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THYROTROPIN INDUCES CHANGES IN THE MORPHOLOGY AND THE ORGANIZATION OF MICROFILAMENT STRUCTURES IN CULTURED THYROID CELLS

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SUMMARY

Thyrotropin (TSH) induces morphological changes in cultures of normal rat thyroid cell lines and in primary bovine thyroid cells. It also induces a specific reorganization of the microfilaments of the thyroid cells. Both effects are fully reversible and are mimicked by 8-bromo-cAMP. These results indicate that the trophic response of TSH involves changes in the organization of the actin-containing filaments, probably mediated through cAMP, followed by changes in cell shape.

Thyrotropin (or TSH), the pituitary stimulator of the thyroid, is required for the organization of follicular structures by cultured thyroid cells of various species [1]. In the absence of TSH thyroid cells grow in a monolayer with an epithelial phenotype [1]. In the presence of TSH follicular structures are formed in vitro. These multicellular structures concentrate iodide and synthesize iodinated thyroglobulin [2]. TSH activates a membrane-associated adenylatecyclase in thyroid cells and many of the responses of TSH can be mimicked by various analogues of cAMP [3]. Therefore, it is largely believed that cAMP acts as a second messenger of TSH in thyroid cells [4].

Ambesi-Impiombato et al. [5–7] recently established several normal rat thyroid cell lines. The thyroid cell line, denoted $FRTL_5$ (Fisher rat thyroid cells in 5% serum), expresses several thyroid functions such as iodide concentration and thyroglobulin synthesis.

In this paper we report that TSH induces morphological changes in cultures of $FRTL_5$ and in primary bovine embryo thyroid cells (BET). It also induces a dramatic reorganization of their microfilament system. Both alterations are reversible and are mimicked by the application of 8-bromo-cAMP. Furthermore, we have observed a concomitant change in the dynamic properties of the cells which results in the formation of follicle-like structures.

MATERIALS AND METHODS

Cell cultures

Fisher rat thyroid cell line established by Ambesi-Impiombato et al. [5–7] was used in this study. We have used a differentiated thyroid cell line called $FRTL_5$ which responds to TSH, synthesizes thyro-

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globulin and accumulates iodide. This cell line is grown in Coon's modified Ham F12 medium supplemented with 5% calf serum and 10 mU/ml TSH, 10 μg/ml insulin, 5 μg/ml transferin, 10 ng/ml somatostatin, 20 ng/ml glycyl-histidyl-L-lysine and 10^{-8} M hydrocortisone. All the thyroid cells are grown at 37°C in a humidified atmosphere of 95% air : 5% CO_2.

Primary cell cultures of bovine embryonic thyroid (BET) were prepared from glands excised from three embryos of 16–18 cm crown–rump length which corresponds to 90–110 days of gestation [8]. At this embryonic stage the thyroid gland is histologically and functionally differentiated [9]. The pituitary contains thyrotrophic cells [9] and TSH [10]. The embryos were collected 20 min after slaughter at the local slaughterhouse with their amniotic sack intact and placed immediately in chilled medium. The thyroid glands were removed aseptically within 2 h after slaughter, washed in medium and cut into small pieces. The tissue was digested enzymatically, as previously reported [5–7]. The tissue pieces were suspended in 1.5 ml of Hanks BSS without Ca^{2+} and Mg^{2+} and with collagenase (20 μg/ml; Worthington), trypsin (1 : 300 dilution; ICN), and 2% heat-inactivated dialysed chicken serum. The tissue was shaken for 20 min at 37°C in a water bath and then was vigorously pipetted until the tissue slices were reduced to a cell suspension. The cell suspension was centrifuged at 1000 g for 5 min and the pellet washed with medium. After another centrifugation and washing the cells were resuspended in Coon's modified Ham F-12 medium and seeded in 35 mm culture dishes. The BET cells were supplemented with 0.5% calf serum and the mixture of the six hormones [5–7].

