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Functional Properties of Normal and Inverted Rat Thyroid Follicles in Suspension Culture

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Rat thyroid follicles in both the normal and the inverted configuration have been cultured in suspension. It has been found that normal follicles (i.e., those having a structural organization similar to the follicles in vivo) express most functional properties of the thyroid gland. They trap iodide, synthesize, secrete, and iodinate thyroglobulin, and secrete thyroid hormone into the culture medium. On the contrary, in inverted follicles (i.e., those having a cell polarity reversed with respect to normal) we were unable to detect expression of some of these functions. We conclude that both the follicular organization and the proper cell polarity are needed for the full expression of thyroid functional properties.

In the last few years there have been several reports on the ability to isolate (Herzog and Miller, 1979; Denef et al., 1980) and to maintain in suspension culture (Mauchamp et al., 1979; Nitsch and Wollman, 1980a; Inoue et al., 1980; Herzog and Miller, 1981) separated thyroid follicles from different species. Rat thyroid follicles in suspension culture can exist in two different configurations: either normal, in which their cells surround an electron-dense lumen and have a proper orientation with microvilli and tight junctions toward the lumen (Nitsch and Wollman, 1980a), or inverted, in which the cells surround an electron-lucent lumen and have an inside-out orientation with microvilli and tight junctions in contact with the culture medium (Nitsch and Wollman, 1980c). We have used this model system to determine whether the organization in follicular structure allows the full expression of the functional properties of thyroid cells in vitro and, furthermore, to investigate the role of a proper orientation of the cells in the follicle in maintaining such properties. Here we report that normal rat thyroid follicles in suspension culture fully express differentiated functional properties and that the inversion of the follicle results in the loss or alteration of some of them.

MATERIALS AND METHODS

Follicle cultures

Suspension cultures were prepared as described (Nitsch and Wollman, 1980a). In brief, thyroids from 4–6-wk-old Wistar rats were minced in Hank's saline and incubated at 37°C for 3–4 h in complete tissue culture medium containing 2 mg/ml of collagenase (CLS II, Worthington). Tissue was dissociated by gentle pipetting and filtered through a 60-μm and a 15-μm Nitex mesh (Tetko, NY). Tissue fragments, mostly consisting of broken follicles, were collected on the 15-μm filter and plated in suspension in agarose-coated dishes in Coon's modified Ham's F12 medium. Calf serum (Flow Laboratories) was added at a concentration of 0.5% to obtain normal follicles and 5% to obtain inverted follicles. Ten thyroids were generally used to prepare ten to twenty 35-mm culture dishes.

Iodide trapping and organification

Follicles were incubated in a shaking bath, at 37°C, in 0.5 ml of complete culture medium containing 10 mM HEPES, 3 mM methimazole, 10^{-6} M $Na^{127}I$, and 5×10^5 cpm of ^{125}I (Amersham, U.K.). At the end of the incubation the follicles were centrifuged, the supernatant was carefully removed, and the radioactivity in the pellet was counted. The amount of DNA in the follicle pellet was determined by a fluorimetric assay. By comparison with a standard curve the number of cells in the follicle pellet was thus determined. To evaluate the amount of organified iodine the same procedure was used but methimazole and $Na^{127}I$ were omitted from the incubation medium and trichloroacetic acid (TCA)-precipitable counts were determined both in the follicles and in the culture media.

DNA fluorimetric assay

The amount of DNA present in follicle preparations was determined by using a modification of the Hinegardner (1971) method. Follicles were resuspended in H_2O and centrifuged. The pellets were dried at 60°C and stored at −20°C until analysed; 20 μl of a DABA solution (diaminobenzoic acid, Aldrich Chemical CO.), 0.4 mg/ml in 4 N HCl, was added to the samples and the DNA standards and incubated at 60°C for 45 min. After the addition of 2 ml cold perchloric acid fluorimetric measurement was made with an excitation wavelength of 408 and an emission wavelength of 508.

