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Growth factors controlling the thyroid gland

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PHYSIOLOGICAL CONTROL OF CELL PROLIFERATION IN THYROID TISSUE IN VIVO

The thyroid tissue is mainly composed of thyroid follicular cells, the thyrocytes (70%), arranged in follicles, and of their supporting mesenchymal tissue and cells, the endothelial cells of the capillaries (20%) and fibroblasts (10%). Scarce calcitonin-secreting parafollicular cells are located at the periphery of the follicles. After its differentiation in the fetus, the tissue grows roughly in parallel with body weight and remains at the same size throughout adult life. As the fetal thyroid weighs about 0.2 g at 20–25 weeks in the fetus and 20–30 g in the adult, and assuming a grossly similar tissue composition, this growth requires at least 6–7 cell divisions. Until 3 years ago, it was still unknown whether the stationary state of the adult human thyroid reflected any cell turnover or not. It had been argued that thyroid cells might not divide any more in adulthood. Of course, it was well accepted that submitted to chronic stimulation in adults the thyrocytes would multiply, i.e. that they had retained the capacity to proliferate (Doniach, 1960). We have shown that human thyroid cells divide about five times in adulthood, which demonstrates that there is a constant, albeit slow, turnover of these cells, with cell division and cell death compensating each other. It is striking that, when corrected for the life of the animal, the evaluated cell turnover is about five in adulthood for man and for animals as different as the dog, rat and mouse (Coclet et al, 1989). The calculation of the cell turnover in the whole gland does not depend on any assumption about the homogeneity of the cell population. However, if only a fraction f of the population was involved in the turnover (e.g. $f = 0.2$) (Smeds et al, 1987), the number n of possible divisions in these replicating cells (life span) would be higher ($n = 5/f$).

Although, under constant conditions, the thyroid maintains its size with a slow cell turnover, in adults it retains the capacity to grow by cell hypertrophy and proliferation in response to a stimulus. The size and function of

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the thyroid are controlled by a physiological negative-feedback mechanism: the thyroid cell secretes thyroid hormones which inhibit the secretion by pituitary thyrotrophs of thyrotropin (TSH), the thyroid-stimulating hormone. Whenever thyroid hormone secretion decreases, as in iodine metabolism defects, iodine deficiency, or after goitrogen or antithyroid drug administration, TSH secretion increases, causing an activation of thyroid function and growth (Doniach, 1960; Dumont, 1971).

In vivo growth, as induced by goitrogen administration in rats, is followed by a progressive increase in thyroid weight which reaches a plateau after 3 months (at 12 times the original volume). In terms of relative components of the tissue, it first involves a fall in follicular lumen space and non-vascular stroma, a rise in epithelial cells and blood vessel space with no further changes after 7–10 days (Wynford-Thomas et al, 1982a). The proliferation of endothelial cells precedes that of the thyrocytes (Many et al, 1984). Capillary vascularization varies more than follicular cell mass with the level of thyroid stimulation (Wollman et al, 1978; Smeds and Wollman, 1983; Imada et al, 1986). Global increase in epithelial cell space is due to cell hypertrophy and cell multiplication; it is accompanied by folliculogenesis (Denef et al, 1981, 1989). The thyroid capsule also grows (Wollman and Herveg, 1978).

The fact that growth stops after 3 months despite persistent high levels of TSH suggests an inherent limitation in the cells or, more simply, a desensitization at any level of the cells to the effect of TSH. The mitogenic effect of wounding in such tissue shows that the desensitization is TSH-specific (Wynford-Thomas et al, 1983, 1985). As the mitotic rate at the plateau level remains elevated, the cell turnover itself has remained higher than in control tissue. However, if goitrogen is withdrawn from the diet for 3 weeks, bringing back TSH levels to normal, the mitotic rate returns to normal and the follicular cell number slightly decreases. Restimulation by goitrogens brings back all parameters to their value before withdrawal. This does not fit in with a simple TSH desensitization mechanism, which should be fully reversible (Wynford-Thomas et al, 1982b). Other effects of TSH are not desensitized, which shows that the desensitization affects only the growth-promoting action of TSH (Wynford-Thomas et al, 1982a; Stringer et al, 1985). The effect is observed in cultured cells from the treated animals, which excludes the hypothesis of a chalone (Stringer et al, 1985). It suggests an inherent, albeit relative, limitation of the growth potential of thyrocytes as of other cells (Goldstein, 1990). Similarly, under constant stimulation after partial thyroidectomy rat thyroids do not recover their normal size (Doniach, 1960). A similar conclusion is drawn from the growth potential in vivo, as evaluated from the number of multifollicular clusters developing after inoculation of 1000 viable cells in a transplanted rat (Mulkahi et al, 1980). Similar results are also obtained in cell cultures in vitro (P. P. Roger and M. Baptist, unpublished results).

The mechanism of co-ordination between parenchymal and stromal cells during growth is unknown, as is the mechanism of endothelial proliferation in thyroid capillaries, in the proximal part of veins and lymphatics, and in the distal segment of thyroid arteries (Smeds and Wollman, 1983; Many et al,

Table 1. Hormones and growth factors reportedly secreted by thyrocytes.

Growth factor	System	Secretion ↗	Secretion ↘	No effect	References
IGF-I	Human			TSH	1
IGF-I, IGF-II	Ovine	GH			2
IGF-II	FRTL-5 line				3
FGF	Porcine		TSH		4
TGF-β	Human		I deficiency		5
	FRTL-5	TSH			6
Glucagon	Human				7
Atrionatriuretic hormone	Human	Epinephrine			8
Interleukin-6	Human	TSH Interferon TNF	Methylmercaptoimidazole propylthiouracil		9

1, Ollis et al (1989); 2, Bachrach et al (1988); 3, Maciel et al (1988); 4, Greil et al (1989); 5, Grubeck-Loebenstein et al (1989); 6, Moris et al (1988); 7, Menendez-Patterson and Blasquez (1984); 8, Sellitti and Hughes (1990); 9, Weetman et al (1990).

1984; Connors et al, 1988). It implies cross-signalling between these cells. The thyrocyte is the obvious site of control of the information about TSH concentration and action, iodine supply and metabolism, and thyroid hormone formation. Presumably, it is the thyrocyte that must co-ordinate the response of the other cell populations of the thyroid (Goodman and Rone, 1987; Denef et al, 1989). This is also suggested by the induction by grafted donor thyrocytes of angiogenesis from cells of the recipient nude mice (Mölne et al, 1987). The intercellular paracrine signals generated by the follicular cells are not known, although several potential candidate molecules are or could be postulated to be secreted by thyroid cells in culture: insulin-like growth factor I (IGF-I) (Bachrach et al, 1988), IGF-II (Maciel et al, 1988), plasminogen activator (Mak et al, 1984), nitric oxide (NO) (Moncada et al, 1989), fibroblast growth factor (FGF) (Greil et al, 1989), adenosine (Adair et al, 1990), etc. (Table 1).

