**Supplementary Materials**

**Sex-specific regulation of neuronal functions in *Caenorhabditis elegans*: the sex-determining protein TRA-1 represses *goa-1/Gα(i/o)***

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Supplementary Materials include:

**Materials and Methods**

**Tables S1-S15**

**Figures S1-S14**

**Supplementary References (1-12)**

**Supplementary Materials and Methods**

**Fluorescent Microscopy**

Transgenic worms were placed on 2% agarose pads, and immobilized by adding 0.1 M levamisole in M9 buffer. Pictures in **Figs. 2**, **3B** and **S3** were taken by an Olympus BX-51 microscope. Pictures in **Fig. S6** were obtained with a LSM710 Zeiss confocal microscope, Axio-observer Z1 controlled by the ZEN software package (ZEN2011). Pictures in **Figs. 3C-D** and **S2**, **S8** and **S9** were taken by a Zeiss AxioImager Z1 epifluorescence microscope equipped with objective Plan-Neofluar 20x/0.50 M27. Images in **Fig. 4** were taken by a Zeiss AxioImager Z1 epifluorescence microscope equipped with an ApoTome semiconfocal setup with objective Plan-Neofluar EC Plan-Neofluar 20x/0.50 M27. In **Fig. S9**, pictures were taken on F1 males derived from crosses between *goa-1(sa734); vtIs1* and *goa-1(sa734)/+; vtIs1*, as well as *goa-1(syIs9); vtIs1* and *goa-1(syIs9)/+; vtIs1*. Fluorescence intensity was analysed in the head (**Figs**. **4** and **S6**) and tail (**Figs. 4** and **S9**) of adults. In **Fig. 3**, fluorescence intensity was analysed in whole embryos. Photos were analysed by using ImageJ software.

**Male mating assay**

Male mating assay was performed as described(Lipton et al. 2004). Onto plastic dishes of 3 cm diameter, filled with NGM, 1 cm diameter (~16 μl) of *E. coli* OP50 culture was inoculated. 5 young *unc-31(-)* mutant (paralyzed) hermaphrodites were placed on the bacterial lawn, and 1 virgin male was placed on the edge of the bacterial lawn and monitored for 10 min. Time was measured when the male touched and found the vulva of the mating partner. Measurements were stopped when the male inserted its spiculum into a hermaphrodite. If males could not find hermaphrodites within 10 min as in the case of *goa-1(-)* mutants, the former were moved next to the latter by a platinum wire to assay response reaction.

**Phylogenetic tree construction**

Protein sequences NP\_492108.1 (GOA-1) and NP\_490790.1 (GPA-16) of *Caenorhabditis elegans* were used in BLAST searches against the nonredundant protein database of NCBI with default settings. Only sequences from nematodes (taxid:6231) and fruit-fly (taxid:7227) were included to the search. For further analysis, only the best hits from each species were retained. 32 sequences from nematodes and 1 sequence from *Drosophila melanogaster* were aligned using Clustal Omega (PMID: 21988835). Since these sequences are highly conserved and 51% of the sequences is completely constant, a high quality alignment was produced. PAUP (Swofford 2002) was used to perform simple heuristic search for a phylogenetic tree using parsimony criteria. The best tree with score 604 was visualized using R statistical environment and ape package (Paradis 2012). The tree was rooted using the fruit-fly sequence as outgroup.

**Sequence Analysis of Human Gα(i/o) Genomic Regions to Identify Conserved GLI Binding Sites**

GLI binding motifs: The GLI family of zinc finger transcription factors have been intensively studied and their DNA binding specificity was described in different conditions. Hallikas et al. (2006) published a consensus GLI binding site with a sequence of GACCACCCA as the most representative motif (Hallikas et al. 2006). Due to a slightly degenerated nature of this binding site, a more specific representation can be achieved using the following position-specific weight matrix (PSWM):

A 9 758 30 0 813 0 166 25 964

C 4 136 971 1000 74 1000 823 941 9

G 945 106 0 0 24 0 0 2 6

T 42 0 0 0 89 0 11 32 22

This motif was used as a starting point in the following analysis. As this motif is rather short, it is expected to deliver many false positive hits during searching. To circumvent this problem, the motif was used to query MotifDB for further, more exact binding site information. Based on the results of this search, two further binding patterns were identified: Gli2 and GLI2-2 (**Fig. S13**) (Jolma et al. 2013). The three patterns were all used in the binding site prediction calculations.

Target genes: Four potential orthologues of the *C. elegans goa-1* gene were identified in the human genome: *GNAO1*, *GNAI1*, *GNAI2* and *GNAI3*. The genomic region of these genes, together with their up- and downstream regulatory sequences, was used in the motif prediction analysis.

Genomic regions of these genes were obtained from the ENSEMBL database (Flicek et al. 2014). Intergenic genomic regions upstream and downstream of the *GNA* genes were considered as shown in **Table S14**. Eutherian orthologue alignments of these regions were obtained from ENSEMBL.

For motif search, Motif Occurrence Detection Suite (Korhonen et al. 2009) was applied on human sequences from each alignment, using custom written BioPerl scripts (Stajich et al. 2002). **Table S15** summarizes the number of hits from the genomic regions of these genes.

