & Co. (Oxnard, CA); 24-well culture plates from Costar (Cambridge, MA); isobutyl methylxanthine (MIX), bovine TSH (bTSH), and RITC-conjugated phalloidin from Sigma Chemical Co. (St. Louis, MO); highly purified bovine TSH (TSH) was kindly provided by National Pituitary Program, National Institutes of Health (Bethesda, MD), [methyl-^{3}H]thymidine (1.0 mCi/ml) and ^{125}I-IGF-I from Amersham Corp. (Arlington Heights, IL); ^{125}I from Dupont-New England Nuclear Corp. (Boston, MA). All other chemicals and reagents were obtained from commercial sources and were of reagent grade or higher.

Culture Techniques

In these experiments FRTL5 clone 2 cells (Ambesi-Impiombato et al., 1982; Ambesi-Impiombato and Villone, 1987), FRTL5 HGPRT$^-$, BRL 30E TK$^-$ and several hybrid clones TxL (Ambesi-Impiombato et al., 1985) were used. FRTL5 HGPRT$^-$ cells were obtained by chemical mutagenesis of FRTL5 cells as already reported elsewhere (Ambesi-Impiombato et al. 1985). BRL 30E TK$^-$ cells are a line of Buffalo rat liver cells bromo deoxiuridine resistent kindly provided by Dr. H. G. Coon (National Institutes of Health). Techniques and methods for cell fusion and hybrid cells selection have been extensively described elsewhere (Ambesi-Impiombato et al. 1985). All the cell strains were routinely cultured in Coon's modified Ham F-12 (mF12) medium supplemented with 5% calf serum, in 100-mm tissue culture plates or 24-well Costar plates at 37°C in an atmosphere of 95% air/5% CO_2 in humified incubator. The medium of the FRTL5 and the FRTL5 HGPRT$^-$ was supplemented with TSH (1 mU/ml), insulin (1 μg/ml), and transferrin (5 μg/ml) (3H medium). Techniques for the subculture of cells have been described in detail previously (Ambesi-Impiombato et al., 1982). Techniques and methods for cell fusion and hybrid cells selection have already been reported (Ambesi-Impiombato et al., 1985). In some studies, cells were maintained in the absence of bTSH, insulin, or transferrin (H-free medium).

Growth Curves

For long-term experiments, cells were plated in 60-mm dishes at a concentration of 1 × 10^5/dish, in medium supplemented with serum as indicated in the legend to the figure. Replicate dishes were periodically (every 2–3 d), trypsinized, and counted in a Neubauer chamber (Saaringa, Federal Republic of Germany).

[^{3}H]Thymidine Incorporation

For studies of [^{3}H]thymidine incorporation into DNA, cells were sparsely seeded in H-free medium in 24-well culture plates, 5 d later, medium was removed, and cells were washed two times with mF12 medium devoid of serum and then they were incubated in the same medium for 24 h. The medium was removed and 500 μl of the same medium containing 0.1% BSA and the appropriate concentrations of the agents to be tested were added. After the appropriate incubation time, medium was removed, [^{3}H-methyl]-thymidine (5 μCi/ml) was added in 250 μl of DME, and cells were incubated for an additional 2 h. The labeling was stopped by aspirating the medium and washing the cells twice with PBS and three times with 10% ice-cold TCA. TCA-precipitable material was solubilized with 500 μl of 2% SDS. Cell-associated radioactivity was then counted in a scintillation spectrometer.

Radioreceptor Assay

TSH was labeled by a gentle cloramine-T technique as previously described (Goldfine et al., 1974). Final specific activity of ^{125}I-bTSH was 150 μCi/μg. Final specific activity of ^{125}I-IGF-1 was 280 μCI/μg. Binding studies were performed as previously described (Tramontano and Ingbar, 1986). Briefly, for studies of the binding of ^{125}I-IGF-I and ^{125}I-TSH, cells were grown to confluence in 24-well Costar plates in ^{3}H medium replaced for an additional 5 d by H-free medium. Immediately before the binding studies, media were aspirated, and monolayers were washed three times with buffer. ^{125}I-TSH or ^{125}I-IGF-I and varying concentrations of unlabeled peptides were then added to each well in 250 μl of modified Krebs-Ringer bicarbonate buffer (KRB) containing 0.1% BSA (Tramontano and Ingbar, 1986c). Steady-state binding experiments were carried out overnight at 4°C. At the end of the incubation cells were washed three times with ice-cold modified KRB. Cells were then solubilized in 1 N NaOH and ^{125}I content of the cell lysates was determined. Protein concentration was measured in aliquot of cell lysate by the method of Lowry et al. (1951). Nonspecific binding was determined in the presence of 26 nM IGF-1 or 1 μM bTSH.

Measurements of Cyclic AMP Concentration

Measurements of the effects of TSH on AMP generation were performed as previously described (Tramontano and Ingbar, 1986). Hybrids and BRL cells were grown to confluence in H-free medium in 24-well Costar plates. FRTL5 cells were grown to confluence in 3H medium in 24-well Costar plates and were then maintained in H-free medium for 7 d. Monolayers were then washed three times with KRB containing 0.1% BSA, pH 7.2, and cells were then incubated at 37°C in 250 μl of the same buffer containing the appropriate concentrations of TSH. After 30 min, the supernatants were collected and frozen at −20°C. To extract intracellular cAMP, monolayers were treated overnight at −20°C with 500 μl of ice-cold absolute ethanol. Extracts were then dried, and each pellet was reconstituted with its original supernatants. Aliquots of appropriate volume were then taken for RIA of cAMP concentration, and results were expressed as pmol/10^5 cells. Cells were counted in a Neubauer chamber after release from plates by trypsinization. In all experiments, quadruplicate wells were studied for each experimental point.