PHYSIOLOGICAL AND PATHOLOGICAL EXTRACELLULAR SIGNALS INVOLVED IN THE CONTROL OF PROLIFERATION OF THYROID CELLS

Whereas compensatory hypertrophy after thyroidectomy and goitrous hyperplasia in iodine deficiency or after goitrogens administration are caused by the operation of the classical thyroid hormone–pituitary–TSH feedback and thus prevented by thyroid hormone treatment, other controls of thyroid cell prolifération exist (Doniach, 1960).

In the embryo the thyroid differentiates and develops before TSH secretion and even in anencephalic, i.e. hypopituitary, fetuses (Jost, 1953; Stranicky and Mess, 1967; Hilfer and Searl, 1980). Similarly, a lesion of the thyroid (wound or cell death and necrosis) provokes an important local wave of cell divisions (Stringer et al, 1983) even in thyroids desensitized to TSH action (Wynford-Thomas et al, 1985). Growth occurs in some cases (iodine treatment after iodine deficiency) when TSH levels are actually decreasing or in other cases in the absence of TSH (Rognoni et al, 1987). Compensatory hyperplasia in hemithyroidectomized mice also takes place, though at a reduced level, in dwarf mice which have a hereditary lack of GH, prolactin and TSH and in hypophysectomized animals (Denef et al, 1980; Lewinski, 1981; Lewinski et al, 1983). These growth processes are obviously independent from pituitary control.

Iodine deficiency in hypophysectomized animals also induces some thyroid growth. Iodine, as such, thus exerts a negative endogenous control on thyrocyte growth. Moreover, thyroids from iodine-deficient animals are more sensitive to TSH. This effect partly accounts for the goitrogenic action of antithyroid drugs in vivo (Stübner et al, 1987). This inhibitory effect of iodide has been reproduced in FRTL-5 cells (Saji et al, 1988). An iodolactone has been proposed as a putative intermediate in iodide action, as it inhibits the proliferation of porcine thyroid cells (Dugrillon et al, 1990). However, as these cells require exogenous arachidonate to synthesize the iodinated derivative, the role of iodolactone remains debatable.

In general, however, the major element controlling thyroid growth in vivo is the level of TSH (Dumont, 1971). Proliferation as evaluated by mitotic activity in young rats follows TSH levels:

1. It increases by a factor of 5 in goitrogen-treated rats (TSH × 54) (Wynford-Thomas et al, 1982a).
2. It follows the circadian rhythm of TSH (Wynford-Thomas et al, 1982c).
3. Its circadian rhythm disappears with the TSH rhythm in goitrogen-treated rats (Wynford-Thomas, 1982c). The growth of thyroid grafts in recipient animals greatly increases in thyroidectomized animals (greatly decreasing the influence of the donor's age); it increases then decreases with age of the recipient in parallel with serum active TSH levels; it increases in male versus female recipients, but not in gonadectomized animals, in parallel with TSH levels (Mulkahi et al, 1980). TSH-increasing treatments markedly enhance the growth of human thyroid tissue transplanted in nude mice (Peter et al, 1985, 1988). In several species, including man, TSH promotes the proliferation of thyrocytes in culture (Roger et al, 1988) (Table 2). The lack of stimulatory or even the inhibitory effects observed on porcine and beef thyroid cells remain an exception (Gärtner et al, 1985; Watanabe et al, 1985; Heldin et al, 1988; Gérard et al, 1989), perhaps due to an artefact of the culture system in the absence of comitogenic factors. There is some in vivo evidence that the stimulation of thyroid growth by TSH is partly dependent on other hormones. Growth effects of TSH are reduced in hypophysectomized (Isler, 1974) or adrenalectomized rats (Jolin et al, 1974). In vitro, optimal growth effects of TSH require IGF, or insulin, as a comitogenic factor in several systems (Roger et al, 1983, 1987a, 1988; Smith et al, 1986; Tramontano et al, 1986a, 1988a; Williams et al, 1988).

It would be interesting to investigate whether thyroid hormones by themselves exert a direct control on the growth of the thyroid gland. Thyroxine certainly increases the growth of transplanted autonomous thyroid tumours in rats in vivo (Sisson et al, 1964) and thyroid cells contain many T3 receptors (DeGroot et al, 1989). Growth hormone, perhaps through IGF-I as an intermediate (Bachrach et al, 1988), induces thyroid growth but does not markedly enhance function, as demonstrated in acromegaly (Miyakawa et al, 1988). In Snell dwarf mice it induces cell proliferation, but contrary to TSH no cell hypertrophy (Denef et al, 1980).

Other plasma signals appear only in disease, such as the autoimmune immunoglobulins directed against thyroid cell membrane receptors. TSAb (thyroid-stimulating antibodies) and TBAb (thyroid-blocking antibodies) bind to the adenylate cyclase-coupled TSH receptor. TSAb activate (Adams, 1980) and TBAb block the stimulation by this receptor of function and growth. TSAb are responsible for Graves' disease hyperthyroidism; TBAb for some iodiopathic myxoedemas (Lu et al, 1990).

The concept of specific thyroid growth immunoglobulins (TGI) arose in the 1980s (Drexhage et al, 1980; Wilders-Trushnig et al, 1990) from the acknowledgement that some TSH effects were not mediated by cAMP, which might be explained by the existence of different TSH receptors or

Table 2. Hormones, drugs, growth factors reported to influence thyroid cell proliferation and differentiation expression.

Factor	System	Growth	Differentiation expression	Function	References
TSH	Human (in vitro, nude mice), dog, FRTL-5, rat, WRT, OVNI	↗	↗	↗	1
TSH	Human neoplasms in nude mice	↗	↗	↗	2
TSH	Pig	↘	↗	↗	3
TSH	Sheep, calf	—	↗	↗	4
Forskolin cholera toxin cAMP analogues	Human, dog, FRTL-5, rat	↗	↗	↗	5
Forskolin DB cAMP	Pig	↘	↗	↗	6
EGF	Human, dog, pig, sheep, calf (WRT)	↗	↘	↘/—?	7
	Sheep in vivo, rat in nude mice	↗	↘	↘	8
	FRTL-5	0			9
	Rat cells	0			10
bFGF	Dog, calf, FRTL-5	↗	↘/0 ?	?	11
Prolactin	Mice, in vivo	↗			12
Somatostatin	FRTL-5	↘			13
Hydrocortisone	Dog, calf, OVNI	0	↗		14
	FRTL-5	↗	↘		15
TSAb	Human in nude mice, FRTL-5	↗	↗	↗	16
IGF-I	Human, sheep, OVNI, pig, FRTL-5, rat cells	↗	↗		17
IGF-II	Sheep, FRTL-5 (TSH)	↗			18
TGF-β	FRTL-5, pig, human	↘			19
Phorbol myristate ester	Pig, sheep, dog, human, FRTL-5	↗	↘	↗ ↘	20
IL-1	Human, FRTL-5, human (in vivo)	↗	↘	↘	21
TNF	Human (in vivo, in vitro), mice, FRTL-5 (TSH, IGF-I)	↘	↘	↘	22
IFN-γ	Human	↗	↘	↘	23
	FRTL-5 (TSH)	↗	↗		24
I^-	Pig	↗		↘	25
	FRTL-5	↗			26
T3		?	?	?	