The conservation of hits in the eutherian orthologues with scores above 8.5 was investigated further using the software Jalview (Waterhouse et al. 2009) and custom BioPerl scripts. Hits showing more than 90% identity among the 17 eutherian orthologues were assessed further manually. [*GNAI1* region](http://www.ensembl.org/Homo_sapiens/Share/919b52e30f2230b74e008e76a42114ad164673938115637#_blank) contains candidates with much lower scores (below 9 in case of all motifs) (**Fig. S14**). No site reaching the defined thresholds was detected in the genomic region of *GNAI2*. In [the *GNAI3* region,](http://www.ensembl.org/Homo_sapiens/Share/7b30c900dcd938832ddf69e1406922bd164673938115637) there are only low score hits mainly because long stretches of the alignment are specific to primates. Interestingly, many of these sites are very similar to each other with the following consensus sequence: GGCCTCCCAA. This is different only at the second position from the described TRA-1/GLI binding site in *C. elegans* (Gentleman et al. 2004). These sequences likely represent real, primate-specific binding sites. This is supported by the fact that one of the sites (109550785-109550798) is significant from two motifs (GLI2 consensus: 9.03, GLI2 Jolma2013: 8.82), and falls to the annotated promoter region of *GNAI3*.

**Supplemental References**

Flicek P, Amode MR, Barrell D, Beal K, Billis K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fitzgerald S, Gil L, Girón CG, Gordon L, Hourlier T, Hunt S, Johnson N, Juettemann T, Kähäri AK, Keenan S, Kulesha E, Martin FJ, Maurel T, McLaren WM, Murphy DN, Nag R, Overduin B, Pignatelli M, Pritchard B, Pritchard E, Riat HS, Ruffier M, Sheppard D, Taylor K, Thormann A, Trevanion SJ, Vullo A, Wilder SP, Wilson M, Zadissa A, Aken BL, Birney E, Cunningham F, Harrow J, Herrero J, Hubbard TJ, Kinsella R, Muffato M, Parker A, Spudich G, Yates A, Zerbino DR, Searle SM (2014) Ensembl 2014. Nucleic Acids Res 42:D749–755.

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5(10):R80.

Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27(2):221–224.

Hallikas O, Palin K, Sinjushina N, Rautinainen R, Partanen J, Ukkonen E, Taipale J (2006) Genome-wide prediction of mammalian enhancers based on analysis of transcription-factor binding affinity. Cell 124(1):47–59.

Jolma A, Whitington T, Toivonen J, [Toivonen](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) J, [Nitta](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) KR, [Rastas](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) P, [Morgunova E,](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) [Enge M](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!), [Taipale](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) M, [Wei G,](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) [Palin K](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!), [Vaquerizas](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) JM, [Vincentelli](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) R, [Luscombe](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) NM, [Hughes](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) TR, [Lemaire](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) P, [Ukkonen](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) E, [Kivioja](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) T, [Taipale](https://www.sciencedirect.com/science/article/pii/S0092867412014961#!) J (2013) DNA-binding specificities of human transcription factors. Cell 152(1-2):327–339.

Korhonen J, Martinmäki P, Pizzi C, Rastas P, Ukkonen E (2009) MOODS: fast search for position weight matrix in DNA sequences. Bioinformatics 25(23):3181–3182.

Lipton J, Kleemann G, Ghosh R, Lints R, Emmons SW (2004) Mate searching in Caenorhabditis elegans: a genetic model for sex drive in a simple invertebrate. J Neurosci 24(34):7427–7434.

Paradis E (2012) Analysis of phylogenetics and evolution with R. Springer Science and Business Media.

Stajich JE, Block D, Boulez K, Brenner SE, Chervitz SA, Dagdigian C, Fuellen G, Gilbert JG, Korf I, Lapp H, Lehväslaiho H, Matsalla C, Mungall CJ, Osborne BI, Pocock MR, Schattner P, Senger M, Stein LD, Stupka E, Wilkinson MD, Birney E (2002) The Bioperl toolkit: Perl modules for the life sciences. Genome Res 12(10):1611–1618.

Swofford DL (2002) PAUP\* version 4.0 b10. Phylogenetic analysis using parsimony (\* and other methods). Sinauer, Sunderland, MA.

Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ (2009) Jalview Version 2-a multiple sequence alignment editor and analysis workbench. Bioinformatics 25(9):1189–1191.

Yamagishi MEB, Shimabukuro AI (2008) Nucleotide frequencies in human genome and fibonacci numbers. Bull Math Biol 70(3):643–653.

**Supplementary Tables**

**Table S1. Statistics for quantifying *goa-1* transcripts levels by qRT-PCR in adult animals.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Background** | **Trials** | **Relative mRNA level (mean)** | **±SD** | **Mann-Whitney U-test P value versus control** |
| *goa-1* | wild-type | 3 | 1.000 | 0.800 | control |
| wild-type male *(50%)* | 3 | 9.338 | 8.346 | 0.027 |
| *tra-1(e1099)* | 3 | 8.869 | 9.144 | 0.030 |
| *tra-1(e1488)* | 3 | 3.875 | 3.099 | 0.085 |
|  | | | | |
| *tra-3(e1767)* | 3 | 1.000 | 0.230 | control |
| *tra-1(e1575gf)/+; tra-3(e1767)* | 3 | 0.529 | 0.137 | 0.014 |

