The TSH receptor as a paradigm for G-protein coupled receptors in endocrinology

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G-protein coupled receptors

Glycoprotein hormone receptors (TSHR)

Role of TSHR in endocrine physiology

Role of TSHR in endocrine pathology

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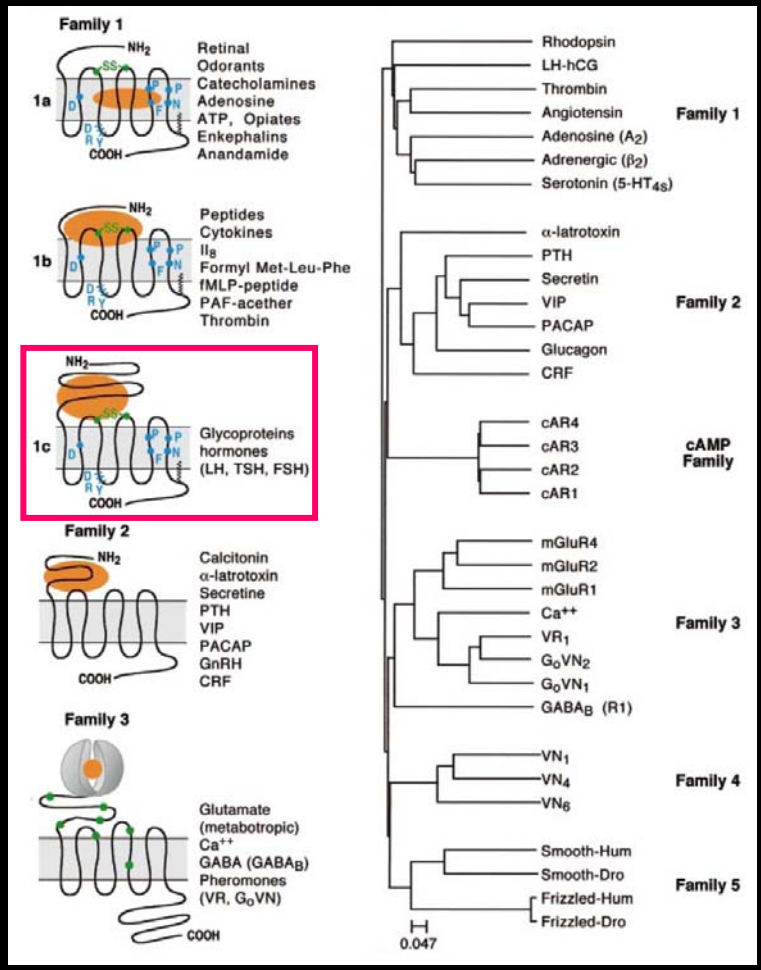
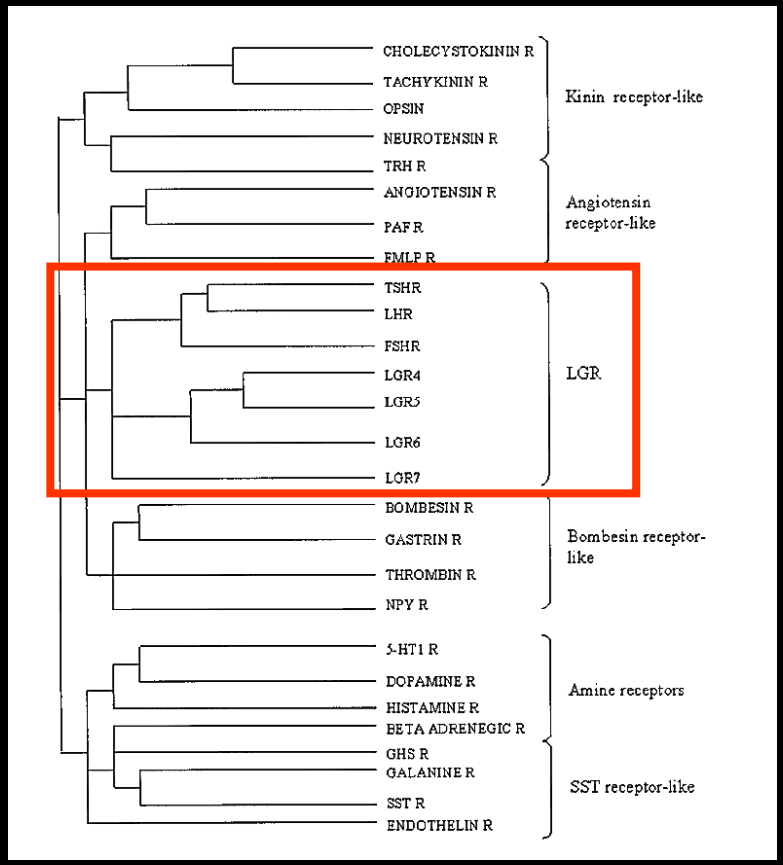
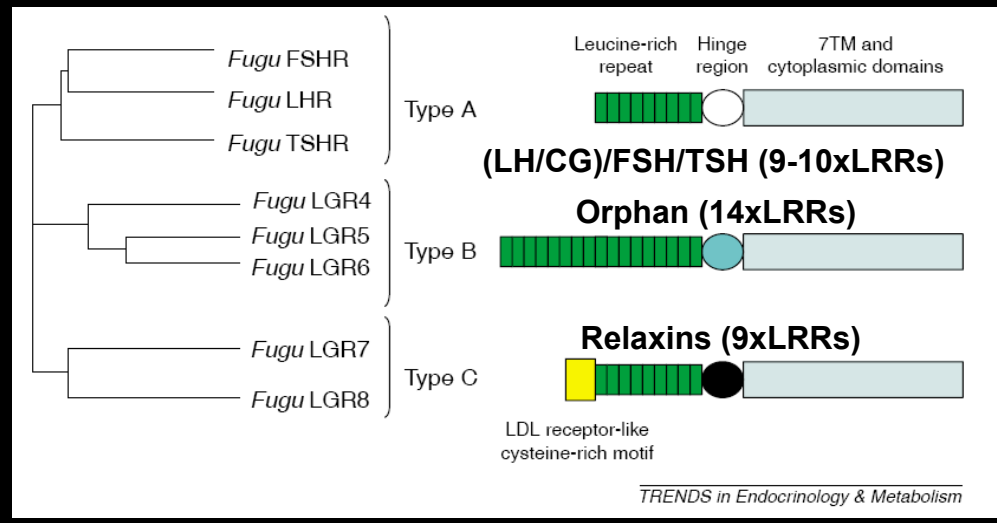
G protein coupled receptors



G protein coupled receptors are divided into 5 sub families
Transduce light, Ca^{2+}, odorants, amino acids, nucleotides, peptides, proteins
Ligands bind at different locations in the receptor

Glycoprotein hormone receptors (Subfamily 1c)





Characteristically GpHRs have a large N-terminal ectodomain 350-400αα containing leucine rich repeats (LRRs)