1, Nitsch and Wollman (1980), Roger et al (1982, 1988), Williams et al (1987), Huber and Davies (1990), Jin et al (1986), Peter et al (1988), Smeds et al (1989), Dere and Rapoport (1986), Brandi et al (1987), Fayet and Hovsépian (1985); 2, Müller-Gärtner et al (1989), Smeds et al (1989); 3, Westermark et al (1986), Heldin and Westermark (1988), Gärtner et al (1985), Fayet and Hovsépian (1979), Watanabe et al (1985); 4, Eggo et al (1984), Gérard et al (1989a); 5, Roger et al (1982, 1983, 1987b, 1988), Dere and Rapoport (1986), Jin et al (1986), Wynford-Thomas et al (1987); 6, Gärtner et al (1985), Watanabe et al (1985); 7, Westermark and Westermark (1982), Westermark et al (1983), Eggo et al (1984), Waters et al (1987), Takasu et al (1988), Roger and Dumont (1982, 1984), Gérard et al (1989a), Lamy et al (1990), Errick et al (1986), Rottella et al (1989); 8, Thorburn et al (1981), Corcoran et al (1986), Ozawa and Spaulding (1990); 9, Eggo et al (1984); 10, Smith et al (1986); 11, Roger and Dumont (1984), Gérard et al (1989a), Black et al (1990); 12, Mayerhofer et al (1990); 13, Tsuzaki and Moses (1990); 14, Roger and Dumont (1983), Gérard et al (1989a), Fayet and Hovsépian (1985); 15, Saji and Kohn (1990); 16, Tramontano et al (1986), Jin et al (1986); 17, Roger et al (1983, 1988), Williams et al (1987), Eggo et al (1984), Maciel et al (1988), Bachrach et al (1988), Santisteban et al (1987), Takasu et al (1989), Saji et al (1987), Smith et al (1986), Tramontano et al (1986a, 1988a); 18, Bachrach et al (1988), Maciel et al (1988); 19, Tsushima et al (1988), Moris et al (1988), Grubeck-Loebenstein et al (1989); 20, Roger et al (1986, 1988), Haye et al (1985), Bachrach et al (1985), Lombardi et al (1988); 21, Kung and Lau (1990), Pang et al (1990), Rasmussen et al (1990), Sato et al (1990), Mine et al (1987), Yamashita et al (1989), Kawabe et al (1989), Enomoto et al (1990); 22, Sato et al (1990), Zakarija and McKenzie (1989), Mooradian et al (1990); 23, Kraiem et al (1990b), Sato et al (1990), Huber and Davies (1990), Kung et al (1990), Ashizawa et al (1986); 24, Zakarija and McKenzie (1989), Mizaki et al (1988); 25, Heldin et al (1987); 26, Becks et al (1988), Saji et al (1988).

effectors (Dumont et al, 1978). Since then, TGI activities have been reported by some groups, but the methodologies used raise questions (Dumont et al, 1987). Although the concept may remain valid, its demonstration would require an unquestionable double-blind study using accepted methodologies (Zakarija and McKenzie, 1990). If accepted the concept should provide an explanation for the thyroid specificity of TGI. Indeed, all known growth factors and their receptors are remarkably ubiquitous, specificity being insured by local delivery through autocrine or paracrine mechanisms.

Human chorionic gonadotropin, and thus luteinizing hormone, at high concentrations activates the cAMP cascade and consequently proliferation in FRTL-5 cells (Davies and Platzer, 1986; Yoshimura et al, 1990) and thyroid cells. The concentrations reached in patients with trophoblastic tumours or even in pregnancy (Pekonen et al, 1988) are sufficient to activate the human thyroid (Hershman et al, 1988; Kasagi et al, 1989; Yoshikawa et al, 1989).

Thyrocytes, as other cells, also respond in vitro to a number of paracrine factors, i.e. factors secreted by neighbouring cells. Some of these factors are also synthesized and secreted by the thyrocytes themselves (autocrine secretion) (Table 1). Several growth factors have been shown to be mitogenic or comitogenic (permissive) for thyrocytes (Roger and Dumont, 1982, 1984; Westermark and Westermark, 1982; Roger et al, 1983, 1987a, 1988; Westermark et al, 1983; Fayet and Hovsépian, 1985; Errick et al, 1986; Ollis et al, 1986; Smith et al, 1986; Tramontano et al, 1986a; Brandi et al, 1987; Maciel et al, 1988; Williams et al, 1988; Gérard et al, 1989a): epidermal growth factor (EGF), FGF, IGF-1, the secondary factor secreted in response to growth hormone, IGF-II and insulin, even at physiological concentrations. IGF-I is produced by sheep thyroid (Bachrach et al, 1988) and IGF-II by FRTL-5 cells (Maciel et al, 1988) and FGF by porcine thyrocytes (Greil et al, 1989). A possible role of insulin and/or IGF-I in modulating thyroid growth response in vivo has been known for a long time (Jolin et al, 1970). The action of growth hormone on thyroid cell proliferation in Snell dwarf mice congenitally deficient in pituitary hormones is probably mediated by IGF-I (Lewinsky et al, 1984). The removal of submaxillary glands in mouse, which presumably greatly decreases serum EGF, has been reported to lead to thyroid regression (Suarez-Nunez, 1970) while perfusion of fetal sheep with EGF results in a considerable enlargement of the thyroid gland (Thorburn et al, 1981). At least one local hormone has been shown to inhibit growth: transforming growth factor β (TGF-β) (Grubech-Loebenstein, 1989; Bidey, 1990). The panel of factors active on thyrocyte growth may vary from one species to another (see Table 2). Of course, such local hormones (IGF-I, FGF, etc.) also act on the endothelial cells and fibroblasts of the thyroid and may thus represent the mediators of the cross-signalling that must exist to allow balanced growth of the gland. As the number of known growth factors increases one may expect the progressive unravelling of a complex network of cell cross-signalling. In pathological situations, cytokines such as interleukin-1, interferon γ (IFN-γ), tumour necrosis factor (TNF) might be secreted in the gland by cells of the immune system. These cytokines strongly influence FRTL-5 and human thyroid cell proliferation and metabolism (Mine et al, 1987; Zakarija et al, 1988; Kawabe et al, 1989; Zakarija and McKenzie, 1989; Enomoto et al, 1990; Kraiem et al, 1990b; Kung and Lau, 1990; Rasmussen et al, 1990).