**Table S2. Statistics for quantifying relative expression intensities of the GFP-tagged translational fusion GOA-1 reporter used in this study, at early embryonic stages**.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **GOA-1::GFP in embryos** | **„Faint” embryos** | | **„Bright” embryos** | | **Kruskal-Wallis H test with Dunn’s Post Hoc Test *P* value** |
| **Mean of relative expression level** | **±SEM** | **Mean of relative expression level** | **±SEM** |
| wild-type | 1.123 | 0.049 | 4.915 | - |  |
| *him-5(e14909* | 1 | 0.085 | 5.752 | 0.399 | faint vs. bright embryos, P<0.000 |
| *tra-1(e1099)/+* | 0.961 | 0.11 | 5.354 | 0.179 | faint vs. bright embryos, P<0.000 |
| *fem-3(e2006)* | 0.54 | 0.028 | 6.06 | - | vs. wild-type, P<0.000 |
| **mutGOA::GFP in embryos** | **„Faint” embryos** | | **„Bright” embryos** | | **Kruskal-Wallis H test with Dunn’s Post Hoc Test *P* value** |
| **Mean of relative expression level** | **±SEM** | **Mean of relative expression level** | **±SEM** |
| wild-type | 0.470 | 0.034 | - | - |  |
| *him-5(e1490)* | 1.175 | 0.104 | 3.583 | 0.001 | faint vs. bright embryos, P=0.002 |
| *tra-1(e1099)/+* | 0.317 | 0.035 | - | - | - |
| *fem-3(e2006)* | 0.389 | 0.032 | - | - | vs. wild-type, *P*=0.148 |

**Table S3. The ratio of strongly glowing embryos transgenic for GOA-1::GFP.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***GOA-1::GFP*** | **Number of embryos** | **Number of highly bright embryos** | **% embryos with intense expression** | **±SD** |
|
| wild-type | 125 | 1 | 0.80% | 1.09% |
| *him-5(e1490)* | 32 | 9 | 28.13% | 1.94% |
| *tra-1(e1099)/+* | 80 | 15 | 18.75% | 1.57% |
| *fem-3(e2006)* | 65 | 1 | 1.54% | 2.08% |
| ***GOA-1::GFP*** | **Number of embryos** | **Number of highly bright embryos** | **% embryos with intense expression** | **±SD** |
|
| wild-type | 56 | 0 | 0.00% | 0.00% |
| *him-5(e1490)* | 42 | 2 | 4.79% | 1.54% |
| *tra-1(e1099)/+* | 36 | 0 | 0.00% | 0.00% |
| *fem-3(e2006)* | 43 | 0 | 0.00% | 0.00% |

**Table S4. Statistics for the quantification of relative expression intensities in embryos by qRT-PCR.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Background** | **Trials** | **Relative mRNA level (mean)** | **±SD** | **Mann-Whitney U-test P value versus control** |
| *goa-1* | wild-type | 3 | 1.000 | 0.400 |  |
| wild-type male (50%) | 3 | 1.702 | 0.375 | 0.020 |
| *him-5(e1490)* | 3 | 1.654 | 0.205 | 0.004 |
| *tra-1(e1099)/+* | 3 | 3.284 | 0.994 | 0.011 |
| *tra-1(e1488)* | 3 | 1.091 | 0.330 | 0.549 |

**Table S5. Statistics for the quantification of relative expression intensities of the GFP-tagged translational fusion GOA-1 reporter (GOA-1::GFP) used in this study, at adult stages**.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **GOA-1::GFP** | **Number of worms** | **Mean of relative expression level** | **±SEM** | **Independent *t*-test *P* value versus control** |
| herm. | 26 | 1.000 | 0.024 | control |
| male | 18 | 1.096 | 0.019 | 0.004 |
| **GOA-1::GFP** | **Number of worms** | **Mean of relative expression level** | **±SEM** | **One -way ANOVA** |
| *wild-type* | 32 | 1.000 | 0.012 | control |
| *fem-3(e2006)* | 31 | 0.495 | 0.009 | vs. control, *P*=8.972E-41 |
| *tra-1(e1099)* | 27 | 1.175 | 0.019 | vs. control, *P*<0.000  vs. *fem-3*(e2006),  *P*= 5.826E-28 |
| **mutGOA-1::GFP** | **Number of worms** | **Mean of relative expression level** | **±SEM** | **Independent *t*-test *P* value versus control** |
| herm. | 26 | 1.000 | 0.033 | control |
| male | 12 | 1.044 | 0.026 | 0.401 |

**Table S6. Statistics for the quantification of relative expression intensities of the GFP-tagged translational fusion GOA-1 reporter (GOA-1::GFP) used in this study, at larval stages**.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **GOA-1::GFP** | | **Number of embryos** | **Mean of relative expression level** | **±SEM** | **Kruskal-Wallis H test with Dunn’s Post Hoc Test and with Bonferroni correction P value vs control** |
|
| L1 stage | wild-type | 89 | 1.000 | 0.027 | control |
| *him-5(e1490)* | 210 | 1.356 | 0.026 | 0.00 |
| *tra-1(e1099)/+* | 210 | 1.356 | 0.026 | 0.00 |
|  |  |  |  |  |  |
| L2 stage | wild-type | 19 | 1.000 | 0.061 | control |
| *him-5(e1490)* | 26 | 1.279 | 0.073 | 0.081 |
| *tra-1(e1099)/+* | 39 | 1.330 | 0.029 | 0.003 |
|  |  |  |  |  |  |
| L4 stage | wild-type | 48 | 1.000 | 0.039 | control |
| *him-5(e1490)* male | 46 | 2.129 | 0.110 | 0.000 |
| *tra-1(e1099)* | 36 | 1.318 | 0.075 | 0.435 |

