G-PROTEIN COUPLED RECEPTORS (GPCRS)

Annalaura Sabatucci

molecules

MDPI

Communication

G-Protein Coupled Receptors in Human Sperm: An In Silico Approach to Identify Potential Modulatory Targets

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Abstract: G protein-coupled receptors (GPCRs) are involved in several physiological processes, and they represent the largest family of drug targets to date. However, the presence and function of these receptors are poorly described in human spermatozoa. Here, we aimed to identify and characterize the GPCRs present in human spermatozoa and perform an in silico analysis to understand their potential role in sperm functions. The human sperm proteome, including proteomic studies in which the criteria used for protein identification was set as <5% FDR and a minimum of 2 peptides match per protein, was crossed with the list of GPCRs retrieved from GLASS and GPCRdb databases. A total of 71 GPCRs were identified in human spermatozoa, of which 7 had selective expression in male tissues (epididymis, seminal vesicles, and testis), and 9 were associated with male infertility defects in mice. Additionally, ADRA2A, AGTR1, AGTR2, FZD3, and GLP1R were already associated with sperm-specific functions such as sperm capacitation, acrosome reaction, and motility, representing potential targets to modulate and improve sperm function. Finally, the protein-protein interaction network for the human sperm GPCRs revealed that 24 GPCRs interact with 49 proteins involved in crucial processes for sperm formation, maturation, and fertilization. This approach allowed the identification of 8 relevant GPCRs (ADGRE5, ADGRL2, GLP1R, AGTR2, CELSR2, FZD3, CELSR3, and GABBR1) present in human spermatozoa that can be the subject of further investigation to be used even as potential modulatory targets to treat male infertility or to develop new non-hormonal male contraceptives.

Keywords: G-protein coupled receptors; spermatozoa; male fertility; bioinformatics

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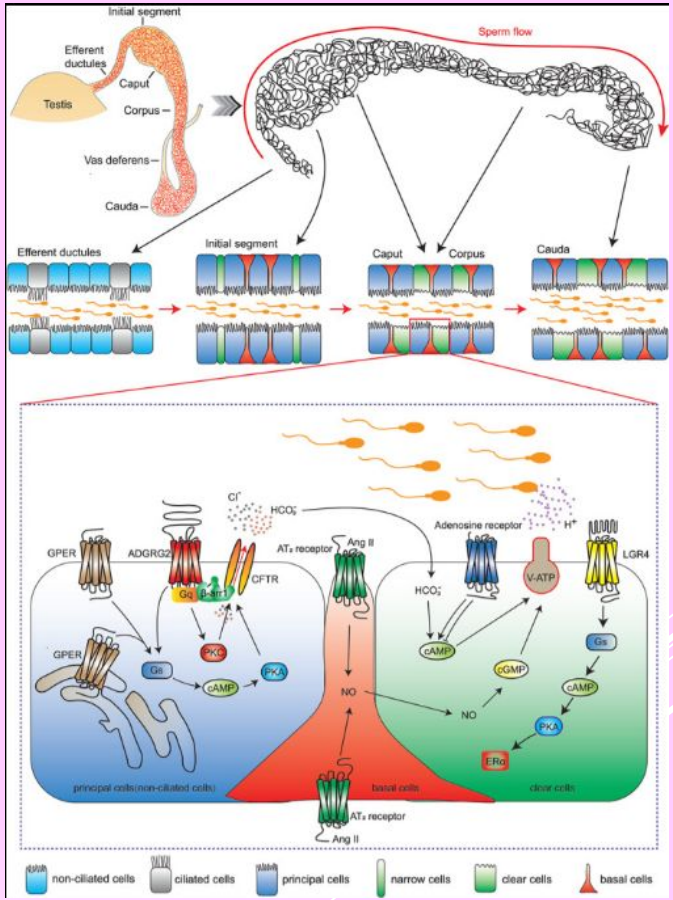


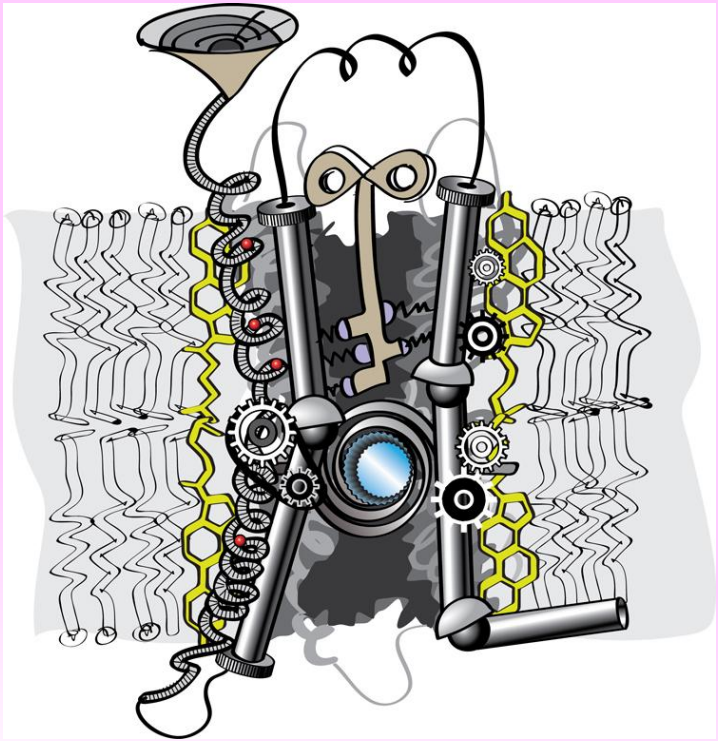
Some GPCRs involved in Reproduction and fertility:

1, 2) (endo)cannabinoid receptors CB1, CB2
3) Kisspeptin receptor (GPR54)
4) Relaxin Receptor 2
5) G protein-coupled estrogen receptor 1 (GPER1), 6
6) gonadotropin-releasing hormone receptor (GnRHR)
7) Lutropin-choriogonadotropic hormone receptor (LHR)
8) GPR64

INTRO TO GPCRs

https://youtu.be/NL_YbPigDzg (min 0-1:40)

GPCR REPRESENT A VERY COMPLEX MACHINERY



Mechanical view of GPCR workings (Image: Scripps Research Institute)

Membrane receptors for hormones, odours, peptides, lipids…
Transmission of a variety of signals through the cell membrane
Activation of secondary metabolic patterns

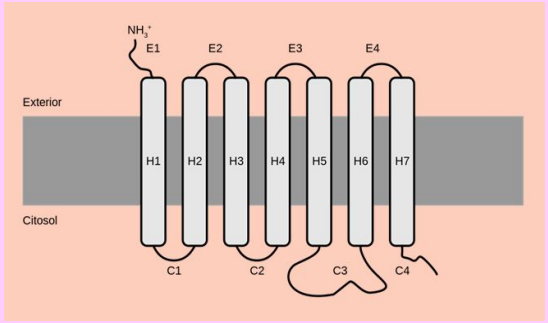
Scientific Background on the Nobel Prize in Chemistry 2012

STUDIES OF G-PROTEIN–COUPLED RECEPTORS

The Nobel Prize in Chemistry 2012 is awarded to Brian K. Kobilka and Robert J. Lefkowitz for studies of G-protein–coupled receptors.

GPCR ARCHITECTURE (signature)

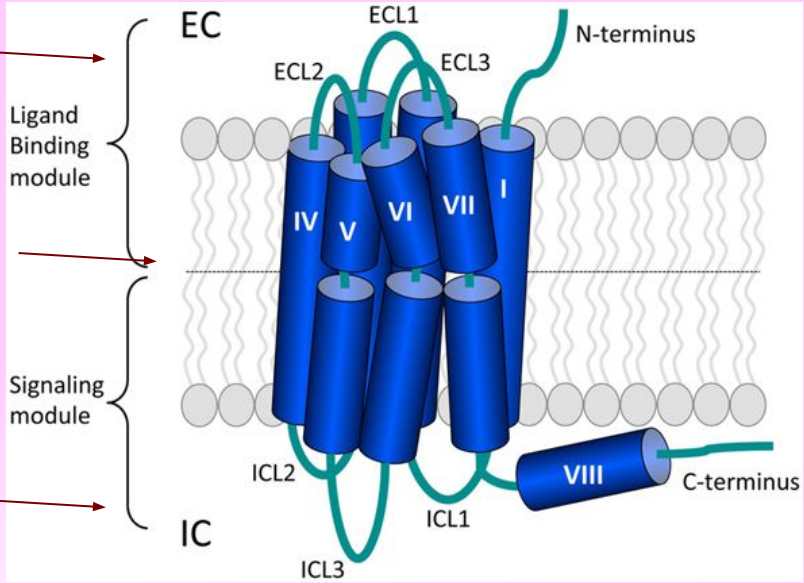
7 transmembrane helices (TMH) separated by loops divide the protein in 3 Regions:



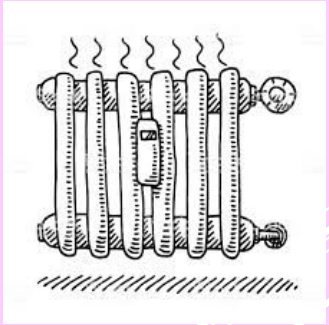
Extracellular (EC)

Transmembrane (TM)

Cytoplasmic (intracellular, IC)

