

The TSH receptor reveals itself

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Commentary

Immunology

The thyrotropin receptor (TSHR), one of the primary antigens in autoimmune thyroid disease, is a target of both antigen-specific T cells and antibodies in patients with this condition (1). Autoantibodies to the TSHR (TSHR-Ab) act as thyroid stimulating factor (TSH) agonists in autoimmune hyperthyroidism (Robert Graves disease) but as TSH antagonists in autoimmune hypothyroidism (Hashimoto thyroiditis). The TSHR antigen is primarily expressed in the epithelial cells of the thyroid follicles, but TSHR mRNA and protein have been reported in a variety of cell types, some of which show evidence of receptor activity (Table 1). The TSHR gene, cloned in 1989 (2–5), maps to human chromosome 14q and encodes a predicted seven-transmembrane, G protein–coupled glycoprotein. Although it is similar to the luteinizing hormone receptor and the follicle-stimulating hormone receptor, the TSHR is the largest of the glycoprotein hormone receptors, due primarily to 8– and 50–amino acid insertions in its ectodomain (residues 38–45 and 317–367) (6). As predicted from its cDNA, the TSHR has an unglycosylated molecular weight of 84 kDa but the glycosylated holoreceptor runs on SDS-PAGE with an apparent molecular weight of 95–100 kDa. There are six potential N-linked glycosylation sites on the TSHR, and it was recently shown that the TSHR is also palmitoylated (7). The minimal 5' promoter region required to confer thyroid-specific expression and cAMP autoregulation extends [...]

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The thyrotropin receptor (TSHR), one of the primary antigens in autoimmune thyroid disease, is a target of both antigen-specific T cells and antibodies in patients with this condition (1). Autoantibodies to the TSHR (TSHR-Ab) act as thyroid stimulating factor (TSH) agonists in autoimmune hyperthyroidism (Robert Graves disease) but as TSH antagonists in autoimmune hypothyroidism (Hashimoto thyroiditis). The TSHR antigen is primarily expressed in the epithelial cells of the thyroid follicles, but TSHR mRNA and protein have been reported in a variety of cell types, some of which show evidence of receptor activity (Table 1).

The *TSHR* gene, cloned in 1989 (2–5), maps to human chromosome 14q and encodes a predicted seven-transmembrane, G protein-coupled glycoprotein. Although it is similar to the luteinizing hormone receptor and the follicle-stimulating hormone receptor, the TSHR is the largest of the glycoprotein hormone receptors, due primarily to 8- and 50-amino acid insertions in its ectodomain (residues 38–45 and 317–367) (6). As predicted from its cDNA, the TSHR has an unglycosylated molecular weight of 84 kDa but the glycosylated holoreceptor runs on SDS-PAGE with an apparent molecular weight of 95–100 kDa. There are six potential N-linked glycosylation sites on the TSHR, and it was recently shown that the TSHR is also palmitoylated (7). The minimal 5' promoter region required to confer thyroid-specific expression and cAMP autoregulation extends from 220 to 39 bp upstream of the transcription start site, but there are multiple transcription start sites between –89 and –68 bp (8).

TSHR signal transduction, regulation, and maturation

The TSHR, long known to signal via cAMP (9), can induce both the phospholipase C (PLC) and the protein kinase A signal transduction systems. Intracellular Ca^{2+} and PLC regulate iodide efflux, H_2O_2 production, and thyroglobulin iodination, while adenylyl cyclase and cAMP regulate iodide uptake and transcription of thyroglobulin (Tg), thyroid peroxidase (TPO), and the sodium-iodide symporter (10, 11). Photoaffinity labeling of the TSHR with azido-GTP followed by immunoprecipitation suggests that all four $\text{G}\alpha$ subtypes are involved (12), but only $\text{G}\alpha_s$ and $\text{G}\alpha_q$ have been shown to mediate TSHR signals.

TSHR levels are positively regulated by TSH in normal cells (13), secondary to prolongation of the mRNA half-life, while exposure to high concentrations of ligand causes the receptors to be downregulated (14). Posttranslational proteolysis clips the TSH receptor (TSHR) into two subunits (referred to as α , or A, and β , or B) (15, 16), linked to each other by disulfide bonds. These α and β subunits are formed by intramolecular cleavage,

apparently at multiple sites, with removal of an intervening polypeptide segment of approximately 50 amino acids (amino acids 317–367) (17, 18). Disulfide bonds that link the two subunits are then reduced, presumably by protein disulfide isomerase leading to release of the α subunit from the membrane-bound receptor (17, 19). This phenomenon of releasing the α subunit is referred to as receptor shedding and explains the approximately 3:1 excess of β subunits found in thyroid membrane preparations (20). These observations also explain the early data indicating that the long-acting thyroid stimulator (LATS) activity in Graves patients' sera, now attributed to activating TSHR-Ab's autoantibodies, can be absorbed using the supernatant from frozen and thawed thyroid tissues (21). Moreover, such ectodomain shedding may also account for the puzzling size diversity of TSHRs detected by Western blot (20). Crucially, shedding also leads to a structural change in the α subunit once it is released from the receptor – a change whose consequences are considered by Chazenbalk et al. in this issue of the *JCI* (22).