Reagents

Bovine TSH (21 IU/mg, NIAMDD, bTSH-9), was a generous gift from the pituitary hormone distribution program of the National Institute of Arthritis, Metabolism, and Digestive Diseases. 8-Bromo-cAMP was purchased from Sigma. 7-Nitrobenz-2-oxa-1,3,diazole-phallacidin (NBD-phallacidin) was used as previously reported [11]. Actin antibodies were prepared in rabbits by injections of pure chicken back muscle actin. The animals were immunized with 2 mg of pure actin emulsified in Complete Freund's Adjuvant and challenged 3 and 5 weeks later with the same dose of the antigen. Antibodies were purified on G-actin immunosorbent.

Visualization of cells

To label actin the cells were fixed with 3% paraformaldehyde for 10 min and permeabilized with 0.1% Triton X-100 for 10 min at room temperature, washed with PBS and incubated with either 2 ng/ml of NBD-phallacidin for 20 min or with 30 μg/ml anti-actin antibodies followed by 7 μg/ml rhodamine-goat anti-rabbit. In some experiments the permeabilized cells were labelled with both NBD-phallacidin and anti-actin antibodies. The fluorescence preparations were observed with a Zeiss inverted microscope IM-35 equipped with filters for selective observation of either NBD (or fluorescein) and rhodamine fluorescence. Photographs were taken on Kodak Tri-X film.

Time-lapse cinematography of thyroid cells was performed under low illumination conditions using a Silicon Intensified Target (SIT) camera (RCA TC/1030 H). Photographs were taken from the television screen with a Polaroid camera as described earlier [12–14].

RESULTS AND DISCUSSION

The thyroid cells were grown in Coon's modified Ham F12 medium supplemented with 5% calf serum and the six hormones (see Materials and Methods). $FRTL_5$ cells, grown under these conditions, are shown in fig. 1*A*. The cells grew in groups of round, closely-associated cells ('rounded up' phenotype). When the $FRTL_5$ cells were grown in the absence of TSH for one week, they acquired a different type of morphology (fig. 1*B*). The cells appeared epithelial-like, growing in groups of flat, closely associated cells ('well spread' phenotype). The flattening was already noticeable after 2 h of TSH deprivation.

The 'rounded up' phenotype of the $FRTL_5$ cells was fully restored when the cells were exposed to either 10 mU/ml of TSH (fig. 1*C*) or to 1 mM 8-bromo-cAMP (fig. 1*D*) for 1 h at 37°C. Both changes were reversible, i.e., the removal of either TSH or 8-bromo-cAMP restored the formation of the 'well spread' phenotype. Hence, the effect of TSH on cell morphology was mimicked by 8-bromo-cAMP and, as with TSH, was reversible.

It is generally believed that cellular morphology and dynamics are controlled by the interactions of cytoskeletal elements. Thus, TSH- and cAMP-induced changes in thyroid cell morphology may be the consequence of alterations in the organization of certain cytoskeletal elements. We have examined the effect of TSH on the organization of the three major cytoskeletal filaments: microtubules, intermediate filaments and microfilaments of the thyroid