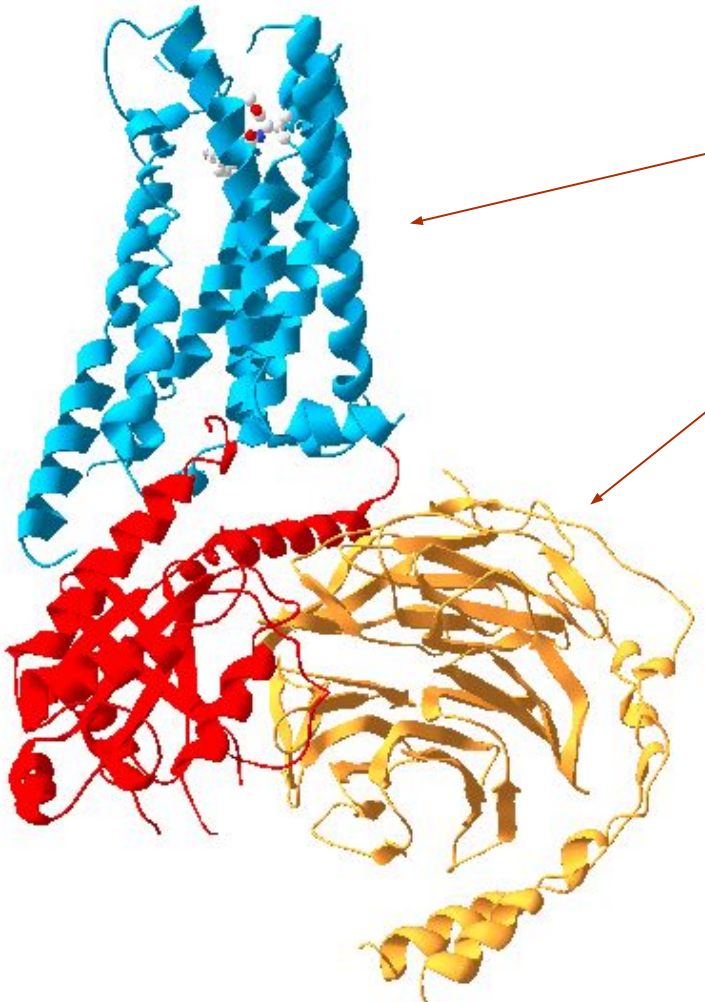


RADIATOR



Eventual intracellular amphipathic helix 8

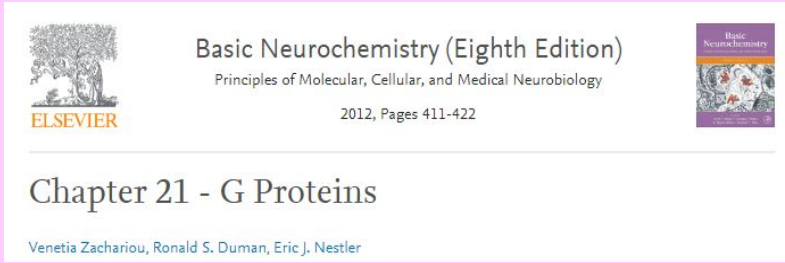
from "Modulation of cellular signaling by herpesvirus-encoded G protein-coupled receptors".



Receptor-
G protein

Complex

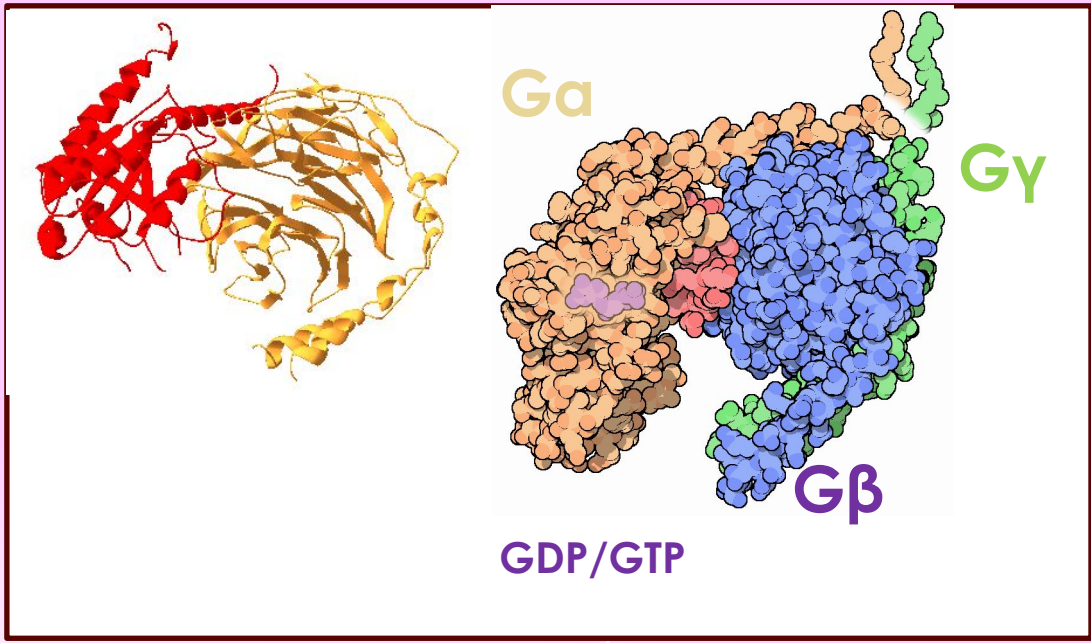
G- PROTEINS



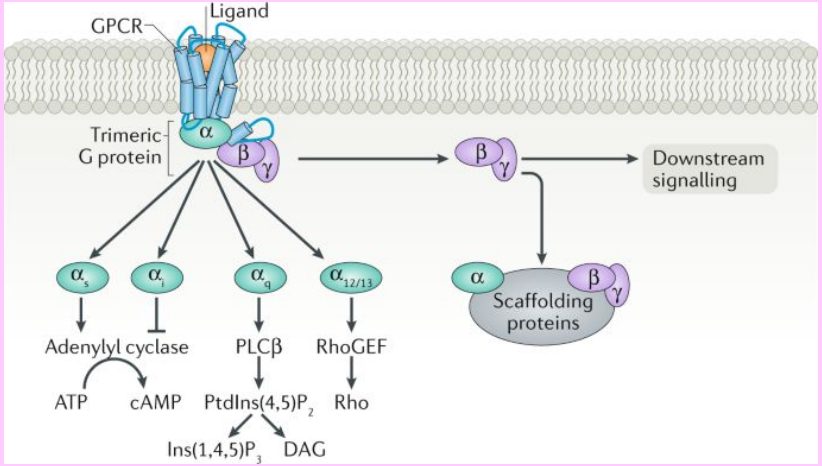
The name: **G** proteins bind **G**TP and **G**DP and possess intrinsic **G**TPase activity.

Role in: Signal transduction
- Membrane vesicle transport
- Cytoskeletal assembly
- Cell growth
- Protein synthesis

Coupled to GPCRs are only **heterotrimeric** G proteins: trimers composed of 3 different subunits: Gα Gβ Gγ

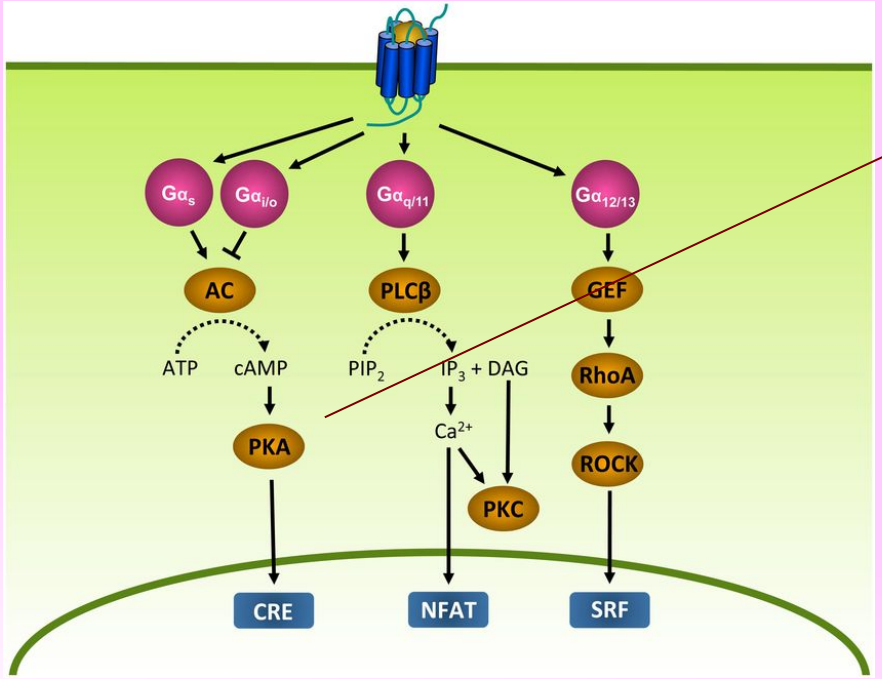


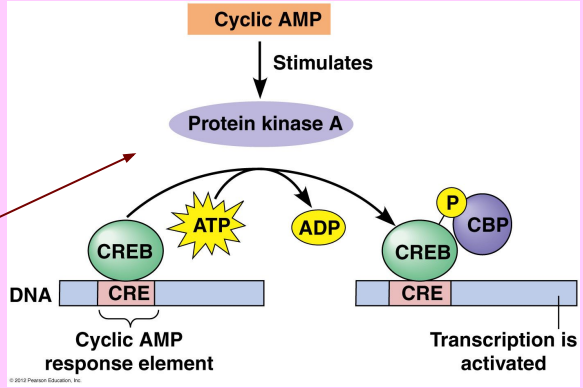
G-protein specificity depends on Gα subunit classes



