THE CYS-LOOP LIGAND-GATED ION CHANNEL GENE FAMILY OF THE SPIDER *CUPIENNIUS SALEI* NERVOUS SYSTEM

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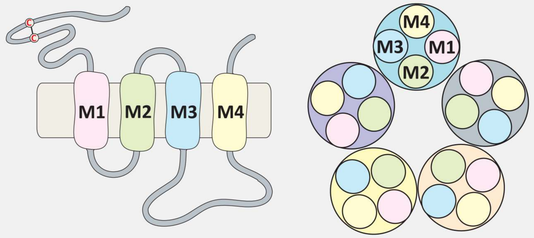
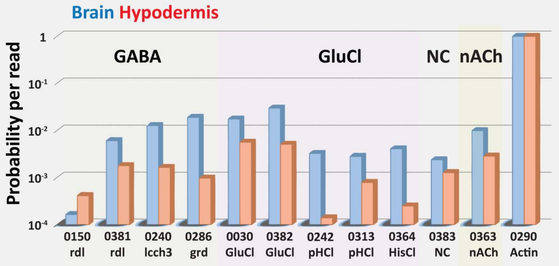
CYS-LOOP RECEPTORS

Cys-loop receptors are ligand-gated ion channels that mediate rapid inhibitory or excitatory neurotransmission in vertebrates and invertebrates. They include cation permeable nicotinic acetylcholine (nACh) and serotonin (5-HT3) receptors as well as anion permeable γ-aminobutyric acid (GABA) and glycine receptors. In invertebrates the anion permeable family also includes glutamate (GluCl), histamine (HisCl), pH (pHCl) and serotonin gated Cl-channels. Invertebrate cys-loop receptors are important targets of insecticides and antiparasitic agents, such as avermectins, fipronil and neonicotinoids. Therefore, many invertebrate cys-loop receptor subunits have been sequenced, and some crystal structures resolved.

Cys loop receptors are pentameric and composed of five of either the same or homologous subunits, but in most cases it is not clear which subunits form the functional receptor. Each subunit has four membrane spanning helices (M1-M4). The M2 helices from each subunit are located centrally and form the channel pore. The amino acid composition of M2 helix determines the ion selectivity and conductance of the channel.



ABUNDANCE



Abundance of mRNAs encoding putative cys-loop receptor genes in the *C. salei* brain and leg hypodermis transcriptomes. Values are shown relative to the abundance of actin. GABA= γ-aminobutyric acid, GluCl=glutamate-gated Cl-channels, NC=non-characterized, nAChn=nicotinic acetylcholine.

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CUPIENNIUS SALEI

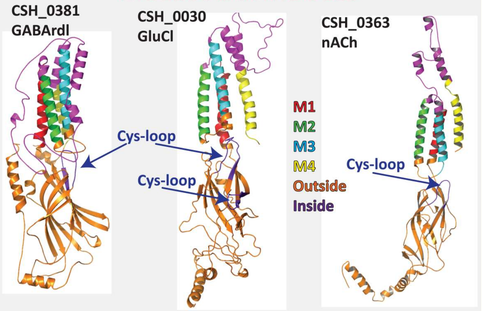
The tropical wandering spider (Cupiennius salei) central and peripheral nervous systems have GABA, glutamate, ACh and histamine containing neurons. Their mechanosensory neurons have several types of receptors for these transmitters but their molecular structures or subunit compositions are not known in any spiders.

C. SALEI CYS-LOOP RECEPTORS

We searched *C. salei* central and peripheral nervous tissue transcriptomes for homologous sequences to other arthropod cys-loop receptors and assembled genes using the transcriptome walking method (French 2012). We found ten genes that are putative cloride channel subunits and one putative cation channel subunit. Nomenclature is based on homologous genes in *Drosophila*.