Staining of Actin Filaments

Cells were sparsely seeded on glass coverslips. After 3 d, appropriate concentrations of the agents to be tested were added, and cells were cultured for additional 24 h. At the end of the incubation time, cells were fixed for 15 min at room temperature with a solution of 3.7% paraformaldehyde in PBS in the presence of 2% sucrose to reduce the background. Coverslips were then washed five times with PBS; the cells were permeabilized with 0.1% Triton X-100 for 5 min in PBS and then washed five times in PBS. Cells were incubated in the dark for 45 min with RITC-conjugated phalloidin (5 μg/ml) and washed five times with PBS. Coverslips were mounted on microscope slides using a 50% solution of glycerol in PBS and examined with a Zeiss photofluor microscope.

Iodide Trapping

Studies of radioiodine uptake were performed as previously described (Lombardi et al., 1988). Cells were brought to confluence and maintained for 4–6 d in H-free medium. Then they were incubated for additional 48 h in medium supplemented with 0.1% BSA in the presence or in the absence of TSH (0.01–10 nM) and IGF-I (0.01–1 nM). Cells were washed once with KRB and incubated with Na^{125}I (0.1 μCi/well) and 10 μM NaI in 250 μl of KRB 37°C for 20 min. The reaction was stopped by washing cells with ice-cold KRB. Intracellular ^{125}I was extracted with 1 ml TCA 10% for 20 min at room temperature and the extract was counted in a gamma scintillation counter.

Statistical Analyses

In all studies at least three separate samples were studied for each experimental point. Analysis of differences in the values obtained in the various experimental groups was conducted by analysis of variance followed by Neuman-Keuls test for the significance of differences among multiple ex-

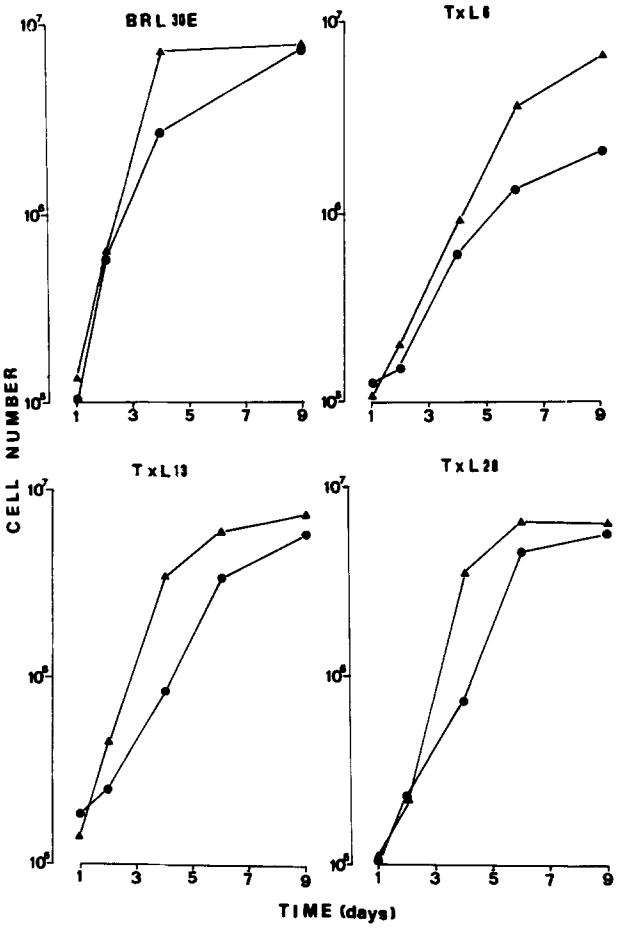

Figure 1. Growth curves of liver parental and hybrid cells performed in mF12 culture medium supplemented with 0.5 (●) or 5% (▲) calf serum. SD ⩽ 2% per each experimental point.

perimental groups (Zar, 1974). All experiments were performed at least twice, and usually more often, with good agreement between the results obtained.

Results

Effect of Serum on the Proliferation of TxL Clones

Among several clones obtained by fusing FRTL5 cells and BRL, four have been chosen for this study. Clones TxL6, TxL13(w/oH), and TxL20 have been selected in HAT medium supplemented with 5% calf serum, whereas TxL13(wH) have been selected in HAT medium supplemented with 5% calf serum and the 3H medium (see Materials and Methods). The average number of chromosomes for the above mentioned clones was 78 vs. 42 of the parental liver and thyroid cells. To ascertain whether serum could substain the growth of TxL hybrid clones, cells were seeded in the presence of 0.5 or 5% calf serum. Similarly to the BRL cells, TxL hybrid clones grew vigorously in the presence of either 0.5 or 5% calf serum (Fig. 1). As it has already been reported (Ambesi-Impiombato et al., 1982; Tramontano et al., 1986*a*), FRTL5 cells did not proliferate in the presence of serum alone at any of the concentrations tested (data not shown).