- $G\alpha_s$ (G stimulatory), activates the cAMP-dependent pathway by direct stimulation of Adenylate cyclase.
- $G\alpha_i$ (G inhibitory): inhibits the production of cAMP from ATP
- $G\alpha_o$ (G other: $G\alpha_q$; $G\alpha_{12/13}$) involved in different signaling pathways

EXAMPLE OF DOWNSTREAM SIGNALING: GENE Expression Regulation





REACTOME:

https://reactome.org/PathwayBrowser/#/R-HSA-388396

De Munnik et al, Frontiers in pharmacology 2015, vol6, p40

8 June 2012

CREB Binding Protein (CBP) Activation Is Required for Luteinizing Hormone Beta Expression and Normal Fertility in Mice

Authors: Ryan S. Miller, Andrew Wolfe, Ling He, Sally Radovick, Fredric E. Wondisford | AUTHORS INFO & AFFILIATIONS

DOI: https://doi.org/10.1128/MCB.00394-12

https://journals.asm.org/doi/10.1128/MCB.00394-12

ABSTRACT

Normal function of the hypothalamic-pituitary-gonadal axis is dependent on gonadotropin-releasing hormone (GNRH)-stimulated synthesis and secretion of luteinizing hormone (LH) from the pituitary gonadotroph. While the transcriptional coactivator CREB binding protein (CBP) is known to interact with Egr-1, the major mediator of GNRH action on the Lhb gene, the role of CBP in Lhb gene expression has yet to be characterized. We show that in the LβT2 gonadotroph cell line, overexpression of CBP augmented the response to GNRH and that knockdown of CBP eliminated GNRH responsiveness. While GNRH-mediated phosphorylation of CBP at Ser436 increased the interaction with Egr-1 on the Lhb promoter, loss of this phosphorylation site eliminated GNRH-mediated Lhb expression in LβT2 cells. *In vivo*, loss of CBP phosphorylation at Ser436 rendered female mice subfertile. S436A knock-in mice had disrupted estrous cyclicity and reduced responsiveness to GNRH. Our results show that GNRH-mediated phosphorylation of CBP at Ser436 is required for Egr-1 to activate Lhb expression and is a requirement for normal fertility in female mice. As CBP can be phosphorylated by other factors, such as insulin, our studies suggest that CBP may act as a key regulator of Lhb expression in the gonadotroph by integrating homeostatic information with GNRH signaling.

cAMP response element-binding protein 1 controls porcine ovarian cell proliferation, apoptosis, and FSH and insulin-like growth factor 1 response

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https://www.publish.csiro.au/rd/rd17508

Abstract

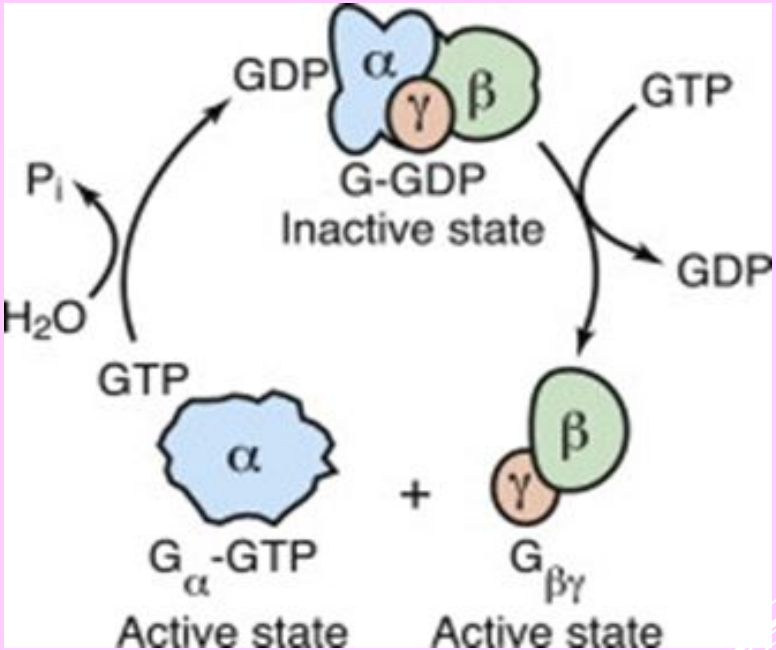
The aim of the present study was to examine the role of cAMP response element-binding protein (CREB) and its phosphorylation in the regulation of ovarian cell proliferation and apoptosis, and of the response of proliferation and apoptosis to the upstream hormonal stimulators FSH and insulin-like growth factor (IGF) 1. In the first series of experiments, porcine ovarian granulosa cells, transfected or not with a gene construct encoding wild-type CREB1 (CREB1WT), were cultured with and without FSH (0, 1, 10 or 100 ng mL^{-1}). In the second series of experiments, these cells were transfected or not with CREB1WT or non-phosphorylatable mutant CREB1 (CREB1M1) and cultured with and without FSH (0, 1, 10 or 100 ng mL^{-1}) or IGF1 (0, 1, 10 and 100 ng mL^{-1}). Levels of total and phosphorylated (p-) CREB1, proliferating cell nuclear antigen (PCNA), a marker of proliferation, and BAX, a marker of apoptosis, were evaluated by western immunoblotting and immunocytochemical analysis. Transfection of cells with CREB1WT promoted accumulation of total CREB1 within cells, but p-CREB1 was not detected in any cell group. Both CREB1WT and CREB1M1 reduced cell proliferation and apoptosis. Addition of 10 and 100 ng mL^{-1} FSH to non-transfected cells promoted CREB1 accumulation and apoptosis, whereas cell proliferation was promoted by all concentrations of FSH tested. FSH activity was not modified in cells transfected with either CREB1WT or CREB1M1. IGF1 at 100 ng mL^{-1} promoted cell proliferation, whereas all concentrations of IGF1 tested reduced apoptosis. Transfection with either CREB1WT or CREB1M1 did not modify the effects of either FSH or IGF1, although CREB1M1 reversed the effect of IGF1 on apoptosis from inhibitory to stimulatory. These observations suggest that CREB1 is involved in the downregulation of porcine ovarian cell proliferation and apoptosis. The absence of visible CREB1 phosphorylation and the similarity between the effects of CREB1WT and CREB1M1 transfection indicate that phosphorylation is not necessary for CREB1 action on these processes. Furthermore, the observations suggest that FSH promotes both ovarian cell proliferation and apoptosis, whereas IGF1 has proliferation-promoting and antiapoptotic properties. The effect of FSH on CREB1 accumulation and the ability of CREB1M1 to reverse the effects of IGF1 on apoptosis indicate that CREB1 is a mediator of hormonal activity, but the inability of either CREB1WT or CREBM1transfection to modify the primary effects of FSH and IGF1 suggest that CREB1 and its phosphorylation do not mediate the action of these hormones on ovarian cell proliferation and apoptosis.

G-protein activation

INACTIVE STATE:
GDP bound to Gα, intact trimer

ACTIVE STATE:
Gα binds to GTP , dissociates to the Gβγ complex

=> interacts with other membrane proteins like Adenylate cyclase

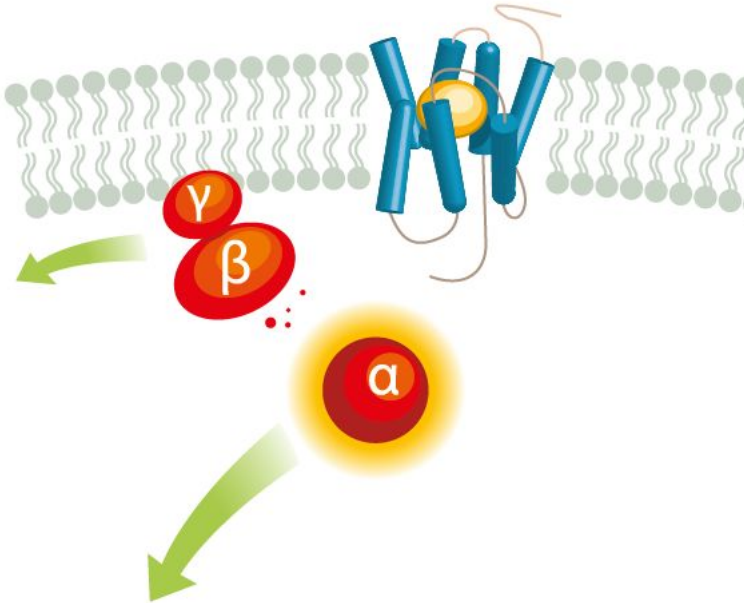


GPCR SIGNALING MECHANISM

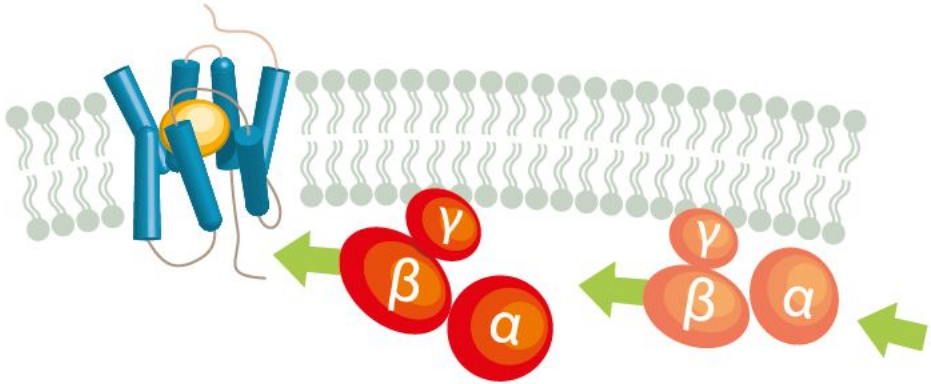
1 A LIGAND binds to the receptor.

