Thyroid hormone induces the generation of a novel putative protein in piscine ovarian follicle that stimulates the conversion of pregnenolone to progesterone

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Ovarian follicles were collected from the perch belonging to the vitellogenic stage and incubated in vitro for 4 h in the absence (control) or presence of triiodothyronine (T3). Addition of T3 (40 ng/ml) to the follicle incubation caused a two-fold increase of [3H] pregnenolone conversion to radiolabelled progesterone (P4) as compared to the control. The increase in P4 formation in the ovarian follicle could be blocked completely by the inhibitors of protein synthesis, actinomycin D and cycloheximide (50 μg/ml), suggesting a protein or peptide mediator of the T3 stimulatory effect. To search for this mediator, ovarian follicles from the control or T3 incubate were homogenized and ultracentrifuged and different fractions were added separately to fresh follicle incubations. Only the 100000 g supernatant from T3 incubate showed a significant (p < 0.01) increase in P4 formation, while the corresponding supernatant from control follicle incubations had no such stimulatory effect. Trypsin or heat destroyed this augmentary effect. Based on its ability to stimulate the conversion of radiolabelled pregnenolone to P4 in the ovarian follicle, the T3-induced protein (TIP) was purified to homogeneity by employing Sephadex G-75 gel filtration. FPLC Mono-Q and FPLC Superose-6 chromatography. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of purified TIP showed it to be a 52 K monomer protein. Addition of TIP in increasing concentrations to follicle incubations caused a linear increase in P4 formation. Experiments with radiolabelled TIP ([125I] TIP) indicate its entry through the follicular cell membrane within the limited period of incubation. Results suggest that TIP activates ovarian 3β-hydroxysteroid dehydrogenase enzyme, thus effecting a greater conversion of pregnenolone to P4.

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Thyroid hormone has long been implicated in the reproduction of vertebrates (1–6) but the precise nature of this influence is still unclear. The existence of thyroid hormone receptors in gonadal cells of different vertebrates and reproductive abnormalities due to deficiency in thyroid hormone indicates its direct role in reproductive physiology (5, 7–11). Binding of T3 to mouse granulosa cell nuclear receptor greatly augmented both protein synthesis and progesterone (P4) release. Inhibition of this protein synthesis by actinomycin D or cycloheximide also blocked the stimulation of P4 release from granulosa cells, indicating the requirement of thyroid hormone-induced protein synthesis in this stimulation (10). In a recent report from this laboratory it has been shown that T3 has a specific nuclear receptor in Leydig cells of goat testis: occupation of this receptor by T3 induces the synthesis of a proteinaceous factor, which in turn stimulates androgen release (12). However, the precise nature of this factor is still unclear.

The aims of this study were to describe the nature of the material that is induced by thyroid hormone in ovarian follicles. Stimulation of P4 formation by T3 was found to be mediated by a putative protein. Characterization of this protein and the probable path of its action is described in this report.

Materials and methods

Animals

Anabas testudineus (Bloch) is a seasonally breeding freshwater perch and its annual reproductive cycle may be divided into four phases: preparatory (from late February to April), prespawning (May and June), spawning (July to mid-September) and postspawning (late September to early February). Perch belonging to the prespawning stage were used for all the experiments. Adult female perch (25–30 g) about 10–12 cm long were acclimatized in laboratory aquaria at 28–30°C for at least 7 days prior to the experiment. They were fed ad libitum with commercial fish food (Shalimar
fish food; Bird and Fish Food Manufacturer, Bombay, India).

