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Low TSH Requirement and Goiter in Transgenic Mice Overexpressing IGF-I and IGF-I Receptor in the Thyroid Gland

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Through the cAMP signaling pathway, TSH stimulates thyroid follicular cell proliferation, differentiation, and function. Although the autocrine production of IGF-I in the thyroid gland suggests an important physiological function for this factor in these processes, the exact role of the IGF-I/IGF-I receptor system *in vivo* remains unclear. Although the mitogenic action of TSH requires the presence of IGF-I or insulin in primary culture of dog and human thyroid cells, IGF-I has an effect equal to and independent of the effect of TSH on cell proliferation in rat thyroid cell lines and may even be the main growth regulator in this case. To investigate the *in vivo* function of the IGF-I/IGF-I receptor system, transgenic mice overexpressing human IGF-I, IGF-I receptor, or both in the thyroid were generated. Adult transgenic mice did not present external signs of thyroid dysfunction, but mice overexpressing both transgenes had significantly increased gland weight and follicular lumen area. A decreased TSH level together with a slightly increased serum T_4 concentration and increased thyroidal iodine uptake were also observed, suggesting that IGF-I and IGF-I receptor stimulate thyroid function to some extent *in vivo*. (*Endocrinology* 142: 5131–5139, 2001)

THYROID FUNCTION AND growth are positively regulated by the pituitary thyrotrophs through TSH. In dogs and humans, the effects of TSH on gland growth are mediated by the adenylate cyclase-cAMP cascade (1). The functional effects are mediated by cAMP alone in dog thyroid cells. In human cells, although cAMP regulates iodide transport and thyroid hormone secretion, H_2O_2 generation, iodination, and the synthesis of thyroid hormones are mediated by Ca^{2+} and the TSH phosphatidylinositol biphosphate-PLC cascade (2). In *in vitro* models, thyroid cell proliferation is routinely studied in the presence of high insulin concentrations in the medium. In fact, insulin is required for the mitogenic action of TSH-cAMP in dog and human thyroid cells in primary culture. This is called the permissive action of insulin. When studied in more detail, it is apparent that the high concentrations of insulin activate both insulin receptors and IGF-I receptors (IGF-IR). The permissive effects of insulin are fully reproduced by low concentrations of IGF-I, and the effects of high insulin concentration are partially inhibited by anti-IGF-IR antibodies (3, 4). Beside its permissive action on TSH mitogenic effects, the IGF-I/IGF-IR system also stimulates Tg expression in dog and rat thyroid cells (5, 6). In FRTL-5 and PCCL3 rat thyroid cell lines, high insulin or IGF-I concentrations have an independent mitogenic effect that is only enhanced by TSH (7). If valid *in vivo*, this would predict a major role for IGF-I in thyroid growth and goitrogenesis. Moreover, autocrine thyroid production of IGF-I in response to TSH has been reported in different species both *in vitro* and *in vivo*, suggesting an important role for this factor in thyroid physiology (8–10). The IGF-I/IGF-IR system has a growth-promoting effect on many cell models, and *in vivo*, knockout mice for IGF-I or IGF-IR exhibit reduced fetal and postfetal growth (11). It is therefore of importance to determine the role of the IGF-I/IGF-IR system in thyroid function and growth *in vivo*. In acromegalic patients in which excess GH causes high IGF-I secretion, no hyperthyroidism is observed, and the thyroid seems to be increased in size, although the TSH level was sometimes reported to be decreased (12–14). We have now generated transgenic mice overexpressing in their thyroids human IGF-I, human IGF-IR, or both. The results show that activation of this system does not lead to hyperthyroidism, but loosens to some extent the dependence of the thyroid on TSH for its growth and function.

Abbreviations: IGF-IR, IGF-I receptor; IGFBP, IGF-binding protein; polyA, polyadenylation sequence; WT, wild-type.

Materials and Methods

Constructs

Human IGF-I cDNA was cloned by RT-PCR on cerebrum polyadenylated RNA isolated with the FastTrack kit (Invitrogen, San Diego, Ca). The following primers were used: forward, 5′-GAT CTG CAG GAT GCA CAC CAT GTC CTC CT-3′; and reverse, 5′-GGT AAC TCG AGC AGA GCA AA-3′.

After sequencing, the human IGF-I- and IGF-IR-coding regions were introduced in *Eco*RI *Bam*HI sites of the pSG5 plasmid containing the β-globin intron II and the simian virus 40 polyadenylation sequences (polyA). Then, a DNA fragment containing the intron, the cDNA, and the polyA was isolated from PSG5 and inserted in a pBluescript plasmid containing the bovine Tg promoter (15). Transgenes were extracted from the plasmid by *Xba*I-*Xho*I restriction.

Production of transgenic mice

Transgenic mice were generated as described by Hogan *et al.* (16) by microinjection of the excised construct into the pronucleus of fertilized eggs from superovulated FVB/N mice. Transgenic mice were screened by Southern blotting on DNA extracted from tail tips.

Histological procedures

Tissues samples were fixed for 18 h in 10% neutral-buffered formalin and embedded in paraffin by standard procedure. Sections (6 μm) were stained with hematoxylin and eosin. The area of each follicle was estimated by measuring the smaller inner diameter (d) and the larger inner diameter (D) of the follicle. The approximate follicular lumen area was calculated by the formula $A = \pi(d/2)(D/2)$. More than 100 follicles were measured in each thyroid gland. For each mouse, cell density per mm^2 was estimated by counting number of cells in 20 areas of 0.0373 mm^2, and follicle density was estimated by counting 10 areas of 0.1485 mm^2.

RT-PCR procedure

Polyadenylated RNA from thyroid glands of wild-type (WT) and transgenic mice were isolated using the FastTrack kit (Invitrogen). RT was performed according to standard procedure using random hexamer primers on 100 ng mRNA. cDNAs were amplified by PCR using the following primers, annealing temperatures, and cycles numbers: IGF-I transgene: forward, 5′-GGC TGC AGG AAT TCG ATA TC-3′; reverse, 5′-GCT GCA ATA AAC AAG TTC TG-3′ (58 C, 35 cycles); IGF-IR transgene: forward, 5′-GGC TGC AGG AAT TCG ATA TC-3′; reverse, 5′-CAC ATC GGC TTC TCC TCC AT-3′ (60 C, 35 cycles); hypoxanthine guanine phosphoribosyl transferase: forward, 5′-GTG ATT GGC GAT GAT GAA CCA G-3′; reverse, 5′-GGC TTT TCC ACT TTC GCT AAT G-3′ (55 C, 35 cycles); IGF-binding protein-1 (IGFBP-1): forward, 5′-GCC GTT CCT GAT TCT CCT GT-3′; reverse, 5′-ATA GGT GCT GAT GGC GTT CC-3′ (55 C, 35 cycles); IGFBP-2: forward, 5′-GTA CCT GTG AAA AGA GAC GC-3′; reverse, 5′-TGT CCG TTC AGA GAC ATC TTG-3′ (58 C, 28 cycles); IGFBP-3: forward, 5′-AGG CAG CCT AAG CAC CTA C-3′; reverse, 5′-TCT GCA CGC TGA GGC AAT G-3′ (58 C, 28 cycles); IGFBP-4: forward, 5′-GAC GAA GCC ATC CAC TGC CC-3′; reverse, 5′-CTG TTT GGG GTG GAA GTT GC-3′ (52 C, 35 cycles); IGFBP-5: forward, 5′-TTG CCT CAA CGA AAA GAG C-3′; reverse, 5′-TTC ATT CCG TAC TTG TCC ACA C-3′ (55 C, 28 cycles).