Table 1
Tissue distribution of the TSHR

Tissue	Gene expressed	mRNA ^A	Protein ^B	References
Lymphocytes	TSHR	+	+	32–34
Thymus	TSHR, NIS, TPO, Tg	+	+	35, 36
Pituitary	TSHR	+	+	37, 38
Testis	TSHR	+	+	39, 40
Kidney	TSHR, Tg	+	+	41
Brain	TSHR	+	NR	42
Adipose/Fibroblast	TSHR	+	+	43–48
Heart	TSHR	+	+	49, 50
Bone	TSHR	+	NR	51, 52

^ART-PCR/in situ hybridization. ^BImmunohistochemistry/ligand binding assay. NIS, sodium-iodide symporter; NR, not reported.

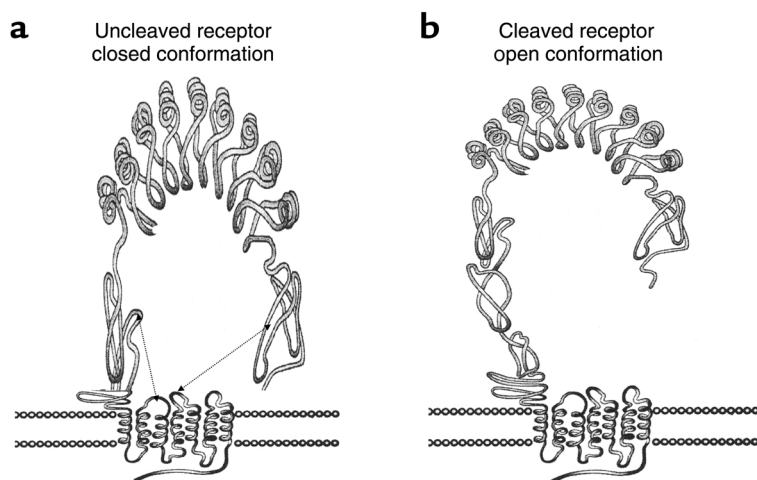


Figure 1
Two possible structural configurations of TSH receptor. (a) The uncleaved receptor is shown with a “buried” structure. The ectodomain and transmembrane interactions result in a “closed” conformation (connecting dotted lines). (b) Cleavage may induce an “open” conformation, a step that is thought to be a precondition for subsequent changes in receptor localization and activity, as described in the text and shown in Figure 2. This figure is adapted from ref. 25 with permission.

Cleavage of TSHR and receptor interactions with pathogenic antibodies

Using modern techniques, Chazenbalk et al. (22) confirm the long-standing suspicion, raised by early work on LATS-absorbing activity, that the fractured TSHR has a higher affinity for TSHR-Ab's than does the intact, membrane-bound receptor. Using both secreted and glycosidylphosphatidyl inositol-anchored (GPI) TSHR ectodomains, their elegant studies confirm that stimulating TSH receptor antibodies, which are directed primarily at the TSHR ectodomain, preferentially bind to these fragments rather than to intact membrane-bound receptor on the cell surface. This preferential binding distinguishes the agonistic TSHR-Ab's found in individuals with Graves disease from the blocking antibody type found in Hashimoto thyroiditis patients. These observations are also consistent with stimulating TSHR-Ab's being directed at a unique conformational epitope, which may be accessed more easily when the ectodomain is shed from the holoreceptor.

Apparently recognizing a conformational epitope on the receptor, activating TSHR-Ab's from susceptible individuals bind to the shed receptor ectodomain, on the so-called open form of the holoreceptor. Accumulating data from mutational analyses indicate that this receptor can shift from a “closed” (inactive) to an “open” (active) form, which can induce signaling when bound by TSH agonists, including activating autoantibodies. While TSHR cleavage and reduction are not necessary for signal transduction (23), interactions between the

ectodomain and the extracellular loops of the transmembrane domains are critical for the maintenance of an inactive state (Figure 1; see also ref. 24). Consistent with this model, removal of the TSHR ectodomain yields a constitutively active receptor, suggesting that its presence inhibits an otherwise constitutively active β subunit (25, 26). This model predicts that only the open form of the recep-

tor should bind ligand and become activated. The observation (23) that a noncleavable TSHR mutant is able to signal could simply be explained by the mutated ectodomain forming an open structure.