As in other cell types the extracellular matrix also probably exerts a local control (presumably negative). This is suggested by the growth response after wounding and by the inverse relation of proliferative response versus cell density in primary thyrocyte culture. Such local controls would have great importance in pathology, generating diverse patterns from one area or one cell to another, i.e. tissue heterogeneity (Ingber and Folkman, 1989).

CASCADES AND MECHANISMS INVOLVED IN THE CONTROL OF THYROCYTE PROLIFERATION

Although there is no doubt that TSH in vivo stimulates the proliferation of thyroid cells, there was in the 1970s no evidence that this was a direct effect. Indeed, the ACTH trophic effect on the adrenal appears to be indirect. We first started to look at early steps of growth in slices (Mockel et al, 1980), and showed that TSH enhances ornithine decarboxylase activity in dog thyroid cells, which is generally considered as a preliminary to growth. The effect was mimicked by cAMP analogues and inhibited by agents inhibiting cAMP accumulation. As these results were against the current dogma, they were generally ignored.

To address the problem of proliferation, we began to use primary cultures and used a technique derived from Kerkof et al (1964), Fayet et al (1971) and Rapoport (1976), with a serum-free medium supplemented as proposed by Ambesi-Impiombato et al (1980). Using several methods, we demonstrated that TSH stimulates proliferation of dog thyroid cells (Roger et al, 1982, 1983). More recently we confirmed this result in normal human thyroid cells (Roger and Dumont, 1987; Roger et al, 1988). Despite earlier negative studies using inadequate culture conditions or pathological tissues (Westermark et al, 1979; Valente et al, 1983b; Errick et al, 1986), the mitogenic effect of TSH on human thyroid cells is now well established in vitro (Williams et al, 1987, 1988; Huber and Davies, 1990). Other results obtained in various culture systems were sometimes contradictory. While in dog thyroid cells in primary culture, in rat thyroid follicles in suspension (Nitsch and Wollman, 1980; Smith et al, 1986), in ovine cell line (OVNI) (Fayet and Hovsépian, 1985), and in rat cell line FRTL-5 (Ambesi-Impiombato et al, 1980; Dere and Rapoport, 1986) and WRT (Brandi et al, 1987), thyrotropin has been demonstrated to enhance or induce cell proliferation, to our knowledge no such effect has been obtained in porcine (Fayet and Hovsépian, 1979; Gärtner et al, 1985; Watanabe et al, 1985; Heldin et al, 1988), calf (Gérard et al, 1989a) or ovine (Eggo et al, 1984) thyroid cells in primary culture. Whether this is due to inaccessibility of the TSH receptor(s), lack of an essential element in the culture medium, alteration of the cell programme in culture or true unresponsiveness to direct TSH action is not known. It should be mentioned here that the

stimulating effects of ACTH on the proliferation of adrenal cells in vivo have not been convincingly reproduced in vitro. In this case, there are arguments that the stimulating effect may be indirect: adrenocorticotrophic hormone (ACTH) would induce the synthesis and secretion of growth factors by the adrenal cells which would then, acting as extracellular signals, trigger the cell proliferation.

In porcine thyroid cells, TSH through cAMP induces EGF receptors making these cells more responsive to EGF (Westermark et al, 1986; Atkinson et al, 1987). In rat thyroid, propylthiouracil-induced goitrogenesis is accompanied by an increased concentration of IGF-II receptors (Polychronakos et al, 1986). In the control of thyroid cell proliferation complementary mechanisms as well as differences of strategy from one species to another are possible.

In the thyroid at least three distinct pathways have been well defined: (1) the hormone receptor–adenylate cyclase–cAMP protein kinase system, (2) the hormone receptor–tyrosine protein kinase pathway and (3) the hormone receptor–phospholipase C cascade (Dumont et al, 1989; Maenhaut et al, 1990). The receptor–tyrosine kinase pathway may be subdivided into two branches: some growth factors, such as EGF, induce proliferation and repress differentiation expression, others, like FGF or IGF-I and insulin, are either mitogenic or are necessary for the proliferation effect of other factors without being mitogenic by themselves, but they do not inhibit differentiation expression in dog thyroid (Pohl et al, 1990). In dog (Roger et al, 1983, 1987a) and human (Roger et al, 1988; Williams et al, 1988) thyroid cells IGF-I or insulin are generally required by the mitogenic action of TSH or EGF but do not by themselves stimulate proliferation. In FRTL-5 and rat cells IGF-I is weakly stimulatory *per se* (Smith et al, 1986; Tramontano et al, 1986a). In pig thyroid cells, IGF-I produces a stronger mitogenic signal (Saji et al, 1987).

It should be noted that, in dog thyroid cells, TSH directly stimulates proliferation while maintaining the expression of differentiation. Differentiation expression, as evaluated by iodide transport, or thyroperoxidase and thyroglobulin mRNA content or nuclear transcription, is induced by TSH, forskolin, cholera toxin and cAMP analogues (Roger and Dumont, 1984; Roger et al, 1985; Gérard et al, 1989b). Similar results, albeit partial, have been obtained in human cells (Roger et al, 1988; Lamy et al, 1990). These effects are obtained in all the cells of a culture, as shown by in situ hybridization experiments (Pohl et al, 1990). They are reversible; they can be obtained either after the arrest of proliferation or during the cell division cycle (Pohl et al, 1990). Moreover, the expression of differentiation, as measured by iodide transport, is stimulated by lower concentrations of TSH than those required for proliferation (Roger and Dumont, 1984; Roger et al, 1988).

EGF also induces proliferation of dog thyroid cells (Roger and Dumont, 1984; Roger et al, 1987a) (Table 2). It also stimulates the growth of thyroid cells from other species in culture (e.g. porcine, ovine, bovine and human, but not of the FRTL cell line which lacks EGF receptors) (Westermark and Westermark, 1982; Westermark et al, 1983; Errick et al, 1986; Gérard et al, 1989a). This effect is often weaker than the effect of TSH. However, the action of EGF is accompanied by a general and reversible loss of differentiation expression (Roger and Dumont, 1982, 1984; Westermark et al, 1983; Eggo et al, 1984; Roger et al, 1985, 1988; Pratt et al, 1989; Lamy et al, 1990) assessed as described above. Similar results have been obtained in sheep in vivo (Thorburn et al, 1981; Corcoran et al, 1986) and in newborn rat thyroids transplanted in nude mice (Ozawa and Spaulding, 1990). The effects of EGF on differentiation can be dissociated from their proliferative action. Indeed, they are obtained in cells that do not proliferate in the absence of insulin (Lamy et al, 1989; Pohl et al, 1990) and in human cells in which the proliferative effect is weaker (Lamy et al, 1990) or in pig cells at concentrations lower than the mitogenic concentrations (Waters et al, 1987).