Western blotting analysis

Proteins (40 μg/lane) were separated by SDS-PAGE and transferred to nitrocellulose membranes for 16 h at 30 V and 4 C. Membranes were incubated in a blocking solution containing 0.5% BSA and 2% dry milk. Primary antibodies were rabbit polyclonal against human IGF-I (mouse cross-reactive) and human IGF-IR (mouse cross-reactive; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). After washing, peroxidase-labeled donkey antirabbit Igs were used to detect primary antibodies. Finally, blots were incubated with enhanced chemiluminescence reagents and autoradiographed.

Tests of thyroid function

Thyroidal radioiodide uptake was measured by counting (Packard 2900TR counter) whole thyroid glands 2 d after ip injection of 50 μCi Na ^{131}I. Blood samples were obtained by retroorbital puncture under ether anesthesia. Circulating TSH and T_4 hormones were measured in serum samples kept frozen at −70 C. T_4 was measured by double antibody precipitation RIA (Diagnostic Products, Los Angeles, CA) modified to measure T_4 in mouse serum with a sensitivity of 0.25 μg/dl. Mouse TSH was measured by a disequilibrium, heterologous RIA described in detail (17). The sensitivity of the assay is 5–10 mU/liter.

Methimazole treatment

Mice received a solution containing 0.2% methimazole (Sigma-Aldrich Corp.) and 10% glucose in drinking water. Treatment was conducted during 3 months.

Statistical analysis

Statistical significance was analyzed with unpaired *t* test excepted for Fig. 5D where the Mann-Whitney test was used.

Results

Production of IGF-I and IGF-IR transgenic mice

To overexpress IGF-I and IGF-IR specifically in the thyroid gland, two transgenes were constructed containing the thyroid specific promoter region of bovine Tg, followed by the second intron of the rabbit β-globin gene, the coding sequence of either the human IGF-I or the human IGF-IR and the polyadenylation signal of simian virus 40 large T (Fig. 1, A and D). Both transgenes were purified and injected separately into the pronucleus of one-cell FVB/N embryos. Three founders transgenic mice were identified to carry either the IGF-I or the IGF-IR transgenes by Southern blotting. Five of these founders transmitted the transgene to their progeny in a Mendelian fashion: three independent IGF-I (IGF-Iα, IGF-Iβ, and IGF-Iγ) and two independent IGF-IR transgenic lines (IGF-IRα and IGF-IRβ) were thus obtained. IGF-Iα and IGF-Iβ transgenic mice were bred, respectively, with IGF-IRα and IGF-IRβ transgenic mice to obtain double transgenic mice overexpressing both transgenes in the thyroid gland (IGF-I/IGF-IR transgenic mice).

The expression of the transgenes in the thyroid gland was confirmed at the mRNA and the protein levels. RT-PCR was performed on mRNA isolated from the thyroid glands of IGF-I or IGF-IR transgenic mice. Primers complementary to the transgene sequences on each side of the intron were designed allowing to distinguish between genomic amplification (1.4 kb for IGF-I and 1.45 kb for IGF-IR) and cDNA amplification (0.75 kb for IGF-I and 0.8 kb for IGF-IR). PCR amplifications corresponding to the cDNA sequence of IGF-I and IGF-IR transgenes were obtained with RNA isolated from the thyroid glands of IGF-I and IGF-IR mice, but not with RNA from nontransgenic mice (Fig. 1, B and E). No PCR fragment of a size corresponding to genomic amplification was ever seen, confirming the purity of the mRNA preparations.

Overexpression of the transgenes at the protein level was demonstrated by Western blotting performed on extracts from WT, IGF-I, IGF-IR, and IGF-I/IGF-IR transgenic thyroid glands using antibodies against human IGF-I or IGF-IR (Fig. 1, C and F, respectively).

The three types of transgenic animals (IGF-I, IGF-IR, and IGF-I/IGF-IR) showed no obvious abnormalities and a had normal life span. In particular, no visible goiter and no obvious signs of hyper- or hypothyroidism were seen; transgenic animals have normal body weight and normal behavioral activity, two characteristics that are expected to be impaired in severe thyroid disease.

Thyroid gland weight in IGF-I, IGF-IR, and IGF-I/IGF-IR transgenic mice

Thyroid gland weight was measured at different ages (Fig. 2A). In 7-, 12-, and 16-wk-old IGF-I and IGF-IR transgenic mice, no significant difference in thyroid gland weight was observed compared with WT mice. However, when both IGF-I and IGF-IR transgenes were expressed in the same mouse, the gland weight was significantly increased. Moreover, the thyroid gland of IGF-I/IGF-IR transgenic mice continues to enlarge, whereas in 12-wk-old control, IGF-I, and IGF-IR mice, growth slows down. The glands of 12- and

