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Activation of the cyclic AMP cascade as an oncogenic mechanism: the thyroid example

C Maenhaut, PP Roger, S Reuse, JE Dumont

Institut de Recherche Interdisciplinaire, Faculté de Médecine, Hôpital Erasme, Université Libre de Bruxelles, Campus Erasme, 808 route de Lennik, B-1070 Brussels, Belgium

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Summary — Three cascades activate thyroid cell proliferation: the EGF–protein tyrosine kinase pathway, the phorbol ester–protein kinase C pathway and the thyrotropin–cyclic AMP pathway. While the first 2 cascades converge early, they remain distinct from the cyclic AMP cascade until very late in G1. The cyclic AMP cascade is characterized by an early and transient expression of c-*myc*, which may explain why it induces proliferation and differentiation expression. Constitutive activation of this cascade causes growth and hyperfunction, *ie*, hyperfunctioning adenomas. The various possible defects that could lead to such a constitutive activation are discussed.

cyclic AMP / oncogenicity / proliferation / thyroid

Cyclic AMP in cell proliferation

It is probable that the key machinery in the immediate control of the decision of cells to divide will prove to be very general. Indeed, the first studies of complementation of proliferation deficient yeasts with mammalian genes have already allowed the identification in mammalian cells of genes able to perform the same function. One of these genes, *cdc2*$^+$, has been shown to be a part of the well-known maturation promoting factor involved in the triggering of meiosis in oocytes [1]. On the other hand, the regulatory circuits allowing the control of mammalian cells by extracellular signals vary in importance and significance from one cell type to another, and within one cell type from one species to another. It is therefore highly improbable that the role of these circuits in the control of the decision to divide will be conserved in all mammalian cells. Indeed, examples of opposite effects of the same cascade on proliferation and differentiation in different cell types abound: the phorbol ester protein kinase C pathway, which in the thyroid induces proliferation and dedifferentiation, exerts the opposite effects in keratinocytes. The cyclic AMP cascade which negatively regulates proliferation in fibroblasts and lymphocytes stimulates it in keratinocytes and thyrocytes, etc. Thus the role of a given cascade in the control of cell proliferation and differentiation has to be considered for each cell type of each species. Generalizations are unwarranted in this field.

Reviews on the control of vertebrate cell proliferation generally consider 2 signal cascades as the main pathways between an extracellular mitogenic signal and mitogenesis itself: the growth factor receptor protein tyrosine kinase pathway and the phosphatidylinositol Ca^{2+}-diacylglycerol protein kinase C cascade [2]. In such reviews, the first demonstrated signal cascade, the cyclic AMP system, is either not considered, or merely ascribed the role of an inhibitory pathway [3]. This general picture derives from the fact that growth and proliferation have mostly been studied on 'easy experimental objects', fibroblasts and cell lines of mesenchymal origin. As is often the case in science, the choice of experimental subject has biased the outlook on the whole subject. In fact, in several epithelial cells as in yeast cells, the cyclic AMP cascade is a positive regulatory pathway of cell growth and proliferation [4]. In the thyroid we shall consider at least 3 well defined distinct pathways: the hormone–receptor–adenylate cyclase–cyclic AMP protein kinase system, the hormone receptor–tyrosine protein kinase pathway and the hormone–receptor–phospholipase C–diacylglycerol protein kinase C–calcium calmodulin protein kinase cascade. The receptor tyrosine kinase pathway may be subdivided into 2 branches: some growth factors, such as EGF induce proliferation and repress

differentiation expression, others like FGF or IGF_1, are mitogenic or are necessary for the proliferation effect of other factors without being mitogenic by themselves, but they do not inhibit differentiation expression.

As we might have assumed by looking at the present complexity of the regulation of a simple organism like the phage, this simple scheme is now expanding daily and becoming more sophisticated. First, new regulatory pathways are being defined, *eg*, the ANF guanylate cyclase-cyclic GMP, and the hormone-phospholipase A_2-arachidonate cascades. Second, at each level, any of the regulatory pathways may be directly controlled by any step in this or the other pathways. Thus, in the dog thyroid, calcium through calmodulin activates a cyclic nucleotide phosphodiesterase, diacylglycerol inhibits phospholipase C and the prostaglandin E_2 stimulation of adenylate cyclase and potentiates TSH activation of this enzyme, etc. Third, the level of the proteins involved in each pathway may be controlled at gene transcription or distal to it, by the same or by other pathways. Fourth, the stimulation of a regulatory pathway in one cell may stimulate the production and release of factors that control these same cells (autocrine mechanisms) or neighboring cells (paracrine mechanisms). A mechanism of such interactions has been provided by Gärtner when he demonstrated the secretion by pig thyroid cells of a fibroblast growth factor [5]. Therefore, different mechanisms functioning to the same end will probably coexist in each system, and the demonstration of one such mechanism does not exclude the possibility of another. Moreover, at each level the circuits may differ from one species to another [6]. Therefore, although data may be coherent within one system they may vary from one system to another.