Effect of TSH and IGF on the Mitogenesis of TxL Hybrids

Although the proliferation of TxL clones is not dependent upon the presence of hormones in their culture medium, experiments were performed to determine whether their proliferation was still sensitive to hormones or growth factors. TxL cells were cultured in the presence of TSH and IGF-I, alone or in combination, for 36 h, then [^{3}H]thymidine incorporation into DNA was measured. TSH was totally ineffective on the proliferation of all hybrid clones tested as it was on the BRL cells, while it did stimulate the incorporation of [^{3}H]thymidine into the DNA of FRTL5 cells (Fig. 2). Conversely, IGF was able to induce DNA synthesis in all the hybrid clones and in FRTL5 cells but not in the BRL cells (Fig. 2). The mitogenic effect of IGF-I was dose dependent in the TxL cells as it is in the FRTL5 cells. However, as it

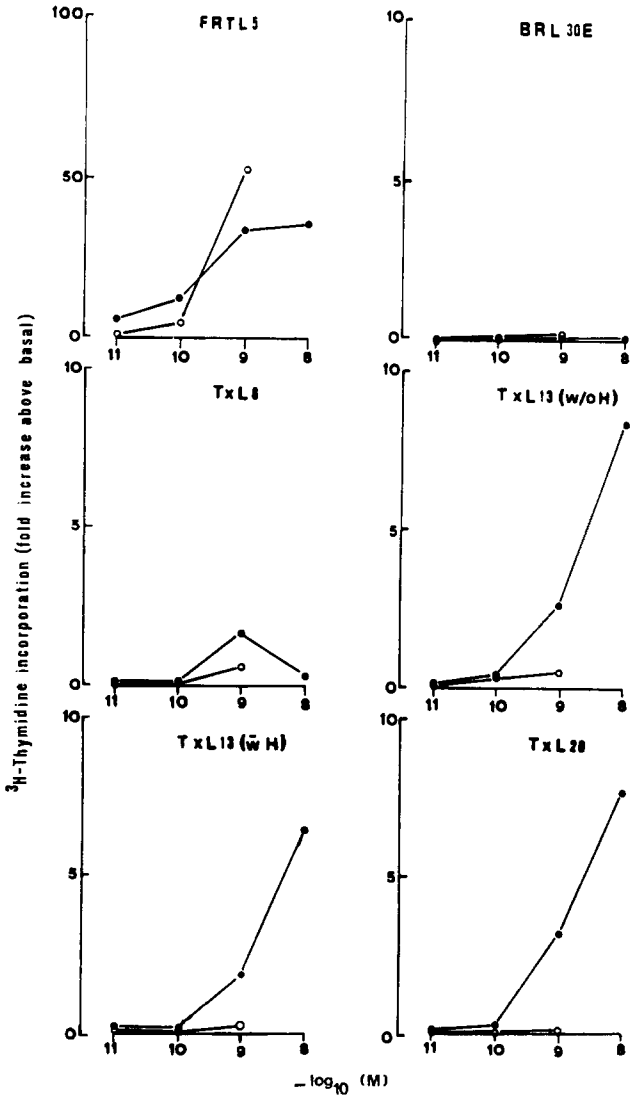

Figure 2. [^{3}H]Thymidine incorporation into DNA of parental and hybrid cells performed in mF12 culture medium supplemented with 0.1% BSA and TSH (○) or IGF-I (●). TxL13 (w/oH) clones have been selected and subcultured in HAT medium without hormones, while TxL13 (wH) clones have been selected and subcultured in HAT medium supplemented with 3H.

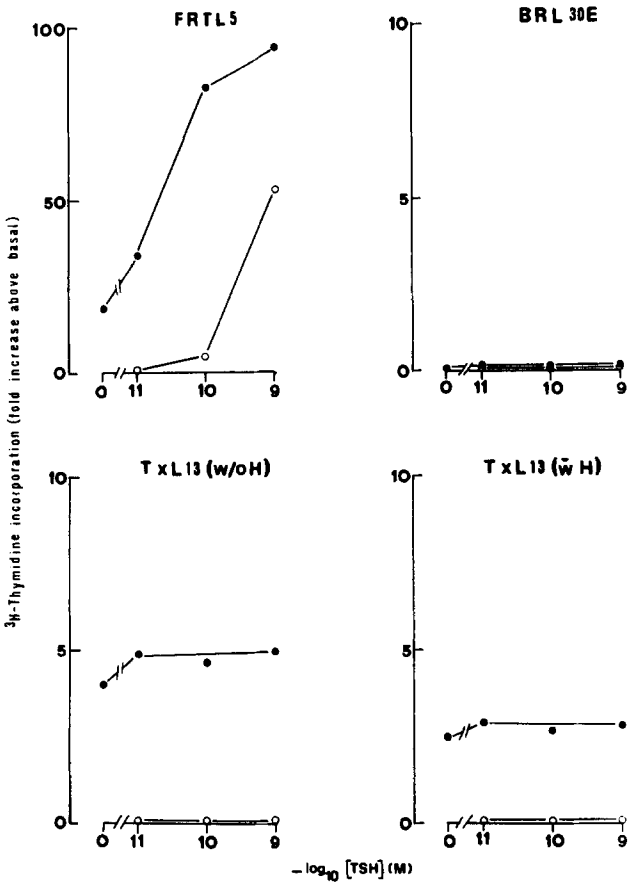

Figure 3. [^{3}H]thymidine incorporation into DNA of parental and hybrid cells performed in mF12 culture medium supplemented with 0.1% BSA and TSH alone (○) or in combination with 1.3 nM IGF-I (●).

is shown by the different scale on the *y* axis (Fig. 2), the magnitude of the stimulation of the [^{3}H]thymidine incorporation into DNA induced by IGF-I in the FRTL5 cells was by far greater than in the hybrid clones (Fig. 2). This difference can be ascribed to the high basal level of [^{3}H]thymidine incorporation observed in the hybrid cells (TxL6 80,601 ± 5,300; TxL13(w/oH) 17,574 ± 4,658; TxL13(wH) 11,444 ± 3,544; TxL20 6,975 ± 1,890; cpm mean ± SD of three separate experiments performed in triplicates) compared to the parental cells (FRTL5 257 ± 20; BRL 452 ± 39; cpm mean ± SD of three separate experiments performed in triplicates).