Gel electrophoresis

Follicles were starved for 4 h in methionine-free medium and incubated for 24 h in fresh medium containing

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150 μCi/ml of ^{35}S-methionine (sp. act. 1050 Ci/mmol; Radiochemical Co., Amersham, U.K.) and 90 μg/liter of cold L-methionine. Alternatively, follicles were incubated for 4 h in medium containing 1×10^6 cpm of ^{125}I (Amersham, U.K.). After either labelling procedure follicles were collected by centrifugation and lysed in a 0.01 M Tris-HCl buffer, pH 7.2, containing 0.1 M NaCl, 0.001 M EDTA, and Nonidet-P40. Both the supernatant of lysed follicles and the culture medium, if enough TCA-precipitable counts were present, were immunoprecipitated with affinity-purified antithyroglobulin antibodies (Alvino et al., 1978). As a control for the specificity of the immunoprecipitation either an excess of cold thyroglobulin was added to part of the samples before the addition of antithyroglobulin antibodies or nonimmune serum was used. After denaturation samples were loaded onto a 6.5% polyacrylamide slab gel according to the method of Laemmli (1970).

Thyrotropin (TSH) stimulation and hormone secretion

Bovine TSH (National Pituitary Agency, 21 U/mg) was dissolved in 0.1% bovine serum albumin (Calbiochem, A grade) and used at a final concentration of 10 mU/ml. Four 35-mm dishes of follicles cultured for 3 days in the presence of 10^{-8} M NaI were pooled and stimulated for 24 h with TSH in 1.0 ml of culture medium. The amount of T_3 and T_4 in the culture medium was determined by radioimmunoassay.

RESULTS

Rat thyroid follicles in suspension culture were tested for the expression of several functional properties, such as the ability to trap iodide, to synthesize, secrete, and iodinate thyroglobulin, and to secrete thyroid hormone.

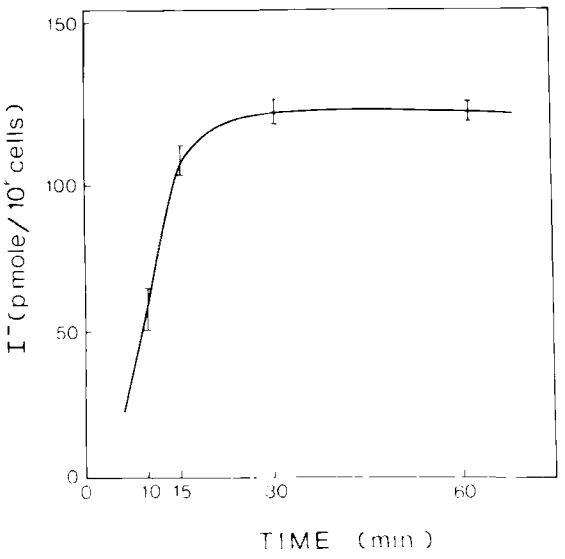

Fig. 1. Trapping of I^- in normal follicles. Follicles were cultured for 3 days, collected by centrifugation, and resuspended in 0.5 ml of ^{125}I-containing medium (see Materials and Methods). Following incubation for different lengths of time the radioactivity and the DNA content in the follicle pellet were determined. Data refer to samples of 3×10^5 cells in triplicates.

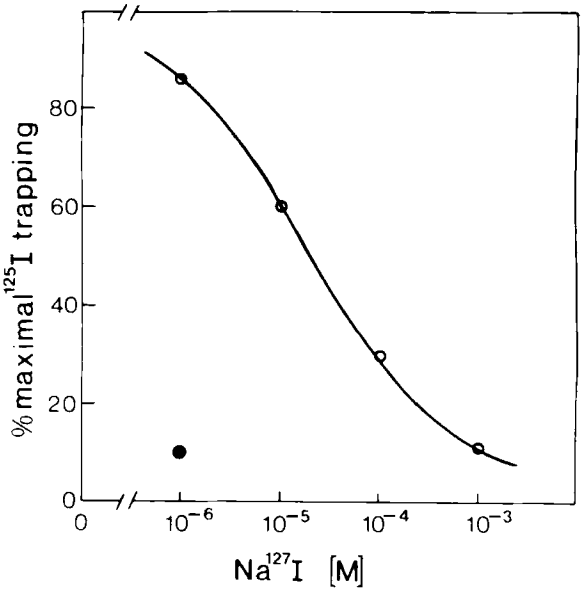

Fig. 2. Inhibition of ^{125}I trapping by sodium perchlorate (●) and by increasing concentrations of $Na^{127}I$ (○). The experiments were performed as in Figure 1. Sodium perchlorate was at a concentration of 10^{-3} M.

Properties of Normal Follicles

Iodide trapping. Kinetics of iodide trapping was analysed in normal follicles cultured in suspension for 3 days. The amount of I^- trapped within the follicles increased with time and plateaued after about 30 min (Fig. 1). Trapping of ^{125}I was dependent upon the concentration of $Na^{127}I$ in the incubation medium and was completely inhibited by sodium perchlorate (Fig. 2). These findings are in agreement with a saturable iodide concentration mechanism similar to the one present in vivo (Wolff, 1964). The C/M after 1 h of incubation was about 15.