Finally, the tumour promoting phorbol esters, the pharmacological probes of the protein kinase C system and analogues of diacylglycerol also enhance the proliferation and inhibit the differentiation of dog as well as other thyroid cells (Bachrach et al, 1985; Haye et al, 1985; Roger et al, 1986, 1988; Lombardi et al, 1988) (Table 2). These effects are transient owing to desensitization of the system by protein kinase C inactivation (Roger et al, 1986). The activation of the PIP2 cascade by physiological agents, such as carbamylcholine and bradykinin in dog thyroid cells, does not reproduce all the effects of phorbol esters. In particular, prolonged stimulation of the cascade inhibits rather than stimulates proliferation (E. Raspé et al, in preparation) as well as the induction of ornithine decarboxylase (Mockel et al, 1980). Thus, we cannot necessarily equate effects of phorbol esters and prolonged stimulation of the PIP2 cascade. Similarly, prolonged enhancement of intracellular Ca^{2+} level might explain the mitogenic effects of IGF-I on FRTL-5 cells (Takada et al, 1990) but does not stimulate growth in dog thyroid cells (E. Raspé, unpublished). The dedifferentiating effects of phorbol esters do not require their mitogenic action either. Thus, the effects of TSH, EGF and phorbol esters on differentiation expression are largely independent of their mitogenic action.

In several thyroid cell models, very high insulin concentrations are necessary for growth even in the presence of EGF (Ambesi-Impiombato et al, 1980; Roger et al, 1983, 1987, 1988; Smith et al, 1986; Tramontano et al, 1986a). We now know that this mainly reflects a requirement for IGF-I (Czech, 1989). It is interesting that in the FRTL-5 cell line (Maciel et al, 1988), as in cells from thyroid nodules (Williams et al, 1989), this requirement may disappear as the cells secrete their own somatomedins and thus become autonomous with regard to these hormones. However, low physiological concentrations of insulin can replace IGF-I to allow growth stimulation by TSH in dog (Roger et al, 1987a) but not in human thyrocytes (Roger et al, 1988). Serum and FGF also induce growth in dog and calf thyroid and in FRTL-5 cells (Roger and Dumont, 1984; Gérard et al, 1989a; Black et al, 1990). IGF-I *per se* also stimulates the proliferation of FRTL-5 cells (Tramontano, 1986a). Although serum fully inhibits differentiation expression in calf thyrocytes (Gérard et al, 1989a) and partially in dog thyroid cells, IGF-I and insulin have no such effect. In fact, IGF-I and insulin acting through IGF-I receptors have some positive effects on specialized

gene expression in FRTL-5 cells (Santisteban et al, 1987), and insulin even at low concentrations is a moderate inducer of thyroglobulin gene expression in dog cells (Gérard et al, 1989b; Pohl et al, 1990). This therefore represents another type of receptor–tyrosine protein kinase pathway which leads to mitogenesis and to some extent to differentiation expression.

The question arises of the role of cAMP in the TSH effects. Thyrotropin induces within minutes a striking morphological change in dog thyroid cells in culture: a rounding up following the disruption of the actin network (Rapoport, 1976; Roger and Dumont, 1984; Nielsen et al, 1985; Roger et al, 1989). All the cells are affected. TSH also enhances the accumulation of cAMP in these cells within less than 5 min. cAMP remains elevated for 48 h in the continuous presence of the hormone. In the dog thyroid cells, analogues of cAMP as well as general cyclase activators (forskolin, cholera toxin) reproduce all the effects of TSH: acute morphological changes, proliferation, expression of differentiation (Roger et al, 1982, 1983, 1985, 1987b). Moreover, combinations of cAMP analogues which are synergistic on the two cAMP-dependent kinase isoenzymes are also synergistic on these effects (Van Sande et al, 1989). cAMP is therefore a general intracellular positive signal for function, proliferation and differentiation in the dog thyroid cells. For proliferation, similar results have been obtained with human (Roger et al, 1988; Kraiem et al, 1990) and rat thyroid cells in culture (Wynford-Thomas et al, 1987) and despite a first contradictory report (Valente et al, 1983b), in FRTL-5 cells (Dere and Rapoport, 1986; Jin et al, 1986; Ealey et al, 1987). In the latter cells, TBAb inhibit in parallel TSH binding and TSH-induced cAMP accumulation and [^{3}H]thymidine uptake (Brown et al, 1990). It is interesting that in cloned, dedifferentiated tumourigenic FRTL-5-derived cells, cAMP, as in fibroblasts, inhibits proliferation (Endo et al, 1990). Thus, changing the phenotype of these cells may reverse the role of cAMP. In other cloned mutated FRTL-5 cells growth becomes independent of TSH and cAMP (Tramontano et al, 1988b).

One argument that cAMP may be the mediator of rat thyroid cell proliferation in vivo is the fact that methylxanthines, inhibitors of cAMP phosphodiesterases, even at doses which do not further enhance serum TSH levels or decrease serum thyroid hormones, greatly potentiate the goitrogenic action of propylthiouracil (Wolff and Varrone, 1969). The action of methylxanthines is abolished by a high-iodine diet or hypophysectomy (Wolf, 1969). Analogues of cAMP or cAMP injected in rats or mice in vivo have also been reported to cause thyroid growth (Pisarev et al, 1970; Lewinski, 1980).

That TSH can act on the growth of human thyroid cells by other pathways than the cAMP cascade is suggested by the facts that (1) in human thyroid cells in primary culture the mitogenic effect of the hormone under some conditions is not fully reproduced by cAMP enhancers (Roger et al, 1988) and (2) the hormone is mitogenic in a human hybrid cell line GEJ (Karsenty et al, 1988) in which it does not enhance cAMP accumulation. Similarly, clones of FRTL-5 cells with low cAMP but normal mitogenic response to TSH have been reported (Davies et al, 1987). If these results cannot be explained otherwise, another pathway of TSH action might be involved. We know that TSH in human thyrocytes but not in dog thyroid cells nor in the FRTL-5 cell line activates both the cAMP and the phosphatidylinositol cascades (Laurent et al, 1987).