In vitro incubation of perch ovarian follicles

Ovaries collected from the perch belonging to pre-spawning (vitellogenic) phase were removed after killing the fish by decapitation and immediately placed in ice-cold oxygenated Earl's minimum essential medium (MEM) obtained from Gibco Laboratory, USA. With the help of fine scissors and forceps, the mesovarian covering was cut from the posterior to the anterior region and peeled off. The Tunica albuginea and germinal epithelium were then removed. Loosely attached ovarian follicles were easily separated out without enzyme treatment. Histological examination of the prespawning stage ovarian follicles showed that the majority belong to stage II; the average diameter of the follicles varied between 0.2 to 0.25 mm, the follicular layer was prominent with theca and granulosa and the onset of vitellogenesis was evident from the presence of yolk vesicle in the periphery of egg cytoplasm. From each perch of prespawning stage, 700–800 mg of ovarian tissue was available and therefore to examine the effect of T3 or T3-induced protein (TIP) on ovarian follicles from one fish was sufficient because each incubation was conducted with 25 mg of ovarian follicle (about 300 follicles). Different experiments were performed in a similar manner, i.e. when “five experiments” is mentioned this means that follicles were taken from five individual fish, each for one experiment. Isolated ovarian follicles were washed three times with MEM and a 25 mg quantity was placed into a 5 ml sterile beaker containing 1.0 ml of MEM. The medium was supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml) and was gassed with 95% O2/5% CO2. Viability of the ovarian follicles was at least 90%, as estimated by using the 0.1% trypan blue dye exclusion technique. Incubation of ovarian follicles was performed at 30°C with gentle shaking under an atmosphere of oxygen. Follicles were initially incubated for 1 h and at 1 h T3 (Sigma Chemical Co., St Louis, MO) or other chemicals were added. Incubation with T3 or other chemicals was then continued for 3 h. Hence, the total incubation time was 4 h.

The following experiments were performed with the above mentioned in vitro incubation system: T3 stimulation of P4 formation: localization of T3 stimulatory factor in different fractions from ovarian follicles: incubation in the presence of T3 to trigger the T3-induced factor and its purification; examination of TIP activity, i.e. addition of TIP to the follicular incubation and then determination of P4 formation in the ovarian follicle.

Determination of progesterone formation in perch ovarian follicles in response to T3

Follicles were incubated in the absence (control) or presence of T3 (40 ng/ml) in a similar manner to that described above. Radioactive pregnenolone (Δ5 [4, 7-3H] pregnenolone, specific activity 11.0 Ci/mmol. Amersham, UK) was added to the follicle incubation (0.7 ng/ml). At the end of incubation, follicles were collected, homogenized in the homogenizing buffer (mentioned below) and subjected to steroid extraction. Extracted steroid was chromatographed on TLC aluminium sheet precoated with silica gel (Alufolien Kieselgel 60 F254, E Merck, Germany). Each plate was developed in a solvent system containing chloroform and methanol (99:1, v/v) for 40–45 min at a controlled room temperature (24–28°C). To identify the position of pregnenolone and P4, a standard run was given. The plate was dried and spots were identified under UV light. Pregnenolone had an Rf of 0.76–0.80 while P4 had an Rf of 0.92–0.96. For the sample, the silica gel plate was scraped out, depending on the marker and the Rf value, and the scraped material was added to scintillation fluid (toluene-based cocktail containing PPO, POPPOP and methyl cellosolve) and counted in a liquid scintillation counter. Results are expressed as pmol radioactive P4 formed from Δ5 [4, 7-3H]pregnenolone per mg protein.

Isolation and purification of TIP

Follicles were incubated as described previously in the absence (control) or presence of T3. After terminating the incubation, ovarian follicles were homogenized in the homogizing buffer (0.01 mol/l Na-PO4 buffer (pH 7.4) and 0.05 mol/l NaCl) and homogenate was centrifuged at 1000 g for 10 min in a refrigerated centrifuge. Supernatant of 1000 g was subjected to centrifugation at 100 000 g for 1 h at 4°C in a Beckman ultracentrifuge (model L7-55), the supernatant of which is designated as 100 K sup. To examine the TIP activity at each step of isolation, a test fraction was added to ovarian follicle incubations and the ability of the fraction to convert radio-labelled pregnenolone to radioactive P4 was determined according to the above-mentioned method.

The 100 K sup from T3-treated follicles was chromatographed through a Sephadex G-75 (Pharmacia United Limited, Sweden) column (33 x 1.5 cm) that was equilibrated with phosphate-buffered saline (0.01 mol/l Na-PO4 buffer (pH 7.4) and 0.05 mol/l NaCl). The flow rate of the column was maintained at 20 ml/h and the volume eluted per fraction was 2.0 ml. To monitor whether the protein peak in Sephadex G-75 chromatography was due to the newly synthesized protein during incubation, [14C]leucine and seventeen other unlabelled amino acids (medium was supplemented with 1 mmol/l amino acid mixture containing Lys, His, Arg, Asp, Thr, Glu, Pro, Ala, Val, Met, Ile, Leu, Tyr, Phe, Ser, Cys and Trp at 1:1 ratio) were added to the follicle incubation in the absence (control) and presence of T3. Each fraction eluted through the column was then monitored for protein synthesis by following
the same procedure as described previously from this laboratory (7).