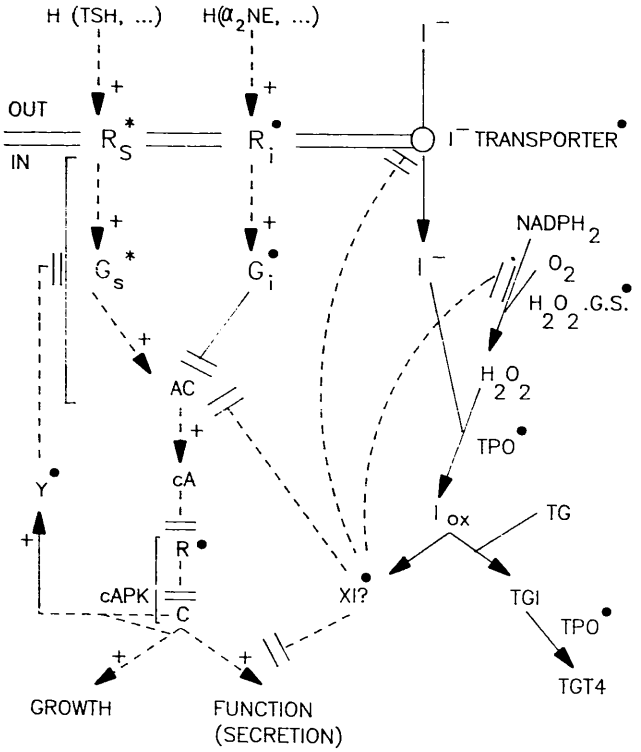

Fig 1. Control of thyroid cyclic AMP cascade: inactivating (•) and activating (*) mutations that can lead to constitutive activation. H = hormone; R = receptor; NE = norepinephrine; AC = adenylate cyclase; cA = cyclic AMP; cAPK = cAMP-dependent protein kinase; → transformation or transport; ---± positive control; —// negative control; G_s, G_i: stimulating and inhibiting G proteins; R_s, R_i: stimulating and inhibiting receptors; H_2O_2.G.S.: H_2O_2 generating system; Y: feedback inhibition on receptor cyclase system; R, C: regulatory, C catalytic unit.

The mitogenic action of TSH

Although there is no doubt that *in vivo* TSH stimulates the proliferation of thyroid cells, in the 1970s there was no evidence that this effect was direct. Indeed, the ACTH trophic effect on the adrenal appears to be indirect. We first started to study early steps of growth in slices [7], 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 analogs and inhibited by agents inhibiting cAMP accumulation. As these results were contrary to current opinion, they were generally ignored. We as well as several others showed that TSH increases [^{3}H] uridine incorporation into RNA in sheep thyroid slices. Did this show that TSH increased RNA synthesis? No: increased [^{3}H] uridine incorporation into RNA only reflected an effect on the radioactivity of the precursor pool [8]. We realize from such an experiment why measurements of [^{3}H] thymidine incorporation into DNA do not measure DNA synthesis either [9].

To investigate the problem of proliferation, we used primary cultures and a technique derived from Kerkof [10], Fayet [11] and Rapoport [12], with a serum-free medium supplemented as proposed by Ambesi [13]. Using several methods, we demonstrated that TSH and cAMP analogs or enhancers stimulate proliferation of dog thyroid cells [14, 15]. More recently we confirmed this result in normal human thyroid cells [16]. Other results obtained in various culture systems were sometimes contradictory. While in dog thyroid cells in primary culture, in rat thyroid follicles in suspension [17], in ovine cell lines (OVNI) [18], and in a rat cell line (FRTL) [13], thyrotropin has been

demonstrated to enhance or induce cell proliferation, to our knowledge, no such effect has been obtained in porcine [19], calf [20] or ovine [21] 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 cell program in culture or real 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: ACTH may induce the synthesis and secretion of growth factors by the adrenal cells which would then, acting as extracellular signals, trigger cell proliferation. It is therefore quite possible, and this has been proposed, that in the species for which no direct proliferative action of TSH has been demonstrated, such a mechanism might operate. In porcine thyroid cells, TSH through cyclic AMP induces EGF receptors making these cells more responsive to EGF [22]. In the control of thyroid cell proliferation differences in strategy from one species to another are possible.

