The first non-chordates QRFP-like peptide receptor gene in the cephalopod *Sepiella japonica*: identification, characterization and possible role in orexigenic activity

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Abstract: The novel identified receptor, GPR103, now renamed QRFPR (also referred to as SP9155 or AQ27), is the endogenous receptor for the neuropeptide QRFP (also referred to as 26RFa). The distribution pattern, structure, and biological actions of QRFPR have been largely described in chordate species, while no knowledge of QRFPR has been reported in non-chordates. Here, the first non-chordates QRFP-like peptide receptor gene in the cephalopod *Sepiella japonica* (*Sj_QRFPLR*) was identified and characterized. Evidence from multiple alignments, phylogenetic analysis, and *in vitro* subcellular localization analysis indicated that *Sj*_QRFPLR is a class A GPCR and it belongs to the QRFPR family. Meanwhile, QRFPR is likely to be structurally conserved in cephalopod species. *In situ* hybridization and RT-PCR data revealed a widespread distribution pattern of *Sj*_QRFPLR in multiple function lobes of the female brain and numerous peripheral tissues in both male and female cuttlefish. Subsequently, a food deprivation and refeeding experiment showed that *Sj*_QRFPLR is likely to exhibit orexigenic properties. Additionally, a possible link between *Sj*_QRFPLR and immune response was briefly detected in cuttlefish. Findings made in this study will contribute to our understanding of QRFPR in the cephalopod, and further understanding the peptidergic regulation of the QRFP/QRFPR system in invertebrates.

Sepiella japonica

① *Sj*_QRFPLR displays typical characteristic features of class A GPCRs

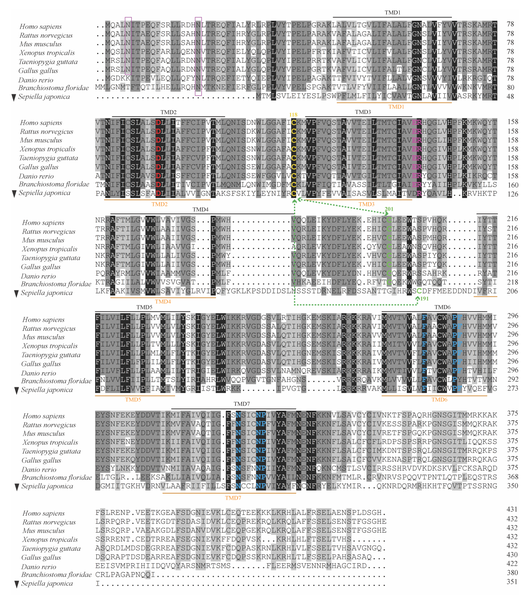

Figure 1. Multiple alignments of *Sj*_QRFPLR amino acid sequences and verified QRFPRs. Amino acid sequence alignments of *Sj*_QRFPLR and QRFPR from different species. Fully conserved and highly conserved residues are shaded in black and dark gray, respectively. Identity with at least one of the ten sequences is indicated by light grey shading. Seven TMDs of vertebrate QRFPRs and *Sj*_QRFPLR are overlined in black and underlined in orange, respectively. The Asp (D) residue in TMD2 implicated in G protein coupling is highlighted in red. Two Cys (C) residues situated in the first and second extracellular loops are highlighted by yellow and green letters, respectively. The disulfide bridge between two C was marked by green dashed lines. The conserved Arg (R) residue in the second intracellular loop, and the conserved Phe (F), Pro (P), and Asn (N) residues in TMD6 and TMD7 are represented by colored letters. Two N-linked glycosylation sites in the N-terminus of chordates QRFPR were boxed by purple frames.

Table 1. Amino acids identities of QRFPR among different species.

Species	*Sepiella japonica*	*Homo sapiens*	*Rattus norvegicus*	*Mus musculus*	*Xenopus tropicalis*	*Taeniopygia guttata*
Sepiella japonica	-					
Homo sapiens	21	-				
Rattus norvegicus	19	84	-			
Mus musculus	19	83	96	-		
Xenopus tropicalis	20	77	73	72	-	
Taeniopygia guttata	18	78	74	74	83	-
Gallus gallus	17	77	74	74	80	91
Danio rerio	18	52	53	52	54	53
Branchiostoma floridae	22	38	37	38	38	37

The highest and lowest identities between *Sj*_QRFPLR and QRFPR corresponds to the representative species were bolded.