Fast-performance liquid chromatography (FPLC) was used with a Mono-Q (HR 5/5) column (Pharmacia, Sweden), an anion exchanger, for further purification of TIP. The Mono Q column was equilibrated by using 5.0 ml of starting buffer (buffer A) in 20 m mol/l Tris (pH 8.0) and then eluting the column with 10 ml of eluting buffer (buffer B) containing 20 m mol/l TRIS (pH 8.0) and 1.0 m mol/l NaCl and finally washed with buffer A until the baseline of the recorder was stable. The gradient programme was started with 4.0 ml of buffer A when buffer B was 0% and the percentage of buffer B was gradually increased by continuous mixing with buffer A at a flow rate of 0.5 ml/min. For each run, the total elution time and volume were 24 min and 12.0 ml, respectively. Fractions were collected in an automatic fraction collector (1 ml/tube).

Because the Mono Q peak II showed TIP activity, fractions under this peak were pooled, lyophilized and then redissolved for loading onto an FPLC Superose-6 column. The column was equilibrated with 0.5 m mol/l phosphate buffer (pH 7.5) and the Mono Q peak II was eluted through this column at a flow rate of 2.0 ml/min. A molecular weight calibration kit with a range of 6500–445 000 (Sigma Chemical Co. St Louis, MO) was used to determine the molecular weight of TIP.

Measurement of protein

The protein content of each chromatographic fraction was determined by measuring the optical density (OD) at 280 nm in a UV spectrophotometer (Shimadzu, Japan). The protein of pooled fractions was estimated by following the method of Lowry et al. (13), taking bovine serum albumin as the standard. The protein content of the follicle homogenate was also determined by Lowry’s method.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

To determine the purity of TIP and its molecular weight, the pooled and lyophilized fraction under peak I of FPLC superose-6 chromatography was subjected to SDS-PAGE, which was performed by following the method of Laemmli (14). The SDS-PAGE was carried out in a similar manner reported previously from this laboratory (15).

Radiiodination of TIP

Triiodothyronine-induced protein was radiiodinated with 125I (Bhabha Atomic Research Centre, Trombay, Bombay) using chloramine T (Sigma Chemical Co., St Louis, MO) following the procedure of Greenwood et al. (16). Separation of radiolabelled TIP from free radioiodine was performed on a Sephadex G-75 column by following the procedure described earlier from this laboratory (15). Specific activity of the radiolabelled TIP was determined according to the method of Jacobs (17) and found to be 35.4 Ci/µg.

Determination of 3β-hydroxysteroid dehydrogenase (3β-HSD) activity

The 3β-HSD activity was determined spectrophotometrically by following the description of Wiebe (18), except for minor modifications required to assay fish ovarian enzyme. Follicles were homogenized, centrifuged at 10 000 g for 20 min and the supernatant fraction was used as the enzyme preparation. Briefly, the assay solution contained 6.0 nmol of NAD, 0.1 m mol/l sodium pyrophosphate buffer (pH 8.9) and 0.125 µmol of pregnenolone dissolved in a propylene glycol ethanol mixture (1:1, v/v). The volume of this mixture was reduced to 10 µl to avoid turbidity. The enzyme was added last to initiate reaction, and the rate of formation of reduced NAD was determined at 340 nm in a Shimadzu double-beam spectrophotometer with the recorder against the blank containing all ingredients except the pregnenolone. Enzyme activity was expressed as nmol NAD reduced min⁻¹ mg⁻¹ protein in a cuvette of 1 cm light path.

Table 1. Location of T₄-induced P₄ stimulatory factor in different fractions of perch follicle homogenate.