2 The receptor alters shape. Inside the cell, the G-protein binds and is activated.

3 Activated G-protein breaks apart. The free α-subunit will trigger a chain of reactions that alters the cell's metabolism.

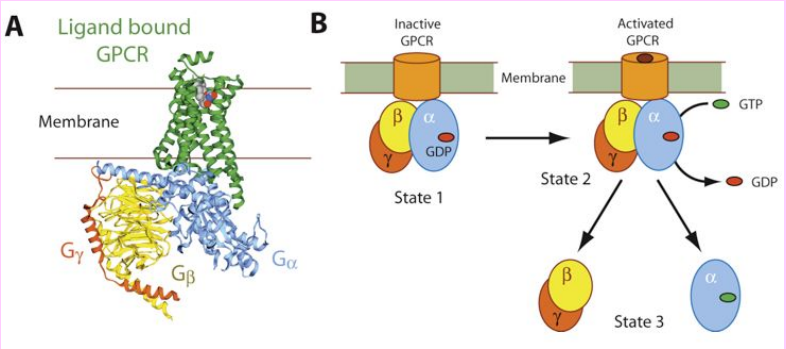


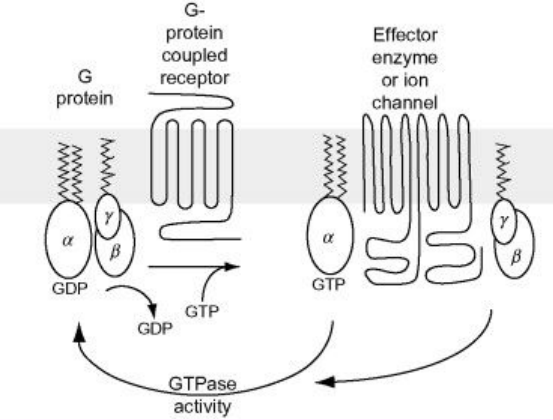
4 A new G-protein binds. The receptor can activate hundreds of G-proteins before the hormone on the outside detaches.



new G-protein

GPCR activation





- **Scheme (NOT NICE):**
https://youtu.be/xT0mAQ4726s

- **More complex scheme (3' 09"):**
https://www.youtube.com/watch?v=Glu_T6DQuLU

- **SHORT 3D animation with crystal structures:**
3D animation: https://youtu.be/nQfaTvV9D5s

- **Nice video (no audio):**
https://www.youtube.com/watch?v=jmYn1jJZ9BE

Review > Mol Cell Endocrinol. 2020 Sep 15;515:110886. doi: 10.1016/j.mce.2020.110886. Epub 2020 Jun 20.

The wonderful and masterful G protein-coupled receptor (GPCR): A focus on signaling mechanisms and the neuroendocrine control of fertility

Andy V Babwah [1]

Affiliations + expand

PMID: 32574585 DOI: 10.1016/j.mce.2020.110886

Abstract

Human GnRH deficiency, both clinically and genetically, is a heterogeneous disorder comprising of congenital GnRH deficiency with anosmia (Kallmann syndrome), or with normal olfaction [normosmi idiopathic hypogonadotropic hypogonadism (IHH)], and adult-onset hypogonadotropic hypogonadism. Our understanding of the neural mechanisms underlying GnRH secretion and GnRH signaling continues to increase at a rapid rate and strikingly, the heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptors (GPCRs) continue to emerge as essential players in these processes. GPCRs were once viewed as binary on-off switches, where in the "on" state they are bound to their Gα protein, but now we understand that view is overly simplistic and does not adequately characterize GPCRs. Instead, GPCRs have emerged as masterful signaling molecules exploiting different physical conformational states of itself to elicit an array of downstream signaling events via their G proteins and the β-arrestins. The "one receptor-multiple signaling conformations" model is likely an evolved strategy that can be used to our advantage as researchers have shown tha targeting specific receptor conformations via biased ligands is proving to be a powerful tool in the effective treatment of human diseases. Can biased ligands be used to selectively modulate signaling by GPCR regulators of the neuroendocrine axis in the treatment of IHH? As discussed in this review, the grand possibility exists. However, while we are still very far from developing these treatments, th exciting likelihood can happen through a much greater mechanistic understanding of how GPCRs signal within the cell.

Keywords: Agonism; DYN; Dimerization; Endosomes; GPCR; GPR83; GRK; GnRH; IHH; KISS1R; KOR; Kisspeptin; Megacomplex; Megaplex; NK(3); NKA; NKB; Neuroendocrine; Nucleus; Oligomerization; Orphan; Signaling; Substance P; β-arrestin.

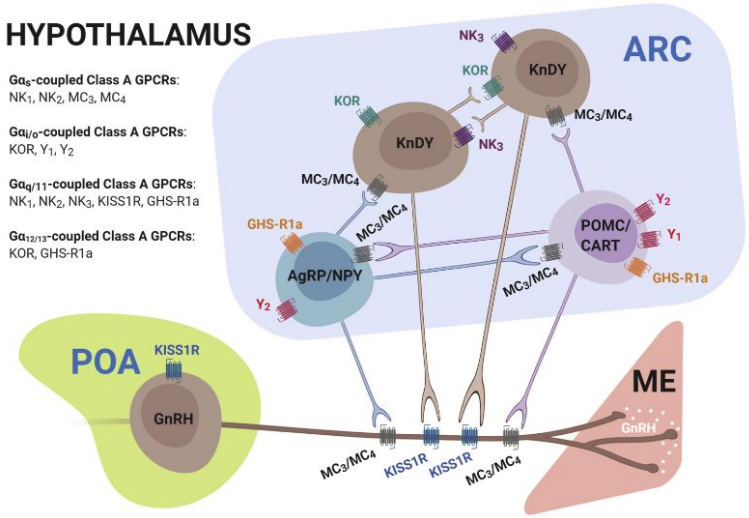


Fig. 2. The cartoon illustrates an intricate GPCR-expressing neuronal network that is localized in the hypothalamic arcuate (ARC) nucleus and which plays a major role in regulating GnRH secretion from the terminals of the preoptic area (POA) GnRH neurons. These GnRH neurons terminate in the median eminence (ME) where GnRH is released and transported to the anterior pituitary (not shown). NK_1, NK_2 and NK_3: tachykinin receptors; MC_3 and MC_4: αMSH receptors; KOR: κ opioid receptor; Y_1 and Y_2: NPY receptors; GHS-R1a: ghrelin receptor and KISS1R: KISS1 receptor. POMC/CART: pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript; AgRP/NPY: agouti-related protein/neuropeptide Y.
KNDy: Kisspeptin/Neurokinin/Dynorphin neuron.

GPCR LIGANDS

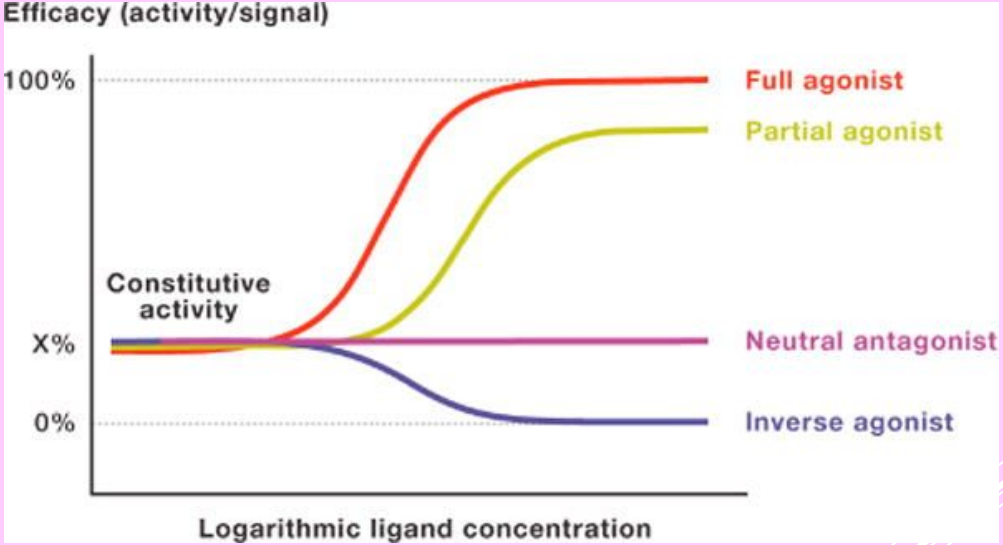
GPCRs are modulated by a variety of endogenous and synthetic **ligands** => represent the largest family of **druggable targets** in the human genome.