② *Sj*_QRFPLR belongs to the QRFPR family

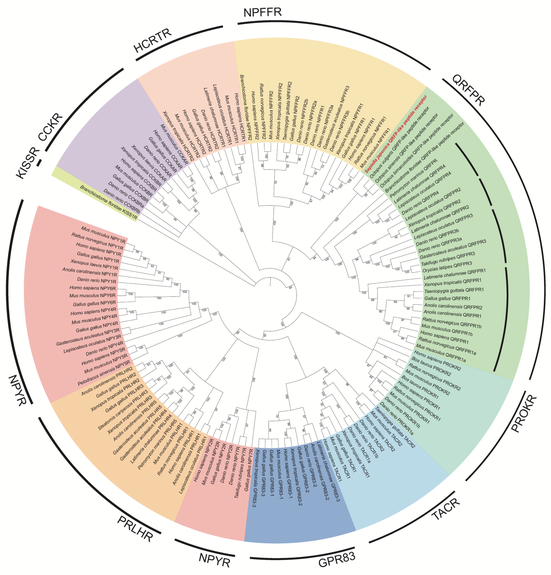

Figure 2. Bayesian phylogenetic tree of the class A GPCR superfamily. Amphioxus KISSR1 was used to root the tree. Representative sequences of various class A GPCR members from different species are downloaded from Uniprot and NCBI.

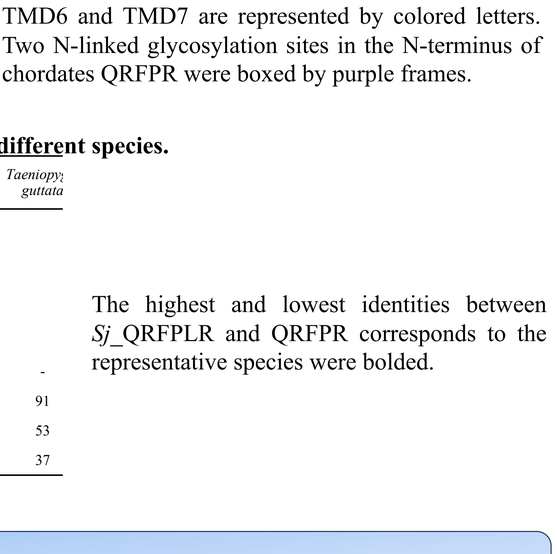

Figure 3. *In vitro* subcellular localization of *Sj*_QRFPLR in HEK293 cells. Arrowheads in different panels mark the nucleus (visualized by DAPI staining, blue in A) and cytomembrane (visualized by DiI staining, green in B), respectively.

③ Widespread distribution of *Sj*_QRFPLR in cuttlefish implies its functional diversity

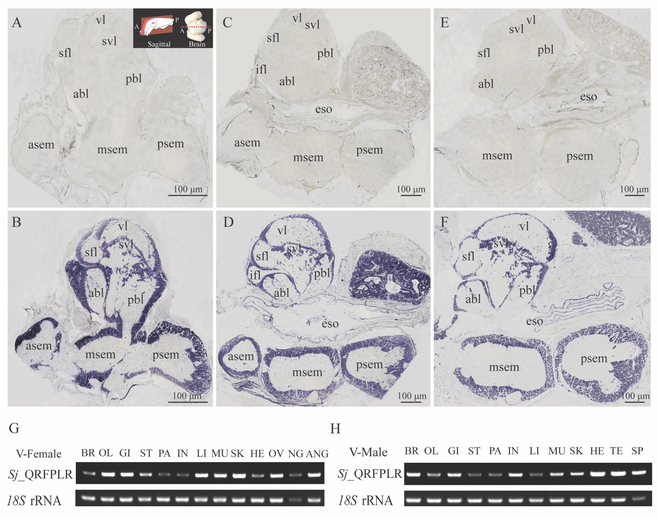

Figure 4. Tissue distribution pattern of *Sj*_QRFPLR in cuttlefish. (A-F) *In situ* hybridization analysis of *Sj*_QRFPLR mRNA in cuttlefish brain at stage I-II (A-B), IV (C-D), and V (E-F). The cuttlefish brain was sectioned in the sagittal plane. A, anterior; P, posterior. The schematic of the section orientation was modified from Montague et al (2023). The brain sections were stained with *Sj*_QRFPLR sense probe (A, C, E) and antisense probe (B, D, F), respectively. abl, anterior basal lobe; asem, anterior suboesophageal mass; eso, esophagus; ifl, inferior frontal lobe; msem, middle suboesophageal mass; psem, posterior suboesophageal mass; sfl, superior frontal lobe; svl, subvertical lobe; vl, vertical lobe; pbl, posterior basal lobe. (G-H) RT-PCR performed on various tissues in female (G) and male (H) cuttlefish. BR, brain; OL, optic lobe; GI, gill; ST, stomach; PA, pancreas; IN, intestine; LI, liver; MU, muscle; SK, skin; He, heart; OV, ovary; NG, nidamental gland; ANG, accessory nidamental gland; TE, testis; SP, spermatophore.