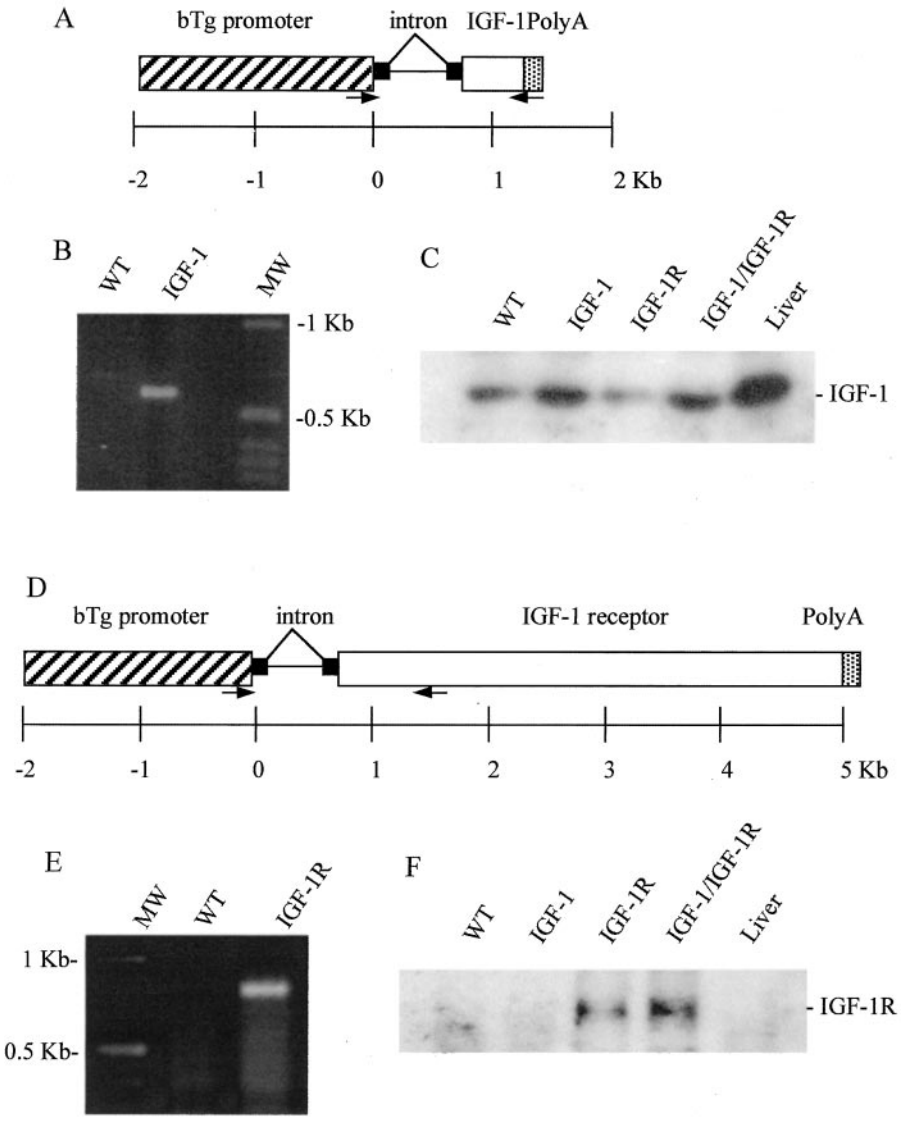

FIG. 1. Transgene structure and expression. A, Structure of the IGF-I transgene, comprising the bovine Tg (bTg) promoter, the rabbit β-globulin second intron (intron), the human IGF-I-coding sequence, and the simian virus 40 large T polyA. *Arrows* represent the primers designed for RT-PCR. B, RT-PCR analysis of mRNA extracted from WT and IGF-I thyroid glands. A 1-kb ladder was used as the mol wt marker (MW). C, Western blot analysis of proteins isolated from WT, IGF-I, IGF-IR, and IGF-I/IGF-IR thyroid glands and WT liver probed with a rabbit anti-IGF-I antibody. D, Structure of the IGF-IR transgene. E, RT-PCR analysis of mRNA extracted from WT and IGF-IR thyroid glands. F, Western blot analysis of proteins isolated from thyroid glands of WT, IGF-I, IGF-IR, and IGF-I/IGF-IR mice and from liver of WT mice probed with a rabbit anti-IGF-IR antibody.

16-wk-old IGF-IR transgenic mice showed a propensity to increase in size without reaching statistical significance compared with WT thyroid gland. The difference reached statistical significance only in 21-wk-old IGF-IR mice.

To increase the TSH-dependent thyroid stimulation, transgenic mice were submitted to treatment with the goitrogen methimazole, a drug that blocks thyroperoxidase, decreases thyroid hormone synthesis, and, in turn, increases TSH secretion. After 12 wk of methimazole treatment, thyroid gland weights were increased in all types of mice (control and transgenic) compared with their untreated controls, but IGF-I/IGF-IR mice conserved the same proportional increase in thyroid gland weights compared with WT mice (Figs. 2B and 3A).

Histological analysis of IGF-I, IGF-IR, and IGF-I/IGF-IR transgenic mice

Cross-section and hematoxylin/eosin staining of 7-, 12-, and 21-wk-old transgenic and WT thyroid gland revealed that the follicular structure was preserved, and the morphology of cells and nuclei was perfectly normal. Overexpression of IGF-I and/or IGF-IR in thyroid gland does not induce visible hyperplasia, and no signs of tumor induction were seen in response to IGF-I and/or IGF-IR overexpression at any age.

Electron microscope analysis of the transgenic thyroid glands confirmed the normal structure of the thyroid cells and the normal organization of the gland. Aspect and size of the nuclei were not different from those observed in WT mice, and abundance and structure of the different organelles were normal (data not shown). In 7-wk-old transgenic mice, the number of cells and follicles per mm^2 were in the normal range, and follicles sizes were not significantly different between control and transgenic mice (data not shown). However, in 16-wk-old IGF-I/IGF-IR mice, the average follicular lumen area was increased (Figs. 3, B and C, and 4A) and the number of cells per mm^2 was slightly decreased when compare to WT mice (Fig. 4B). The number of follicles per mm^2 in IGF-I/IGF-IR mice showed also a slight decrease compared with WT mice (Fig. 4C).

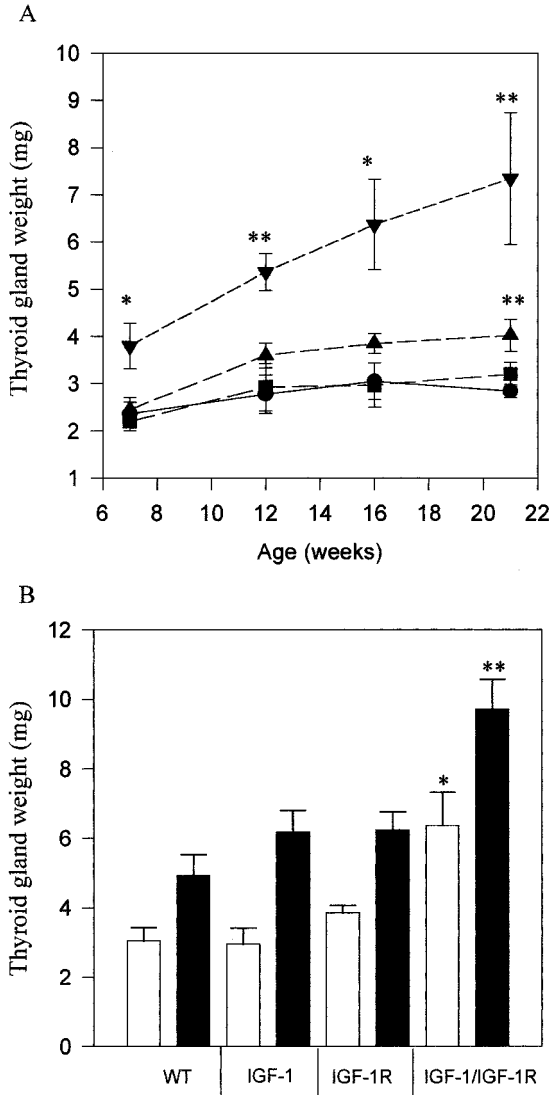

FIG. 2. Thyroid gland weight in transgenic mice as a function of age. ●, WT mice (n = 4–7); ■, IGF-I mice (n = 3–5); ▲, IGF-IR mice (n = 3–7); ▼, IGF-I/IGF-IR mice (n = 3–7). Values are the mean ± SEM. *Asterisks* indicate statistically significant differences compared with WT mice of the same age. *, $P \leq 0.03$; **, $P \leq 0{,}006$. B, Thyroid gland weight of 16-wk-old WT, IGF-I, IGF-IR, and IGF-I/IGF-IR mice receiving a normal diet (□) or a goitrogen diet (■). Values are the mean ± SEM. *Asterisks* indicate statistically significant differences compared to WT mice. *, $P \leq 0.02$; **, $P \leq 0.002$.