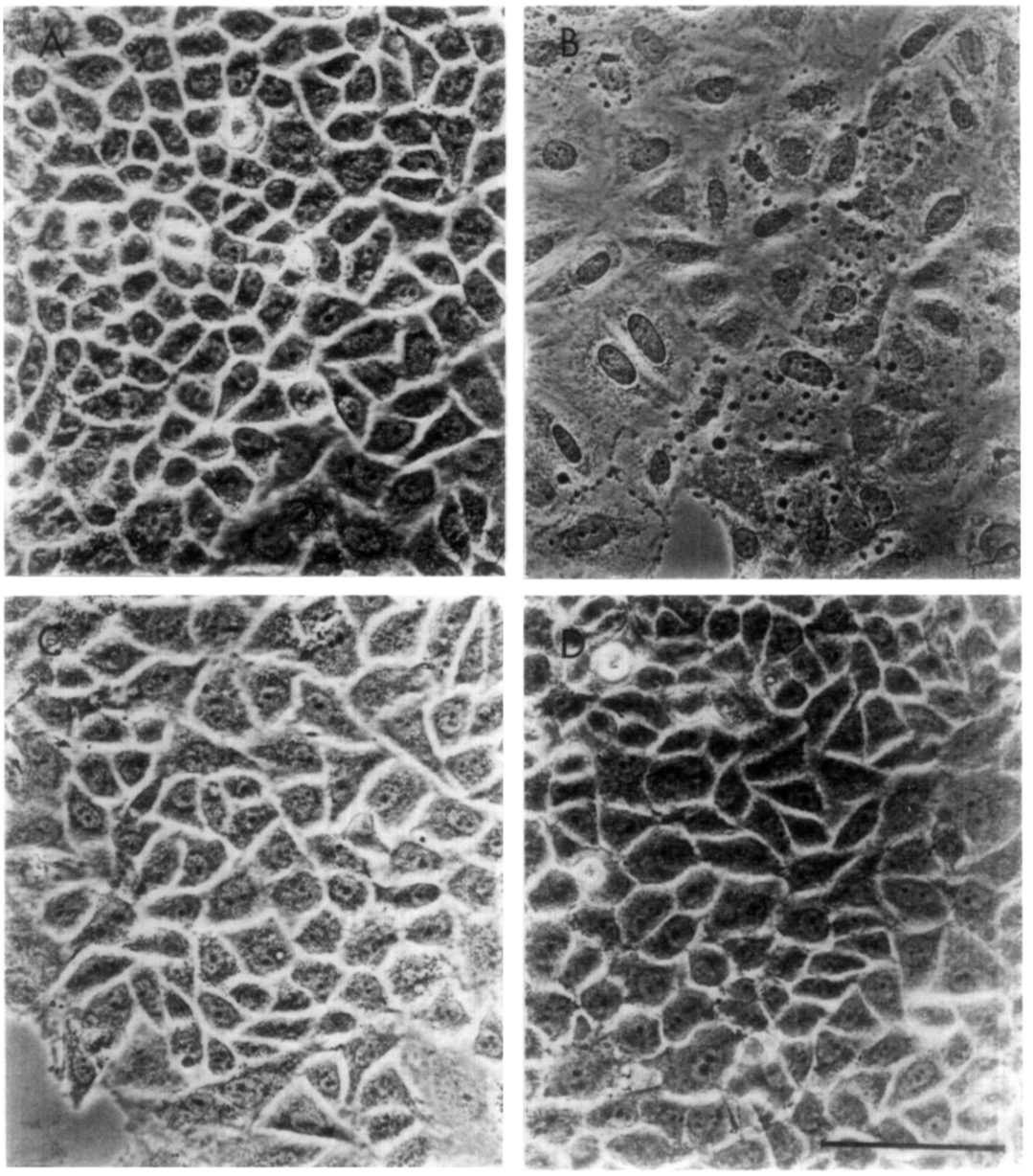


Fig. 1. The morphology of $FRTL_5$ cells in the presence or absence of either TSH or 8-bromo-cAMP. $FRTL_5$ cells grown in (*A*) presence, (*B*) absence of TSH, for one week; effect of exposure to (*C*) TSH for 24 h; (*D*) to 8-bromo-cAMP for 1 h on the morphology of $FRTL_5$ cells grown in the absence of TSH. Bar, 10 μm.

cells. We did not observe significant changes in cells immunofluorescently labelled for intermediate filaments or microtubules (data not shown). However, we observed striking changes in the organization of the actin-containing microfilaments as a consequence of the exposure of the cells to either TSH or 8-bromo-cAMP.

Actin was labelled in Triton-permeabilized $FRTL_5$ cells by two specific reagents: NBD-phallacidin or by indirect immunofluorescent labelling with actin antibodies.