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 cyclic AMP analogs, in dog thyroid cells [23–25]. Similar results, albeit partial, have been obtained in human and calf cells [20, 26]. These effects are obtained in all the cells of a culture, as shown by *in situ* hybridization experiments [27]. They are reversible and can be obtained either after the arrest of proliferation or during the cell division cycle [28].

The question arises as to the role of cyclic AMP in the TSH effects. Within minutes thyrotropin induces a striking morphological change in dog thyroid cells in culture: a rounding up following the disruption of the actin network [23, 28]. All the cells are affected. TSH also enhances the accumulation of cyclic AMP in these cells within < 5 min. Cyclic AMP remains elevated for 48 h in the continuous presence of the hormone. In the dog thyroid cells, analogs of cyclic AMP as well as general cyclase activators (forskolin, cholera toxin) reproduce all the effects of TSH: acute morphological changes, proliferation, expression of differentiation [23, 25]. Moreover, combinations of cyclic AMP analogs which are synergistic on the 2 cyclic AMP dependent kinases isoenzymes are also synergistic on these effects [29]. Cyclic AMP is therefore a general intracellular signal for function, proliferation and differentiation in dog thyroid cells. Similar results have been obtained for proliferation with human [16] and rat thyroid cells in culture [30] and, despite a first contradictory report [31], in FRTL-5 cells [32]. It is interesting that in cloned, dedifferentiated tumorigenic FRTL-5 derived cells, cyclic AMP, as in fibroblasts, inhibits proliferation [33]. Thus changing the phenotype of these cells may reverse the role of cyclic AMP.

One argument that cAMP may be the mediator of rat thyroid cell proliferation *in vivo* is provided by the fact that methylxanthines, inhibitors of cyclic AMP phosphodiesterases, even at doses which do not further enhance serum TSH levels or decrease serum thyroid hormones greatly potentiate the goitrogenic action of propylthiouracil. By blocking thyroid hormone formation propylthiouracil, chronic increase in TSH levels and thyroid stimulation is induced. The action of methylxanthines is abolished by a high iodine diet or hypophysectomy [34]. Analogues of cyclic AMP injected in rat *in vivo* have also been reported to cause thyroid growth [35].

The kinetics of biochemical effects in the various 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 phorbol esters (TPA) [36]. Whatever the stimulant, there is a similar minimal delay of 16 to 20 h before the onset of labelling, *ie* 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 the entire prereplicative period: any interruption of stimulation (*eg* by forskolin washing) greatly delays the onset of DNA synthesis [37]. What happens between the onset of stimulation and the beginning of DNA synthesis? The time sequence of biochemical phenomena suggests a causal sequence. However, many of such postulated 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 of identifying steps in this action. Three biochemical aspects of the proliferative response occurring at different times during the prereplicative phase have been considered. The pattern of protein phosphorylation induced within minutes by TSH is reproduced by cyclic AMP analogs [38]. 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 5 proteins is stimulated, 2 of them phosphorylated on tyrosines (42 kDa). These 2 proteins are similar (isoelectric points, approximate molecular weight,

composition in phosphorylated amino acids) [38] to the two 42 kDa proteins described in other systems, which have been implicated in the mitogenic response to diverse agents and recently identified as MAP-kinase [39]. This kinase phosphorylates S_6 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 cyclic AMP enhancers on the one hand, and by EGF and phorbol esters on the other hand.

The expression of c-*myc* and c-*fos* has been studied by Northern analysis of RNA extracts [40]. As in other types of cells, EGF and TPA first enhance c-*fos*, then c-*myc* mRNA concentrations. On the other hand, TSH or forskolin enhance strongly but for a limited time c-*myc* mRNA concentration with the same kinetics as for EGF/TPA, c-*fos* mRNA concentration. In fact, cyclic AMP first enhances, then decreases c-*myc* mRNA accumulation. This second phenomenon is akin to what has been observed in fibroblasts in which cyclic AMP negatively regulates growth [41].