After methimazole treatment, the thyroid gland of WT, IGF-I, and IGF-IR mice showed a important reduction of the average follicular area with sometimes nearly complete disappearance of the follicular structure, as normally seen after such goitrogen treatment (Fig. 3, B and D, and data not shown). However, the thyroids of IGF-I/IGF-IR transgenic mice kept large follicular lumina despite goitrogen treatment compared with WT mice (Figs. 3, D and E, and 4A). Moreover, after methimazole treatment, the number of cells per mm^2 was lower in IGF-IR and IGF-I/IGF-IR mice compared with WT mice (Fig. 4B). The increased gland weight observed in these mice therefore does not correspond to a parallel increase in thyroid follicular cell density.

Thyroidal functions in IGF-I, IGF-IR, and IGF-I/IGF-IR transgenic mice

As thyroid gland weight was increased in IGF-I/IGF-IR mice, the presence of abnormal thyroid function was considered. Thyroidal radioiodide uptake 48 h after administration of the isotope was not significantly different in IGF-I and IGF-IR transgenic mice compared with that in WT mice, but was increased by a factor of 2 in IGF-I/IGF-IR transgenic mice (Fig. 5A).

IGF-I/IGF-IR mice showed thyroid enlargement and increased iodide uptake, suggesting that the regulation of thyroid hormone synthesis is impaired. Serum T_4 in 7-wk-old IGF-I and IGF-IR transgenic mice was not significantly different compared with that in WT mice, but IGF-I/IGF-IR transgenic mice had a mean T_4 level slightly, but significantly, higher than that in WT mice (Fig. 5B). Methimazole treatment reduced the serum T_4 concentration in all mice, but IGF-I/IGF-IR transgenic mice conserved a significantly higher T_4 level compared with WT mice receiving the same antithyroid treatment (Fig. 5B).

The serum TSH level is the most sensitive indicator of thyroid function state and therefore is required to understand the action of the IGF-I/IGF-IR system in our transgenic mice. The TSH concentration in 7-wk-old mice was significantly lower in IGF-IR and IGF-I/IGF-IR, compared with WT, mice (Fig. 5C). IGF-I transgenic mice also showed a propensity to decreased TSH level, but the mean value did not reach statistical significance. Serum TSH was also measured after 3 months of methimazole treatment (Fig. 5D). TSH levels increased in all types of mice during methimazole treatment. This increase was only moderate in IGF-IR and IGF-I/IGF-IR transgenic mice (in particular for IGF-I/IGF-IR mice) compared with WT mice.

IGFBP expression in IGF-I transgenic mice

IGFBP expression was previously shown to be modified in some transgenic animals in response to IGF-I overexpression (18, 19). As our IGF-I simple transgenic mice show no alteration in thyroid morphology and function, we investigated whether such a compensatory mechanism is present in their thyroid. Expression of IGFBP-1 to -5 in thyroid was investigated by semiquantitative RT-PCR on thyroid mRNA. No expression of IGFBP-1 and IGFBP-4 was detected in the mouse thyroid. IGFBP-2 and IGFBP-5 were expressed in the mouse thyroid, but in a similar way in IGF-I and WT mice (Fig. 6). However, IGFBP-3 expression was significantly reduced in the thyroids of IGF-I transgenic mice to 50% of the level observed in WT animals (Fig. 6). When RT-PCR analysis was performed on mRNA from IGF-I/IGF-IR double transgenic thyroid glands, a similar down-regulation of IGFBP-3 expression was also observed (data not shown).

Discussion

Results from *in vitro* studies of the role of IGF-I/IGF-IR system in thyroid follicular cell proliferation, differentiation, and function are still unclear or contradictory. They could represent a peculiarity of the immortalized thyroid cell lines or of the species used in the primary culture experiments. The