When cells were concomitantly treated with increasing concentrations of TSH and one single concentration of IGF-I, the expected synergistic effect induced by the two mitogens in the FRTL5 cells was not observed in the TxL cells, while the effect of IGF-I alone was maintained and unaffected by TSH (Fig. 3).

Binding of ^{125}I-TSH and ^{125}I-IGF-1 to TxL Cells

To ascertain whether the lack of effect of TSH on the proliferation of the TxL cells was due to a lack of receptors for TSH, steady-state and competition binding studies were performed (Fig. 4). ^{125}I-TSH binds the TxL cells with kinetic characteristics similar to that of the FRTL5 cells (Table I). Analysis of saturation studies revealed an upward concave configuration of the Scatchard plots (Scatchard, 1949), suggesting that TxL cells contain more than one binding site for TSH as do the thyroid parental cells. When the analyses were based on the assumption that two classes of binding sites were present and data were resolved in two linear functions, the affinities of the two sites differed widely as did their respective binding capacities. Specific binding of ^{125}I-TSH to the BRL cells could not be demonstrated. ^{125}I-IGF-I binds to specific membrane receptors of both the hybrid clones and the parental cells (Fig. 5). Scatchard analyses of saturation studies conducted on the hybrid clones and on the parental cells (Table II), revealed a single apparent binding site whose affinity is consistent with that of type 1 IGF receptor found in other tissues (Beguinot et al., 1985; Pilistine et al., 1984).

TSH-stimulated Production of cAMP in TxL Cells

Since the lack of mitogenic effect of TSH could not be ascribed to lack of TSH receptors, it has been investigated whether the TSH receptors present on the membrane of TxL cells were biologically active. In two separate experiments it was observed that the basal level of cAMP in the TxL cells was as low as that of quiescent FRTL5 cells, suggesting that in the hybrid cells the adenylate cyclase is not constitutively activated. More importantly, TSH did significantly increase the intracellular levels of cAMP into the hybrid cells in a dose-dependent fashion, although the increase of cAMP in the TxL cells was by far lower than that in the FRTL5 cells (Fig. 6).

Cytoskeletal Organization in Hybrid Cells

In FRTL5 cells, as in other thyroid cells (Westermark and Porter, 1982), TSH profoundly influences cell morphology and the organization of cytoskeletal elements (Tramontano et al., 1982). The latter effect is manifested by the depolymerization of actin filaments, and is mediated by cAMP (Tramontano et al., 1982; Westermark et al., 1983). As

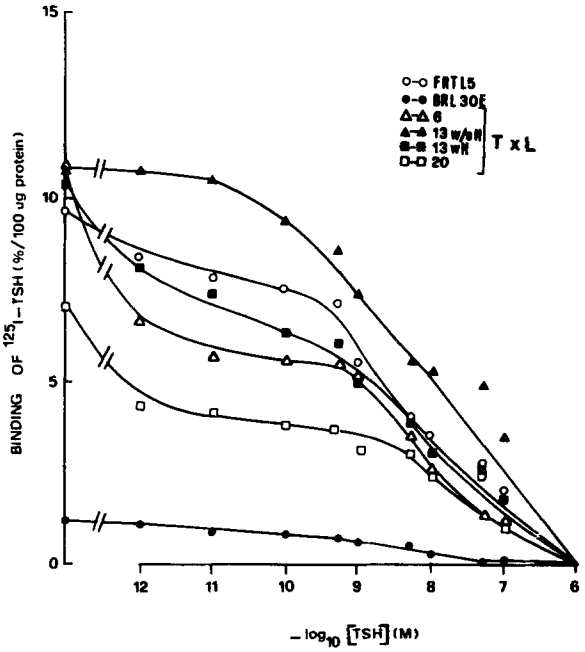

Figure 4. Steady-state binding of ^{125}I-TSH and competition study with unlabeled bTSH in parental and hybrids clones.

Table I. Kinetic Properties of the TSH Receptor in the Hybrids

	Low affinity binding sites		High affinity binding sites	
	K_a*	MBC‡	K_a	MBC
	M^{-1}	M	M^{-1}	M
FRTL5	1.0×10^{7}	3.9×10^{-9}	4.9×10^{8}	9.2×10^{-11}
TxL6	2.3×10^{7}	1.5×10^{-9}	1.5×10^{8}	1.8×10^{-10}
TxL13 (w/oH)	7.1×10^{6}	8.3×10^{-9}	5.0×10^{8}	1.1×10^{-10}
TxL13 (wH)	1.2×10^{7}	3.2×10^{-9}	5.6×10^{8}	7.3×10^{-11}
TxL20	8.1×10^{6}	3.4×10^{-9}	3.4×10^{8}	4.4×10^{-11}

Scatchard analysis of data shown in Fig. 4. The values reported are the mean of those obtained in three separate experiments. Standard deviation is < 2%.
* K_a, affinity constant.
‡ MBC, maximum binding capacity.