<table>
<thead>
<tr>
<th>Fractons</th>
<th>Pellet</th>
<th>Supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control follicle incubation (C)</td>
<td>0.055 ± 0.002</td>
<td>0.071 ± 0.003</td>
</tr>
<tr>
<td>C + 1000 µg sup from control follicles</td>
<td>0.059 ± 0.004</td>
<td>0.070 ± 0.002</td>
</tr>
<tr>
<td>C + 1000 µg sup from T₄-treated follicles</td>
<td>0.064 ± 0.003</td>
<td>0.11 ± 0.005**</td>
</tr>
<tr>
<td>C + 100 K sup from T₄-treated follicles</td>
<td>0.057 ± 0.004</td>
<td>0.23 ± 0.007**</td>
</tr>
<tr>
<td>C + 100 K sup from T₄-treated follicles pretreated with trypsin</td>
<td>–</td>
<td>0.064 ± 0.003</td>
</tr>
<tr>
<td>C + 100 K sup from T₄-treated follicles heated to 100 °C for 5 min</td>
<td>–</td>
<td>0.055 ± 0.002</td>
</tr>
</tbody>
</table>

*Oocytes from control and T₄-treated incubations were homogenized and subjected to differential ultracentrifugation. The pellet and supernatant of each fraction were collected and added to another set of follicle incubations (10 µg protein/ml) and formation of P₄ was determined by TLC as described in the text. Trypsin (300 µg) treatment of the 100 000 g supernatant (100 K sup. 150 µg) was terminated at 90 min by the addition of Soya bean trypsin inhibitor (20 µg/ml). Heat treatment was carried out by taking the 100 K sup (150 µg) in a glass test-tube keeping it under a boiling water-bath for 10 min, cooling it and then adding it to the follicle incubations. Values are means ± sas of three observations. **p < 0.01 as compared to 1000 µg sup from control follicles.
Data were analysed by one-way analysis of variance (ANOVA). Where the F value indicated significance, means were compared by a post hoc multiple range test. All values are expressed as means ± SEM.

Results

Triiodothyronine-induced protein stimulates $P_4$ formation

Incubation of perch ovarian follicles with $T_3$ caused about a twofold increase ($p < 0.01$) in the formation of radioactive $P_4$ from radioactive pregnenolone as compared to the control. Stimulation of $P_4$ formation by $T_3$ could be blocked completely by actinomycin D or cycloheximide, indicating that $T_3$ augmentation of $P_4$ formation in the follicle has a protein(s) mediator. Actinomycin D and cycloheximide also inhibited $P_4$ formation in the control follicles. When the formation of progesterone from radiolabelled cholesterol or the formation of androstenedione via $17\alpha$-hydroxyprogesterone from radiolabelled progesterone was examined in response to $T_3$ simulation, conversion of these radiolabelled steroid precursors was not detected in the ovarian follicle, suggesting $T_3$ specificity for pregnenolone conversion to $P_4$ formation (data not shown). Because $T_3$ stimulation of $P_4$ formation is via a protein(s) mediator, evidence for such a mediator was searched for by homogenizing $T_3$-incubated follicles and subjecting them to differential ultracentrifugation. Each fraction was examined for its stimulatory effect on $P_4$ formation by adding the fraction to the follicle incubation. Table 1 demonstrates the results of this experiment. The 1000$g$ supernatant from $T_3$-
incubated follicles had a significant \((p < 0.1)\) stimulatory effect on \(P_4\) formation as compared to the control. Stimulatory activity was considerably increased when the 100 K sup from \(T_3\) incubate was added to the follicle incubation, suggesting removal of extraneous proteins. To check the proteinaceous nature of this factor, the 100 K sup from \(T_3\)-incubated follicles was treated with trypsin or heated to 100°C. Both of these treatments destroyed the ability of this factor to stimulate \(P_4\) formation, suggesting it to be a protein or proteins.