The effects of EGF on dog thyroid cell (proliferation, inhibition of differentiation expression) are mimicked by phorbol esters tumour promoters (Roger et al, 1986). However, these compounds also inhibit EGF action: the effect of phorbol esters which is lower than the effect of EGF is not increased by EGF. In several cell types, EGF not only activates a tyrosine-specific protein kinase, but also induces a rapid rise in cytoplasmic free Ca^{2+} concentration. This rise in Ca^{2+} concentration following EGF stimulation has been linked to an activation of the phosphatidylinositol Ca^{2+} cascade, although it has been suggested recently that it might result from an entry of extracellular Ca^{2+} through the plasma membrane. It would therefore be conceivable that EGF action on the thyroid cell might result from an increase in Ca^{2+} entry or from an activation of the Ca^{2+} phosphatidylinositol cascade with generation of diacylglycerol, the action of which is mimicked by phorbol esters. However it should be noted that this activation of the PIP2 cascade by EGF apparently only occurs in cells in which EGF receptors are overexpressed (Levitzki, 1990). EGF induces a rise in intracellular Ca^{2+} in porcine thyroid cells (Takasu et al, 1988), but such an effect, if it exists, is minor in dog cells. Moreover, carbamylcholine, the most potent activator of the Ca^{2+} phosphatidylinositol cascade in these cells is not mitogenic (E. Raspé, unpublished). On the other hand, neither EGF nor phorbol esters enhance cAMP accumulation in these cells. It is therefore likely that EGF acts through the phosphorylation of key proteins on tyrosyl residues. The three cascades are therefore fully distinct at the level of their primary intracellular signal and/or of the first signal-activated protein kinase (Contor et al, 1988). It is interesting that iodide, through an as yet unknown oxidized derivative(s), acutely inhibits the cAMP and the Ca^{2+} phosphatidylinositol cascades and in a more delayed and chronic effect decreases the sensitivity of the thyroid to the TSH growth response. In FRTL-5 cells it inhibits the TSH-, IGF-I- and tumour promoters (TPA), i.e. phorbol myristate ester-induced cell proliferation (Becks et al, 1988; Saji et al, 1988); these effects are relieved, according to our general paradigm (Van Sande et al, 1975), by perchlorate and methimazole.

THE KINETICS AND INTERACTIONS OF THE THYROID MITOGENIC PATHWAYS

The kinetics of the induction of thymidine incorporation into nuclear DNA of dog thyroid cells is very similar for TSH, forskolin, EGF and TPA (Roger et al, 1987a). Whatever the stimulant, there is a similar minimal delay of 16–20 h before the beginning of the labelling, i.e. of DNA synthesis. This is the minimal time required to prepare the necessary machinery. For the cAMP pathway, the stimulatory agent has to be present during this whole prereplicative period: any interruption of the activation (e.g. by washing out the stimulatory forskolin) greatly delays the start of DNA synthesis (Roger et al, 1987b).

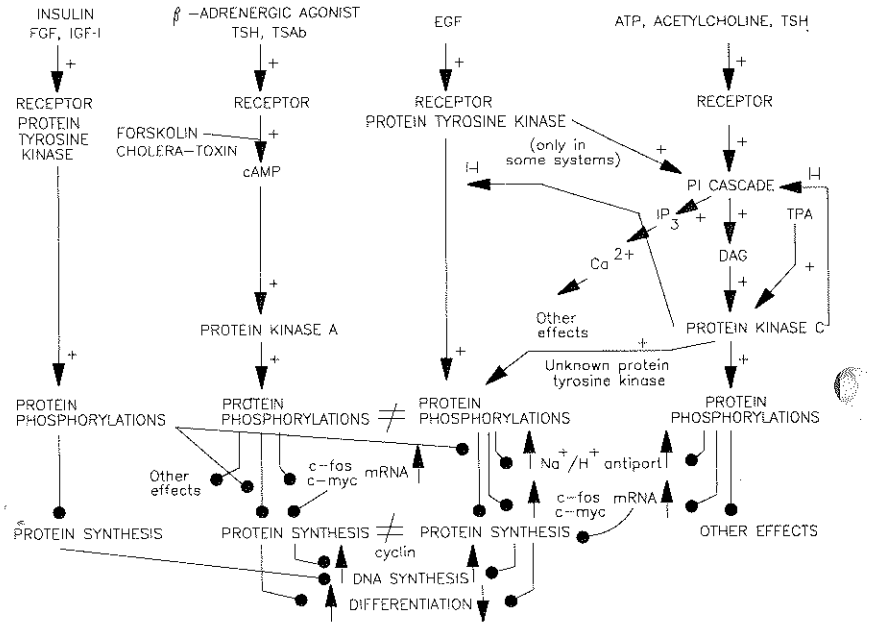

Figure 1. Mitogenic pathways in the thyroids: data from the thyroid cells system are integrated into the present scheme of cell proliferation cascades. DAG, diacylglycerol; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin like growth factor (somatomedin); IP_3, inositol 1,4,5-trisphosphate; Pi, phosphatidylinositol; TPA, phorbol ester; TSI, thyroid-stimulating immunoglobulin; ≠, not overlapping patterns; ——⁺→, stimulation; ——●, time sequence for which the causal relationships remain to be proved.

We have thus studied the phenomenology of EGF, TPA and TSH proliferative action on dog quiescent cells with the aim to identify steps in this action. Three biochemical aspects of the proliferative response occurring at different times of the prereplicative phase have been considered. The pattern of protein phosphorylation induced within minutes by TSH is reproduced by forskolin and cAMP analogues (Contor et al, 1988). The phosphorylation of at least 11 proteins is increased or induced. NaOH treatment of the gels does not reveal any remaining phosphorylation on these proteins suggestive of tyrosine phosphorylation. In EGF-stimulated cells, the phosphorylation of five proteins is stimulated, two of them phosphorylated on tyrosines (42k). These two proteins are similar (isoelectric points, approximate molecular weight, composition in phosphorylated amino acids) (Contor et al, 1988) to the two 42k proteins described in other systems, which have been implicated in the mitogenic response to diverse agents and recently identified as the MAP-kinase (Rossomondo et al, 1989). This kinase phosphorylates the S6 kinase II which is involved in the control of protein synthesis at ribosome level. Phorbol esters induce the phosphorylation of 19 proteins, including the tyrosine phosphorylated proteins mentioned above. There is no overlap in the patterns of protein

phosphorylation induced by TSH and cAMP enhancers on the one hand, and by EGF and phorbol esters on the other (Contor et al, 1988).