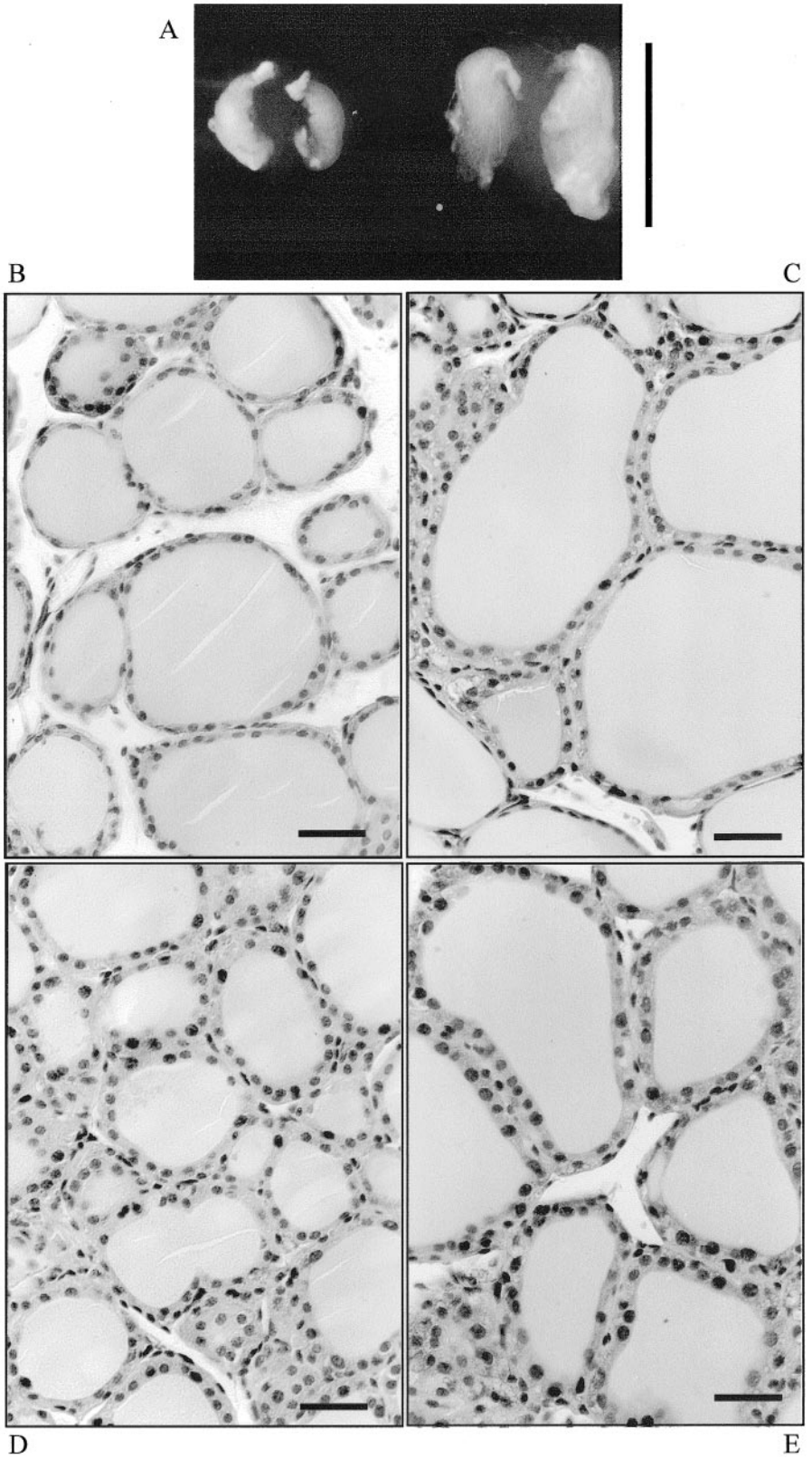

FIG. 3. Histological features of transgenic thyroids. A, Two thyroid lobes from WT mice (*left*) and IGF-I/IGF-IR transgenic mice (*right*) after treatment with methimazole (*scale bar*, 5 mm). Cross-section of thyroid gland from a WT (B) and a IGF-I/IGF-IR (C) mouse maintained on a normal diet and from a WT (D) and a IGF-I/IGF-IR (E) mouse receiving methimazole treatment. *Scale bars* in B, C, D, and E, 30 μm.

goal of this study was to analyze the role of the IGF-I/IGF-IR system in a more physiological way *in vivo* by generating and analyzing transgenic mice overexpressing IGF-I and/or its receptor specifically in thyroid follicular cells.

The expression of IGF-I and IGF-IR was successfully targeted to the thyroid glands of transgenic mice using the bovine Tg promoter. Transgene overexpression at the mRNA and protein levels was demonstrated by RT-PCR and by

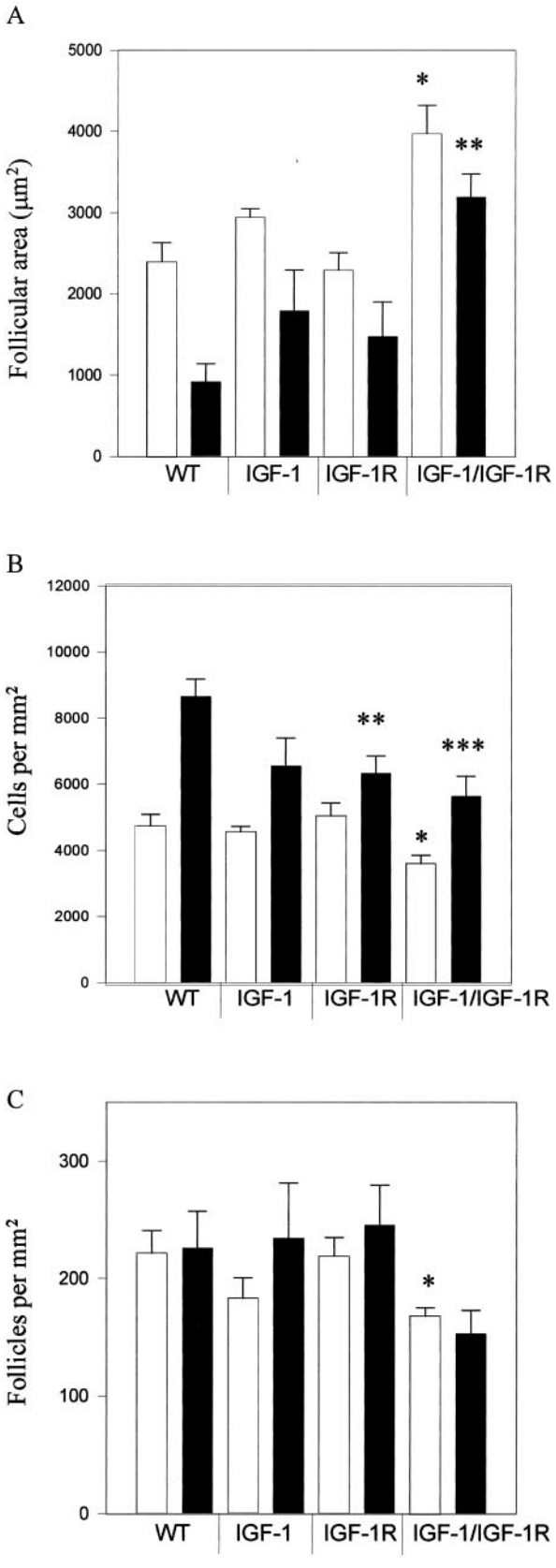

FIG. 4. Morphological parameters measured in thyroid glands of 16-wk-old WT, IGF-I, IGF-IR, and IGF-I/IGF-IR mice maintained on a normal diet (□; n = 3) and after methimazole treatment (■; WT, n = 4; IGF-I, n = 5; IGF-IR, n = 5; IGF-I/IGF-IR, n = 6). Values are the mean ± SEM. *Asterisks* represent statistical significance compared with WT mice in the same diet condition. A, Average follicular area in the different types of mice. *, $P \leq 0.02$; **, $P \leq 0.0005$. B, Cellular density. *, $P \leq 0.05$; **, $P \leq 0.02$; ***, $P \leq 0.008$. C, Follicular density. *, $P \leq 0.04$.

Western blotting, respectively. IGF-I and IGF-IR mice were crossed to obtain transgenic mice overexpressing both transgenes. None of the transgenic mice produced showed external signs of thyroid dysfunction, such as a visible goiter, or signs of hyper/hypothyroidism. The absence of obvious phenotype in these mice may result from the existence of compensatory mechanisms.