**Table S7. Statistics for the quantification of relative expression levels of PKD-2::GFP at adult stages.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **PKD-2::GFP(*bxIS14*)** | | **Number of worms** | **Mean of relatíve expression level** | **±SEM** | **Kruskal-Wallis H test with Dunn’s Post Hoc Test and with Bonferroni correction *P* value** |
| tail | *him-5(e1490)*  XO male | 19 | 1.000 | 0.064 | control |
| *goa-1(sa734);him-5(e1490)*  XO male | 16 | 0.236 | 0.064 | vs. control *P*<0.0001 |
| *goa-1(syIS9gf);him-5(e1490)*  XO male | 15 | 1.971 | 0.329 | vs. control  *P*=0.390 |
|  |  |  |  |  |  |
| head | *him-5(e1490)*  XO male | 12 | 1.000 | 0.102 | control |
| *goa-1(sa734);him-5(e1490)*  XO male | 16 | 0.326 | 0.058 | vs. control,  *P*=0.001 |
| *goa-1(syIS9gf);him-5(e1490)*  XO male | 10 | 1.116 | 0.232 | vs. control, *P*=0.246 |

**Table S8. The ratio of hermaphrodites showing ectopic PKD-2::GFP expression.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **PKD-2::GFP(*bxIS14*)** | **Trials** | **Number of worms with ectopic expression** | **Number of worms** | **Rate of worms with ectopic expression** | **Chi-squared test P value** |
| *him-5(e1490)* XX herm. | 3 | 1 | 815 | 0.001 |  |
| *goa-1(syIs9gf);him-5(e1490)* XX herm. | 3 | 12 | 1176 | 0.010 | 0.015 |

**Table S9. Statistics for the quantification of relative expression levels of *dat-1::gfp* in the male tail.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***dat-1::gfp (vtIs1)*** | | **Number of worms** | **Mean of relatíve expression level** | **±SEM** | **Mann-Whitney U-test *P* value versus control** |
| tail | *XO male* | 26 | 1.000 | 0.041 | control |
| *goa-1(sa734) XO male* | 16 | 0.605 | 0.046 | vs. control, *P*<0.001 |
|  |  |  |  |  |
| *XO male* | 15 | 1.000 | 0.184 | control |
| *goa-1(syIS9gf) XO male* | 30 | 1.474 | 0.165 | vs. control, *P*=0.084 |

**Table S10. Statistics for the quantification of food leaving (mate searching) behaviour**.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Genotype** | **Number of worms** | **Friedman test with Wilcoxon Signed Ranks Post Hoc Test *P* value** | **PL** | **Kruskal-Wallis H test with Dunn’s Post Hoc Test *P* value** |
| **Figs. 4A** and **S10** | | | | |
| *him-5(e1490)* XXherm*.* | 104 | χ2(9)=71.79 *P*<0.0001 | 0 |  |
| *him-5(e1490)* XOmale | 50 | vs. *him-5(e1490)* XX herm., *P*=0.008 | -0.057 | vs. *him-5(e1490)*  XX herm., *P*=0.012 |
| *goa-1(sa734)* XXherm*.* | 40 | vs. *him-5(e1490)* XX herm, *P*=0.317 | 0 | vs. *him-5(e1490)* XX herm, *P*=0.908 |
| *goa-1(sa734)* XOmale | 41 | vs. *him-5(e1490)* XX h*erm.*, *P*=1.000 | -0.001 | vs. *him-5(e1490)* XX herm., *P*=0.645 |
| vs. *him-5(e1490)* XO male*, P*=0.008 | vs. *him-5(e1490)* XO male, *P*=0.091 |
| *goa-1(syIs9gf)* XXherm*.* | 86 | vs. *him-5(e1490)* XX herm., *P*=0.008 | -0.034 | vs. *him-5(e1490)* XX herm., *P*=0.061 |
| *goa-1(syIs9gf)* XOmale | 40 | vs. *him-5(e1490)* XO male, P=0.008 | -0.101 | vs. *him-5(e1490)* XO male*, P*=0.382 |
| *tra-1(e1099)* XXmale | 20 | vs. *him-5(e1490)* XX herm., *P*=0.018 | -0.071 | vs. *him-5(e1490)* XX herm., *P*=0.094 |
| *goa-1(sa734);tra-1(e1099)* XXmale | 34 | vs. *tra-1(e1099)* (XX) male, *P*=0.017 | -0.009 | vs. *tra-1(e1099)* (XX) male, *P*=0.382 |
| *egl-30(ad806)* XX herm*.* | 56 | vs. *him-5(e1490)* XX herm., *P*=0.012 | -0.027 | vs. *him-5(e1490)* XX herm., *P*=0.303 |
| *egl-30(ad806)* XO male | 40 | vs. *him-5(e1490)* XO male, *P*=0.028 | -0.193 | vs. *him-5(e1490)* XO male, *P*=0.111 |
| **Fig. S11** | | | | |
| **Genotype** | **Number of worms** | **Wilcoxon Signed Ranks Test P value** | **PL** | **Mann-Whitney U-test *P* value versus control** |
| *him-5(e1490)* XO male L3 | 10 |  | -0.005 |  |
| *goa-1(syIs9gf)* XX herm. L3 | 20 | vs. *him-5(e1490)* XO male L3, *P*=0.655 | -0.002 | vs. *him-5(e1490*) XO male L3, *P*=0.317 |