The pattern of proteins synthesized in response to the various proliferation stimuli has been studied [42]. Again 2 patterns emerge. TSH and forskolin induce the synthesis of at least 8 proteins and decrease the synthesis of 5 proteins. Epidermal growth factor, phorbol ester and serum induce the synthesis of at least 1 protein and decrease the synthesis of 2 proteins. The only overlap between the 2 patterns concerns the decrease in the synthesis of a protein (18 kDa) which is also reduced by EGF after proliferation has stopped. Only one protein has been shown to be synthesized in response to the 3 pathways: PCNA, the auxiliary protein of DNA polymerase δ; but the kinetics of this synthesis are very different, with an early synthesis in the cyclic AMP cascade (consistent with a signal role) and a late S phase synthesis in the other cascades [42]. Thus, obviously 2 different phenomenologies are involved in the proliferation response to TSH through cyclic AMP on the one hand, and epidermal growth factor 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 2 largely independent pathways.

The studies of protein phosphorylation, proto-oncogene expression and protein synthesis in dog thyrocytes allow discrimination between 2 models of cyclic AMP 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 cyclic AMP involved such an autocrine loop one would expect faster kinetics of action of the growth factor, at least some common parts in the patterns of protein phosphorylation and protein synthesis induced by cyclic AMP and the growth factor. The results do not support such a hypothesis, at least for the growth factors we have tested.

IGF_2 which is secreted by FRTL-5 cells and is mitogenic for them [43] 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 appear to saturate the IGF_1 receptor [37]. For protein kinase C activators and EGF, the kinetics of action of TSH or forskolin are similar for the end point of DNA synthesis for the 3 types of agents. Moreover, the kinetics of proto-oncogene c-*myc* and c-*fos* expression are not delayed for TSH and cyclic AMP. 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 cyclic AMP action. Thus there is no evidence to support the involvement of an autocrine loop with a growth factor in the action of TSH and cyclic AMP on dog thyroid cells in primary culture. This does not exclude such a mechanism for the thyroid of other species as suggested by the induction by TSH of EGF receptors in porcine thyroid cells and of IGF_2 in FRTL-5 cells.

The paradox of TSH and cyclic AMP and their apparently opposite effects: stimulation of proliferation, induction of differentiation expression

The incompatibility at the cell level of a proliferation and differentiation program 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 tumor 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 [44]. The effects of the cyclic AMP cascade are in striking contrast to this general concept. Indeed, TSH and cyclic AMP induce proliferation of dog thyrocytes while maintaining differentiation expression: both proliferation and differentiation programs can be

triggered by TSH in the same cells at the same time [27]. 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 stimulation, leading to either proliferation or differentiation expression [45]. 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 [46–48]. 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 c-*myc* gene appear tightly controlled. After a first phase of 1 h of higher level c-*myc* mRNA, c-*myc* expression is decreased below control levels [40]. In this second phase, cyclic AMP decreases c-*myc* mRNA levels, as it does in proliferation inhibited fibroblasts. It even depresses EGF-induced expression [40]. 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 regulates at least at a post-transcriptional level 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 therefore hypothesized that the first rise in c-*myc* mRNA expression reflects a very high induction of transcription combined with a destabilization mechanism. Later, the positive transcription effect is repressed and the destabilization mechanism persists, leading to a resulting down-regulation of the c-*myc* mRNA level. The transcription could be repressed either at the initiation [49–51] or at the elongation level [52]. It would be interesting to test whether cloned tumorigenic FRTL-5 cells, in which cyclic AMP inhibits proliferation [33], 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 cyclic 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* [51].

Goitrous growth and mitogenic pathways

The disease in which goiter is easiest to explain is Graves' disease. In this disease autoantibodies directed against the TSH receptor (TSAb) activate this receptor and consequently the whole cyclic AMP cascade. At the highest concentrations reached in pathology, these TSAb do not activate, as TSH does, the Ca^{2+} phosphatidylinositol cascade (Laurent E *et al*, 1991, *J Clin Invest* (in press)). Thus, hyperthyroidism in Graves' disease appears to result from a chronic hyperstimulation of the cyclic AMP cascade. The effects of this cascade on cultured thyroid cells enhance function and proliferation while maintaining differentiation, *ie* they represent the *in vitro* counterparts of what is observed in Graves' diseases thyroids *in vivo*. It is interesting to note that, as *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 goiter. Also, in these chronically stimulated thyroids, in which proliferation and the increasing number of mitoses and bound to allow the fixation of more mutations, cancer incidence is increasing [53]. Thus, even though the cyclic AMP 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 goiter resulting from congenital defects in iodine metabolism by the gland is also simply explained by classical concepts of thyroid regulation. Deficiency of 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, the lack 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 cyclic AMP accumulation. 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 iodination defects which most severely affect the iodide inhibitory pathway lead to the severest goiters and to a great incidence of thyroid cancers.