ID	Accession Number	Name	Number of Amino Acids	MW (kDa)
CSH_0150	GAKT01000019	GABArdl	504	57
CSH_0381	GBFC01000040	GABArdl	478	54
CSH_0240	GAKT01000067	GABAlcch3	460	53
CSH_0286	GAKT01000104	GABAgrd	509	59
CSH_0030	GAKT01000005	GluCl	399	46
CSH_0382	GBFC01000041	GluCl	401	47
CSH_0242	GAKT01000068	pHCl	468	54
CSH_0313	GAKT01000128	pHCl	469	53
CSH_0364	GBFC01000023	HisCl	422	48
CSH_0383	GBFC01000042	NC	450	51
CSH_0363	GBFC01000022	nACh	417	46

MOLECULAR MODELS



Molecular models of *C.salei* cys-loop receptor subunits were prepared using I-TASSER server (Roy et al 2010). *C.elegans* GluCl channel was the closest structural analogue for all spider GluCl and GABA group channels. *Torpedo marmorata* nACh channel has highly similar structure to the spider nACh channel.

PERCENTAGE IDENTITY/SIMILARITY

	C.salei GABArdl 0150	*R.microplus* NC C8CGT5	*N.vitripennis* GABArdl K7LR9	*T.castaneum* GABArdl A8DMUO	*A.aegypti* GABArdl Q16896	*T.urticae* GABArdl T1L3N8	*D.melanogaster* GABArdl P25123	*H.sapiens* $GABA_A\alpha2$ P47869	*H.sapiens* $GABA_A\beta3$ P28472	*H.sapiens* Glyα2 P23416
C.salei GABArdl 0150		61.8/69.7	57.7/64	57/64.9	54.1/61.5	51.5/58.4	51/59	29.8/41.9	33.5/46.6	30.6/42.5
C.salei GABArdl 0381	67.5/75.8	61.8/68.5	57.6/64.8	55.3/63.9	52.9/60.6	51.6/59.6	49.3/57.4	30.9/42.3	34.1/46.1	31.3/42.3

	T.castaneum GABAlcch3 A8DMU3	*M.occidentalis* GABAR XP003747411	*D.melanogaster* GABAlcch3 Q08832	*N.vitripennis* GABAlcch3 D3UAF7	*I.scapularis* GABAR B7Q4M3	*S.affoinalis* GABAR Q9GYU4	*L.stagnalis* GABAR P26714	*H.sapiens* $GABA_A\alpha2$ P47869	*H.sapiens* $GABA_A\beta3$ P28472	*H.sapiens* Glyα2 P23416
C.salei GABAlcch3 0240	65.4/73.6	63.3/72.2	62.9/70.6	61.3/68.9	59.7/65.7	55.3/65	53.8/63.9	30.4/43.1	49.5/60.6	31.5/45.7

	I.scapularis GABAR R9S1A7	*M.occidentalis* GABAR XP003747426	*A.mellifera* GABAgrd H9K1K6	*P.humanus* GABAR EOVY06	*T.castaneum* GABAgrd A8DMU2	*B.mori* GABAgrd EOX9H5	*I.scapularis* GABAR B7Q4N8	*H.sapiens* $GABA_A\alpha2$ P47869	*H.sapiens* $GABA_A\beta3$ P28472	*H.sapiens* Glyα2 P23416
C.salei 0286 GABAgrd	49.1/60.4	41/48.5	31.4/41.6	30.4/41.7	30.1/41.8	28.2/39.5	28.2/37.7	31.6/42.3	30/40.7	25.1/38.6

	C.salei pHCl 0313	*M.occidentalis* GluCl XP003748445	*T.urticae* pHCl T1K9D2	*T.castaneum* pHCl A8DMU9	*N.vitripennis* pHCl D3UAF2	*D.melanogaster* pHCl Q5D6W1	*S.maritima* NC T1JD00	*H.sapiens* $GABA_A\alpha2$ P47869	*H.sapiens* $GABA_A\beta3$ P28472	*H.sapiens* Glyα2 P23416
C.salei pHCl 0242	61.1/68.9	48.9/56.5	44.4/54.7	42.4/54.5	41.5/52.2	39.8/50.9	37.8/45.9	19.2/30.7	19.6/33.1	19.8/32.5
C.salei pHCl 0313		50.9/58.6	45.5/56.8	43.5/54.7	41.3/51.1	40.9/51.6	37.7/45.2	19.7/31.9	18.5/34.4	20.7/34.2