**Table S11. Statistics for the quantification of male mating behaviour.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Phase of mating** | | **Genotype** | **Number of worms** | | | **Time of performing the phase (min:s)** | | **Kruskal-Wallis H test with Dunn’s Post Hoc Test and with Bonferroni correction *P* value**  **(to the time of performing the phase)** |
| **start** | **perform** | **perform% vs start** | **Mean** | **±SEM** |
| first touch→ spiculum insertion | | *him-5(e1490)* XO male | 21 | 13 | 61.9% | 00:46 | 00:07 |  |
| *tra-1(e1099)*  XX male | 20 | 8 | 40.0% | 01:56 | 00:33 | vs. *him-5(e1490)* XO male, *P*=0.120 |
| *goa-1(sa734); him-5(e1490)* XO male | 21 | 3 | 14.3% | 02:37 | 00:36 | vs. *him-5(e1490)* XO male, *P*=0.036 |
| *goa-1(syIs9gf)* XO male | 20 | 11 | 55.0% | 00:56 | 00:12 | vs. *him-5(e1490)* XO male, *P*=2.952 |
|  | | | | | | | | |
| getting to screen  →  first touch | *him-5(e1490)* XO male | | 21 | 18 | 85.7% | 00:21 | 00:06 |  |
| *tra-1(e1099)* XX male | | 20 | 16 | 80.0% | 00:38 | 00:11 | vs. *him-5(e1490)* XO male, *P*=1.992 |
| *goa-1(sa734); him-5(e1490)* XO male | | 21 | 14 | 66.7% | 01:42 | 00:20 | vs. *him-5(e1490)* XO male, *P*<0.000 |
| *goa-1(syIs9gf)* XO male | | 20 | 14 | 70.0% | 00:46 | 00:10 | vs. *him-5(e1490)* XO male, *P*=0.114 |
|  | | | | | | | | |
| first touch → response | *him-5(e1490)* XO male | | 21 | 17 | 81,0% | 00:09 | 00:02 |  |
| *tra-1(e1099)* (XX) male | | 20 | 11 | 55.0% | 00:15 | 00:03 | vs. *him-5(e1490)* XO male, *P*=0.606 |
| *goa-1(sa734); him-5(e1490)*  XO male | | 21 | 9 | 42.9% | 00:21 | 00:04 | vs. *him-5(e1490)* XO male, *P*=0.030 |
| *goa-1(syIs9gf)* XO male | | 20 | 14 | 70.0% | 00:12 | 00:03 | vs. *him-5(e1490)* XO male, *P*=2.508 |

**Table S12. Statistics for quantifying chemotaxis to sex pheromone.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Genotype** | **Number of worms** | **Number of "plates"** | **Median of chemotaxis** | **Mean of chemotaxis** | **±SEM** | **Kruskal-Wallis H test with Dunn’s Post Hoc Test *P* value** |
|
| *him-5(e1490)* XX herm. | 269 | 13 | 0.000 | 0.060 | 0.041 |  |
| *him-5(e1490)* XO male | 270 | 14 | 0.553 | 0.510 | 0.059 | vs. *him-5(e1490)* XX herm. *P*<0.0001 |
| *goa-1(sa734); him-5(e1490)* XX herm. | 191 | 10 | 0.074 | 0.086 | 0.037 | vs. *goa-1(sa734); him-5(e1490)*, XO male, *P*=0.453 |
| *goa-1(sa734); him-5(e1490)* XO male | 184 | 10 | 0.000 | 0.024 | 0.019 | vs. *him-5(e1490)* XO male P<0.0001 |
| *goa-1(syIs9gf); him-5(e1490)* XX herm. | 199 | 11 | 0.238 | 0.208 | 0.053 | vs. *him-5(e1490)* XX herm., *P*=0.071 |
| *goa-1(syIs9gf); him-5(e1490)* XO male | 78 | 4 | 0.250 | 0.315 | 0.101 | vs. *goa-1(syIs9gf); him-5(e1490)* XX herm. *P*=0.413 |
| *tra-1(e1099)* XX male | 236 | 13 | 0.550 | 0.467 | 0.092 | vs. *him-5(e1490)* XO male, *P*=0.526 |
| vs. *goa-1(syIs9gf); him-5(e1490)* XO male *P*=0.682 |
| *goa-1(sa734);tra-1(e1099)* XX male | 119 | 7 | 0.000 | 0.001 | 0.027 | vs. *goa-1(sa734); him-5(e1490)* XO male P=0.723 |
| vs. *tra-1(e1099)* XX male P<0.0001 |