Table II. Kinetic Properties of the IGF-I Receptor in the Hybrids

	K_a*	MBC‡
	M^{-1}	M
BRL	1.4×10^{9}	9.3×10^{-11}
FRTL5	1.4×10^{9}	2.7×10^{-10}
TxL6	1.1×10^{9}	2.4×10^{-10}
TxL13 (w/oH)	1.4×10^{9}	1.5×10^{-10}
TxL13 (wH)	1.3×10^{9}	1.6×10^{-10}
TxL20	1.2×10^{9}	1.8×10^{-10}

Scatchard analysis of data shown in Fig. 5. The values are the mean of those obtained in three separate experiments. Standard deviation is > 2%.
* K_a, affinity constant.
‡ MBC, maximum binding capacity.

shown in Fig. 7 the addition of TSH (1 nM) to quiescent FRTL5 cells induced the expected disruption of microfilaments. In the BRL cells actin is organized in microfilaments spanning the whole cell and this pattern is not influenced by the addition of TSH (Fig. 7, *c* and *d*). The organization of the cytoskeletal elements in the hybrid clones was examined in basal conditions or under the stimulation of TSH. Only in clone TxL13(w/oH), where microfilaments were organized similarly to the FRTL5 cells, was the effect of TSH evident (Fig. 7, *e* and *f*).

As for the other hybrids (Fig. 8), clone TxL20 was composed of cells with no polymerized actin, clone TxL6-included cells with microfilaments and cells with depolymerized actin and clone TxL13(wH) displayed well defined microfilaments, and TSH did not influence cell morphology and cytoskeletal organization (data not shown).

Effect of Iodine on the Proliferation of TxL Clones

It is generally accepted that iodine transport into thyroid cells is stimulated by TSH via cAMP and that the inhibitory effects of iodine on thyroid cell functions is to be ascribed to its ability to inhibit adenylate cyclase system. On the other hand it has been recently reported that iodine inhibits thyroid cell growth stimulated by either cAMP dependent and cAMP independent pathways.

Since TSH stimulated increase of cAMP intracellular levels in the TxL clones, the effect of TSH on iodine uptake in the hybrid clones was studied. Cells were treated for 48 h with increasing concentrations of TSH or IGF-I and then ^{125}I uptake was measured. In the absence of TSH a small amount of iodine was present in all the cells tested. TSH failed to increase basal uptake of radiolabeled iodine in the TxL cells or in the liver parental cells at any concentration, whereas TSH stimulated a dose-dependent increase of iodine uptake in the FRTL5 cells. Finally, IGF-I did not influence iodine uptake in any of the cells tested (Table III).

Experiments have been performed to investigate whether

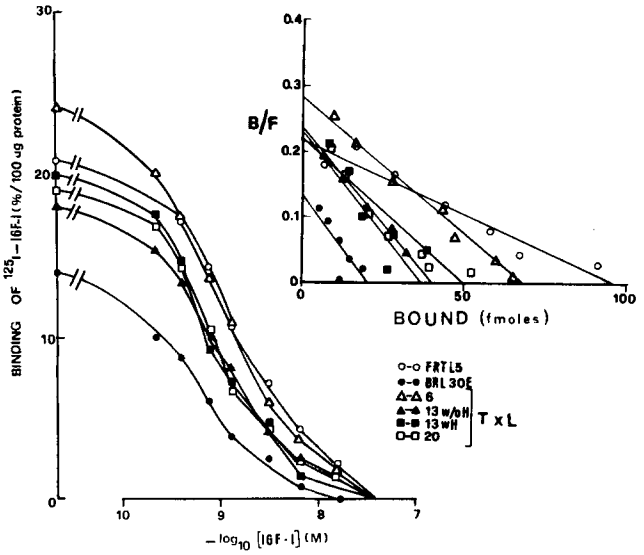

Figure 5. Steady-state binding of ^{125}I-IGF-I and competition study with unlabeled IFG-I in parental and hybrids clones. Inset on top, Scatchard plot; *B/F*, bound to free ratio.

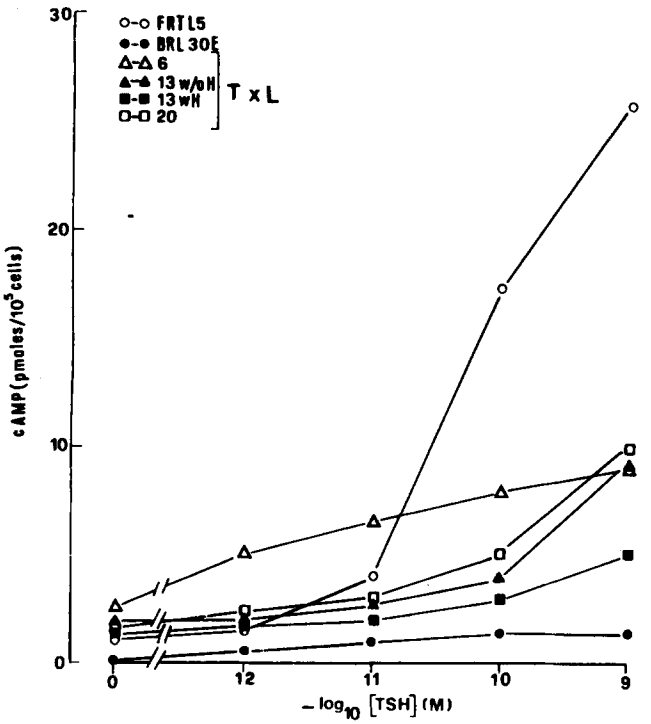

Figure 6. Effect of TSH on total cAMP production in parental and hybrid cells. SD ≤ 3% per each experimental point.