The expression of c-*myc* and c-*fos* has been studied by Northern analysis of RNA extracts (Reuse et al, 1990). As in other types of cells, EGF and TPA enhance first c-*fos*, then c-*myc* mRNA concentrations also (Heldin and Westermark, 1988). On the other hand, TSH or forskolin enhances strongly but for a short period c-*myc* mRNA concentration and with the same kinetics as for EGF/TPA, c-*fos* mRNA concentration. In fact, cAMP first enhances, then decreases c-*myc* mRNA accumulation. This second phenomenon is akin to what has been observed in fibroblasts in which cAMP negatively regulates growth (Heldin et al, 1989). The enhancement but not the decrease has been observed for TSH and agents increasing intracellular cAMP in FRTL-5 cells (Dere et al, 1985; Tramontano et al, 1986b). In pig thyroid cells TSH and cAMP are not mitogenic and do not enhance c-*myc* and c-*fos* gene expression (Heldin and Westermark, 1988). The effect of TSH and cAMP on c-*fos* gene expression in FRTL-5 cells is transcriptional (Damante and Rapoport, 1988).

The pattern of proteins synthesized in response to the various proliferation stimuli has been studied (Lamy et al, 1989). Again two patterns emerge. TSH and forskolin induce the synthesis of at least eight proteins and decrease the synthesis of five proteins. Epidermal growth factor, phorbol ester and serum induce the synthesis of at least two proteins and decrease the synthesis of two proteins. The only overlap between the two patterns concerns the decrease in the synthesis of a protein (18k) which is also reduced by EGF after proliferation has stopped. Only one protein has been shown to be synthesized in response to the three pathways: PCNA, the auxiliary protein of DNA polymerase δ but the kinetics of this synthesis are very different, with an early synthesis in the cAMP cascade (consistent with a role of signal) and a late, S phase synthesis in the other cascades (Lamy et al, 1989). Thus, obviously two different phenomenologies are involved in the proliferation response to TSH through cAMP on the one hand, and EGF and phorbol ester, presumably through protein tyrosine phosphorylation, on the other hand. Although this conclusion needs to be further substantiated, it certainly suggests that the proliferation of dog thyroid cells is controlled by at least two largely independent pathways.

The studies of protein phosphorylation, proto-oncogene expression and protein synthesis in the dog thyrocytes allow discrimination between two models of cAMP action on proliferation in this system: a direct effect on the thyrocyte or an indirect effect through the secretion and autocrine action of another growth factor. If the effect of TSH through cAMP involved such an autocrine loop one would expect faster kinetics of action of the growth factor, and at least some common areas in the patterns of protein phosphorylation and protein synthesis induced by cAMP and the growth factor. The results do not support such a hypothesis, at least for the growth factors we have tested.

It has been suggested that part or all the TSH growth effect on FRTL-5 cells is secondary to their autocrine secretion of IGF-II and other factors (Takahashi et al, 1990). This is not general as IGF-II, which is secreted by

FRTL-5 cells and is mitogenic for them (Maciel et al, 1988) is not a growth factor for dog thyroid cells by itself. Moreover, our experiments are in general carried out in the presence of high concentrations of insulin that would saturate the IGF receptors (Roger et al, 1987a). In the FRTL-5 cells themselves anti-IGF antibody (Sm 1.2) only inhibits the effects of low concentrations of TSH (Maciel et al, 1988) and does not inhibit the synergism between TSH and high concentrations of insulin. In the papers of Takahashi and Maciel, there is no evidence that TSH does stimulate the production of IGF-II or other autocrine factors. An effect of TSH and cAMP through protein kinase C activators or EGF is also very unlikely in dog thyroid cells. The kinetics of action of TSH or forskolin is similar for the end-point of DNA synthesis for the three types of agents. Moreover, the kinetics of proto-oncogene c-*myc* and c-*fos* expression is not delayed for TSH and cAMP. Finally, the patterns of protein phosphorylation and protein synthesis induced by EGF and phorbol esters show partially common responses while there was no overlap with the pattern of TSH or cAMP action. Thus there is no evidence in favour of the involvement of an autocrine loop with a growth factor in the action of TSH and cAMP on dog thyroid cells in primary culture. This does not exclude that such a mechanism may operate in thyroids of other species as suggested by the induction by TSH of EGF receptors in porcine thyroid cells (Westermark et al, 1986; Atkinson et al, 1987).

CHARACTERISTICS OF THE TSH–cAMP CASCADE WHICH MAY EXPLAIN A DUAL ROLE IN THE STIMULATION OF PROLIFERATION AND THE INDUCTION OF DIFFERENTIATION EXPRESSION

The incompatibility at the cellular level of a proliferation and differentiation programme is commonly accepted in biology. In general, cells with a high proliferative capacity are partly differentiated and during development such cells lose this capacity as they progressively differentiate. Some cells even lose all potential to divide when reaching final differentiation; this is called terminal differentiation. Conversely, in tumour cells there is an inverse relationship between proliferation and differentiation expression. It is therefore not surprising that in thyroid cells, the general mitogenic agents and pathways, phorbol esters and the protein kinase C pathway, EGF and the protein tyrosine kinase pathway induce both proliferation and the loss of differentiation expression (Roger and Dumont, 1984; Roger et al, 1986; Gérard et al, 1989a). The effects of the cAMP cascade are in striking contrast to this general concept. Indeed, TSH and cAMP induce proliferation of the dog thyrocytes while maintaining differentiation expression: both proliferation and differentiation programmes can be triggered by TSH in the same cells at the same time (Pohl et al, 1990). It is tempting to relate this apparent paradox to the role and expression of proto-oncogene in these cells. c-*fos* expression is enhanced in a great variety of cell stimulations, leading to either proliferation or differentiation expression (Müller, 1986).

On the other hand, if there is one generalization that could be made on proto-oncogenes, it is the dedifferentiating role of c-*myc*. A rapid and dramatic decrease in c-*myc* mRNA has been associated with the differentiation of a variety of cell types (Heikkila et al, 1987; Griep and Westphal, 1988; Prochownik et al, 1988). It is therefore striking that in the case of the thyrocyte in which the activation of the cAMP cascade leads to both proliferation and differentiation, the kinetics of the c-*myc* gene appear tightly controlled. After a first phase of 1 h of a high level of c-*myc* mRNA, c-*myc* expression is decreased below control levels (Reuse et al, 1990). In this second phase, cAMP decreases c-*myc* mRNA levels, as it does in proliferation-inhibited fibroblasts. It even depresses EGF-induced expression (Reuse et al, 1990). The first phase could be necessary for proliferation while the second phase could reflect the stimulation of differentiation by TSH. This down-regulation is suppressed by cycloheximide, which suggests the involvement of a neosynthesized (by an autoregulatory mechanism) or a labile protein in the inhibition at the transcriptional level or at the stabilization of the mRNA.