Hsu SYT 2003 TEM 14: 303-309

G-protein coupled receptors

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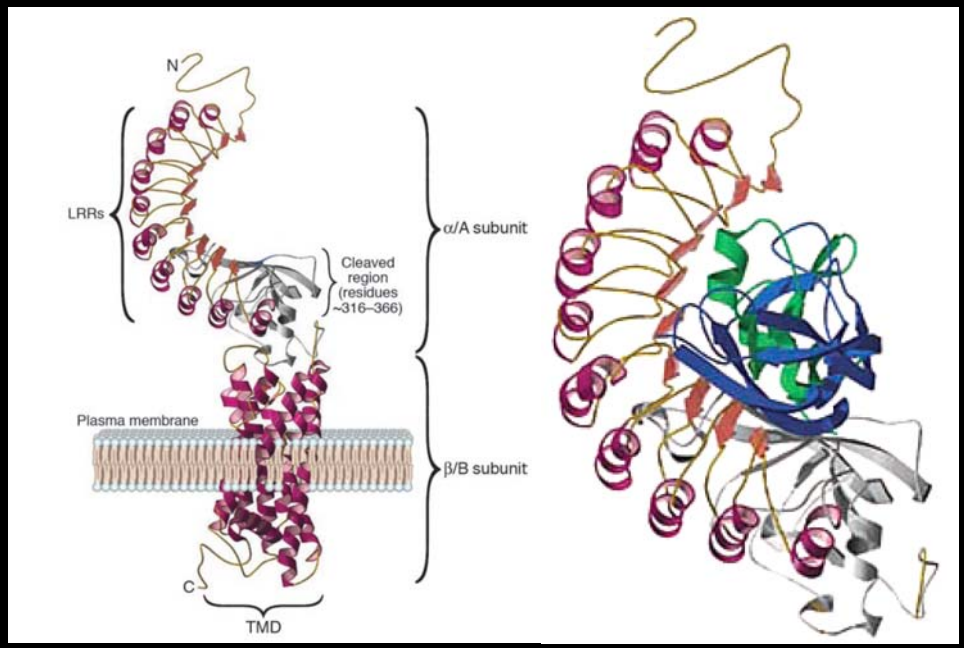
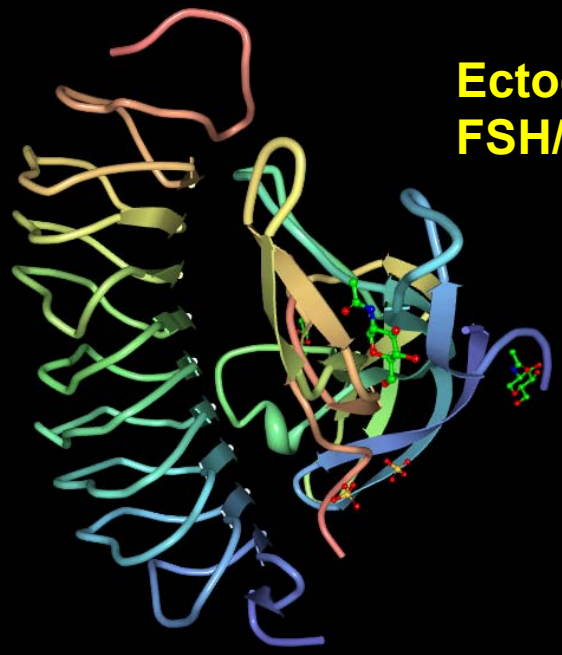
Role of TSHR in endocrine physiology

Role of TSHR in endocrine pathology

Thyroid stimulating hormone receptor





Ectodomain FSH/FSHR

TSHR expression

- **Thyroid, thymus, pituitary, testis, kidney, brain, heart, bone, fat and lymphocytes**

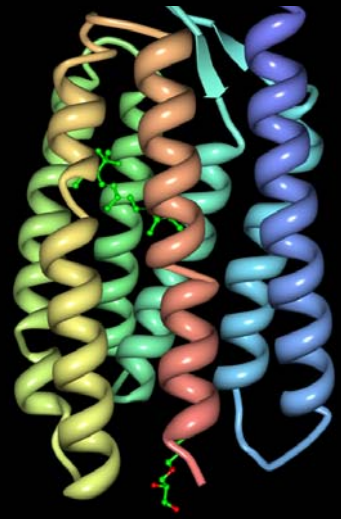
9x Leucine rich repeat ectodomain

- **LRRs are a 20-30αα motif of β-strand and α-helix**
- **40% homology between TSHR, LH/CGR and FSHR**

Cystein rich flanking hinge region

Heptahelical serpentine domain

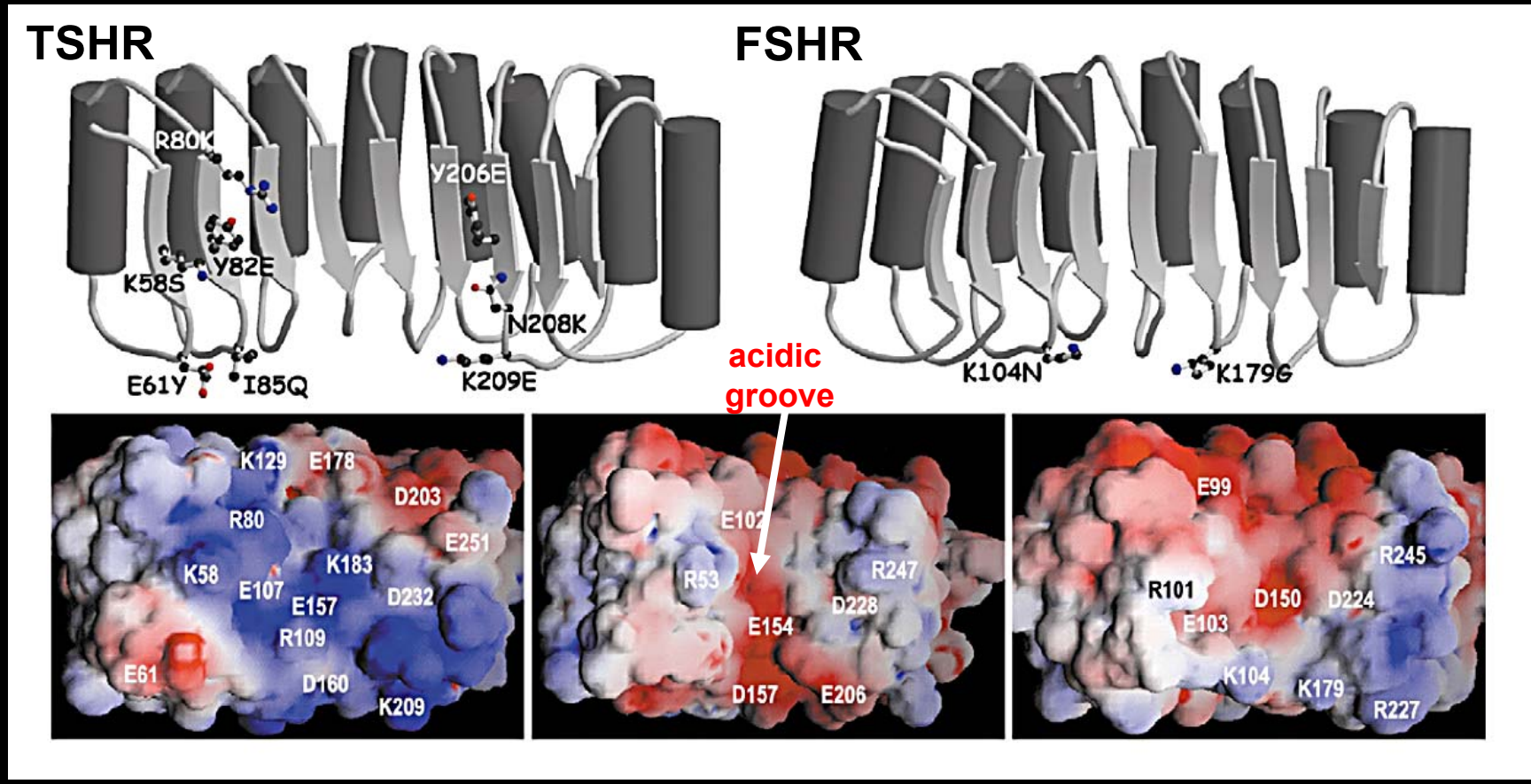
- **70% homology between TSHR, LH/CGR and FSHR**



Serpentine-domain (7TM) Rhodopsin

Luecke H 2001 Science 293:1449, Fan QR Nature 2005 433:269, Davies T 2005 JCI 115:1972