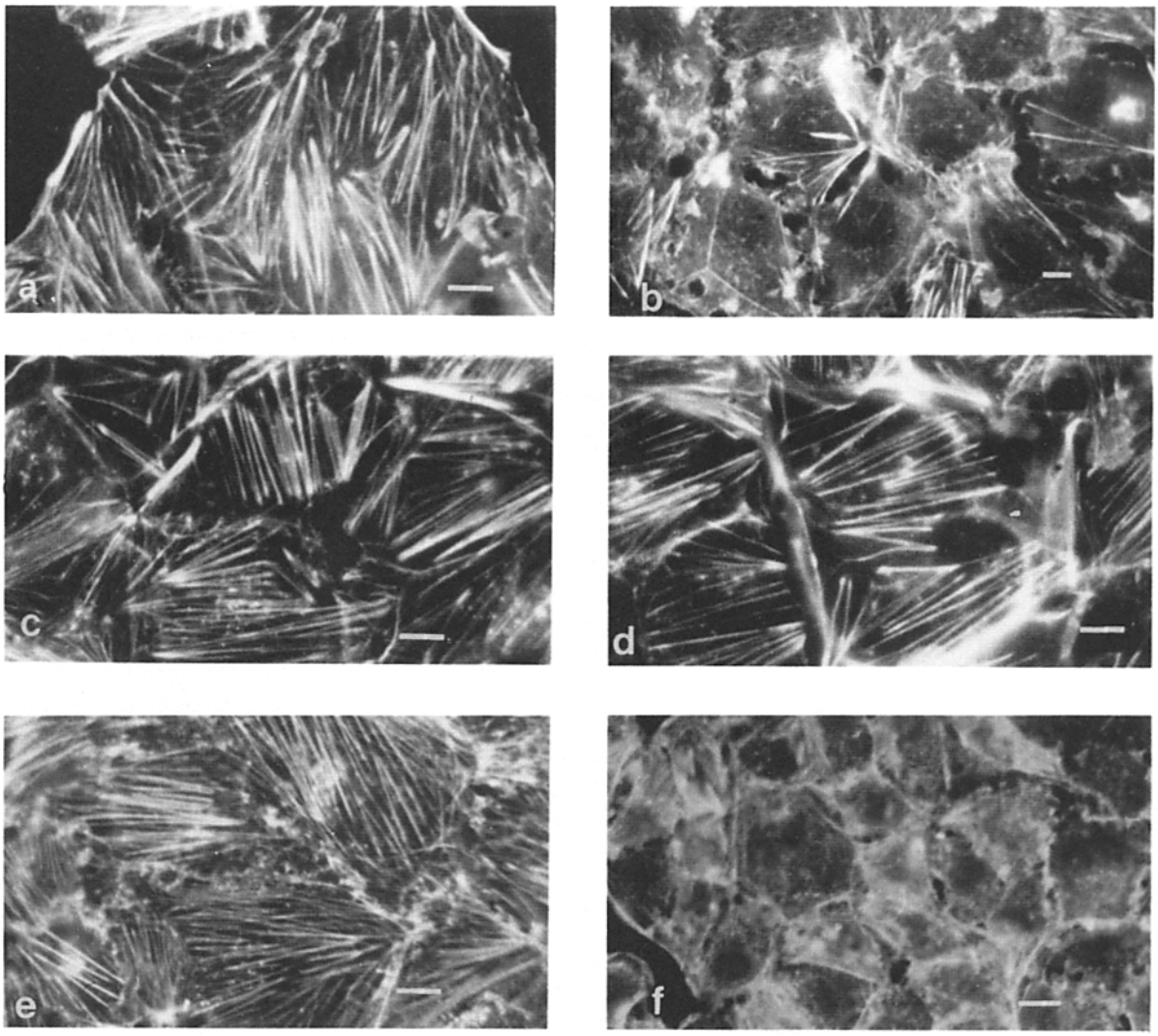

Figure 7. TSH effect on the organization of actin cytoskeleton in the FRTL5 and BRL parental cells and in the hybrid clone TxL13 (w/oH). Cells were cultured on glass coverslips, fixed, and stained with RITC-phalloidin. Actin filaments are prominent in untreated FRTL5 (*a*), BRL (*c*), and TxL13 (w/oH) (*e*) cells. Addition of TSH to the culture medium induced actin depolymerization in the FRTL5 parental cells (*b*) and in the hybrid cells (*f*), but not in the BRL parental cells (*d*). Bar, 10 μm.

the basal level of iodine present in the TxL cells was able to inhibit their proliferation. Hybrid clones and the parental cells were treated for 36 h with IGF-I (1.3 nM) in the presence or the absence of iodide (1 mM) (Fig. 9). As it did for the parental FRTL5 cells, iodine inhibited the incorporation of [^{3}H]thymidine into the DNA of the TxL clones stimulated by IGF-I, while it did not affect the synthesis of DNA of the liver parental cells.

Discussion

The relationship between TSH and IGFs and the inhibitory effect of iodine on the proliferation of thyroid cells is a distinctive "trait" of the thyrocytes and can be looked at as a differentiated function of thyroid follicular cells.