Upon removal of TSH and the appearance of the 'spread out' phenotype, most of the actin appeared in filament bundles oriented in different directions. Similar distribution of actin was observed when the permeabilized cells were labelled with actin antibodies (fig. 2*A*) or with NBD-phallacidin (fig. 2*C*) or with both reagents together (fig. 2*F*, NBD-phallacidin and fig. 2*E*, anti-actin). The distribution pattern of both markers was clearly overlapping.

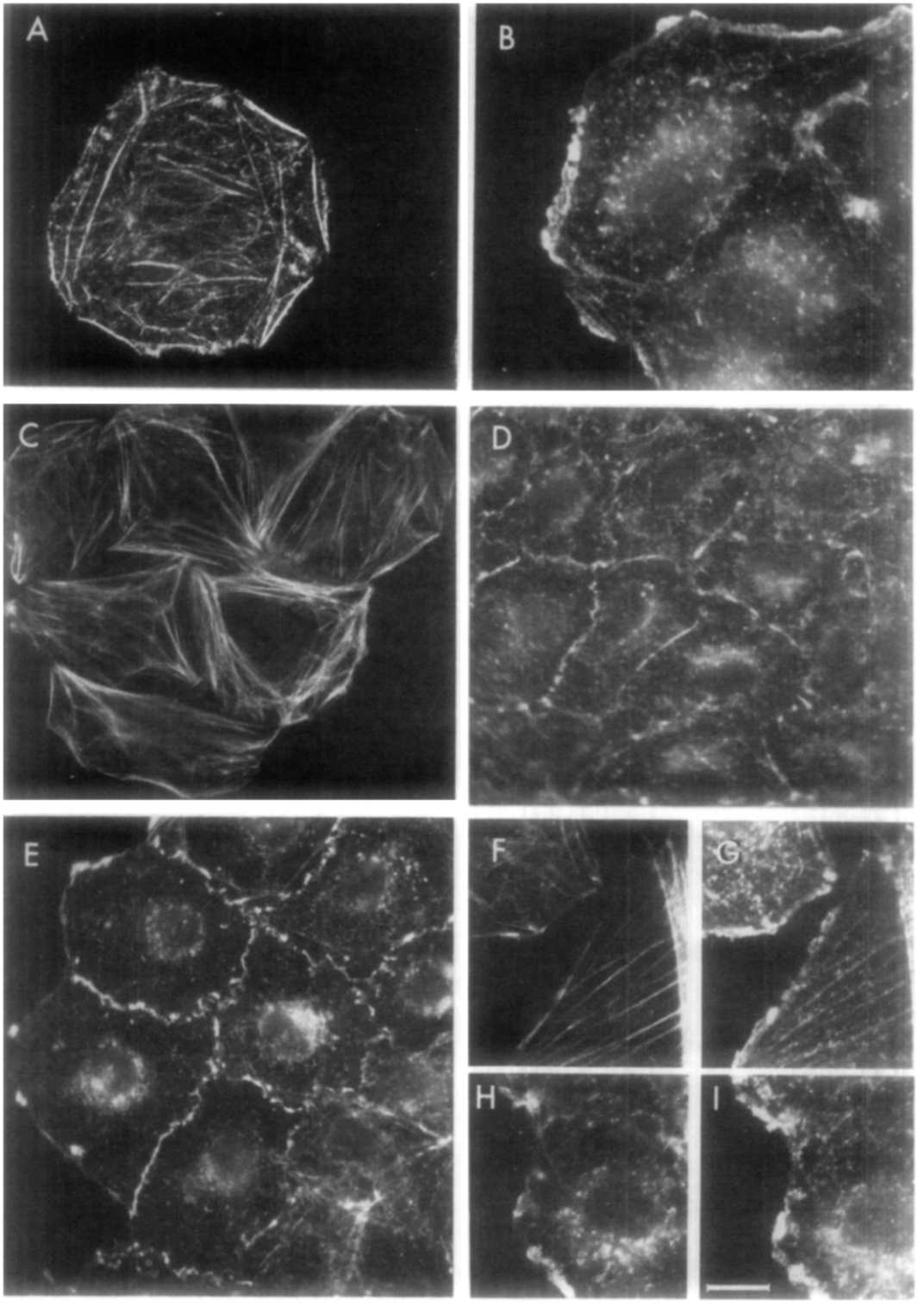
When the $FRTL_5$ cells were exposed to either 10 mU/ml TSH or 1 mM 8-bromo-cAMP a different pattern was observed. Both reagents induced a drastic reduction in the number and organization of the actin bundles. Most of the actin appeared diffuse, or concentrated in dots which did not form a well-defined pattern. Again both NBD-phallacidin and anti-actin antibodies showed similar pattern of labelling. Fig. 2*B* illustrates the immunofluorescent labelling with actin antibodies in cells treated with TSH. Fig. 2*D* shows a similar experiment for cells labelled with NBD-phallacidin. Fig. 2*H* and 2*D* represents cells labelled with both NBD-phallacidin and anti-actin, respectively. Similar results were obtained when $FRTL_5$ cells were treated with 8-bromo-cAMP, fixed permeabilized and then labelled with NBD-phallacidin (fig. 2*E*).