A first compensatory mechanism identified is the down-regulation of TSH in IGF-IR, IGF-I/IGF-IR, and most probably IGF-I mice. This indicates that IGF-I and IGF-IR stimulate thyroid function and overproduction of thyroid hormones, which, in turn, decreases pituitary TSH production by the classical negative feedback effect. Consequently, thyroid function is restored to its normal level. This is the classical response to overproduction of thyroid hormones and explains why in such conditions measurement of serum TSH is the most sensitive test of thyroid function. Indeed, in mild cases of hyperthyroidism, only a decrease in serum TSH is observed with little change in thyroid hormone level. Only in more severe disease do thyroid hormone levels increase. So the slightly increased T_4 level in IGF-I/IGF-IR (but not in simple transgenic mice) could reflect a more severe phenotype due to a synergic effect of both transgenes. Moreover, the thyroidal enhanced iodine uptake in IGF-I/IGF-IR transgenic mice also indicates that IGF-I/IGF-IR system stimulates thyroid functions. This increased function could be due to a direct effect of IGF-I on thyroid function or to an increased sensitivity of the thyroid to TSH in response to IGF-I.

A second compensatory mechanism may result from the modulation of IGFBP expression in transgenic thyroid cells, as previously seen in other transgenic models (18, 19). IGFBP-3, especially when cell associated, enhances IGF-I action in some cultured cells (20). IGFBP-3 also induces a tissue-specific organomegaly when ubiquitously overexpressed in transgenic mice (21). Thus, IGFBP-3 could enhance IGF-I action, and its reduced expression in IGF-I and IGF-I/IGF-IR transgenic mice may decrease the effects of the transgene on thyroid follicular cells.

These two compensatory mechanisms (TSH and IGFBP-3 down-regulation) may explain the normal thyroid gland structure, the absence of significant enlargement, and the lack of severe perturbations of thyroid function despite expression of the single IGF-I or IGF-IR transgene. The presence of a significantly enlarged thyroid in mice expressing both transgenes probably results from a synergic effect of IGF-I and IGF-IR. However, the consequence of this synergic effect is more an increase in colloid volume than an increase in cell number, and therefore does not reflect marked hyperplasia. Indeed, histological analysis showed an increased follicular lumen area in IGF-I/IGF-IR transgenic mice. Such an increased thyroid follicular area was also observed in mice overexpressing GH (22) and in hereditary dwarf mice treated with GH and/or TSH (23). This effect is probably due to

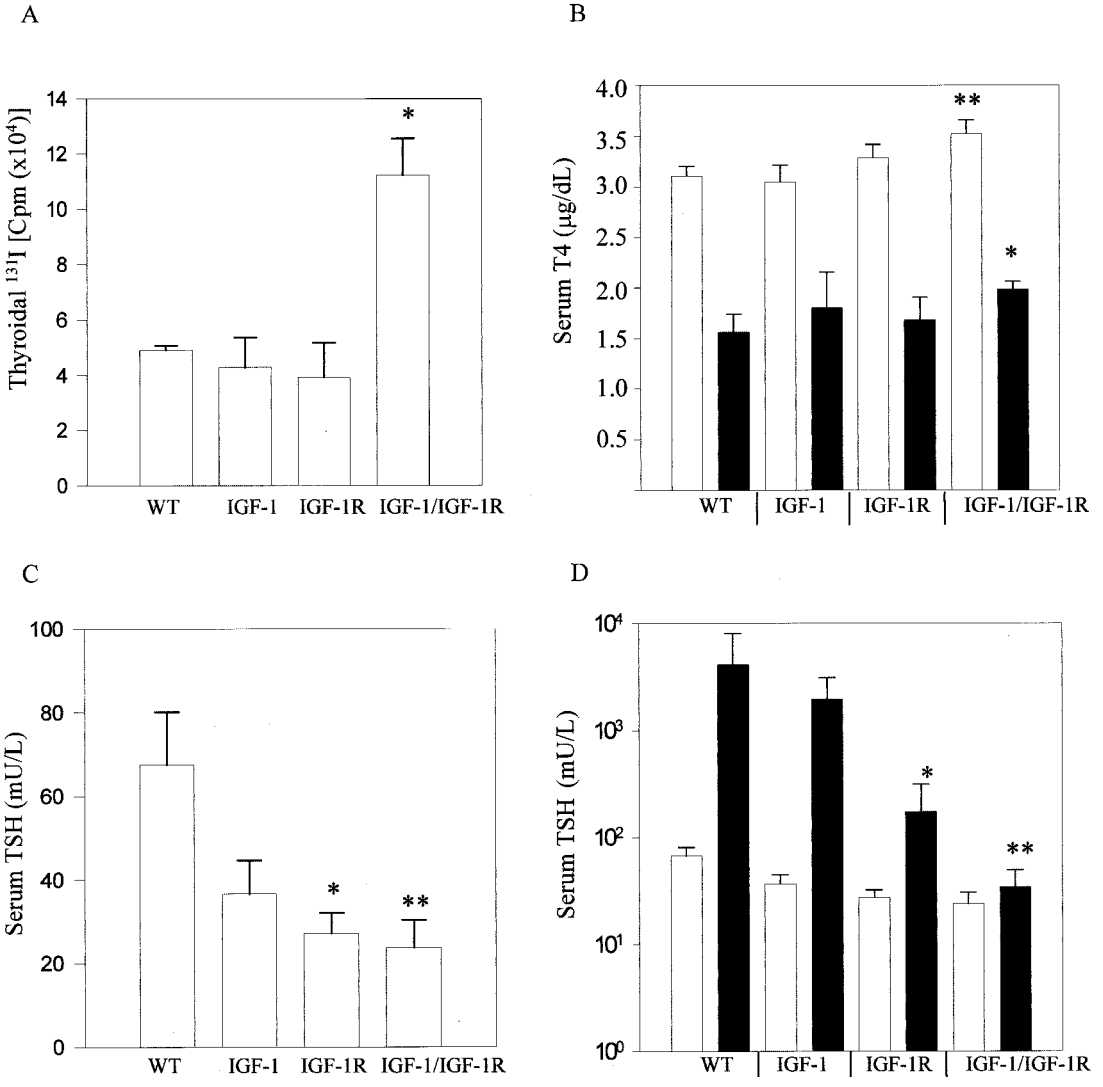

FIG. 5. Thyroidal function of control (WT), IGF-I, IGF-IR, and IGF-I/IGF-IR transgenic mice. Values are the mean ± SEM. A, Thyroidal ^{131}I uptake 2 d after injection of the isotope to 7-wk-old mice (one of two experiments is shown; n = 2). *, $P \leq 0.05$ compared with WT mice. B, Serum T_4 levels in mice maintained on a normal diet (□; n = 15–22) or after methimazole treatment (■; n = 5–9). *, $P \leq 0.05$; **, $P \leq 0.02$ (compared with WT mice). C, Serum TSH level in mice maintained on a normal diet (n = 11–17). *, $P \leq 0.02$; **, $P \leq 0.007$ (compared with WT mice). D, Serum TSH level in mice receiving a normal diet (□; n = 11–17) or after methimazole treatment (■; n = 5–9). *, $P \leq 0.02$; **, $P \leq 0.002$ (compared with WT mice).

increased Tg synthesis and accumulation in the follicular lumen. A similar IGF-I- or insulin-dependent Tg synthesis has also been demonstrated *in vitro* in FRTL-5 cells and dog thyroid cells (5, 6). Thus, the increased colloid volume could explain at least in part the increased thyroid gland weight found in IGF-I/IGF-IR transgenic mice.