TYPES OF LIGANDS:

- AGONISTS (full or partial) bind to the ORTHOSTERIC site

- INVERSE AGONISTS (ANTAGONISTS) compete for the ORTHOSTERIC site

- ALLOSTERIC LIGANDS Positive (PAMs) or Negative (NAMs) bind to allosteric sites



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5560499/

CB1 binding sites

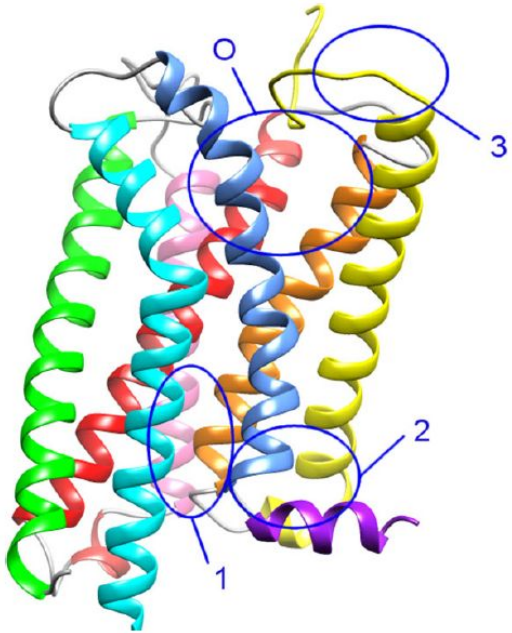


FIG. 1 *Localization of the orthosteric site (O) and the three potential allosteric binding pockets identified in the 3D structure 5TGZ.pdb of CB_1 receptor.*

TABLE 1 *Binding sites of CB_1*

Modulators	*Pocket 1*			*Pocket 2*			*Pocket 3*			*Orthosteric site*		
	Cluster	*Fullfitness*	*ΔG (kcal/mol)*	*Cluster*	*Fullfitness*	*ΔG (kcal/mol)*	*Cluster*	*Fullfitness*	*ΔG (kcal/mol)*	*Cluster*	*Fullfitness*	*ΔG (kcal/mol)*
PAMs/NAMs												
Pregnenolone	0	−1,097.44	−7.43	11	−1,087.74	−6,69	–			–		
Fenofibrate	0	−1,069.62	−7.94	–			5	−1,065.29	−7,84	1	−1,067.24	−8.71
Lipoxine A_4	1	−1,158.34	−9.66	2	−1,157.35	−10,25	0	−1,158.39	−8,17	–		
Cannabidiol	2	−1,141.1	−7.75	–			3	−1,139.94	−7,39	0	−1,141.5	−8.15
ORG27569	9	−1,141.48	−7.39	–			0	−1,156.6	−9,29	–		
Agonists												
THC	–			–			–			0	−2,022.04	−8.34
2-AG	–			–			–			0	−1,132.36	−9.41
AEA	–			–			–			0	−1,148.66	−9.55
Arachydonyl-2-chloroethylamide	–			–			–			0	−1,157.48	−9.5
Antagonists												
Rimonabant	–			–			–			0	−1,949.28	−9.98
Taranabant	–			–			–			0	−1,083.86	−9.32

In silico mapping of allosteric ligand binding sites in type-1 cannabinoid receptor

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Daniel Tortolani[2†]
Enrico Dainese[1,3*,‡]
Mauro Maccarrone[3,4*,‡]

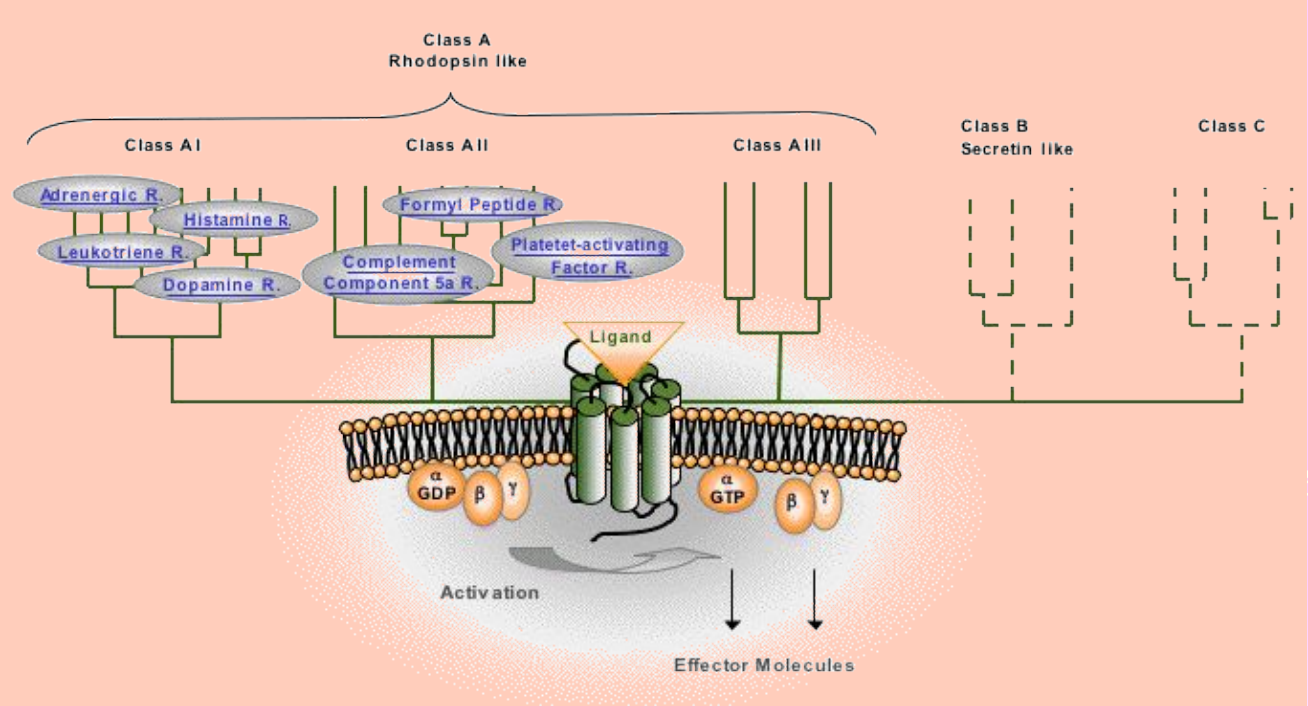
DOI: 10.1002/bab.1589

1) TRADITIONAL GPCR CLASSIFICATION based on the agonists: 3 CLASSES

CLASS A:
Rhodopsin-like (neuropeptides and monoammides)

CLASS B:
Secretin, glucagone and calcitonin

CLASS C:
Glutammate and Ca-regulated

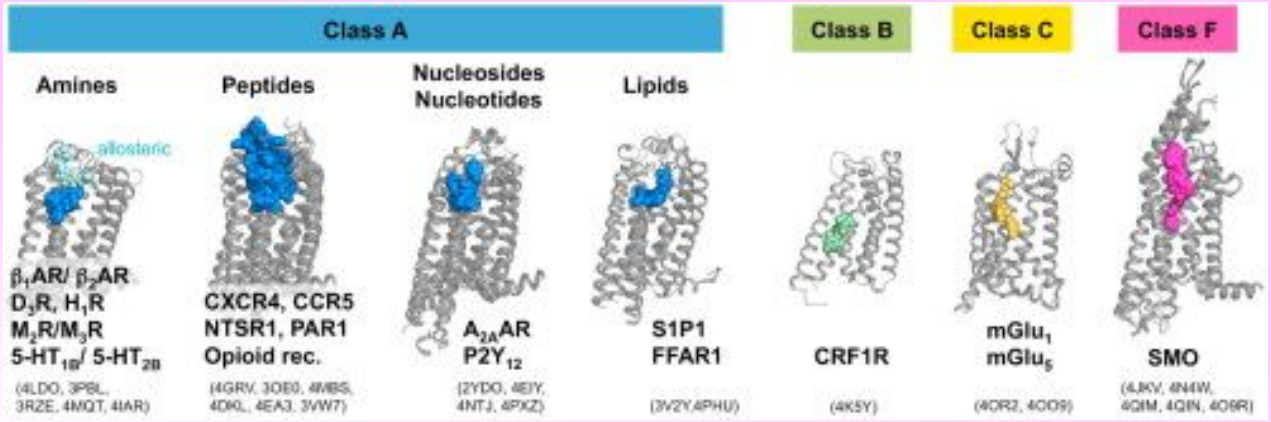


2) CLASSIFICATION ON SEQUENCE/FUNCTIONAL SIMILARITY

On the basis of sequence and functional similarities, GPCRs are categorized into six classes:

Class A (rhodopsin-like receptors),
Class B (the secretin family),
Class C (metabotropic glutamate receptors),
Class D (fungal mating pheromone receptors),
Class E (cyclic adenosine monophosphate (cAMP) receptors)
Class F (Frizzled and Smoothened receptors).