Thyroglobulin synthesis and iodination. When normal follicles were labelled with ^{35}S-methionine, it was found that they synthesized a protein that was immunoprecipitated by antithyroglobulin antibodies and migrated on $NaDodSO_4$ polyacrylamide gel electrophoresis (PAGE) as two major bands of about 300–330 KDa (Fig. 3). Thyroglobulin extracted from rat thyroid glands showed, after reduction, an identical migration pattern (Palumbo and Ambrosio, 1981). The amount of radioactivity in the immunoprecipitate was between 2 and 4% of the total TCA-precipitable counts, in good agreement with data obtained with cultured cell lines (Avvedimento et al., 1985). If follicles were labelled with ^{125}I instead of methionine, together with the two 300–330 KDa bands, a third band of about 70 KDa was sometimes detected (Fig. 3). In the follicle culture media, either by ^{35}S-methionine or by ^{125}I labelling, the TCA-precipitable counts were very low and variable, although significantly above background. By autoradiography of sections of follicles incubated with ^{125}I for 60 min lumina appeared to be labelled, further demonstrating the iodination of the thyroglobulin deposited in the lumen (data not shown).

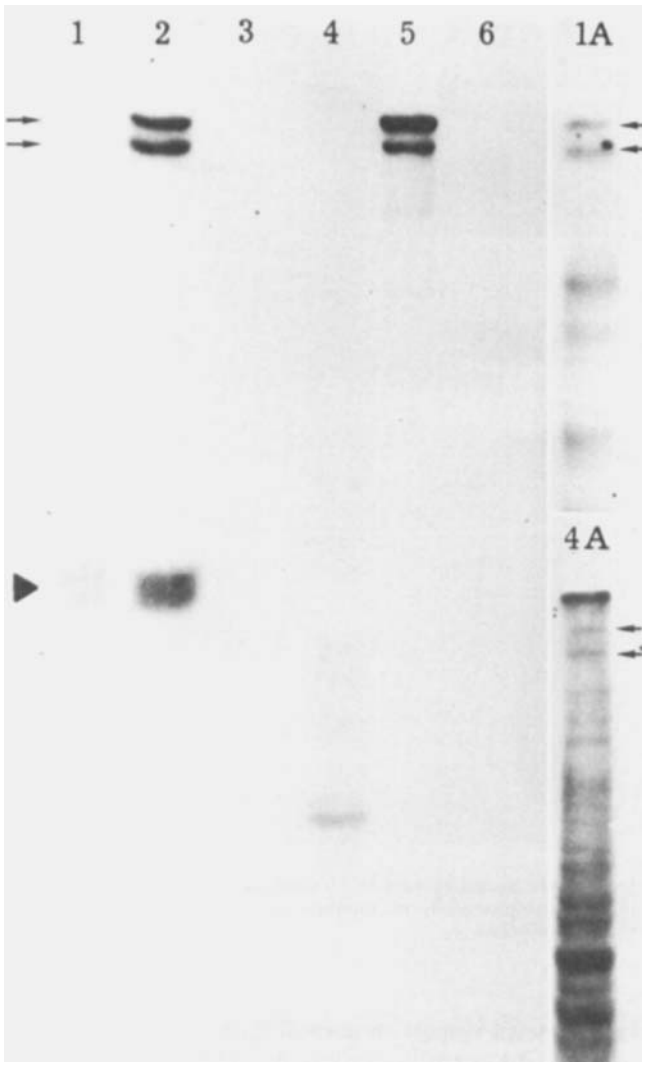

Fig. 3. Fluorography of an $NaDodSO_4$ polyacrylamide gel electrophoresis of total soluble proteins of normal follicles cultured for 3 days (lanes 1 and 4) and proteins immunoprecipitated by antithyroglobulin antibodies (lanes 2 and 5). Lanes 3 and 6 are, respectively, the same as lanes 2 and 5 but excess cold thyroglobulin was present during the immunoprecipitation. Lanes 1–3: ^{125}I labelling; lanes 4–6: ^{35}S-methionine labelling. 1A and 4A are, respectively, longer exposures of 1 and 4. The 300–330 KDa bands (arrows) and the 70 KDa band (arrowhead) are indicated.