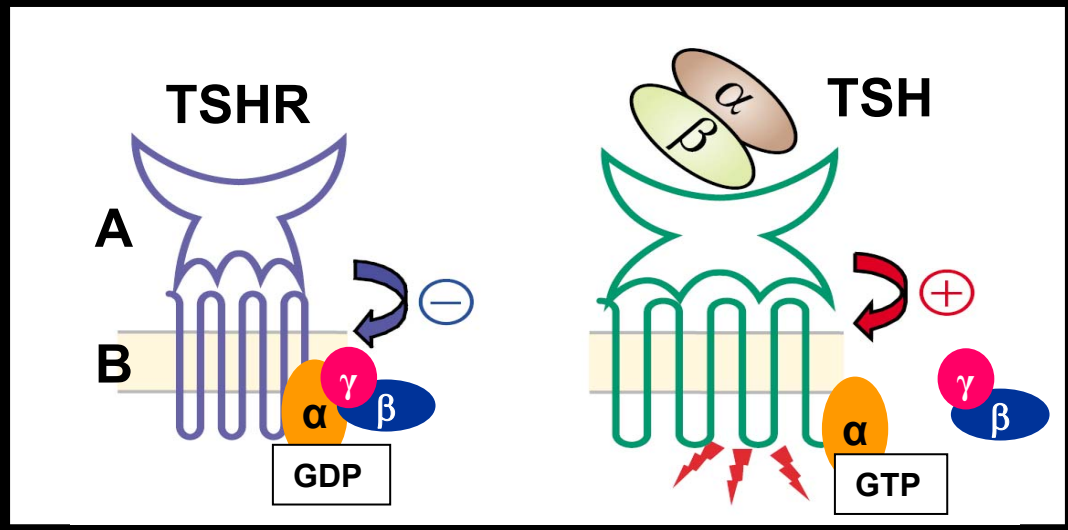
GpHR ectodomain specificity



Electrostatic potential over the accessible β-strand concave surface

Vassart G 2004 Trends Biochem Sci 29:119

TSHR silencing and activation



TSHR has a bipartite structure

- LRR ectodomain mediates ligand specificity (A-subunit)
- Ectodomain transmits signal to serpentine domain (B-subunit)
- Serpentine domain mediates signal transduction to Gs/Gq

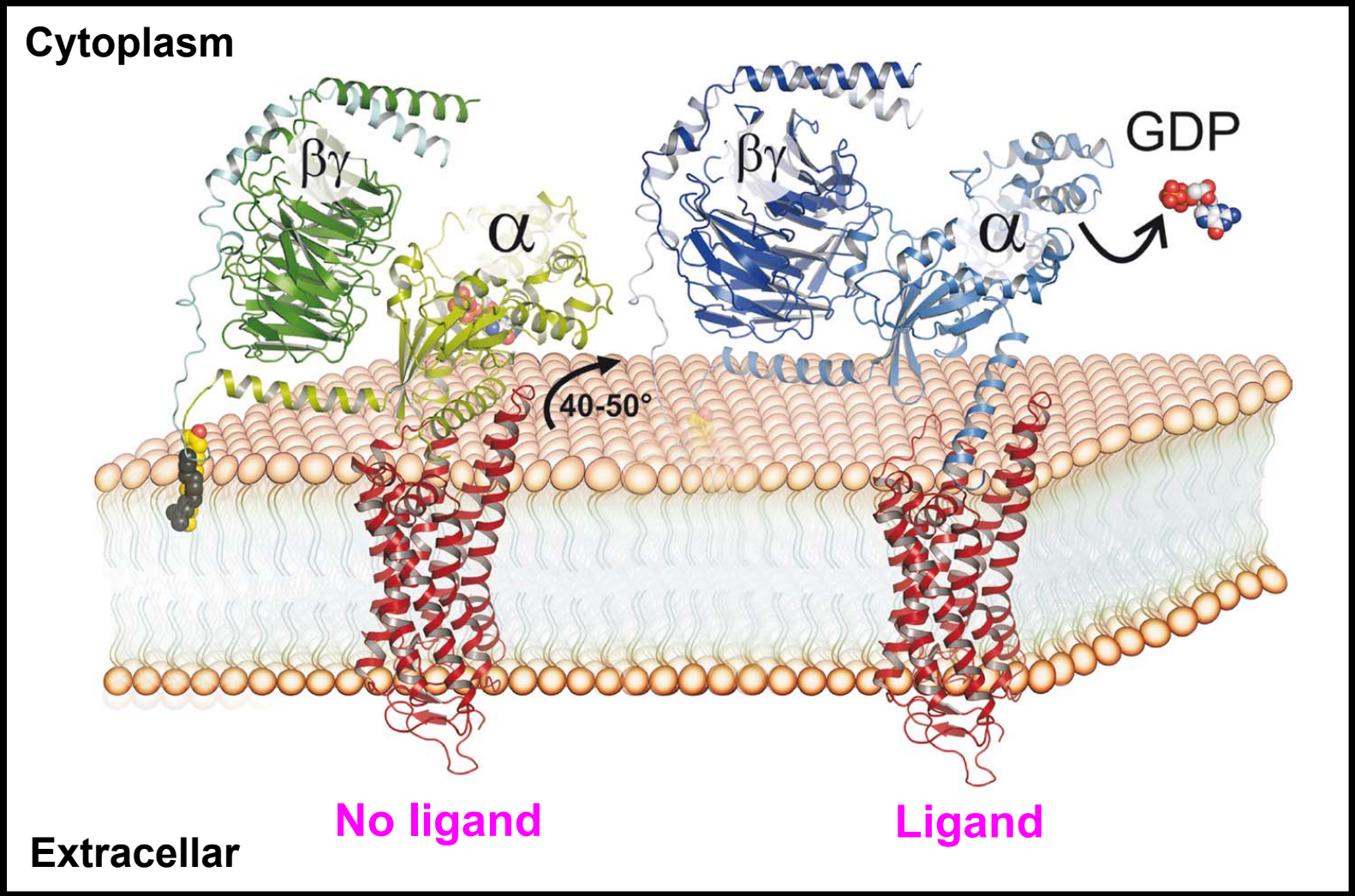
B-subunit heptahelical domain has constitutive activity (noisy)

- Unliganded A-subunit mediates receptor silencing

How conformational changes in the hinge region, following ligand binding, relieves silencing and promotes activation is starting to be elucidated

Ligand binding switches the ectodomain from tethered inverse agonist to full agonist of the serpentine domain

Activation of Gsα



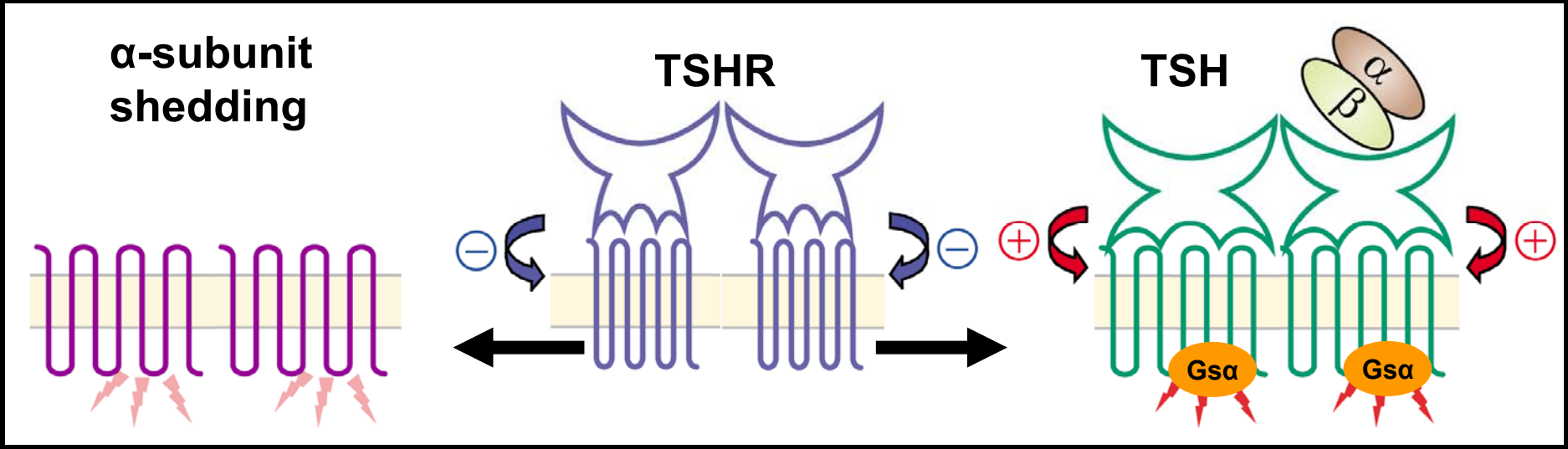
Receptor activation result in exchange of GDP for GTP