**Purification of \(T_3\)-induced putative protein**

Trilodothyronine-induced protein was then purified further by gel filtration. The 100 K sup from \(T_3\) incubate or control follicles was passed through a Sephadex G-75 column. A major initial peak (peak I) and a minor but clear second peak (peak II) was observed in the case of the 100 K sup from \(T_3\) incubate, while this was not marked in the 100 K sup from control follicles (Fig. 1). In a separate set of incubations, [\(^{14}C\)]leucine was added to monitor protein synthesis. The TCA precipitable radioactivity was clearly noticed in the same fractions of peak II of \(T_3\)-treated follicles which was not observed in the case of the control (Fig. 1). Fractions under peak I and peak II from \(T_3\)-treated follicles were lyophilized, redissolved and added to follicle incubations to check for TIP activity. The inset of Fig. 1 shows that peak I had no TIP activity while peak II from \(T_3\) incubate stimulated \(P_4\) formation to...
more than three fold as compared to the control. The peak II fractions from the same region of the control follicles did not show stimulation of P₄ formation (data not shown). Triiodothyronine-induced protein was then purified by employing FPLC Mono Q column chromatography, the column was eluted using a NaCl gradient. Figure 2 depicts that there was an unadsorbed protein peak (Mono Q-I) and another adsorbed protein peak (Mono Q-II) that was eluted with a 37% NaCl gradient. Inset of Fig. 2 shows that Mono Q Peak II fractions had TIP activity. The Mono Q Peak II was subjected to FPLC Superose-6 column chromatography and only peak I (S6-I) demonstrated TIP activity (Fig. 3). Sodium dodecyl sulphate PAGE of TIP showed it to be a 52 K monomer protein (Fig. 4). Extent of purification determined at the end of S6-I indicated a 266.6-fold purification over the starting material (follicle homogenate).

**Functional relevance of TIP**

Addition of increasing concentrations of finally purified TIP to the follicle incubations elicited a linear increase in P₄ formation till 3 μg/incubation and reached saturation at 4 μg/incubation (Fig. 5). The fact that addition of purified TIP to follicle incubation causes a functional response would raise a question about its passage through the follicular cell membrane. To investigate this, experiments were performed with radiolabelled TIP. Results showed that about 36% of total [¹²⁵I]-TIP (60 fmol) added to the incubation was taken up by the follicles within 3 h of incubation (Fig. 5), that about 36% of total [¹²⁵I]-TIP entered into the follicle, about 69% was in the cytosol fraction and 32% was particulate bound (Table 2). The results therefore show that T₃ induces the generation of a 52 K protein in the perch ovarian follicle, i.e. TIP. Because TIP greatly increased pregnenolone conversion to P₄, it probably stimulates the 3β-HSD activity, the enzyme that catalyses this particular step. To examine whether TIP itself is 3β-HSD or it stimulates 3β-HSD activity, a 10 K supernatant (10 K sup) of perch ovarian follicles was used as crude

![Graph](image)

**Fig. 5.** Effect of increasing concentrations of T₃-induced protein (TIP) on progesterone formation in the follicles. Each concentration was added to in vitro incubation of follicles and, after the termination of the incubation, follicles were homogenized and steroids were extracted and subjected to TLC for the determination of progesterone formation. Each point represents the mean ± SEM of five experiments.

![Graph](image)

**Fig. 6.** Effect of T₃-induced protein (TIP) on 3β-hydroxysteroid dehydrogenase activity in the presence of substrate (E + P + TIP) or just on substrate (P + TIP). Enzyme + pregnenolone (E + P) served as the control. A 2 μg quantity of TIP was added where mentioned. Each value is the mean ± SEM of four observations.

### Table 2. Internalization of radiolabelled T₃-induced protein ([¹²⁵I]TIP) into the follicles.

<table>
<thead>
<tr>
<th>System</th>
<th>[¹²⁵I] TIP (fmol/10⁵ follicles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact follicles</td>
<td>22.08 ± 2.3</td>
</tr>
<tr>
<td>Pellet of 10000 g</td>
<td>2.6 ± 0.32</td>
</tr>
<tr>
<td>Pellet of 100000 g</td>
<td>3.1 ± 0.19</td>
</tr>
<tr>
<td>Pellet of 1000000 g</td>
<td>1.2 ± 0.44</td>
</tr>
<tr>
<td>100000 g supernatant</td>
<td>15.0 ± 1.9</td>
</tr>
</tbody>
</table>

*Sixty femtomoles of [¹²⁵I] TIP was added to the follicle incubation after 1 h. Incubation was continued for another 3 h. On terminating the incubation, follicles were washed several times until there was no detectable count in the washing medium. Follicles were then homogenized and subjected to differential ultracentrifugation.*
3β-HSD enzyme preparation. Figure 6 demonstrates that 2 μg of TIP could increase the 3β-HSD activity of 10 K sup to more than threefold, whereas in the absence of the 10 K sup TIP had no 3β-HSD activity in the presence of the substrate (pregnenolone). This suggests that TIP is not the 3β-HSD enzyme but it augments 3β-HSD activity remarkably.