In all the experiments the appearance of the 'rounded up' phenotype was accompanied by diffuse distribution of cellular actin.

When either TSH or 8-bromo-cAMP were removed a well organized actin system reappeared. Furthermore, the 'spread out' phenotype was re-expressed. Hence, the effect of TSH on the organization of the actin-containing bundles was mimicked by a cAMP analogue and both changes in cell morphology and in the organizations of the actin bundles were reversible. Similar effects on cytoskeletal organization and cellular morphology were observed when primary cultures of early bovine-embryo thyroid cells (BET), grown under conditions similar to the $FRTL_5$ cells, were exposed to either TSH or to 8-bromo-cAMP (unpublished results).

Using an image-intensified time-lapse video system [13, 14] we followed the dynamic changes which occurred in TSH-deprived BET cells after the addition of either TSH or 8-bromo-cAMP leading to the formation of 'follicle-like' structures similar to those described by Winand & Kohn [1] (fig. 3*A*). Living cells were maintained at 37°C in a Dvorak chamber containing complete growth medium supplemented with 20 mM Hepes, pH 7.4 in the absence or presence of either TSH or 8-bromo-cAMP. Phase micrographs of the thyroid cells were taken with a Polaroid camera from the television screen that projects the intensified images of the cells. Low intensity of light which does not damage the cells was used to follow the dynamic properties of the thyroid cells. Typical morphological changes induced in TSH-deprived BET cells at various times after the addition of