α-subunit-GTP then dissociates from receptor and βγ dimer

α-subunit-GTP then activates adeylyl cyclase

Scheerer P 2009 PNAS 106:10660

TSHR functional dimerisation

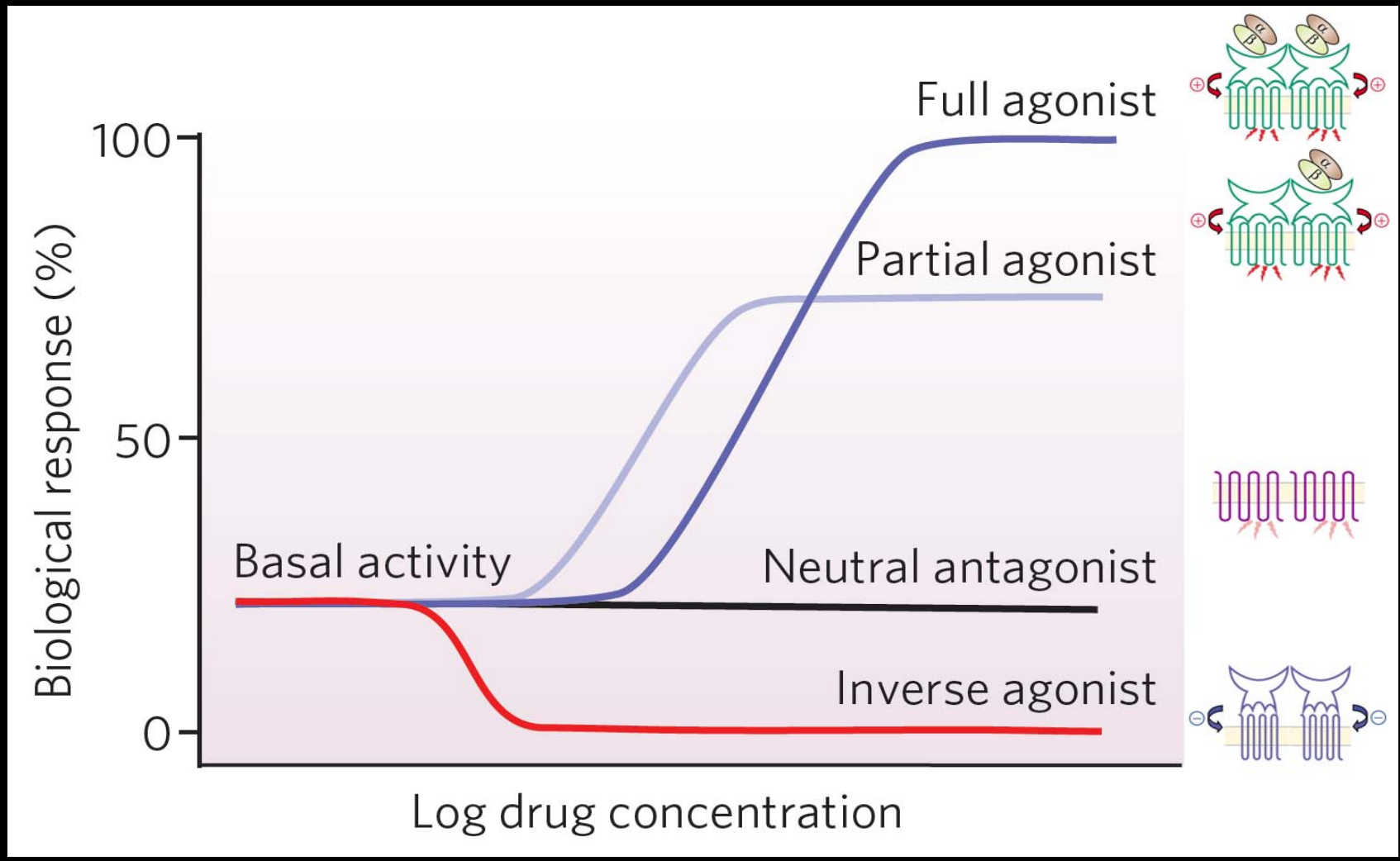


TSHR is a dimer in the plasma membrane

- Interaction between serpentine domains (TM5 and TM6)
- Probably acts as a single functional unit
- Strong negative cooperativity of hormone binding
- Allows responses over wider range of TSH concentration)
- The TSHR may also heterodimerise with other GpHRs

Uriza E 2005 EMBO J 24:1954

Full agonists and inverse agonists

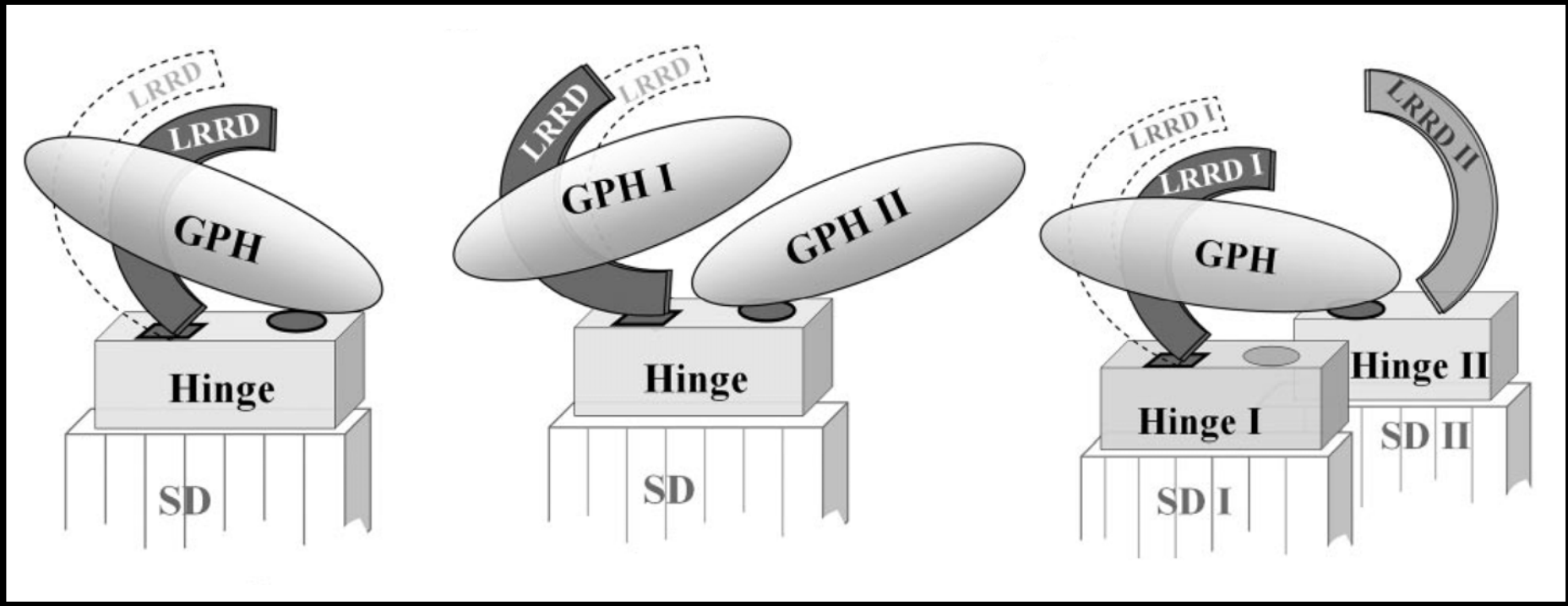


GPCRs are molecular rheostats

Rosenbaum DM et al 2009 Nature 459:356

TSH/TSHR binding and signalling

TSH binding to LRRD exposes a second binding site in the hinge region of TSHR which conducts the signal to the 3 extra cellular loops