**Table S13. Statistics for quantifying rate adaptation of animals with reduced or elevated levels of GOA-1 activity to isoamyl alcohol.** IS: intersex.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Genotype** | **Number of worms** | **Trials** | **Median Rate of adaptation [{Ci(c)-Ci(IAA)}/Ci(c)]** | **Mean Rate of adaptation [{Ci(c)-Ci(IAA)}/Ci(c)]** | **±SEM** | **Independent *t*-test *P* value** |
| *wild-type* herm. | 1576 | 12 | 0.704 | 0.74 | 0.047 |  |
| *wild-type* male | 113 | 4 | 0.708 | 0.75 | 0.345 | vs. *wild-type* herm. *P*=0.0979 |
|
| *him-5(e1490)*  male | 721 | 10 | 0.683 | 0.683 | 0.083 | vs. *wild-type* male *P*=0.789 |
|
| *tra-1(e1099)* male | 186 | 9 | 0.792 | 0.792 | 0.07 | vs. *wild-type* herm. *P*=0.534 |
|
| *tra-1(e1488)*  IS | 695 | 10 | 0.768 | 0.768 | 0.079 | vs. *wild-type* herm., *P*=0.768 |
|
| *tra-1(e1575gf); tra-3(e1767)* | 1624 | 15 | 0.352 | 0.338 | 0.06 | vs. *wild-type* herm. *P*<0.001 |
|
| *fem-3(e2006)* | 893 | 8 | 0.279 | 0.302 | 0.041 | vs. *wild-type* herm. *P*<0.001 |
|
| *goa-1(sa734)* | 1182 | 3 | 0.031 | 0.106 | 0.077 | vs. *wild-type* herm. *P*<0.001 |
|
| *goa-1(n1134)* | 832 | 8 | -0.067 | -0.035 | 0.06 | vs. wild-type herm. *P*<0.001 |
|
| *goa-1(syIs9gf)* | 654 | 9 | 1.054 | 1.077 | 0.131 | vs. *wild-type* herm. *P*=0.014 |
|
| *goa-1(sa734); him-5(e1490)* | 182 | 5 | 0.084 | 0.057 | 0.123 | vs. *him-5(e1490)* *P*<0.001 |
|
| *goa-1(n1134); him-5(e1490)* | 420 | 6 | -0.041 | -0.081 | 0.128 | vs. *him-5(e1490) P*<0.001 |
|
| *tra-1(e1488); goa-1(n1134)* | 932 | 9 | 0.410 | 0.39 | 0.039 | vs. *tra-1(e1488)* IS, *P*<0.001 |
|

**Table S14. Genomic regions selected for motif search.**

| **Alignment** | **Genomic region in GRCh38 genome build** |
| --- | --- |
| GNAO1 block1 | Chromosome 16 56112617-56190248 |
| GNAO1 block7 | Chromosome 16 56190249-56205016 |
| GNAI1 block1 | Chromosome 7 80079059-80136450 |
| GNAI1 block2 | Chromosome 7 80146971-80199230 |
| GNAI1 block3 | Chromosome 7 80219161-80245947 |
| GNAI1 block4 | Chromosome 7 80199231-80219160 |
| GNAI1 block5 | Chromosome 7 80136451-80146970 |
| GNAI2 | Chromosome 3 50220911-50267410 |
| GNAI3 block2 | Chromosome 1 109579210-109619792 |
| GNAI3 block1 | Chromosome 1 109545907-109579209 |

**Table S15**.**Number of hits in the genomic regions of human *Gα(i/o)* genes.** Only hits with *P*<0.0001 were considered. The bottom row shows the number of motifs formed by chance, based on base frequencies. To contrast the number of hits found by this approach, the number of motifs formed by chance was estimated as follows. Chromosome-specific nucleotide frequencies[11] were used to calculate the probability of formation of the consensus binding motif (GACCACCCA) by chance. There are strong functional constrains on these motifs in the GNAO1 region (250-450 x enrichments).

| **Motif** | ***GNAO1*** | ***GNAI1*** | ***GNAI2*** | ***GNAI3*** |
| --- | --- | --- | --- | --- |
| GLI consensus | 44 | 104 | 26 | 40 |
| GLI2 Jolma2013 | 34 | 124 | 18 | 46 |
| GLI2-2 Jolma2013 | 26 | 74 | 4 | 16 |
| GLI consensus by chance | 0.14 | 0.34 | 7.71x10-2 | 0.15 |

**Supplementary Figures and Figure Legends**

**Figure S1.**

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**Figure S1. Phylogenetic tree of GOA-1 and GPA-16 protein sequences from nematode proteomes, constructed by using parsimony criteria in heuristic tree search**. The two proteins represent separated but related subfamilies of Gα(i/o) proteins. The sequence of Gα(i/o) from *Drosophila melanogaster* was used as outgroup to root the tree. *Caenorhabditis elegans* is highlighted.

**Figure S2.**

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**Figure S2. Expression of a *gpa-16* exonic fragment paralogous to a *goa-1* coding region containing a potential TRA-1 binding site does not depend on TRA-1 activity.** Left panel: minimal reporter containing a short *gpa-16* coding fragment with a highly divergent TRA-1 binding site (-bs) is widely expressed in embryos (white arrows) in an otherwise wild-type genetic background. Middle panel: mutated minimal reporter in which the TRA-1 binding sequence was previously restored (bs) fails to be expressed in an otherwise wild-type background. Right panel: mutated minimal reporter with restored TRA-1 binding sequence (bs) is widely expressed in embryos depleted for TRA-1. Free embryos (outside of hermaphrodites) were tested. Small windows show the corresponding Nomarski pictures. Scale bars represent 50 µm**.**

**Figure S3.**

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**Figure S3. GOA-1 accumulation in adult males.** Expression of a translational fusion GOA-1::GFP reporter in the middle (left panel) and posterior (right panel) parts of males.