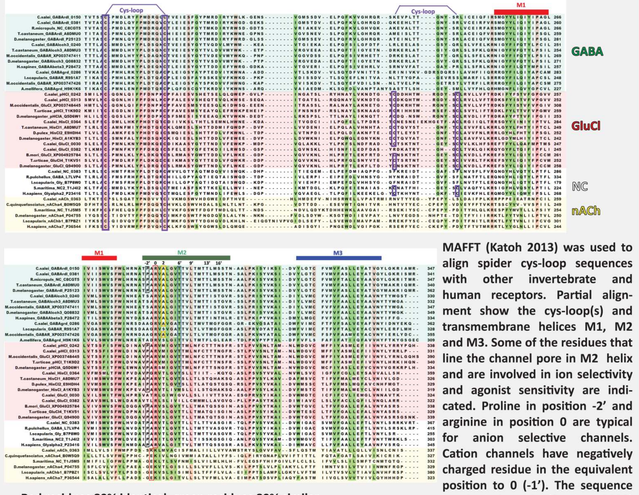
	T.castaneum HisCl1 A8DMU7	*D.pulex* HisCl2 E9HDH4	*N.vitripennis* HisCl1 D3UAF0	*D.melanogaster* HisCl A1KYB3	*T.urticae* HisCl1 T1KWX0	*I.scapularis* GluCl B7Q8K2	*I.scapularis* HisCl1 R9S1B2	*H.sapiens* $GABA_A\alpha$ P47869	*H.sapiens* $GABA_A\beta3$ P28472	*H.sapiens* Glyα2 P23416
C.salei HisCl 0364	30.1/41.6	29.3/40.5	28.9/39.6	28.4/38.9	28.3/40.3	28.2/38.3	23.6/30.4	19.5/33.8	21.2/31.7	24/33.1

	C.salei GluCl 0030	*B.mori* GluCl XP004925784	*A.mellifera* GluCl Q0GQR3	*T.urticae* GluCl4 T1KV51	*L.cuprina* GluCl O76773	*D.melanogaster* GluCl Q94900	*R.sanguineus* GluCl3 D0EGG8	*H.sapiens* $GABA_A\alpha$ P47869	*H.sapiens* $GABA_A\beta3$ P28472	*H.sapiens* Glyα2 P23416
C.salei GluCl 0030		41.1/53.4	41/52.6	40.1/52.5	39.7/52.7	39.6/52.2	33.3/46	26.7/38.6	25.5/38.5	29.6/41.6
C.salei GluCl 0382	28.6/43	34.3/46.5	33.7/45.4	29.5/43.7	33.6/45.1	32.9/44.3	27.7/41.4	23.5/38.4	21.3/35.8	27.3/39.9

	R.pulchellus GABA L7LVP4	*I.scapularis* Gly B7P8W0	*S.maritima* NC2 T1J4I2	*D.variabilis* GluCl 1_11	*M.occidentalis* Gly XP003748161	*I.scapularis* GluCl2 R9S0M9	*I.scapularis* $GABA_A\alpha$ B7PLP1	*H.sapiens* $GABA_A\alpha2$ P47869	*H.sapiens* $GABA_A\beta3$ P28472	*H.sapiens* Glyα2 P23416
C.salei NC 0383	49.7/59.5	43.1/46.4	35.4/46.2	34.4/44.9	34.3/45.4	31.9/42.2	25.2/33.8	29/41.6	27.6/40	33.8/46.3

	C.quinquefasciatus nAChα4 B0WSQ9	*S.maritima* NC T1J5M5	*A.gambiae* nAChα Q7PSA8	*D.melanogaster* nAChα α4 A8JNX5	*C.elegans* nAChβ Q93149	*M.occidentalis* nAChα XP003745665	*H.sapiens* $nAChα7$ P36544	*H.sapiens* $GABA_A\beta3$ P28472
C.salei nACh 0363	24/35.5	23.7/37.4	22.7/36.2	17.8/27.8	17.3/29.3	14.9/29.4	14.2/25.4	20.2/33 / 17.3/28.8