Thyroid hormone secretion. Normal follicles cultured in suspension for 3 days in the presence of 10^{-8} M sodium iodide secreted thyroid hormone into the culture medium when stimulated for 24 h by TSH. Significant amounts of T_3 could be detected by radioimmunoassay in the culture medium (Table 1). T_4 was not present in appreciable amount. No detectable secretion occurred in the absence of thyrotropin stimulation (Table 1).

TABLE 1. Secretion of T_3 and T_4 by normal follicles[1]

	T_3 (pg/10^6 cells/h)	T_4 (pg/10^6 cells/h)
Normal follicles (−TSH)	167 ± 40	4,167 ± 833
Normal follicles (+TSH)	487 ± 40	5,208 ± 1,042
Calf serum (10%)	152 ± 40	3,542 ± 625

[1] T_3 and T_4 were determined by radioimmunoassay in the culture medium of normal follicles that had been cultured in suspension for 3 days and stimulated with TSH for 24 h (see Materials and Methods). Differences in T_4 secretion are not significant due to scatter in the radioimmunoassay. Calf serum was present in all samples at a concentration of 10%.

TABLE 2. Iodine trapping and organification in inverted follicles versus normal follicles[1]

Follicles	^{125}I (cpm × 10^{-3})
Normal (+methimazole)	40 ± 2
Inverted (+methimazole)	4 ± 1
Normal (−methimazole)	200 ± 30
Inverted (−methimazole)	15 ± 10

[1] Iodine trapping and organification were determined as described in Materials and Methods. Each follicle sample consisted of about 3×10^5 cells. The amount of ^{125}I incorporated by inverted follicles represents background counts also detected in normal follicles in the presence of 10^{-3} M sodium perchlorate (see Fig. 2).

Properties of inverted follicles

The general morphological properties of inverted follicles from rat (Garbi and Wollman, 1982) and some functional properties of inverted follicles from pig thyroids have already been reported (Herzog and Miller, 1981). Inverted follicles did not actively trap iodide (Table 2). If methimazole was omitted from the incubation medium some TCA-precipitable counts were found associated with the follicle pellet (Table 2), indicating that a limited amount of iodine organification could occur. Inverted follicles also had the ability to synthesize thyroglobulin. Total protein synthesis and percent of thyroglobulin synthesis were not significantly different from that of normal follicles. Most of the newly synthesized thyroglobulin was found in the culture medium (Fig. 4). This thyroglobulin migrated, after reduction, as a single band of about 330 KDa on $NaDodSO_4$ PAGE (Fig. 4). The thyroglobulin in the culture medium was not significantly iodinated and no free T_3 or T_4 could be detected by radioimmunoassay.

DISCUSSION

Our results indicate that normal rat thyroid follicles reconstructed in vitro not only maintain the morphological appearance (Nitsch and Wollman, 1980a) but also the functional properties of their in vivo counterparts. Moreover, we show that the inversion of polarity of follicles results in the loss or alteration of some functional properties, indicating that it is not enough for thyroid cells to be polarized and organized in three-dimensional structures to be completely functional: a proper orientation is also required. Table 3 summarizes the different properties of normal and inverted follicles. In the interpretation of data on functional properties of inverted follicles it should be considered that, at least in the rat, some of them are indeed "mixed follicles." They consist of inverted follicles that contain, in close contact with their inner wall, a normal follicle. These are thought to arise from cell aggregates with multiple lumina and are stable in culture for several days (Garbi and Wollman, 1982).