**Figure S4.**



**Figure S4. Accumulation levels of a GOA-1::GFP fusion protein and its mutated version lacking a potential TRA-1 binding site in embryos.** (**A**) Expression of *goa-1* in embryos with different genetic backgrounds. In *tra-1(-)* and *him-5(-)* mutant backgrounds the expression becomes elevated as compared to the wild-type background. FEM-3 deficiency lowers expression below levels seen in the wild type. (**B**) Expression of a mutGOA-1::GFP reporter in embryos. The mutations affect the potential TRA-1 binding site. The reporter is not responsive to any genetic background tested. In panels **A** and **B**, data are represented by Dot Plot. Each dot corresponds to the expression level of an individual embryo. Images were captured with the same exposure time. For statistics, see **Tables S2** and **S3**.

**Figure S5.**

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**Figure S5. GOA-1 accumulates at higher levels in male than in hermaphrodite embryos.** Quantitative real-time PCR analysis demonstrates that transcript levels of *goa-1* are lower in hermaphrodites than in wild-type and *him-5* males, and in *tra-1(e1099)* mutant XX animals.Embryos were collected by hypochlorite treatment. “wild-type herm.”: all embryos are hermaphrodites; “wild-type male”: approximately 50% of the embryos are males; “*him-5(e1490)*”: around 30-38% of the embryos are males; “*tra-1(e1099)*”: only a small portion (<16.6%) of the embryos are males; “*tra-1(e1488)*”: intersex animals. Bars represent ±S.D., \*: P<0.05; \*\*: P<0.01; NS denotes not significant. Mann-Whitney U-test P value. For data, see **Table S4**.

**Figure S6.**

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**Figure S6. GOA-1 accumulation is also attenuated by TRA-1 in adult animals.** (**A**) The expression of GOA-1::GFP in a hermaphrodite versus [*him-5(-)* mutant] a male adult animal (first and second panels, respectively). Quantification of relative GOA-1 accumulation in hermaphrodite and male adults (third panel). GOA-1 accumulates at higher levels in males than in hermaphrodites. (**B**) Accumulation of GOA-1 in wild-type (first panel), *tra-1(-)* mutant (second panel) and *fem-3(-)* mutant (third panel) adults. GOA-1 accumulates in virtually all neurons. Quantification of relative GOA-1 accumulation in genetic backgrounds indicated (last panel). GOA-1 protein levels are increased in TRA-1 and decreased in FEM-3 defective backgrounds, as compared with the wild type. (**C**) The binding site mutant version of the reporter (mutGOA-1::GFP) is not capable of responding to the sex of the animal. mutGOA-1::GFP accumulates at similar levels in a hermaphrodite (first panel) and a male (second panel) adult. Quantification of relative reporter expression in hermaphrodite and male adults (last panel). In the last panel of each row, bars represent ±S.E.M. \*\*: P<0.01, \*\*\*: P<0.001, independent Student’s *t*-test. Statistics are included in **Table S5**. NS: not significant.

Figure S7.



**Figure S7. Accumulation levels of a GOA-1::GFP fusion protein at larval stages.** Expression of *goa-1* at L1 (**A, A’**), L2 (**B**, **B’**) and L4 (**C**, **C’**) larval stages in different genetic backgrounds. In *tra-1(-)* and *him-5(-)* mutant backgrounds, the expression becomes elevated compared to the wild-type background at every larval stage examined. “wild-type herm.”: all larvae are hermaphrodites, “*him-5*”: around 30-38% of the larvae are males in L1 and L2 samples, whereas all larvae are male in L4 samples; “*tra-1(e1099)*”: only a small portion (<16.6%) of the larvae are males in L1 and L2 samples, whereas all larvae are male in L4 samples. In panels **A-C**, data are represented by Dot Plot. Each dot corresponds to the expression level of an individual larva. Images were captured with the same exposure time. In panels **A’-C’**, bars represent ±S.E.M. \*\*: P<0.01, \*\*\*: P<0.001, NS denotes not significant. Kruskal-Wallis H test with Dunn’s Post Hoc Test and with Bonferroni correction .For statistics, see **Tables S6.**

**Figure S8.**



**Figure S8. PKD-2 accumulation in XX hermaphrodites hyperactive for *goa-1*.** *pkd-2* (*bxIs14*) is normally expressed in males only. Left panel: XX hermaphrodite does not show PKD-2 accumulation. Right panel: XX hermaphrodite bearing a *goa-1* gain-of-function mutation (*sylS9*) ectopically accumulates PKD-2 in a head neuron (the white arrow). PKD-2 accumulates in several neurons of a male tail (the blue arrow). The penetrance of this ectopic *pkd-2* expression in *goa-1* gf mutant background is less than 1%. Exposure time: 300 msec. For data, see **Table S8.**