Methimazole treatment of WT, IGF-I, and IGF-IR mice results in an important increase in TSH level. However, treatment of IGF-I/IGF-IR mice induced only a slight increase in TSH level compared with WT mice. This resistance to antithyroid drug action in double transgenic mice indicates that a much lower increase in TSH is necessary to achieve a similar relative increase in the weight and function of the thyroid.

In our transgenic animals, IGF-I could replace TSH to some extent, but TSH remains necessary for thyroid stimulation. Indeed, the TSH level is reduced, but not absent. This is in agreement with previous findings in patients with hypopituitarism showing deficiency in both TSH and GH and therefore in IGF-I. In these patients GH replacement, which presumably restores the normal IGF-I level, does not increase thyroid volume (24). A similar TSH requirement is observed in IGF-I/IGF-IR transgenic mice.

IGF-I (or insulin at supraphysiological concentrations) is required for the mitogenic action of TSH in dog and human thyroid cells in primary culture, but IGF-I alone has no mitogenic effect in these systems. In rat FRTL-5 and PCCL3 thyroid cells, however, IGF-I alone stimulates the proliferation to a similar extent as TSH (7). Our *in vivo* results offer new arguments that IGF-I *per se* has little mitogenic effect on the thyroid cell, but acts with TSH to control its proliferation. Indeed, thyroid weight in IGF-I/IGF-IR transgenic mice is moderately increased compared with that in mice with constitutive activation of the cAMP cascade in thyroid (25, 26).

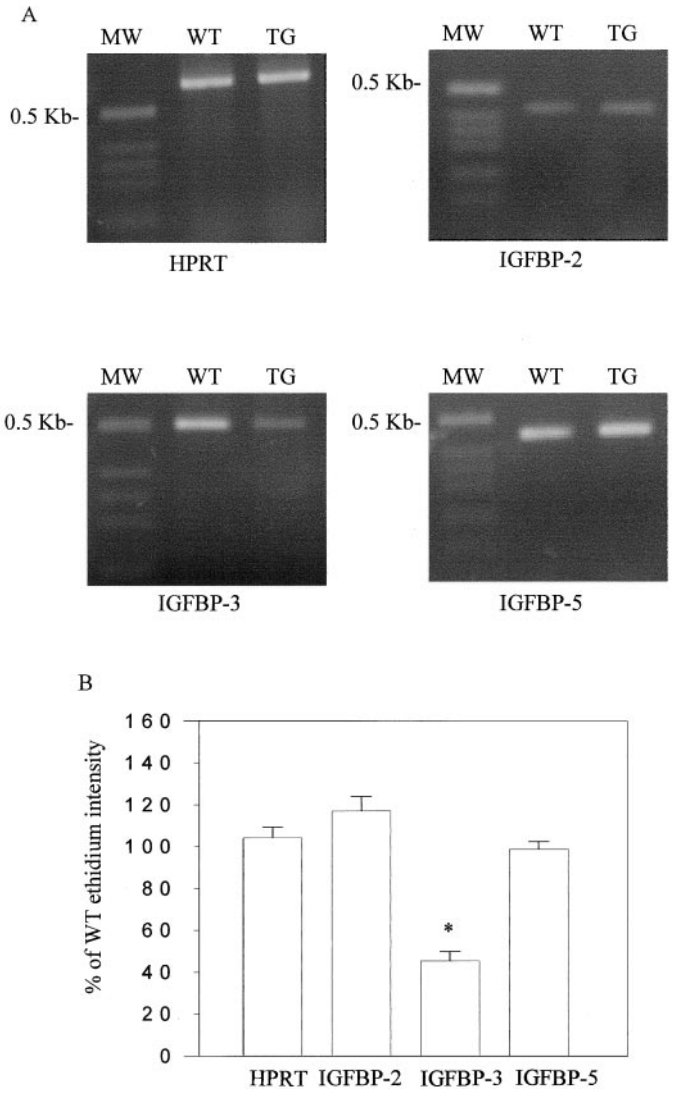

FIG. 6. IGFBP expression in WT and IGF-I thyroid gland. A, Ethidium bromide staining of a typical RT-PCR on thyroid polyA RNA from WT and IGF-I (TG) mice. Hypoxanthine-guanine phosphoribosyl transferase, IGFBP-2, IGFBP-3, and IGFBP-5 cDNAs were amplified using specific primers (see *Materials and Methods*). MW indicates the mol wt marker. B, Results of eight different RT-PCR experiments on mRNA isolated from WT and IGF-I thyroids. Results are expressed as a percentage of the WT intensity of staining measured with the Photocapt 99.01 program. Values are the mean ± SD. *, $P \leq 0.0004$ compared with HPRT relative intensity.

Even in IGF-I/IGF-IR transgenic mice, this increase largely reflects an increased colloid volume, rather than an increase in cell population. Thus, contrary to what could be expected from *in vitro* work on cell lines, but in agreement with work on primary cultures of dog and human thyroid cells, IGF-I has little mitogenic effect *per se* in vivo. Recent work on dog thyroid cells defining the different roles of IGF-I and TSH in the generation of the cyclin D3/CDK4 complex that triggers the mitogenic cascade may explain our findings. Indeed, although IGF-I provides the cell with the cyclin D3 necessary to form the cyclin D3/CDK4 complex, TSH activates this complex (27). Moreover, the comitogenic action of IGF-I and TSH is probably regulated by TSH itself, which stimulates the thyroidal autocrine production of IGF-I (8–10), making TSH the main physiological regulator of thyroid function and growth *in vivo*.

The finding that in our transgenic mice IGF-I/IGF-IR system enhances thyroid function and growth is reminiscent of previous observations made in acromegalic patients (12–14). Thyroid enlargement is present in a majority of patients with acromegaly. An inverse relationship between thyroid volume and TSH level has been reported in such patients, suggesting that thyroid enlargement develops independently of TSH or that GH and IGF-I hyperstimulation may result in partial thyroid autonomy. To explain the discrepancy between the frequent impairment of TSH response and the normal concentration of thyroid hormone, Yoshinari *et al.* (28) suggested that the high IGF-I levels found in acromegaly may have a direct stimulatory effect on the secretion of T_4, independently of TSH action. Our double transgenic mice also present a thyroid enlargement and reduced TSH levels. The later abnormality probably results from the stimulation of thyroid hormone synthesis by the IGF-I/IGF-IR system, as suggested by the slight increase in T_4 levels in these mice. Our IGF-I/IGF-IR transgenic mice may thus represent a model of thyroid problems in acromegalic patients.