The simplest example of a somatic mutation leading to autonomous hyperfunctioning adenomas has been demonstrated by Dr 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 cyclic AMP cascade, which leads to functional activation and growth. In hyperfunctioning autonomous adenomas of somatotrophs, Landis *et al* demonstrated a mutation in Gs which causes constitutive activation of this transducing protein and consequently of the whole cyclic AMP cascade [54]. A

systematic search for similar lesions in other tumors allowed the demonstration of such a mutation in the Gs of autonomous hyperfunctioning thyroid adenomas [55].

Models of constitutive activation of the cyclic AMP mitogenic pathway

Among the mitogenic cascades, the cyclic AMP pathway is the only one that in some cell types induces both proliferation and differentiation and also activates function [4]. As shown above for the thyroid and hypophysis, its permanent stimulation leads to hyperfunctioning hypertrophied lesions. These involve the entire organ in the case of Grave's disease, where the thyroid cascade is constantly stimulated by thyroid stimulating immunoglobins, or an adenoma in cases where a somatic mutation causes constitutive activation of one element of the cascade. It might therefore be warranted at this stage to speculate about other possible somatic mutations that could cause constitutive activation of the cyclic AMP cascade and hyperfunctioning adenomas. Any permanent enhancement of a positive control or suppression of a negative control could give a selective advantage to the cells. This advantage is expressed by a higher mitotic rate and therefore a higher probability of mutations and of further progression of the cells to autonomy and tumorigenesis. However, only constitutive enhancement of a positive control could lead to autonomy from the normal regulatory feedback control (such as the thyroid pituitary feedback). If we take the thyrocytes as an example, the following steps can be considered (fig 1).

Adenylyl cyclase coupled receptors

There are several ways in which receptors could chronically activate adenylate cyclase. Overexpression of the receptor could lead to permanent stimulation if the unoccupied receptor had some basal activity or at least render the thyrocyte more sensitive to TSH and thus give it a selective advantage. Mutation of the receptor could induce or increase its constitutive activity, as has been shown for the EGF receptor in v-*erb* B oncogene transformation [56]. Heterotypic expression of a non-thyroid receptor (such as the ß adrenergic receptor) could make the cells independent of the normal thyroid pituitary feedback. Heterotypic expression of receptors coupled to the phosphatidyl-inositol cascade (serotonin 5HT1c [57], angiotensin receptor *ie* the *mas* oncogene [58]) leads to fibroblast transformation *in vitro*. Similarly expression of a receptor to a metabolite (*eg* adenosine) or a signal (*eg* a prostaglandin) that is constantly produced by the cell could also cause apparently constitutive activation. Such physiologically constitutive activation has been conferred in culture on various types of cells by the expression of RDC8 gene, *ie* of the high affinity A2 adenosine receptor [59]. These causes of chronic activation would of course have to override the normal desensitization mechanism.

Cyclase activating GTP binding protein (GS)

Constitutive activation by mutational impairment of the GTPase activity of G_s or its subunit α_s would, as in the inhibition of this activity by cholera toxin, lead to permanent constitutive activation of adenylate cyclase. This type of somatic mutation, discovered by Landis in pituitary adenomas [54], has been described earlier. An inactivating mutation of the inhibitory subunit ß of G_s would have the same effect. We do not know if overexpression of Gs could induce a permanent activation of adenylyl cyclase.

Cyclic AMP dependent protein kinase

Inactivating mutations of the inhibitory regulatory subunit of cyclic AMP protein kinase lead to constitutive activation of the enzyme. Such mutants have been produced in cell lines [60].

Mutations on negative controlling elements

Mutations in any negative control element could lead to activation of the affected cell and would confer a selective advantage. Negative controls which could be considered are: i), negative feedbacks in cyclic AMP action (Xi [61], phosphorylated protein inhibitors as postulated in autonomous nodules [62]); ii), the receptor-inhibitory GTP binding protein (Gi) pathway; iii), the iodide inhibitory pathway (at the level of iodide transport, oxidation or inhibitory iodinated derivative Xi synthesis) [61], etc. It is interesting in this regard that adenomas with defects in some steps in the latter pathway have been demonstrated [63]. Similar somatic mutations in cells in which cyclic AMP is a negative signal for growth would of course have the opposite effect, and would select against the affected cells.

Conclusion

The study of the control of thyroid growth has thus led us to define the stimulatory role of the cyclic AMP cascade and now allows us to predict alterations in its pathway that may cause hyperfunctioning and hyperplastic lesions.

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