④ *Sj*_QRFPLR is likely to exhibit orexigenic properties in cuttlefish

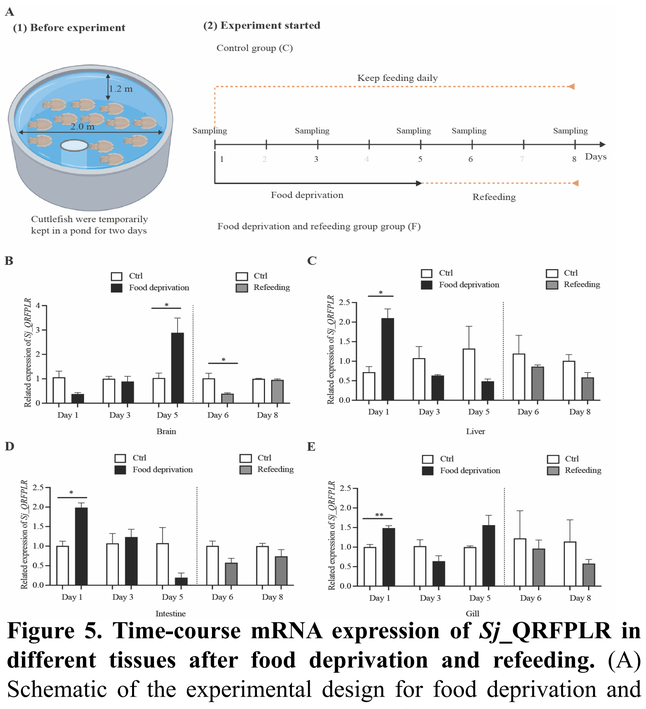

Figure 5. Time-course mRNA expression of *Sj*_QRFPLR in different tissues after food deprivation and refeeding. (A) Schematic of the experimental design for food deprivation and refeeding. (B-E) The expression level of *Sj*_QRFPLR mRNA in cuttlefish Brain (B), Liver (C), Intestine (D), and Gill (E) after food deprivation and feeding resumed. The expression level of *Sj*_QRFPLR mRNA was normalized by *β-actin* and *GAPDH*. Values are shown as means ± SD. *, $p < 0.05$, **, $p < 0.01$, unpaired two-tailed t-test.

⑤ A possible link between *Sj*_QRFPLR and immune response in cuttlefish

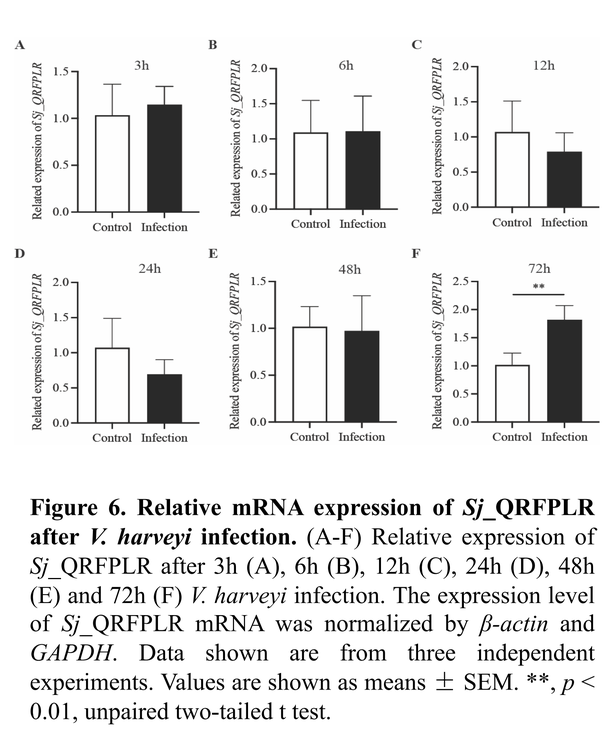

Figure 6. Relative mRNA expression of *Sj*_QRFPLR after *V. harveyi* infection. (A-F) Relative expression of *Sj*_QRFPLR after 3h (A), 6h (B), 12h (C), 24h (D), 48h (E) and 72h (F) *V. harveyi* infection. The expression level of *Sj*_QRFPLR mRNA was normalized by *β-actin* and *GAPDH*. Data shown are from three independent experiments. Values are shown as means ± SEM. **, $p < 0.01$, unpaired two-tailed t test.

Conclusions

In summary, the first non-chordates QRFP-like peptide receptor gene was identified and characterized in the cephalopod *S. japonica*. Evidence from multiple alignments, phylogenetic analysis, and *in vitro* subcellular localization analysis indicated that *Sj*_QRFPLR is a class A GPCR and it belongs to the QRFPR family. Meanwhile, QRFPR is likely to be structurally conserved in cephalopod species. *In situ* hybridization and RT-PCR data revealed a widespread distribution pattern of *Sj*_QRFPLR in multiple function lobes of female brain and numerous peripheral tissues in both male and female cuttlefish, suggesting a functional diversity of *Sj*_QRFPLR. Subsequently, functional analysis reveals that *Sj*_QRFPLR is likely to exhibit orexigenic properties that is to stimulate food intake. Additionally, a possible link between *Sj*_QRFPLR and immune response is suggested in cuttlefish. Findings made in this study will contribute to our understanding of QRFPR in the cephalopod, and further understanding the peptidergic regulation of the QRFP/QRFPR system in invertebrates.