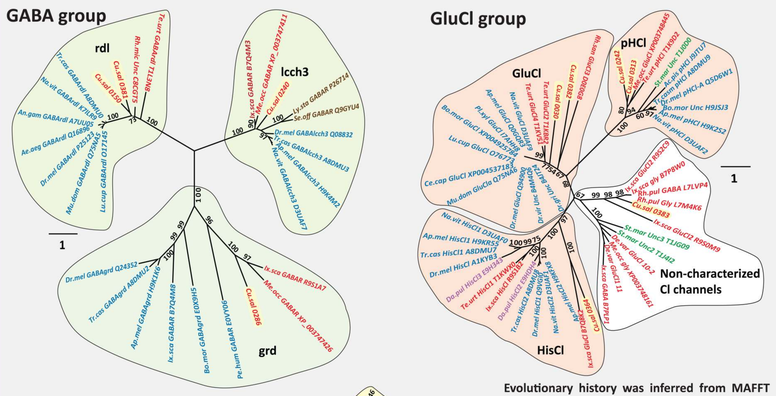
ALIGNMENT OF *C.SALEI* CYS-LOOP SEQUENCES

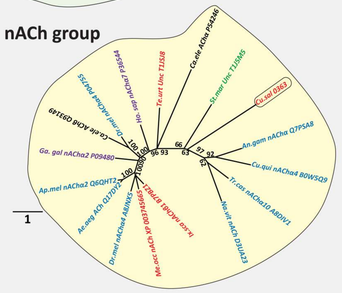


Red residues 80% identical, green residues 80% similar

MAFFT (Katoh 2013) was used to align spider cys-loop sequences with other invertebrate and human receptors. Partial alignment show the cys-loop(s) and transmembrane helices M1, M2 and M3. Some of the residues that line the channel pore in M2 helix and are involved in ion selectivity and agonist sensitivity are indicated. Proline in position -2' and arginine in position 0 are typical for anion selective channels. Cation channels have negatively charged residue in the equivalent position to 0 (-1'). The sequence manipulation suite (Stothard 2000) was used to prepare the images.

MOLECULAR PHYLOGENETIC ANALYSIS





Evolutionary history was inferred from MAFFT aligned sequences using the Maximum Likelihood method. The trees with the highest log likelihood are shown. Initial tree(s) for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using a JTT model. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The trees are drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA6 (Tamura et al 2013). Numbers at each node signify bootstrap values with 1000 replicas. The closest homologs for *C. salei* genes are other arachnid sequences, except the nACh subunit that is closer to a centipede (*Stigmata maritima)* and insect genes.

SUMMARY AND CONCLUSIONS

- We found eleven cys-loop receptor subunits in *C. salei* transcriptomes. Most subunits are predicted to form anion selective channels and include homologs to insect GABA-, glutamate-, histamine-, and pH-gated channels. They are also homologous to vertebrate GABA and glycine receptors. The $GABA_A\beta3$ subunit is especially similar to the vertebrate $GABA_A\beta3$ subunit.
- All subunits were found in both the spider brain and hypodermis transcriptomes, although there were significant differences in the relative abundance of some genes.
- Only one of the *C. salei* genes is predicted to form a cation permeable ACh-gated channel. However, this subunit lacks some of the determinants of cation permeability.
- The functional cys-loop receptors in vertebrates are heteropentamers with each receptor composed of two or three different subunits. So far, no native subunit compositions for any invertebrate cys-loop channels have been determined.
- Phylogenetic analysis placed the *C. salei* putative anion selective subunits closest to other arachnid cys-loop subunits. The nACh sequence was closer to insect and chilopoda subunits.
- Three-dimensional molecular models were predicted using I-TASSER server. The closest structural analogs whose crystal structures were available in the protein data bank were the *C. elegans* GluCl channel and the *T. marmorata* nACh receptor.
- *C. salei* is an important model for research into mechanotransduction and its synaptic modulation. Molecular identification of its neurotransmitter receptors is a crucial step in understanding the functions of these receptors. Since cys-loop receptors are targets of insecticides and antiparasitic agents, it is essential to identify their molecular structures in beneficial arthropods.