Binding of 1x TSH to 1x TSHR

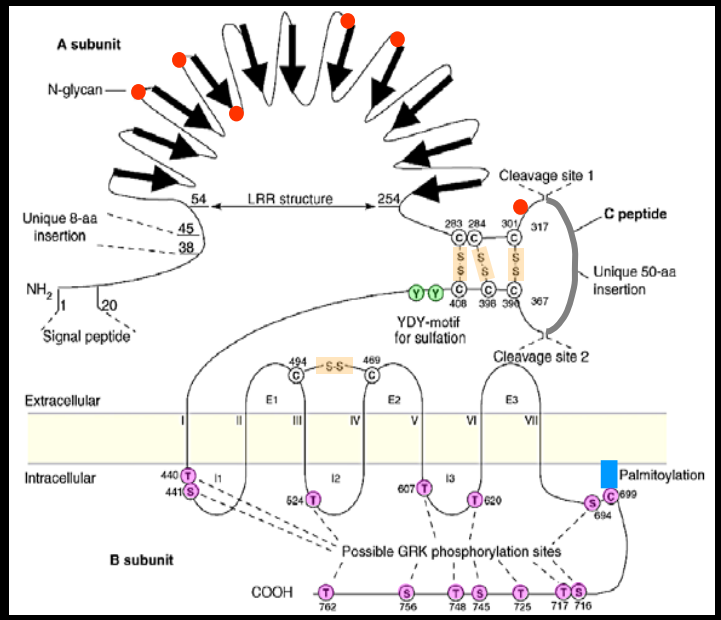
Binding of 2x TSH to 1x TSHR

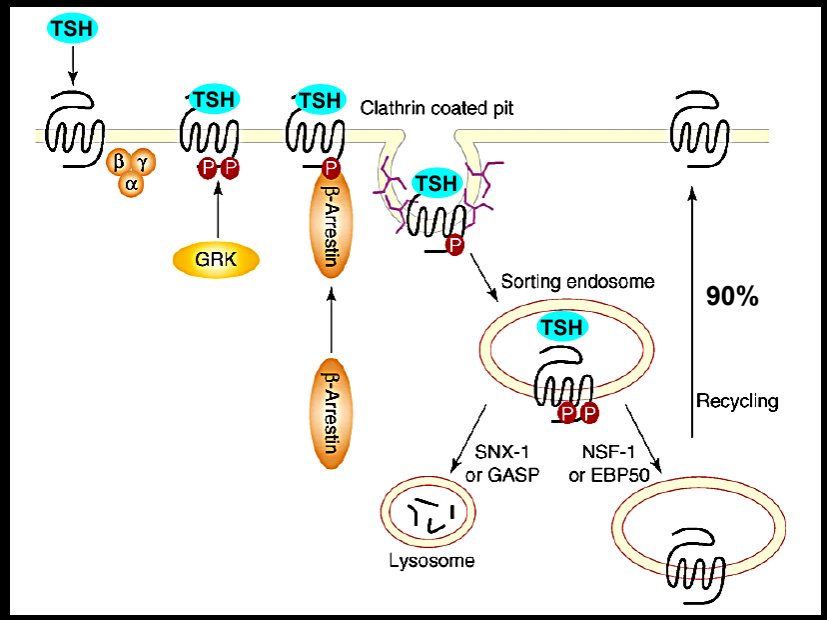
Binding of 1x TSH to TSHR dimer

Kleinau G et al 2009 Endo Reviews 30:133

TSHR post-translational modification

Protein folding, trafficking, ligand binding and signalling





Disulphide bonds	Folding and tetramerization
Cleavage	Activation? α-subunit shedding, 3:1 excess of β-subunit
Oligomerisation	Mediated by β-subunit heptahelical domain
Palminolation	Formation of a 4th ICL (cell surface expression and lipid raft entry)
Sulphation	High affinity binding requires sulphated tyrosine in β-subunit
Glycosilation	Folding, surface expression, effective TSH binding (40% mol wt)
Sialylation	Increase and prolong cell surface expression
Phosphorylation	GRKs: agonist dependent phosphorylation and desensitization

Kursawe R 2007 TEM 18:199

G-protein coupled receptors

Glycoprotein hormone receptors (TSHR)

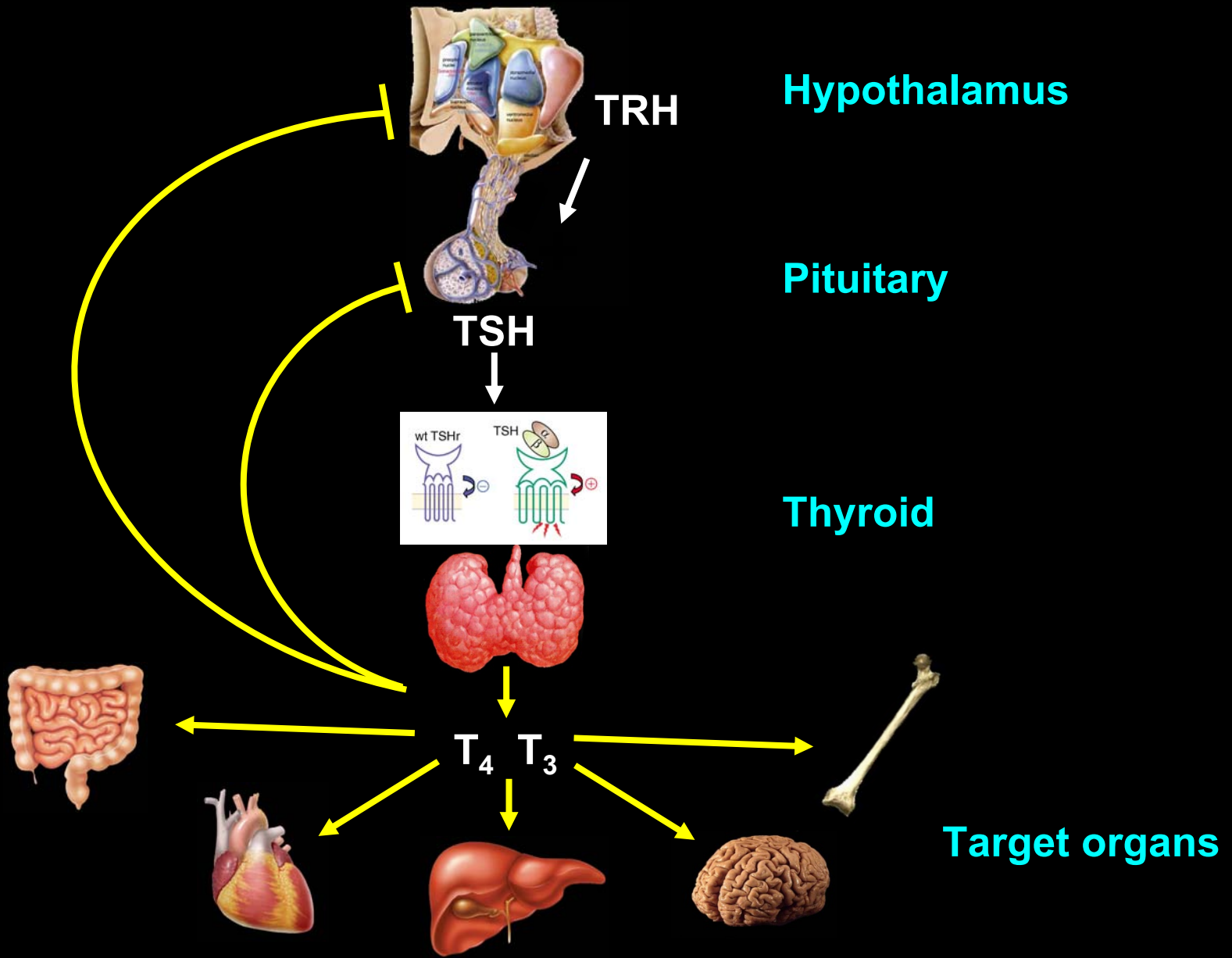
Role of TSHR in endocrine physiology

Role of TSHR in endocrine pathology

TSHR in endocrine physiology

The thyrotropin receptor plays a pre-eminent role in thyroid physiology and disease