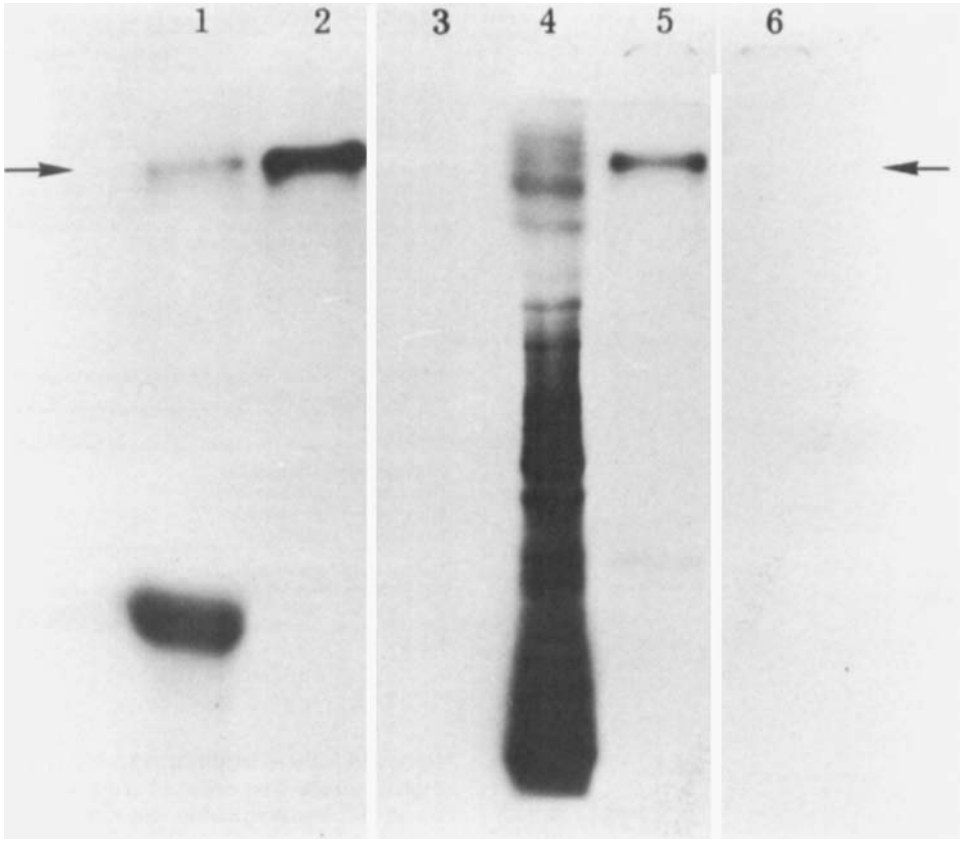

Fig. 4. Fluorography of an $NaDodSO_4$ gel electrophoresis of ^{35}S-methionine-labelled proteins in the culture medium (lanes 1–3) and in the cells (lanes 4–6) of inverted follicles that had been cultured for 3 days. Lanes 1 and 4: total soluble proteins; lanes 2 and 5: proteins immunoprecipitated by antithyroglobulin antibodies; lanes 3 and 6: proteins immunoprecipitated by nonimmune serum. The 330 KDa band (arrows) is indicated.

TABLE 3. Summary of the functional properties of normal and inverted follicles

	Normal follicles	Inverted follicles
Iodine trapping	+	–
Thyroglobulin synthesis	+	+
Thyroglobulin secretion	Into the lumen	Into the medium
Thyroglobulin iodination	+	–
Hormone secretion	+(T_3)	–
Fluid transport	(?)	Into the lumen[1]
Response to TSH		
acute	+[2]	(?)
chronic	+[3]	+[4]

[1]Mauchamp et al. (1979).
[2]Nitsch and Wollman (1980a).
[3]Nitsch and Wollman (1980b).
[4]Herzog and Miller (1981).

Iodide trapping

Normal follicles actively trap iodide by a specific pumping system. Inverted follicles, instead, lack this ability. A substantial decrease of iodide uptake in porcine thyroid follicles, after 3 days of suspension culture, has been reported by Karlsson et al. (1982). Our results, however, cannot be directly compared with theirs because it is not clear which is the percentage of inverted follicles with respect to normal follicles in their preparations. One possible explanation for the lack of iodide trapping in inverted follicles might be that when inversion occurs the active pumps involved in iodide trapping are moved to the luminal side of the cells and are therefore unable to get in contact with their substrate present in the medium. The possibility that the principal site of the mechanism for the concentration of the radioiodide by the thyroid follicle is at the basal end of the cells was already proposed by Andros and Wollman (1967). More recently it has been suggested that the polarized distribution of iodide pumps is responsible for the loss of trapping ability in monolayer cultures of thyroid cells grown on filters when iodide is present from the apical side (Chambard et al., 1983). An alternative explanation for the loss of iodide trapping ability in inverted follicles could be the modifications that cells undergo following inversion of polarity. One major change is cell attenuation due to swelling of inverted follicles (Garbi and Wollman, 1982). It has been proposed by Studer et al. (1978) that cell shape modifications could determine the loss of some functional properties in vivo.