Preliminary results indicate that TSH down-regulates, at least at a post-transcriptional level, the c-*myc* mRNA expression: as soon as TSH is in the medium, a destabilization of c-*myc* mRNA is observed when transcription is blocked by actinomycin D. We now therefore hypothesize that the first rise of c-*myc* mRNA expression reflects a very high induction of transcription combined to a destabilization mechanism. Later, the positive transcription effect is repressed and the destabilization mechanism would persist, leading to a resulting down-regulation of the c-*myc* mRNA level. The transcription could be repressed either at the initiation (Hay et al, 1989; Takimoto et al, 1989; Penn et al, 1990) or at the elongation level (Miller et al, 1989). It would be interesting to test whether cloned tumourigenic FRTL-5 cells, in which cAMP inhibits proliferation (Endo et al, 1990), have lost the first positive control of c-*myc* expression. In a feedback mechanism, the neosynthetic protein could even be the c-*myc* protein itself, specifically modified at the post-translational level by the c-AMP pathway. Such an autoregulatory mechanism of blockade of transcriptional initiation requires additional transacting factors and could act as a homeostatic regulator of c-*myc* expression in vivo (Penn et al, 1990). It is interesting that transformed FRTL-5 cells have lost the positive effect of TSH and cAMP on proliferation and express only a negative control (Endo et al, 1990). This suggests that in normal cells a dual control exists with a dominant positive regulation.

GROWTH CONTROL AND THYROID DISEASE

The role of the signals and cascades controlling thyroid cell proliferation and differentiation in pathology has been until now little studied. In a few cases thyroid pathology can be explained straightforwardly within the framework presented above.

The disease in which goitre is easiest to explain is Graves' disease. In this disease autoantibodies directed against the TSH receptor (TSAb) activate

this receptor and consequently the whole cAMP cascade. At the highest concentrations reached in pathology, these TSAb do not activate, as TSH does, the Ca^{2+} phosphatidylinositol cascade (Laurent et al, 1991). Thus, hyperthyroidism in Graves' disease appears to result from a chronic hyperstimulation of the cAMP cascade. The effects of this cascade on cultured thyroid cells are to enhance function and proliferation while maintaining differentiation, i.e. they represent the in vitro counterparts of what is observed in Graves' disease thyroids in vivo. It is interesting to note that, as in in vitro or in vivo chronically stimulated thyroids, the growth of thyroid in Graves' disease is generally limited. This apparently simple and unicausal disease may lead in time to heterogeneous goitre. Also, in these chronically stimulated thyroids, in which proliferation and the increasing number of mitoses are bound to allow the fixation of more mutations, cancer incidence is increasing (Mazzaferi, 1990). Thus, even though the cAMP cascade itself maintains differentiation while promoting proliferation, the greater number of mitoses will give a higher probability of occurrence to the rare mutagenic events which lead to carcinogenesis.

The goitre resulting from congenital defects in iodine metabolism by the gland is also simply explained by classical concepts of thyroid regulation. Deficiency in thyroid hormone formation resulting from the defect relieves the thyroid hormone feedback on the hypophysis and leads to increased TSH secretion and stimulation of the thyroid. In addition, a deficiency in iodine metabolism, at the level of trapping or iodination, will relieve the negative feedback of iodide and increase the sensitivity of the gland to the TSH growth-promoting effect. Impaired iodination due to a congenital defect or to inhibition by antithyroid drugs has been shown to relieve the inhibitory effect of iodide on cAMP accumulation (Demeester-Mirkine et al, 1984). Defects of iodotyrosine coupling and iodotyrosine deiodination, which also lead to iodine depletion, will in time have the same effect. It is interesting to note that defects in iodination which most severely affect the iodide inhibitory pathway lead to the severest goitres and to the highest incidence of thyroid cancers. It is also important to note that even in this case where a single identified cause of the disease and its goitrogenic consequences exists, prolonged stimulation of the thyroid will in time generate heterogeneity and its ultimate result, the multinodular goitre. Similar considerations apply to endemic goitre in which iodine deficiency and sometimes goitrogen intake phenotypically reproduce the congenital defects. However, in this case variations of the stimulation, in time, will lead earlier to macroscopic heterogeneity. The death of many cells after iodine administration in iodide-depleted glands, the burst of cell proliferation and the consequent remodelling of the thyroid may explain these phenomena (Denef et al, 1989).

The simplest example of a somatic mutation leading to autonomous hyperfunctioning adenomas has been demonstrated by Bourne's group in the hypophysis of acromegalic rats. In the rat somatotrophs, as in dog and human thyroid cells, the activating hormone GRH acts by activating adenylate cyclase and the cAMP cascade, which leads to functional activation and growth. In hyperfunctioning autonomous adenomas of the

somatotrophs Landis and coworkers (1989) demonstrated a mutation in Gs which causes constitutive activation of this transducing protein and consequently of the whole cAMP cascade. A systematic search for similar lesions in other tumours has allowed the demonstration of several identical mutations in the Gs of thyroid adenomas (Lyons et al, 1990). Of course, any somatic mutation leading to the constitutive activation of the first elements of the cAMP cascade (TSH or other stimulating receptor, cyclase, protein kinase, etc.) or to the inactivation of a negative controlling element (inhibiting receptor, GI, XI) could result in a similar phenotype of constitutive activation of the cAMP cascade and consequently of the function and growth of the affected cells, i.e. in an autonomous thyroid adenoma. Thus, although available data on the cAMP system in the human autonomous thyroid adenoma suggest that the Gs defect demonstrated by Landis will only explain a minority of cases, it provides a useful paradigm for the study of other mechanisms of autonomy. Autonomy in these adenomas corresponds to a loss of control. Moreover, similar mutations inducing constitutive activation in the elements of genes of proteins of the Ca^{2+} PIP2 cascade of cells secreting by an exocytic process could explain autonomous functional adenoma of such exocrine or endocrine organs. It will be of interest to look for such defects in calcitonin-secreting cell tumours.

The isolated defects in iodide trapping and of iodination in a few well-studied 'cold' adenomas could perhaps also explain the growth of these adenomas. In the absence of iodide trapping or oxidation the negative control of iodide is relieved, which might confer a selective advantage to the affected cells, favouring the appearance of new mutations and of tumourigenesis. Undoubtedly, these mechanisms do not account for all thyroid adenomas and others will be found.

Besides these well-defined pathologies, our expanding knowledge on the secretion in the thyroid of growth factors and local hormones and on the effects on thyrocytes of such factors and of cytokines has not been really translated in the study of disease until now. The recent separations and cloning of these factors and of their receptors now give the tools necessary to investigate, by immunohistochemistry and in situ hybridization on individual cells in thyroid sections, the local pathogenetic process involved in such common diseases as simple goitre and thyroiditis. The next review on this subject will certainly involve a lot of such information.

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