Cite this: *J. Med. Chem.* 2018, 61, 1, 1−46
Publication Date: June 28, 2017
https://doi.org/10.1021/acs.jmedchem.6b01453



GPCRs phylogenetic tree (2013)

nature REVIEWS DRUG DISCOVERY

Journal home > Archive > Perspectives > Opinion > Full text > Figure 1

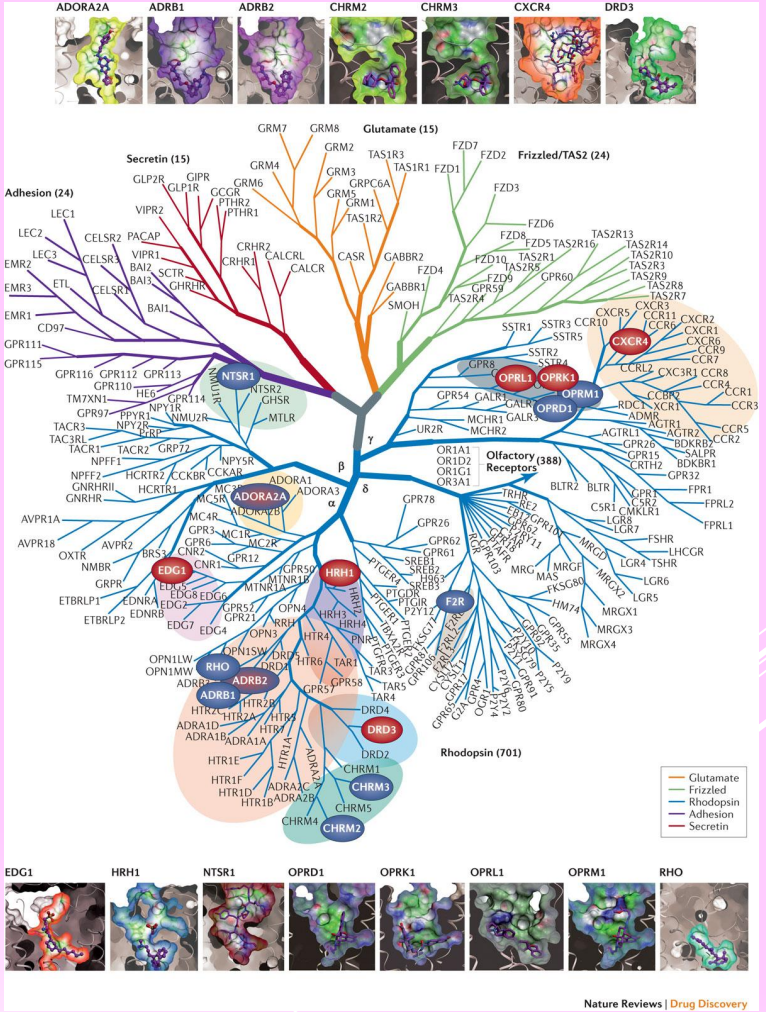
FIGURE 1 | Phylogenetic tree representation of the human GPCR superfamily.

FROM THE FOLLOWING ARTICLE:
The GPCR Network: a large-scale collaboration to determine human GPCR structure and function
Raymond C. Stevens, Vadim Cherezov, Vsevolod Katritch, Ruben Abagyan, Peter Kuhn, Hugh Rosen & Kurt Wüthrich
Nature Reviews Drug Discovery **12**, 25-34 (January 2013)
doi:10.1038/nrd3859

There are more than 1000 human GPCRs.

This phylogenetic tree was constructed using sequence similarity within the 7-TM region.

Family members with determined structures are highlighted within the tree, and their binding pockets with the ligand — as captured in each of the distinct structures — are shown around the tree in the same orientation for ease of comparison.



GPCR B.W. AA NUMBERING SYSTEM

originally described in: Ballesteros and Weinstein (1995) Methods Neurosci 25, 366-428.

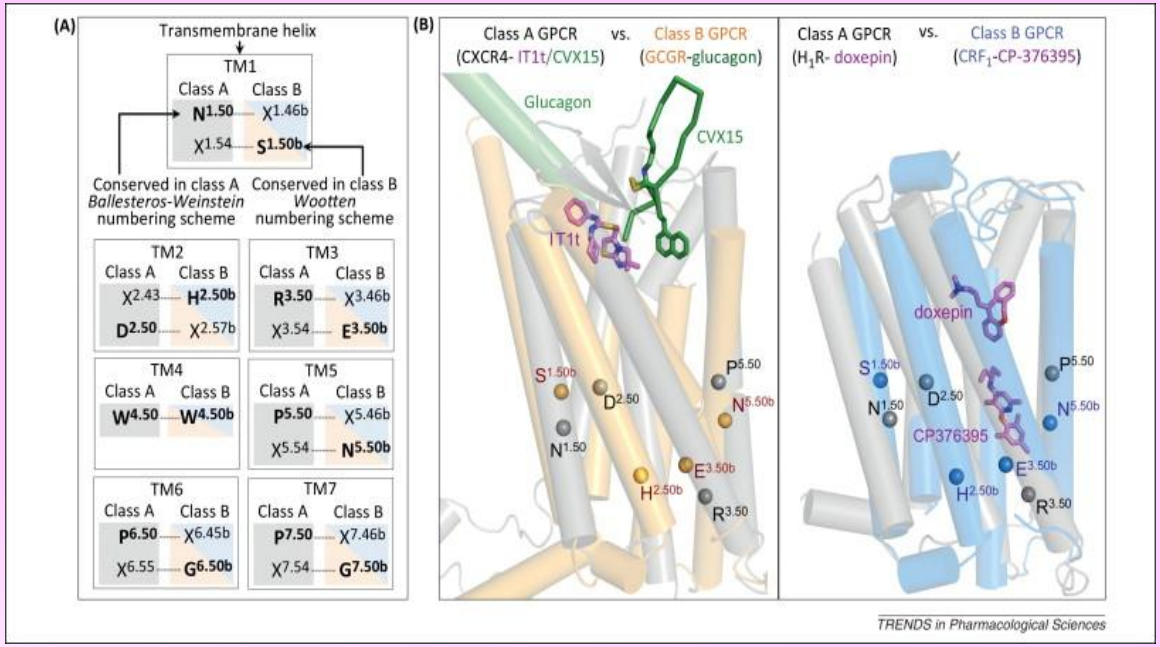
aa residues in the TM domain are assigned two numbers (N1,N2)
N1 = TM number
N2 = number relative to the most conserved residue in this TM, which is assigned **50**, numbers decreasing towards N-terminus and increasing towards C-terminus

for example, 5.42 denotes a residue located in TM5, eight residues before the most conserved residue, Pro5.50.

Class A conservation:
N1.50: 98%, D2.50: 90%,
R3.50: 95%, W4.50: 97%,
P5.50: 78%, P6.50: 99%,
P7.50: 88%

TM Helices	Conserved Residues of Class A GPCRs	Conserved Identifier of Class A GPCRs
TM 1	Asn	N1.50
TM 2	Asp	D2.50
TM 3	Arg	R3.50
TM 4	Trp	W4.50
TM 5	Pro	P5.50
TM 6	Pro	P6.50
TM 7—Helix 8	Pro, Phe	P7.50, F8.50

doi:10.1371/journal.pone.0122223.t003



https://doi.org/10.1016/j.tips.2013.11.001

GPCRs PTMs
(Post Translational Modifications)

MINI REVIEW article

Front. Chem., 10 March 2022
Sec. Chemical Biology
Volume 10 - 2022 | https://doi.org/10.3389/fchem.2022.843502

This article is part of the Research Topic
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Post-Translational Modifications of G Protein–Coupled Receptors Revealed by Proteomics and Structural Biology

Bingjie Zhang[1†], Shanshan Li[1†] and Wenqing Shui[1,2*]

Figure 1

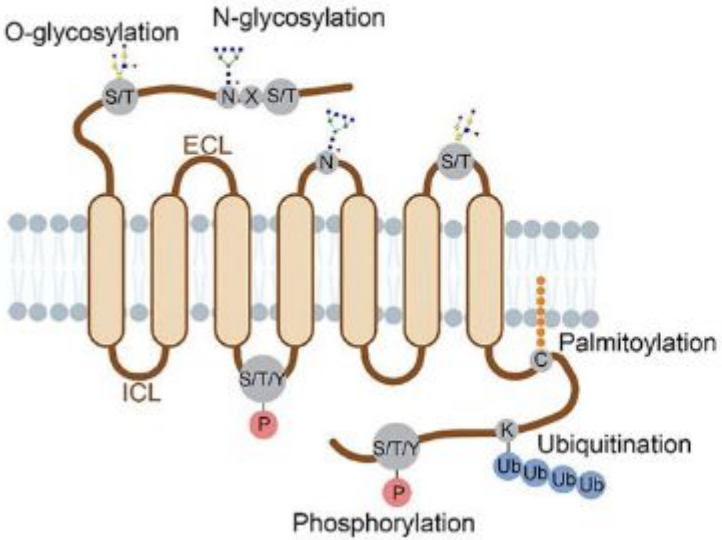


FIGURE 1. Structural localization of GPCR post-translational modifications overviewed in this review. Four major types of PTMs are distributed on the N-terminus, ECLs, ICLs and C-terminus of a GPCR protein. Glycosylation occurs on the N-terminal and ECL domains, with N-glycosylation at N of the sequence motif N-X-S/T (X≠P) and O-glycosylation at S/T residues. Phosphorylation occurs at S, T or Y residues on the C-terminal and ICL domains. Ubiquitination occurs at K residues and palmitoylation at C residues, both on the C-terminus.