Hypothalamic-pituitary-thyroid axis



TSHR and thyroid development

Not required for early organogenesis and migration

Not required for follicle formation

Expressed from 12 weeks of gestation

Essential for terminal thyroid maturation and growth

Hyt/Hyt ($TSHR^{P556K}$) and $TSHR^{-/-}$ mice

Congenital hypothyroidism, thyroid hypoplasia

TSH/TSHR and thyroid function

Thyroid follicular cell proliferation and differentiation

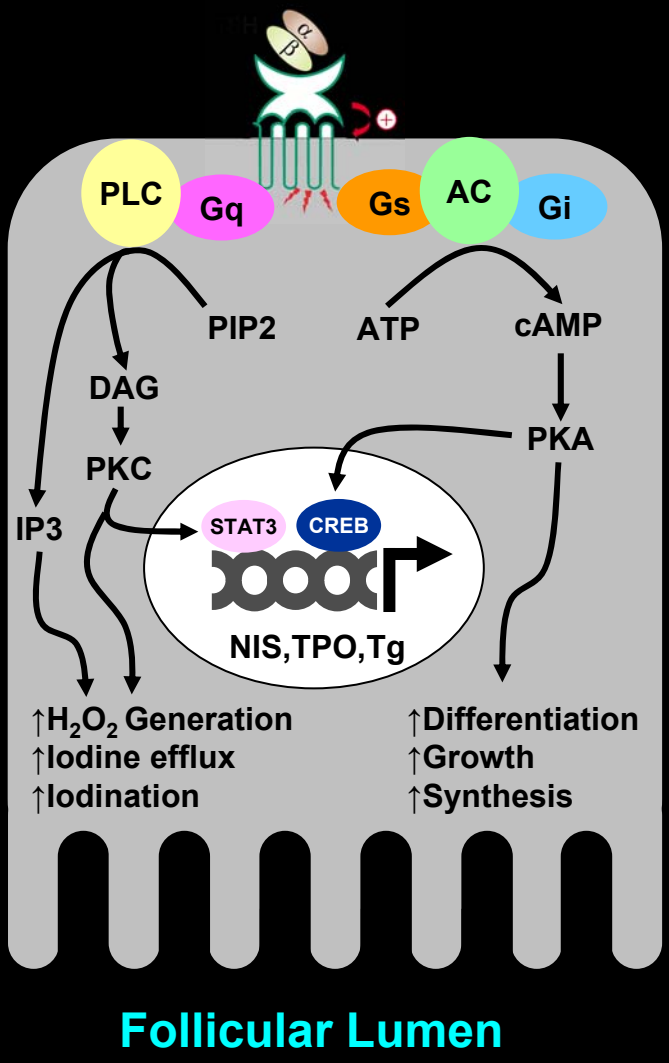
Mitogenic effect of TSH mediated by cAMP

Thyroid hormone synthesis (TPO, NIS) via cAMP

Transcriptional and post-transcriptional

Induces TSHR expression by cAMP at low concentration

TSHR secondary messenger pathways



cAMP is the predominate secondary messenger

Inactive Gs
- **GDP bound α-subunit and βγ dimer**

On receptor activation exchange of GDP for GTP
- **α-subunit-GTP dissociates from receptor and βγ dimer**
- **α-subunit-GTP then activates adeylyl cyclase**

Deactivation is by hydrolysis of GTP to GDP
- **Re-association with βγ dimer**

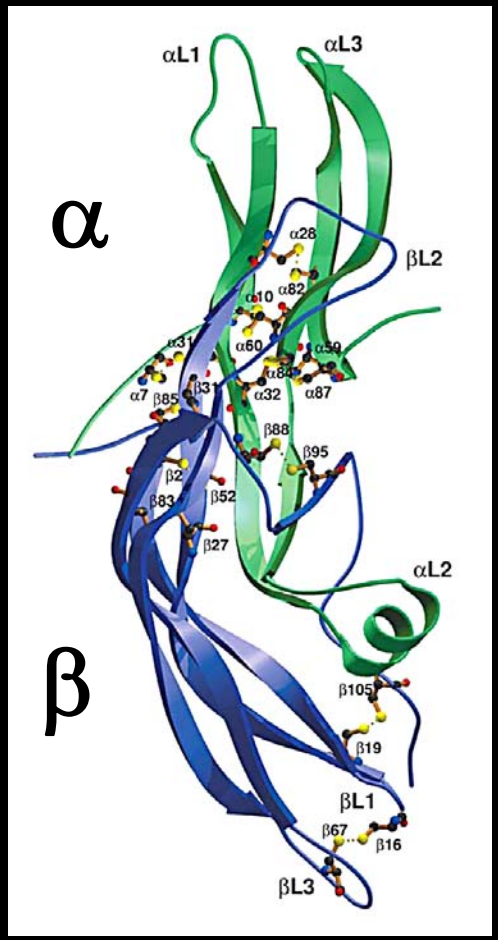
$G\alpha_s$/AC/cAMP/PKA regulates transcription of
- **Sodium-iodide symporter (NIS)**
- **Thyroid peroxidase (TPO)**
- **Thyroglobulin (Tg)**

$G\alpha_q$/PLC/PKC/Ca^{2+} regulates
- **Iodine efflux**
- **Thyroglobulin iodination**
- **H_2O_2 generation**

Kopp P 2001 CMLS 58:1301; Davies T 2002 JCI 110:161

TSHR ligands

Thyrotropin (TSH)



Heterodimeric glycoprotein hormone family

- Common α-subunit
- Unique β-subunit (TSHβ, FSHβ, LHβ and hCGβ)

Thyroid stimulating hormone (TSH)

- Common 42αα α-subunit
- Specific β-subunit 41% identity to hCG
- intrachain disulphide bonds form cysteine knot motif

Thyrostimulin

- Glycoprotein-α2 (GPA2) diverse expression
- Glycoprotein- β5 (GPB5) pituitary, brain
- IHC: Pituitary, brain, adrenal, pancreas, duodenum, testis
- α2β5 heterodimer activates TSHR and cAMP
- 10x higher affinity than TSH (physiological role unknown)

Human chorionic gonadotrophin (hCG)

- Promiscuous activation of TSHR during pregnancy

Glinoer G 1997 Endo Rev 18:404; Nakabayashi K 2002 JCI 109:1145; Miguel RN 2004 Thyroid 12: 991

G-protein coupled receptors

Glycoprotein hormone receptors (TSHR)

Role of TSHR in endocrine physiology

Role of TSHR in endocrine pathology

TSHR in endocrine pathology

Germline inactivating mutations
- TSH resistance (AR) (congenital hypothyroidism)

Germline activating mutations
- Non-autoimmune familial hyperthyroidism (AD)