Fig. 2. Fluorescent labelling of $FRTL_5$ cells for actin in presence or absence of either TSH or 8-bromo-cAMP. In the absence of TSH, both actin antibodies (*A*) and NBD-phallacidin (*C*) decorated actin associated with well organized bundles oriented in different directions. Some of them span the entire cell and terminate in areas of cell-to-cell contact. Similar labelling pattern was obtained when $FRTL_5$ cells were double-labelled with NBD-phallacidin (*F*) and anti-actin antibodies (*G*). In the presence of TSH actin labelled with either specific antibodies (*B*) or NBD-phallacidin exhibited diffuse distribution or was organized in dots which did not form a well-defined pattern. Double labelling with both NBD-phallacidin (*H*) and actin antibodies (*I*) on the same cells yielded similar results. 8-Bromo-cAMP induced a similar effect on the microfilament system (*E*).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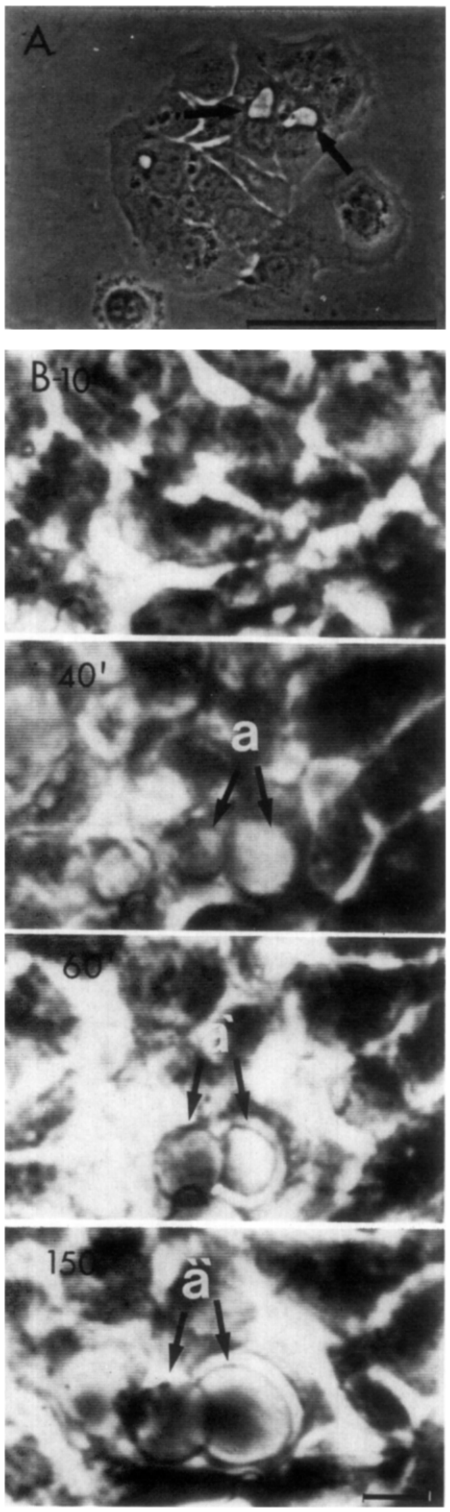

TSH, are shown in fig. 3*B*. Within 10 min after the addition of TSH the cells became gradually more round (fig. 3*B*). After 40 min most of the cells were already round and a 'follicle-like' structure [1, 10] appeared (fig. 3*B*, *a*). The 'follicle-like' structure was enlarged slightly after 60 min (fig. 3*B*, *a'*) and after 150 min (fig. 3*B*, *a''*). TSH induces the formation of follicle-like structures in cultured thyroid cells from various species [1]. Furthermore, the polarization of the follicle-like structures is defined by a polar distribution of microfilaments and microtubules [15].

Using this approach we have correlated the TSH-mediated changes in the cytoskeletal organization and in cellular morphology with the dynamic properties of the cells which lead to the formation of the follicle-like structures in culture. The major conclusions are as follows:

(1) The cultured thyroid cells are poorly motile both in the absence and in the presence of TSH. Thus, the follicular organization mediated by TSH cannot be attributed to aggregation due to increased cell motility.

(2) TSH induces a decrease in cell ruffling and a gradual appearance of the 'rounded up' phenotype. This involves changes in the packing of the thyroid cells which eventually lead to the formation of the follicular structures.

Fig. 3. Dynamics of the morphological changes induced in thyroid cells (BET) after addition of TSH. Phase micrograph of (*A*) BET cells (48 h after plating) forming 'follicle-like' structures (indicated by the arrows); (*B*) thyroid cells grown in a Dvorak chamber taken with a Polaroid camera from the television screen that projects the intensified image of the cells at various times after the addition of TSH. Ten minutes after addition of TSH the cells began to be round; 40 min after addition of TSH the cells changed their shape and two 'follicle-like' structures appeared (see *a*). Sixty minutes after addition of TSH, the 'follicle-like' (see *a'*) continued to grow; 150 min after addition of TSH, the 'follicle-like' continued to grow (see *a''*). Bar, 10 μm.

Our results indicate that the trophic response of TSH involves changes in the organization of actin-containing filaments (probably mediated through cAMP) followed by changes in cell shape. Further research is required in order to elucidate the relationship between these findings and the structural polarity which appears in thyroid cells [1, 15] of the mature thyroid follicles.

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