Recent work from our laboratory has demonstrated that the TSH receptor is constitutively oligomeric (27). Using fluorescent resonance energy transfer between two different fluorescently

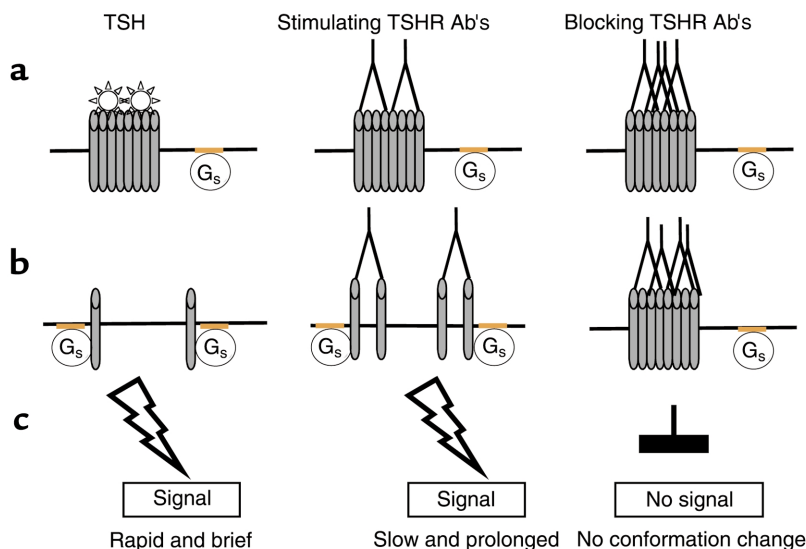


Figure 2
A model for oligomerization in TSH receptor signaling. (a) TSH receptors initially exist in an open conformation as cleaved oligomers residing on the cell surface outside lipid rafts. Signal-transducing G_s proteins, on the other hand, are restricted to lipid rafts. (b) Following TSH binding, the oligomers break apart into monomers. Conversely, stimulating antibodies, as found in sera from patients with the autoimmune disorder Graves disease, are proposed to favor formation of TSH receptor dimers; blocking antibodies, are unable to bring about this conformational change. (c) Dissociated monomers, as well as dimers, move into the lipid rafts and bind to G_s proteins, thereby initiating the signaling cascade. In the case of TSH, the signal is rapid and brief because of faster movement of monomers into the lipid rafts, in contrast to the retarded movement of the dimers. Multivalent blocking TSHR antibodies may cross-link the oligomers, thereby preventing them from dissociating and impeding their entry into lipid rafts.

labeled TSHR constructs, we confirmed the presence of TSHR multimers in cell membranes, as we previously suggested based on molecular size (20). We have now also found that these oligomeric forms rapidly dissociate upon TSH binding (28) (Figure 2), indicating that ligand binding alters the shape of the receptor, promoting dissociation of the oligomeric receptors into active, open monomers. Moreover, murine monoclonal TSHR antibodies, which block TSH action, fail to mimic TSH or to promote TSHR monomer formation (R. Latif et al., unpublished data). If, as we suggest, the monomeric form of the TSHR can be recruited more efficiently into lipid rafts (29) than can the larger complexes, these observations may indicate a mechanism by which the monomer preferentially couples with G proteins (30) to initiate signaling (Figure 2).

Although stimulating TSHR-Ab's are unique to patients with Graves disease, their serum has been shown to most commonly contain a mixture of both blocking and stimulating TSHR-Ab's (1), and the bioactivity of the serum reflects a balance between the two. Unfortunately, the surprising failure of many investigators to isolate high-affinity monoclonal stimulating TSHR antibodies represents a major impediment to understanding the basis of this response. Nevertheless, we hypothesize that stimulating, but not blocking, TSHR-Ab's lead to the formation of TSHR dimers, which may still be able to move into the lipid rafts and initiate signal transduction (Figure 2). While monomers can move quickly within the lipid rafts, as shown by fluorescence recovery after photobleaching experiments, movement of dimeric receptors within the rafts is predicted to be slower, perhaps accounting for the relatively weak but prolonged signaling that results from exposure to LATS sera (31).

The failure to obtain monoclonal stimulating antibodies of high affinity to the TSHR remains a great enigma in modern thyroid research. While blame has always been assigned to insensitive detection systems combined with a small repertoire of TSHR-Ab-secreting B cells, it is also possible that the state of the TSHR, not just the nature of the antibody,

determines whether antibody binding can activate signaling. Combinations of TSHR-Ab's, which on their own may not activate the receptor, may be required to shift the receptor into an activated form — perhaps by promoting TSHR monomer formation. This hypothesis is eminently testable and promises more excitement in this rapidly developing field.

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