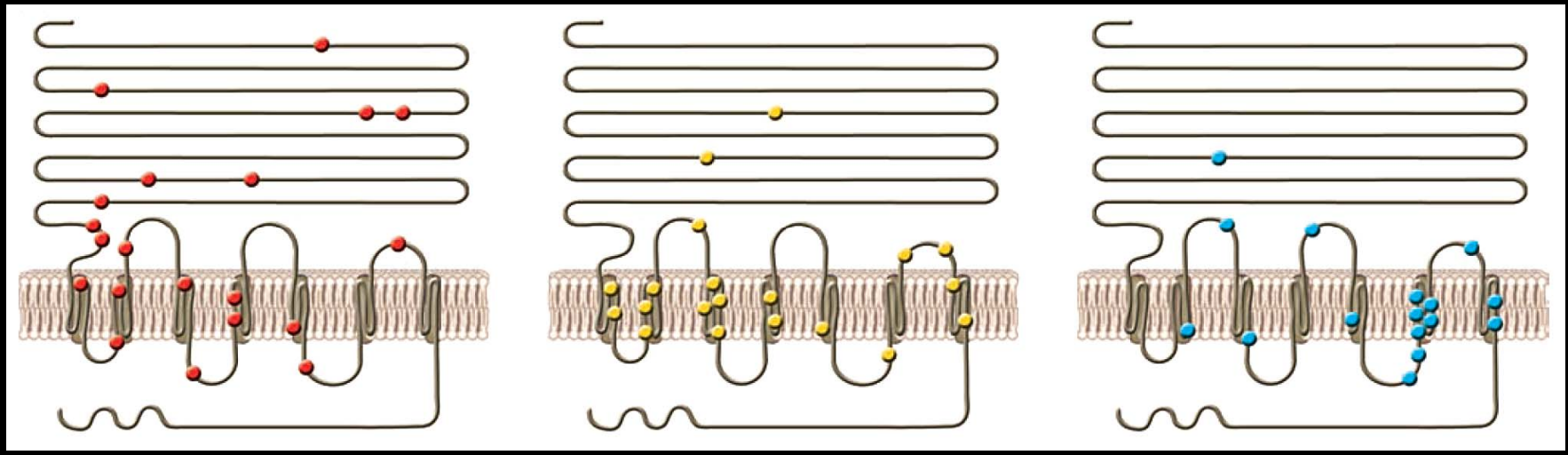
Somatic activating mutations
- Toxic thyroid adenomas

TSHR antibodies
- Graves' Disease (TSHR stimulating antibodies)
- Autoimmune hypothyroidism (TSHR blocking antibodies)

Promiscuous activation by hCG
- Gestational hyperthyroidism
- Trophoblastic tumour induced hyperthyroidsm

$G\alpha_s$ mutations
- Pseudohypoparathyroidism and hypothyroidism
- Toxic thyroid adenomas

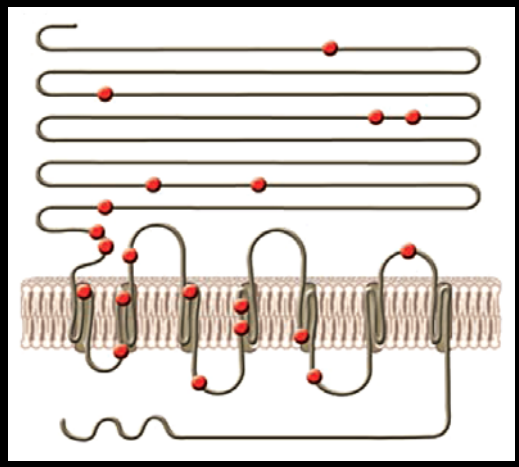
Mutations of the TSHR



Germline inactivating	Germline activating	Somatic Activating
widely scattered	Predominantly in TMD	Predominantly in TMD

Davies T 2005 JCI 115:1972

Germline TSHR inactivating mutations



Congenital hypothyroidism (AR)

- Homozygous or compound heterozygous TSHR mutations
- Mutations scattered throughout the receptor
- Usually result in reduced cell surface expression

Compensated partial TSH resistance (AD/AR)

- Heterozygous TSHR mutations
- May be dominant negative interference due to dimerisation

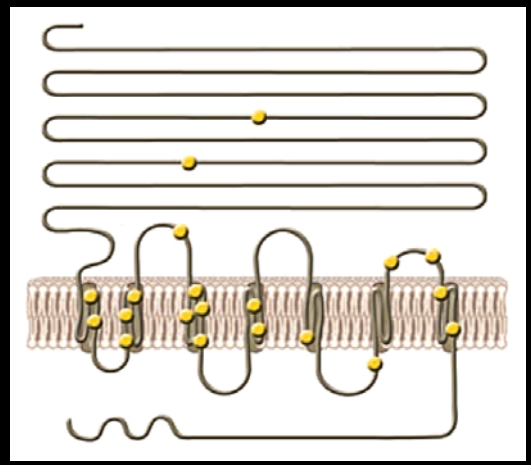
Hyt/Hyt mouse TSHR P556L in TMD IV

- No surface expression, thyroid hypoplasia, severe hypothyroidism

$TSHR^{-/-}$ mouse

- Thyroid hypoplasia and severe hypothyroidism

Germline TSHR activating mutations



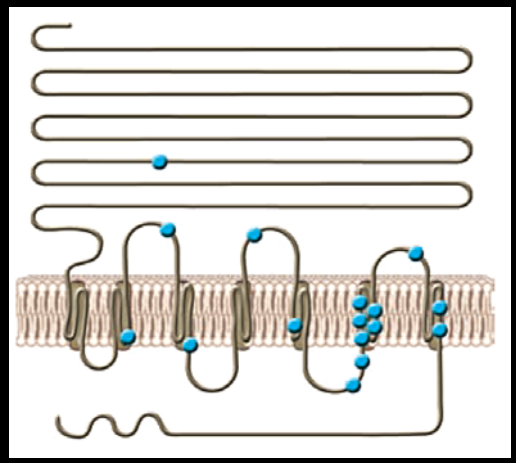
Non-autoimmune familial hyperthyroidism (AD)

- Rare condition diffuse goiter and thyrotoxicosis
- Age of onset may vary
- Heterozygous gain of function mutations often in exon 10
- Rx anti thyroid drug and thyroidectomy after age 5
- May also occur sporadically

The common transient form of congenital hyperthyroidism is due to placental transfer of TSHR stimulating antibodies

Duprez 1994 Nat Genet 7:396

Somatic TSHR activating mutations



Thyroid nodules

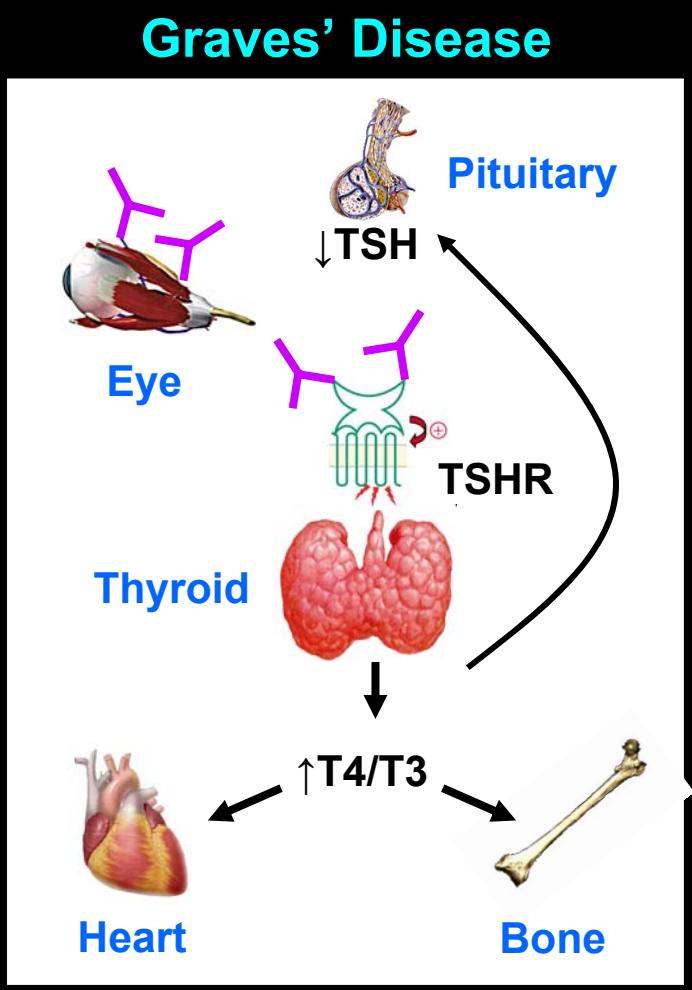
- 50% of population in iodine deficient areas have thyroid nodules due to prolonged TSH stimulation