Discussion
Our findings clearly show that T3 stimulation of P4 formation in perch ovarian follicles is not direct but mediated via the generation of a putative protein factor, i.e. TIP. This information adds a new dimension to the understanding of thyroid hormone influence on reproduction. Although the stimulatory effect of T3 on cellular protein synthesis (19, 20) is known, such an action of T3 on the gonad has not yet been shown except for a few recent reports from our laboratory (7, 10, 12). Findings so far described in these reports lead to an obvious question, i.e. what could be the physiological relevance of T3 nuclear receptor in gonadal cells and the function of the protein(s) synthesized as a result of receptor occupation by T3? There must be some meaningful purpose for this newly synthesized protein because deficiency of thyroid hormone has an adverse effect on gonadal function of animals, including humans (21–23). To investigate this, perch ovarian follicles from the prespawning phase of the annual reproductive cycle were selected for two reasons: in this phase we observed earlier a maximum amount of T1 nuclear receptors as compared to other phases, indicating the prespawning stage perch to be most suitable for studying thyroid influence (8); and binding of T1 to the nuclear receptor of such follicles resulted in a significant increase in protein synthesis (7). Present observations show that a putative protein factor, i.e. TIP, is generated in perch ovarian follicles in response to T1, which remarkably augmented P4 formation in the follicles. Hence, TIP appears to be a novel mediator of thyroid hormone action. We therefore purified it to homogeneity by using FPLC Mono Q and Superox-6 chromatography. Sodium dodecyl sulphate PAGE demonstrated it to be a 52 K protein and a monomer. Purification of this mediator protein was possible because a reliable and dependable assay for TIP could be developed. Addition of test material containing TIP to the follicle incubations always increased P4 formation. In fact, this relationship is concentration dependent, as has been observed in later experiments. Increased concentrations of purified TIP caused a linear increase in P4 formation that is saturable at higher concentrations, a characteristic feature usually common to the biologically active proteins and peptides.

The question is how does TIP, being a 52 K protein, enter the follicular cell membrane barrier. The follicular envelope may permit the entry of a high-molecular-weight protein to pass through it if such a protein is required, e.g. entry of vitellogenin, which is a 400–600 K protein. Experiments with radiolabelled TIP indicate that it enters into the follicle cells. Internalization of 15 fmol of [125I]TIP into the cytosol of 103 follicles out of 60 fmol of [125I]TIP added to the incubation within 3 h indicates a preferential uptake of this protein by ovarian follicles. Neither T1 nor TIP has any effect on the conversion of radiolabelled cholesterol to pregnenolone or radiolabelled progesterone to 17α-hydroxyprogesterone and androstenedione (data not shown). Because TIP specifically augments the conversion of radiolabelled pregnenolone to P4 its site of action is probably around the 3β-HSD enzyme because this enzyme is responsible for catalyzing P4 formation from pregnenolone. Hence, the next question is how does TIP stimulate 3β-HSD? There may be two possibilities: TIP itself may be 3β-HSD and T1 simply increased its synthesis in the ovarian follicle; or TIP, being a putative protein mediator of T3, stimulates the 3β-HSD enzyme. Examination of the first possibility by preparing a crude 3β-HSD enzyme from ovarian follicles shows that addition of purified TIP to the enzyme preparation greatly increased its activity, while TIP alone with the substrate did not exhibit any enzyme activity. This suggests that TIP is not 3β-HSD but it stimulates ovarian 3β-HSD enzyme activity. How does it perform this function? We have no clear answer to this question at present, but because TIP can stimulate ovarian 3β-HSD activity in vitro, it possibly modulates the enzyme activity from a less active to a more active state. In conclusion, it may be stated that T3 can induce the generation of a 52 K putative protein in the vitellogenic ovarian follicles of a freshwater perch, i.e. TIP: TIP in turn stimulates 3β-HSD enzyme activity and this results in a higher conversion of pregnenolone to P4. Addition of this TIP to a mouse granulosa cell or goat testis Leydig cell incubation causes a highly significant increase in P4 or androgen release, respectively (unpub, obs.). These observations have the following implications: lack of species specificity for perch TIP or a TIP-like putative protein may also form in mouse granulosa cells or goat testis Leydig cells in response to T3. The existence of the latter possibility has been suggested in a current publication from our laboratory (12).

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