REVIEW ARTICLE

Front. Endocrinol., 15 August 2013 | https://doi.org/10.3389/fendo.2013.00100

GPCR heterodimerization in the reproductive system: functional regulation and implication for biodiversity

Honoo Satake*, Shin Matsubara, Masato Aoyama, Tsuyoshi Kawada and Tsubasa Sakai

Suntory Foundation for Life Sciences, Bioorganic Research Institute, Osaka, Japan

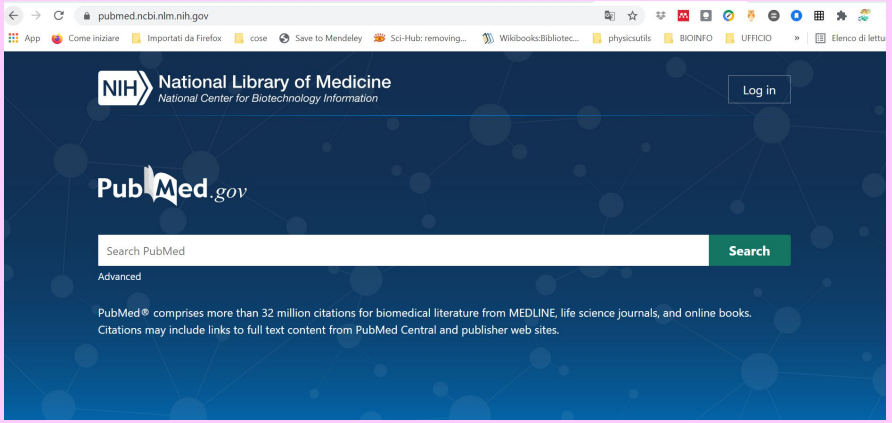
CASE STUDY

- In scientific literature Look for information about a GPCR involved in reproduction .
- Look for protein information: number of aa, MW, function, agonists, modulators, associated G-proteins… .
- Make a sequence alignment of the receptor in different species and with rhodopsin
- Look for the 3D structure

- **In scientific literature Look for information about a GPCR involved in reproduction .**

Some Scientific literature sites:

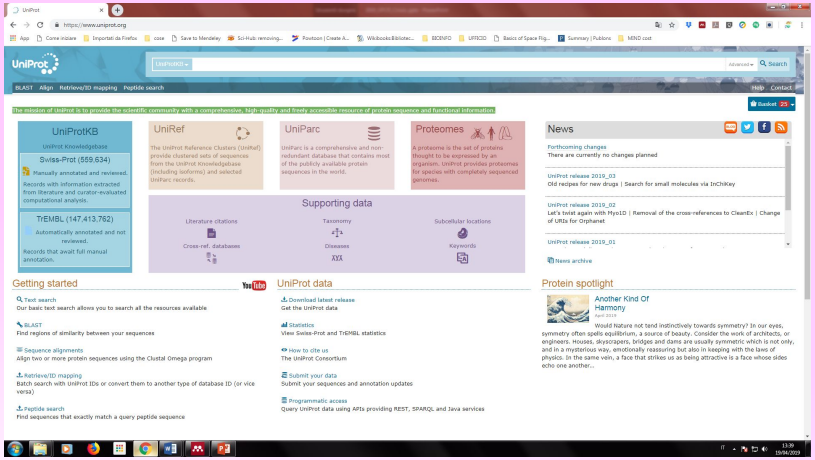
- PUBMED
- GOOGLE SCHOLAR
- Scopus
- Web of Science...



- **Look for protein information: number of aa, MW, function, agonists, modulators, associated G-proteins…**

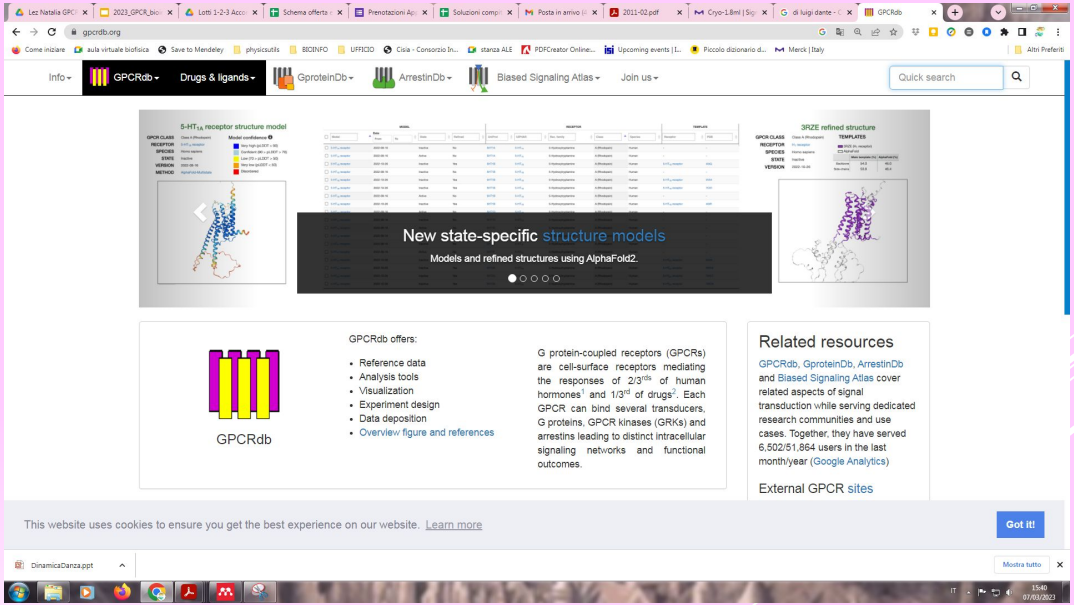
Where to retrieve PROTEIN aa sequence and other information : UNIPROT.org

The mission of UniProt is to provide the scientific community with a comprehensive, high-quality and freely accessible resource of protein sequence and functional information.

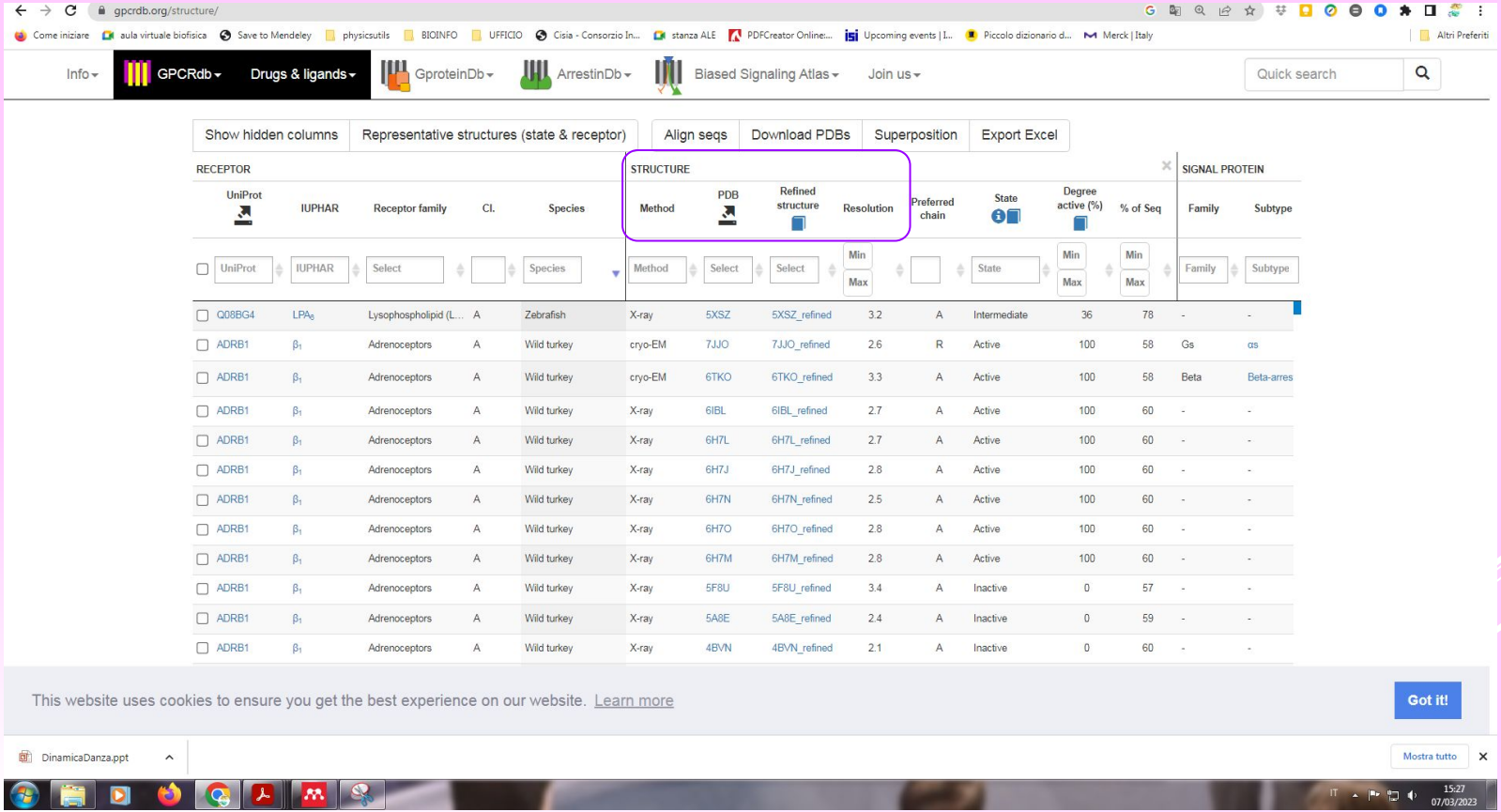


- Make a sequence alignment of the receptor in different species
- make a structural comparative analysis with rhodopsine