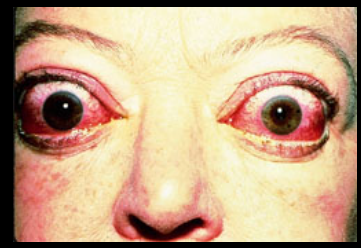
5-10% are autonomous nodules ("hot" nodules)

- Result in hyperthyroidism
- 60% have constitutively activating mutations of TSHR
- most commonly in serpine TMD
- Mutation induces clonal expansion and nodule formation
- Treatment is with radioactive iodine (^{131}I)

Kron K 2002 Mol Gen Metab 75:202

TSHR autoimmunity





TSHR antibodies

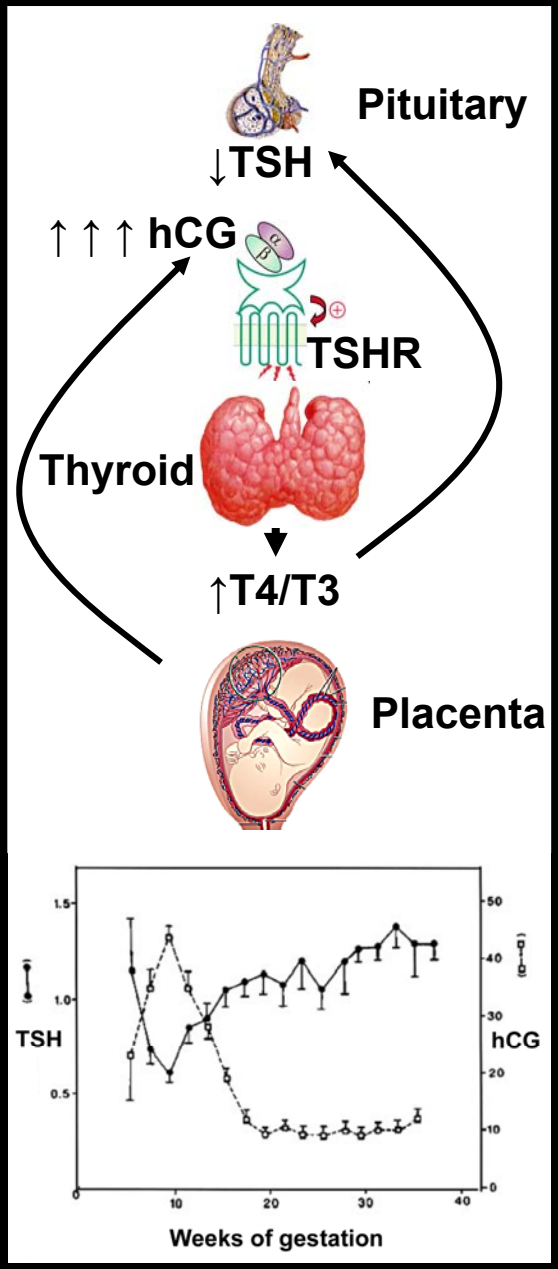
Graves' disease

- Autoimmune hyperthyroidism
- Opthalmopathy
- TSHR stimulating antibodies (ectodomain)
- Stimulates growth and hormone secretion
- Shedding of α-subunit may be antigenic
- Genetic component DZ twins 35% concordant (HLA DRβ1, CTLA4, PTNP22, CD40 and Tg)
- Transient congenital hyperthyroidism
- Treated with anti-thyroid medication or ^{131}I

Atrophic Hasimoto's disease

- Autoimmune hypothyroidism
- TSHR blocking antibodies in 15% (ectodomain)
- Transient congenital hypothyroidism

hCG induced hyperthyroidism



Promiscuous activation of TSHR by hCG

hCG in µmol/l range activates WT TSHR

- **1st trimester gestational hyperthyroidism**
- **Inverse relationship between TSH and hCG**
- **hCG is µmol/l (TSH/FSH/LH are pmol/l)**
- **Occurs in 4% of pregnancies (twins)**
  - **(hyperemesis gravidarium)**
- **Trophoblastic tumours**
  - **(Choriocarcinoma/Hydatidiform mole)**

Familial gestational hyperthyroidism

- **K183R mutations of TSHR ectodomain**
- **increase TSHR sensitivity to hCG but still**
- **1000x less sensitive than LH/CGR**
- **No change in TSH sensitivity**

Symptoms

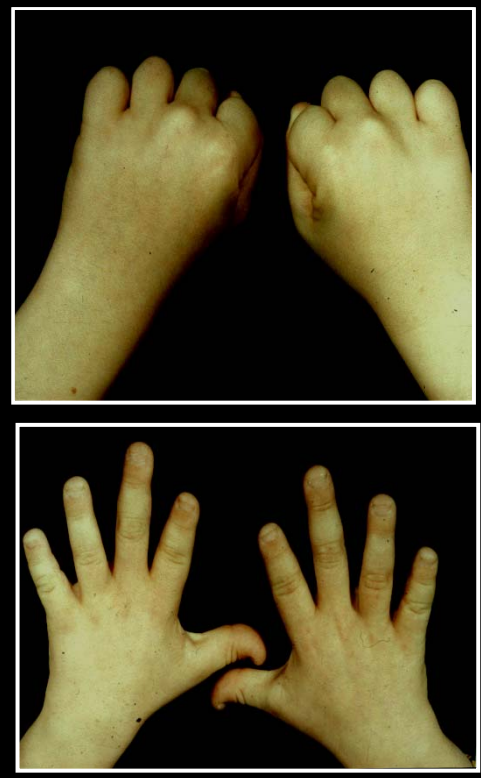
- **Hyperemesis, hyperthyroidism throughout preganancy, multiple miscarriages**

Rodien P 1998 NEJM 339:1823; Glinoer G 1997 Endo Rev 18:404

$G\alpha_s$ mutations and thyroid pathology

Resistance to PTH, TSH, FSH and LH

- Pseudohypoparathyroidism type 1a (PHP1a)
- Albrights hereditary osteodystrophy
- Germline inactivating mutations of $G\alpha_s$
- TSH cannot stimulate cAMP response
- Often TSH resistance occurs later than PTH
- Treatment is T4 replacement



Autonomous thyroid nodules ("hot" nodules)

- Somatic gain of function mutations in $G\alpha_s$
- Results in hyperthyroidism
- 3% of toxic nodules have mutations of $G\alpha_s$
- Mutations frequently inhibit GTP hydrolysis
- Persistent activation of TSHR signalling pathway and elevated cAMP
- Treatment with ^{131}I

Kron K 2002 Mol Gen Metab 75:202

GPCR Summary

GPCRs are the oldest signal transduction molecules
Most diverse of all membrane receptors

GpHRs
- LRR ectodomain mediates ligand specificity
- Serpine TMD mediates signal transduction to G proteins

TMD is inherently noisy
- Unliganded ectodomain acts as inverse agonist to suppress TMD
- Liganded ectodomain as a full agonist of TMD

GpHR dimerisation
- Negative cooperativity extends range of ligand concentration

GpHRs multiple post-translational modifications are essential for
- Folding, activation, ligand affinity, oligomerisation, cell surface expression and desensitisation

GpHRs ligands
- Heterodimeric glycoproteins common α-subunit, diverse β-subunit

TSHR Summary

Has a critical role in thyroid physiology and pathology

Essential role in the
- Hypothalamic-pituitary-thyroid axis
- Thyroid growth and hormone synthesis

Not essential for thyroid organogenesis or migration

Signals predominantly via cAMP but also PLC

Inactivating mutation
- Spectrum TSH resistance to severe congenital hypothyroidism

Gain of function mutations usually effect the TMD
- Familial hyperthyroidism and sporadic toxic adenomas

Antibodies to TSHR ectodomain
- Graves' Disease and autoimmune hypothyroidism

TSHR in other tissues and the alternative ligand thyrostimulin
- Physiological roles remains uncertain