Thyroglobulin synthesis

Both normal and inverted follicles are able to synthesize thyroglobulin. In normal follicles we have found, by

immunoprecipitation, two bands of about 300–330 KDa in $NaDodSO_4$ PAGE, which represent newly synthesized and iodinated thyroglobulin, and a third band, of about 70 KDa, that is very prominent in the ^{125}I-labelled follicles. Although both the 300–330 KDa bands correspond to thyroglobulin molecules, and they are both in fact immunoprecipitated by antithyroglobulin antibodies, it is not clear what determines the difference in their migration (van der Walt et al., 1978). The presence of one thyroglobulin gene in the haploid genome (van Ommen et al., 1983) and of a single immunoprecipitable band in the cell-free translation of rat thyroid mRNA (Alvino et al., 1982) suggests that the presence of two bands may be due to posttranslational modification(s). The 70 KDa peptide, which is differently labelled with the two isotopes, could be a degradation product of thyroglobulin well represented in ^{125}I-labelled follicles, where older molecules can possibly be labelled. Small peptides with a very high iodine content have been actually found in different species (Dunn et al., 1970; Lejeune et al., 1983). In inverted follicles the newly synthesized thyroglobulin migrates, after reduction, as a single band in $NaDodSO_4$ PAGE. This may indicate the lack of some posttranslational modification of the protein synthesized by cells of inverted follicles with respect to that synthesized in normal follicles.

Thyroglobulin secretion and iodination

Thyroglobulin secreted by cells of normal follicles accumulates in the lumen (Nitsch and Wollman, 1980a) and can be iodinated. In inverted follicles, instead, most of the newly synthesized thyroglobulin is directly secreted into the culture medium. This finding is consistent with that of Herzog and Miller (1981) on inverted pig thyroid follicles and agrees quite well with the cellular polarity observed by morphological analysis. Furthermore, this thyroglobulin is not iodinated. This may be due to the fact that thyroglobulin is very diluted in the culture medium with respect to the follicle lumen in vivo and is possibly at low concentration near the apical border of the cells where iodination is likely to occur (Ekholm and Wollman, 1975). Moreover, the inverted follicles are not even able to trap iodide. When inverted follicles are incubated with ^{125}I in the absence of methimazole, however, some TCA-precipitable counts can be found associated with the follicles. This observation can be explained by the presence of "mixed follicles," in which a normal follicle is found within an inverted follicle (Garbi and Wollman, 1982). Preliminary autoradiographic data indicate that the lumen of normal follicles found within mixed follicles can be labelled with ^{125}I (Nitsch, unpublished results).

Thyroid hormone secretion

Although normal follicles are able, after thyrotropin stimulation, to release the thyroid hormone T_3 into the medium, such ability is not manifested by inverted follicles. This seems to be an obvious consequence of their inability to iodinate thyroglobulin. No T_4 production could be demonstrated by normal follicles after prolonged thyrotropin stimulation. Why follicles did not secrete T_4 is not clear. It should be considered that normally, in the rat, a large fraction of the circulating T_3 is directly secreted into the blood by the thyroid gland (Inoue et al., 1967) while in other species most of the circulating T_3 is produced from T_4 by peripheral deiodinases. Furthermore, if the level of iodination is low T_3 can be preferentially secreted (Greer et al., 1968).

Response to TSH

We have shown that normal follicles respond to acute (Nitsch and Wollman, 1980a) and chronic (Nitsch and Wollman, 1980b) TSH stimulation. Here we find that significant amount of T_3 is secreted after TSH stimulation, thus confirming the functional response of normal follicles to the hormone. It has not been clearly determined yet whether inverted follicles respond to acute TSH stimulation. There is evidence that they respond to chronic stimulation (Herzog and Miller, 1981), indicating the accessibility of the TSH receptor. It has been shown, however, by Chambard et al. (1983) that thyroid cells grown on filters cannot be acutely stimulated by TSH added to the apical side, suggesting the basolateral distribution of the TSH receptor on the plasma membrane. The discrepancy between the two data could be either due to the presence of TSH receptors on the apical surface of inverted follicles or to the transfer of TSH molecules into the follicular lumen, possibly by transcytosis (Herzog, 1983).

Role of the extracellular matrix

The expression of fully differentiated properties by normal follicles, in this system, does not seem to depend on the presence of any organized extracellular matrix (Nitsch and Wollman, 1980a). Normal follicles in suspension culture have, however, a rather unstable polarity and they tend to undergo inversion of polarity, especially if the serum concentration in the culture medium is elevated (Nitsch and Wollman, 1980c). On the other hand, a collagen gel can control the polarity of the follicles either by preventing the occurrence of inversion (Garbi et al., 1984) or by reinverting the polarity of inverted follicles (Chambard et al., 1981). One of the functions of the extracellular matrix could, therefore, consist in determining and/or stabilizing the proper orientation of the follicular cells, which is a prerequisite for the full expression of functional properties.

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