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References

1. **Dumont JE, Lamy F, Roger P, Maenhaut C** 1992 Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and others factors. Physiol Rev 72:667–689
2. **Vassart G, Dumont JE** 1992 The thyrotropin receptor and the regulation of thyrocyte function and growth. Endocr Rev 13:596–611
3. **Burikhanov R, Coulonval K, Pirson I, Lamy F, Dumont JE, Roger P** 1996 Thyrotropin via cyclic AMP induces insulin receptor expression and insulin co-stimulation of growth and amplifies insulin and insulin-like growth factor signaling pathways in dog thyroid epithelial cells. J Biol Chem 271:29400–29406
4. **Deleu S, Pirson I, Coulonval K, Drouin A, Taton M, Clermont F, Roger PP, Nakamura T, Dumont JE, Maenhaut C** 1999 IGF-I or insulin, and the TSH cyclic AMP cascade separately control dog and human thyroid cell growth and DNA synthesis, and complement each other in inducing mitogenesis. Mol Cell Endocrinol 149:41–51
5. **Santisteban P, Kohn LD, Di Lauro R** 1987 Thyroglobulin gene expression is regulated by insulin and insulin-like growth factor I, as well as thyrotropin, in FRTL-5 thyroid cells. J Biol Chem 262:4048–4052
6. **Polh V, Roger PP, Christophe D, Pattyn G, Vassart G Dumont JE** 1990 Differentiation expression during proliferative activity induced through different pathways: in situ hybridization study of thyroglobulin gene expression in thyroid epithelial cells. J Cell Biol 111:663–672
7. **Kimura T, Dumont JE, Fusco A, Golstein J** 1999 Insulin and TSH promote growth in size of PC CI3 rat thyroid cells, possibly via a pathway different from DNA synthesis: comparison with FRTL-5 cells. Eur J Endocrinol 140:94–103

8. **Thomas GA, Davies HG, Williams ED** 1994 Site of production of IGF-I in the normal and stimulated mouse thyroid. J Pathol 173:355–360
9. **Hofbauer LC, Rafferzeder M, Janssen OE, Gärtner R** 1995 Insulin-like growth factor-1 messenger ribonucleic acid expression in porcine thyroid follicles is regulated by thyrotropin and iodine. Eur J Endocrinol 132:605–610
10. **Tode B, Serio M, Rotella CM, Galli G, Franceschelli F, Tanini A, Toccafondi R** 1989 Insulin-like growth factor-1: autocrine secretion by human thyroid follicular cells in primary culture. J Clin Endocrinol Metab 69:639–647
11. **Baserga R, Sell C, Porcu P, Rubin M** 1994 The role of the IGF-I receptor in the growth and transformation of mammalian cells. Cell Proliferat 27:63–71
12. **Miyakawa M, Saji M, Tsushima T, Wakai K, Shizume K** 1988 Thyroid volume and serum thyroglobulin levels in patients with acromegaly: correlation with plasma insulin-like growth factor I levels. J Clin Endocrinol Metab 67:973–978
13. **Kasagi K, Shimatsu A, Miyamoto S, Misaki T, Sakahara H, Konishi J** 1999 Goiter associated with accromegaly: sonographic and scintigraphic findings of the thyroid gland. Thyroid 9:791–796
14. **Cannavo S, Squadrito S, Finocchiaro MD, Curto L, Almoto B, Vieni A, Trimarchi F** 2000 Goiter and impairment of thyroid function in acromegalic patients: basal evaluation and follow-up. Horm Metab Res 32:190–195
15. **Ledent C, Parmentier M, Vassart G** 1990 Tissue-specific expression and methylation of a thyroglobulin-chloramphenicol acetyltransferase fusion gene in transgenic mice. Proc Natl Acad Sci USA 87:6176–6180
16. **Hogan BLM, Costantini F, Lacy E** 1986 Manipulating the mouse embryo: a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory
17. **Pohlenz J, Maqueen A, Cua K, Weiss RE, Van Sande J, Refetoff S** 1999 Improved radioimmunoassay for measurement of mouse thyrotropin in serum: strain differences in thyrotropin concentration and thyrotroph sensitivity to thyroid hormone. Thyroid 9:1265–1271
18. **Wolf E, Jehle PM, Weber MM, Sauerwein H, Daxenberger A, Breier BH, Besenfelder U, Frenyo L, Brem G** 1997 Human insulin-like growth factor 1 (IGF-I) produced in the mammary glands of transgenic rabbits: yield, receptor binding, mitogenic activity and effects on IGF-binding proteins. Endocrinology 138:307–313
19. **Dyck MK, Ouellet M, Gagn M, Petitclerc D, Sirard MA, Pothier F** 1999 Testes-specific transgene expression in insulin-like growth factor-1 transgenic mice. Mol Reprod Dev 54:32–42
20. **Baxter RC** 2000 Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. Am J Physiol 278:E967–E976
21. **Murphy LJ, Rajkumar K, Molnar P** 1995 Phenotypic manifestations of insulin-like growth factor binding protein-1 (IGFBP-1) and IGFBP-3 overexpression in transgenic mice. Prog Growth Factor Res 6:425–432
22. **Mayerhofer A, Easterly S, Amador AG, Gher J, Bartke A, Yun J, Wagner TE** 1990 Studies of the thyroid in transgenic mice expressing the genes for human and bovine growth hormone. Experientia 46:1043–1046
23. **Denef JF, Cordier AC, Haumont S, Beckers C** 1980 The influence of thyrotropin and growth hormone on the thyroid gland in the hereditary dwarf mouse: a morphometric study. Endocrinology 107:1249–1257
24. **Cheung NW, Lou JC, Boyages SC** 1996 Growth Hormone does not increase thyroid size in the absence of thyrotropin: a study in adults with hypopituitarism. J Clin Endocrinol Metab 81:1179–1183
25. **Ledent C, Dumont JE, Vassart G, Parmentier M** 1992 Thyroid expression of an A2 adenosine receptor transgene induces thyroid hyperplasia and hyperthyroidism. EMBO J 11:537–542
26. **Zeiger MA, Saji M, Gusev Y, Westra WH, Takiyama Y, Dooley WC, Kohn LD, Levine MA** 1997 Thyroid-specific expression of cholera toxin A1 subunit causes thyroid hyperplasia and hyperthyroidism in transgenic mice. Endocrinology 138:3133–3140
27. **Van Keymeulen A, Bartek J, Dumont JE, Roger PP** 1999 Cyclin D3 accumulation and activity integrate and rank the comitogenic pathways of thyrotropin and insulin in thyrocytes in primary culture. Oncogene 18:7351–7359
28. **Yoshinari M, Tokuyama T, Kuroda T, Sato K, Okazawa K, Mizokami T, Okamura K, Fujishima M** 1992 Preserved thyroidal secretion of thyroxine in acromegalic patients with suppressed hypophyseal secretion of thyrotropin. Clin Endocrinol (Oxf) 36:355–360