To continue along this line of reasoning, experiments have been performed to examine the question of whether the mechanisms regulating thyroid cells proliferation are so strictly intertwined that they must be inherited as a single functional property or they can be inherited independently. To address this question, somatic hybrid clones, TxL, derived by fusing FRTL5 cells and BRL cells, have been used. At first the proliferative response of TxL clones to calf serum and to TSH or IGF, alone or in combination, has been examined. TxL hybrid cells respond to the mitogenic stimulation of calf serum in that they vigorously proliferate in the presence of serum alone even at concentrations as low as 0.5%. These data indicate that the hybrid clones inherited from the liver parental the ability to grow in the presence of serum, without added hormones, while the thyroid parental are unable to do so.

When examined for their ability to respond to specific factors able to stimulate the proliferation of thyroid cells, the TxL clones showed a differential response to TSH and IGF. Thus IGF, unable to induce the proliferation of BRL cells, did stimulate the DNA synthesis in the hybrid clones in a fashion similar to that of FRTL5 cells. On the contrary, TSH

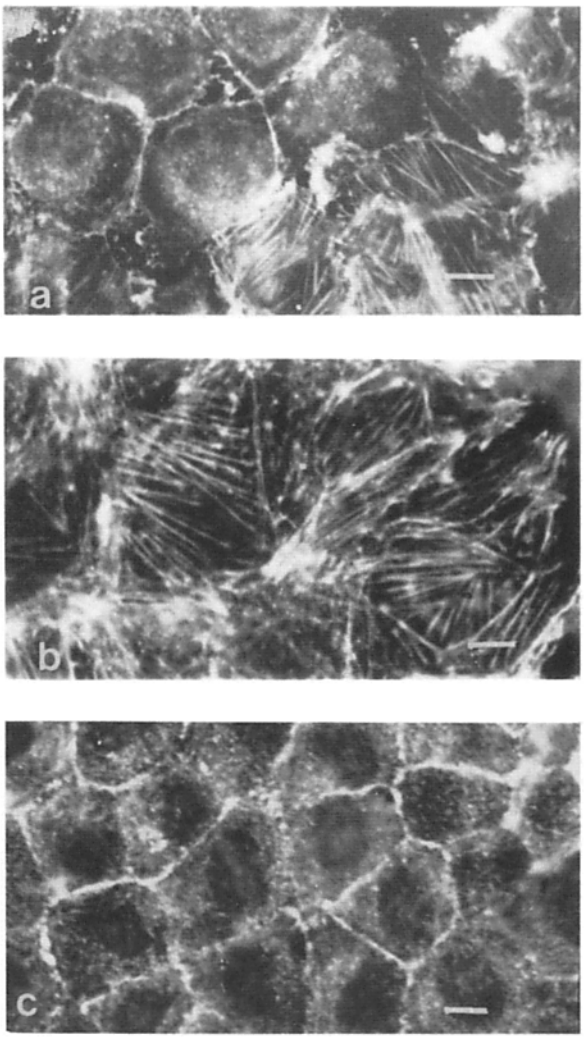

Figure 8. Actin staining in clones TxL6, TxL13 (wH), and TxL20. Clone TxL6 was composed of cells with no polymerized actin and cells containing microfilaments (*a*). In clone TxL13 (wH) cells contained well-developed microfilaments (*b*). In clone TxL20 all cells had no organized microfilaments (*c*). Bar, 10 μm.

was completely ineffective in promoting the proliferation of the hybrid cells, although TxL cells possess, on their plasma membrane, receptors for TSH. Steady-state binding studies and Scatchard analysis demonstrated that the TSH receptors present in the TxL cells were quantitatively and qualitatively similar to those of the FRTL5 cells. In addition, they were able to transduce the appropriate postreceptoral signal as it is demonstrated by the ability of TSH to increase intracellular levels of cAMP in the TxL cells.

Thus, the lack of response to the mitogenic effect of TSH in the TxL cells cannot be ascribed either to an alteration of the receptors for TSH or to a constitutive activation of the adenylate cyclase, since the basal level of cAMP present in the hybrid cell, actively proliferating, is similar to that found in quiescent FRTL5 cells.

Among other biological effects, TSH influences the organization of cytoskeletal elements in the thyroid cells of differing origin. When TSH is withdrawn from the culture medium, FRTL5 cells become quiescent and actin organizes into microfilaments; readdition of TSH restores growth and depolymerizes actin microfilaments and this action of TSH is mediated by cAMP. The hybrid clones are heterogeneous in respect to the organization of actin and, as for clones TxL6, TxL13w/H, and TxL20, TSH does not influence the actin pattern. Only clone TxL13w/oH, in the absence of TSH, presents actin organized in stress fibers that became depolymerized upon readdition of TSH. These results indicate that in the hybrid cells, independently from the pattern of actin presented, contrary to the FRTL5 cells, there is no correlation between growth and microfilament organization. Moreover, the increase of intracellular level of cAMP does not influence actin organization with the exception of clone 13w/oH that in turn, as the other hybrids, is independent from TSH for its proliferation. These results underline the genetic difference existing among the various TxL clones whose behavior is very similar when other parameters are analyzed. In addition they suggest that in various hybrid clones the cAMP dependent pathway can be interrupted at different steps along the way leading to the complete transduction of TSH signal as it is demonstrated by the observation that, although TSH increases intracellular levels of cAMP in all hybrid cells tested, it depolymerizes actin only in clone TxL13(w/oH).

As for proliferation, it cannot be excluded that TxL cells are independent from TSH because the TSH-induced pathway became constitutively activated downstream from the adenylate cyclase. In addition, since the TxL clones similarly to the BRL cells are able to proliferate even in the absence of serum, it cannot be excluded that autocrine factors are being produced and involved in the control of the proliferation. Preliminary observations from our laboratory indicate that the medium conditioned by the TxL clones stimulate [^{3}H]thymidine into the DNA of FRTL5 cells.