- The GPCR database (GPCRdb)
- (www.gpcrdb.org)



- Look for the 3D structure

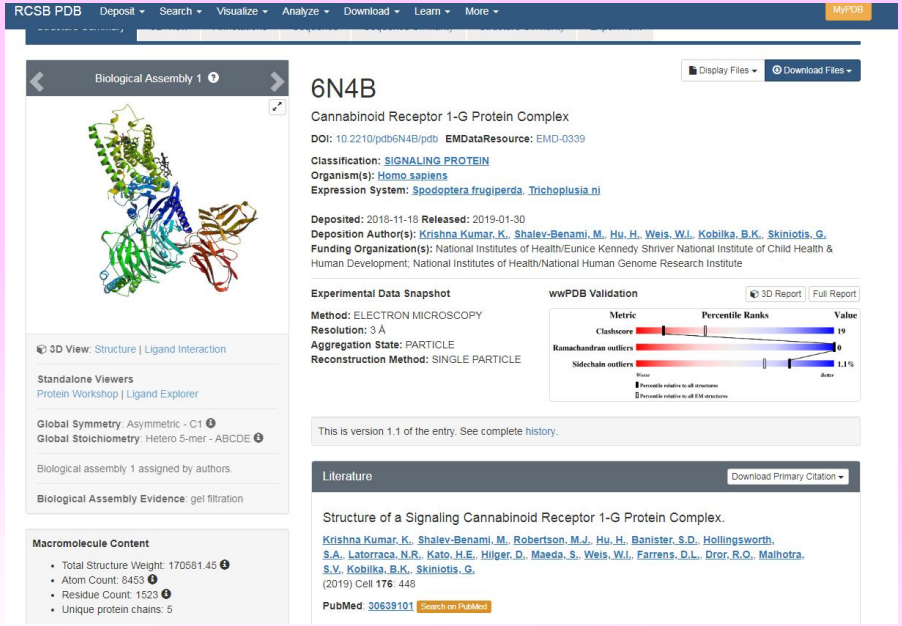


- Look for the 3D structure

GPCR Xtal and EM structures: the PROTEIN DATA BANK (PDB)

www.rcsb.org

REMEMBER: Membrane proteins are difficult to crystallize!



Electron microscopy!

For many years, only the structure of a few GPCRs was solved! In the last years, cryo-electron microscopy allowed the resolution of many structures

A VIDEOLESSON ON GPCRs…

https://www.khanacademy.org/science/ap-biology/cell-communication-and-cell-cycle/changes-in-signal-transduction-pathways/v/g-protein-coupled-receptors

THANK YOU FOR YOUR ATTENTION!

Adhesion-GPCRs pp 179-188 | Cite as

Adhesion-GPCRs in the Male Reproductive Tract

Authors | Authors and affiliations

Ben Davies, Christiane Kirchhoff

Chapter

5 Citations

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Part of the Advances in Experimental Medicine and Biology book series (AEMB, volume 706)

Abstract

The male reproductive tract expresses a diverse array of adhesion-GPCRs, many in a highly specific and regulated manner. Despite this specificity of expression, little is known about the function of this receptor family in male reproductive physiology. Insights into function are beginning to emerge with the increasing availability of genetically modified mice harbouring mutations in these genes. Gpr64 is the best characterised of the adhesion-GPCRs in the male reproductive system and the phenotype of Gpr64 knock-out mice implicates this receptor in the regulation of fluid absorption in the efferent ducts and proximal epididymis. This chapter summarizes recent data concerning this receptor and other family members in the male reproductive system.

Bonger, K.M. (2008)

Dimeric ligands for GPCRs involved in human reproduction: synthesis and biological evaluation

Doctoral Thesis

Dimeric ligands for G-protein coupled receptors that are involved in human reproduction, namely the gonadotropin releasing hormone receptor, the luteinizing hormone receptor and the follicle-stimulating hormone receptor, were synthesized and biologically evaluated.

PLOS BIOLOGY

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

A G protein–coupled receptor mediates neuropeptide-induced oocyte maturation in the jellyfish *Clytia*

Gonzalo Quiroga Artigas, Pascal Lapébie, Lucas Leclère, Philipp Bauknecht, Julie Uveira, Sandra Chevalier, Gáspár Jékely, Tsuyoshi Momose, Evelyn Houliston

Published: March 3, 2020 • https://doi.org/10.1371/journal.pbio.3000614

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Abstract
Introduction
Results
Discussion
Methods
Supporting information
Acknowledgments
References

Reader Comments (0)
Figures

Abstract

The reproductive hormones that trigger oocyte meiotic maturation and release from the ovary vary greatly between animal species. Identification of receptors for these maturation-inducing hormones (MIHs) and understanding how they initiate the largely conserved maturation process remain important challenges. In hydrozoan cnidarians including the jellyfish *Clytia hemisphaerica*, MIH comprises neuropeptides released from somatic cells of the gonad. We identified the receptor (MIHR) for these MIH neuropeptides in *Clytia* using cell culture–based "deorphanization" of candidate oocyte-expressed G protein–coupled receptors (GPCRs). *MIHR* mutant jellyfish generated using CRISPR-Cas9 editing had severe defects in gamete development or in spawning both in males and females. Female gonads, or oocytes isolated from *MIHR* mutants, failed to respond to synthetic MIH. Treatment with the cAMP analogue Br-cAMP to mimic cAMP rise at maturation onset rescued meiotic maturation and spawning. Injection of inhibitory antibodies to the alpha subunit of the G_s heterodimeric protein ($G\alpha_S$) into wild-type oocytes phenocopied the *MIHR* mutants. These results provide the molecular links between MIH stimulation and meiotic maturation initiation in hydrozoan oocytes. Molecular phylogeny grouped *Clytia* MIHR with a subset of bilaterian neuropeptide receptors, including neuropeptide Y, gonadotropin inhibitory hormone (GnIH), pyroglutamylated RFamide, and luqin, all upstream regulators of sexual reproduction. This identification and functional characterization of a cnidarian peptide GPCR advances our understanding of oocyte maturation initiation and sheds light on the evolution of neuropeptide-hormone systems.

Figures

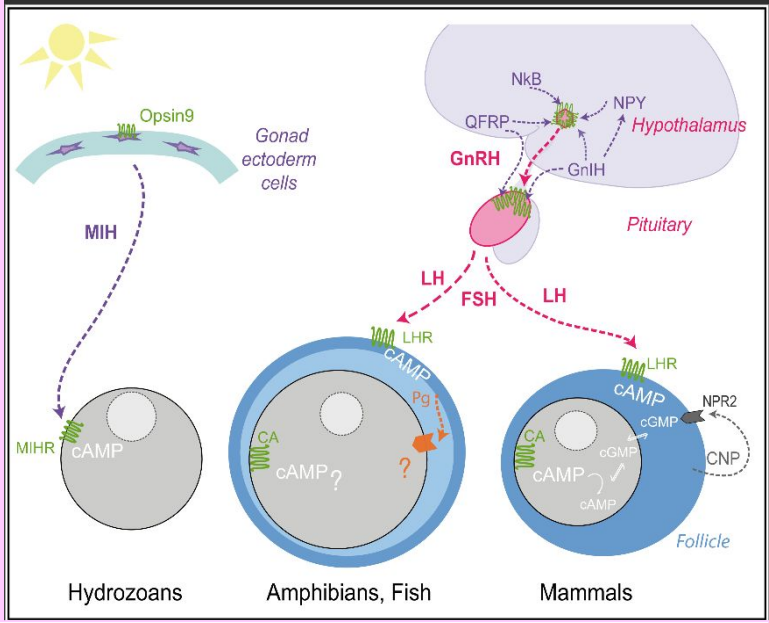

Fig 7. Schematic comparison of GPCR regulation of *Clytia* and vertebrate oocyte maturation. Simplified view of the tissues, hormones, and receptors involved in regulating oocyte maturation in *Clytia* and in fish/amphibians and mammals. For simplicity, we have not included protostome or echinoderm models. The principle peptide hormones of the reproductive hypothalamus-pituitary-gonadal axis (GnRH and LH/FSH) are in pink, and those for which the receptors group phylogenetically with *Clytia* MIHR in "Group A" (Fig 6) are in purple. Peptide hormones: *Clytia* MIH, Neuropeptide Y (NPY), GnIH, GnRH, LH, QRFP, NkB, and C-type natriuretic peptide (CNP). All their receptors, except the guanylyl cyclase natriuretic peptide receptor 2 (NPR2) activated by CNP, are GPCRs (green). Constitutively active (CA) GPCRs in vertebrate oocytes maintain cytoplasmic cAMP levels high prior to maturation. In mouse oocytes, a cAMP decrease upon hormone stimulation triggers maturation; however, in fish and frog oocytes the degree and role of this decrease is debated. Several types of oocytes receptor (orange) may respond to steroid hormones (Pg) in different species of amphibians and fish, but the relative importance of multiple downstream signalling pathways remains to be clarified [1,43,44,45,46]. CNP, C-type natriuretic peptide; FSH, follicle-stimulating hormone; GnIH, gonadotropin inhibitory hormone; GnRH, gonadotropin-releasing hormone; GPCR, G protein–coupled receptor; LH, luteinizing hormone; LHR, lutenizing hormone receptor; MIH, maturation-inducing hormone; MIHR, MIH receptor; NkB, neurokinin B; QRFP, pyroglutamylated RFamide peptide.