Studies on somatic cell hybrids have shown that the fusion of a cell expressing a specific differentiated function with a nonexpressing cell results in the loss of the specific function in the stable hybrid (Chin and Fournier, 1987; Davidson, 1974; Fourgere and Weiss, 1978; Killary and Fournier, 1984; Lincheseiner et al., 1987). In respect to thyroid functions, it has already been reported that the TxL clones lost the ability to produce thyroglobulin and to concentrate iodine (Ambesi-Impiombato et al., 1985), suggesting that they follow the general rule. However, the presence of a biologically active TSH receptor together with the ability of iodine to inhibit the proliferation of TxL clones and finally the ability of IGF-I to induce cell growth in the hybrid cells, all properties of the thyroid cells, indicate that the hybrid clones have inherited and maintained important markers of thyroid differentiation.

The data presented here indicate that in the FRTL5 cells, the two pathways leading to thyroid cell proliferation, one triggered by TSH and one triggered by IGF, are totally independent and they can be independently inherited. That is, the hybrid clones have inherited from the parental FRTL5 cells only the IGF-stimulated pathway but not the TSH-stimulated one. Since the growth pathway regulated by TSH is preferentially lost, an intrinsic difference between the pathway induced by TSH and that induced by IGF-I have to be supposed. In an hypothetical hierarchy of the pathways controlling cell proliferation, the TSH-dependent one represents, in the thyroid cells, the highest level of sophistication in the control

Table III. Iodide Trapping

	FRTL5	BRL	TxL6	TxL13 (w/oH)	TxL13 (wH)	TxL20
			pmol/mg protein/20 min			
Basal	125	60	75	47	67	140
TSH (0.01 nM)	1,522	60	75	62	62	180
TSH (0.1 nM)	5,000	70	100	65	80	110
TSH (1 nM)	4,200	50	100	35	52	180
TSH (10 nM)	3,000	110	67	37	37	130
IGF-I (0.01 nM)	120	62	80	48	92	120
IGF-I (0.1 nM)	110	57	95	52	65	120
IGF-I (1 nM)	180	47	65	47	12	110

Cells were incubated with TSH and IGF-I for 48 h. I^- uptake and protein concentration were measured as described in Materials and Methods. Values are the mean of triplicate samples per each experimental point; standard deviation is <5%.

of growth, in that it behaves as a "luxury function", and it is lost in hybrid clones. The IGF stimulated pathway behaves more like an "housekeeping" pathway and then it is retained by the hybrids.

Finally, it has already been reported (Becks et al., 1988; Tramontano et al., 1989) that iodine specifically inhibits thyroid cell proliferation stimulated by TSH and IGFs; it is to be noted that neither IGFs nor insulin are able to induce iodine uptake above basal level. Nevertheless, the iodine taken up by the FRTL5 cells in basal condition (i.e., in the absence of TSH) is sufficient to inhibit the IGF-I-stimulated FRTL5 cell proliferation. This effect of iodine, like other autoregulatory effects, is blocked by compounds with antithyroid activity, such as methimazole, that are inhibitors of thyroid peroxidase-catalyzed iodination. This observation suggests that the inhibitory effect of iodine, at least on thyroid cells proliferation, does not depend upon the concentration of iodine within the thyrocytes but upon iodine organification. Thus, the tissue specificity of the inhibitory effect of iodine, manifested by its failure to inhibit the growth of myoblasts or fibroblasts, may reflect a fundamental difference between the mitogenic pathways in the FRTL5 cells and those in the other cell types with respect to their sensitivity to iodine such as a failure of these cells to carry out the requisite organification (Tramontano et al., 1989). In this view, the observation that in hybrid cells iodine inhibits the IGF-stimulated growth but not the serum-stimulated one, the latter being inherited by the hybrid from the liver parental cells, suggests that iodine can inhibit only the thyroid-inherited arm of mitogenic regulation. In addition, it raises the intriguing possibility that in the thyroid cells, along the way leading to cell proliferation that is triggered by IGF-I, thyroid-specific signals are present and they may be the target of the inhibitory action of iodine.

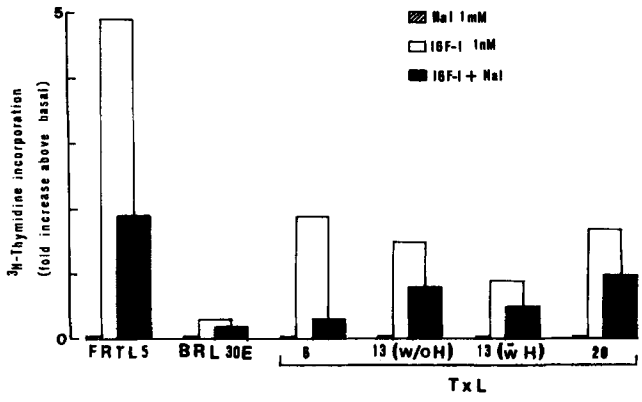

Figure 9. Effect of NaI (1 mM) on [^{3}H]thymidine incorporation into DNA of parental and hybrid cells. In the parental liver cells, IGF-I (1.3 nM) vs. IGF-I (1.3 nM) + NaI (1 mM), $P > 0.5$. In all other cells tested, IGF-I vs. IGF-I + NaI, $P < 0.001$.

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