**Figure S9.**

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**Figure S9. GOA-1 influences the expression level of *dat-1* in the male tail.***dat-1* is expressed in dopaminergic neurons, and in the tail it can be found only in male-specific neurons.(**A**) DAT-1 accumulation in tail neurons of adult males. DAT-1 levels are lower in *goa-1* defective, but higher in *goa-1* hyperactive mutants than in the wild-type background. **(A’-A’’)** Quantification of relative DAT-1::GFP levels in the mail tail. Bars represent ±S.E.M., \*\*\*: P<0.001; NS: NS denotes not significant. Mann-Whitney U-test.For statistics, see **Table S9.**

**Figure S10.**



**Figure S10. Food leaving behaviour in *egl-30(-)* mutants is determined by food perception only.** This is a control experiment for assaying food leaving in *goa-1* and *tra-1* mutant animals (**Fig. 5A**). EGL-30 functions in food searching and acceptance of food quality. In case of hermaphrodites, food leaving can be divided into two phases, before egg-laying and during egg-laying. Young, non-gravid hermaphrodites were tested which start to lay eggs 5 hours later they were transferred to the test plate. These animals finish to leave the food source when initiating egg laying. Note that *goa-1(gf)* mutant XO males continuously leave the bacterial layer (**Fig. 5A**). For statistics, see **Table S10**.

**Figure S11.**



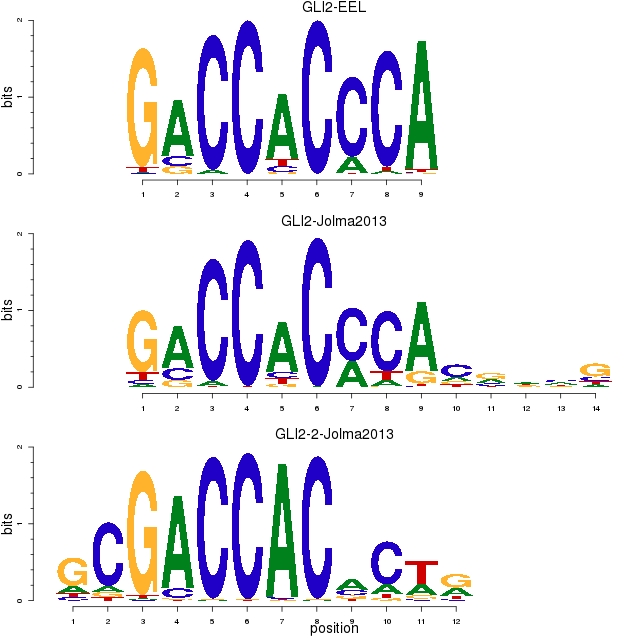
**Figure S11. Food leaving (mate searching) behaviour in XO males and *goa-1(gf)* mutant XX hermaphrodites at the L3 stage.** Before sexual maturation, animals show no sex drive; L3 larvae do not leave the bacterium layer. After 10 hours of initiating the experiment, the first adults appear, and start to leave the food source. For statistics, see **Table S10**.

**Figure S12.**

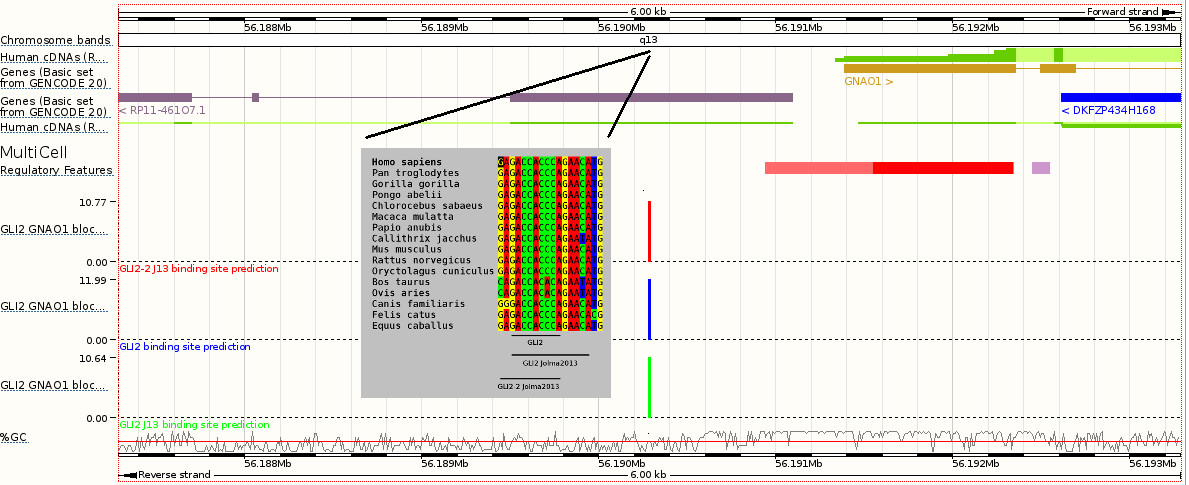
****

**Figure S12. TRA-1 and GOA-1 affect male mating behaviour and chemosensation**. (**A**) Time required for males to find hermaphrodites is assayed. (**B**) Time required for males from the first touch of a hermaphrodite to mating response is measured. For statistics, see **Table S11**. “First touch”, when a male first touches a hermaphrodite; “Response”, when a male realizes that it touches a hermaphrodite, and then tries to find the vulval opening; “Spiculum insertion”, a successful mating. (**C**) Mutational inactivation of *goa-1* hampers mating in XO males (light blue column). “pic. ins.” denotes spiculum insertion. For statistics, see **Table S11**.

**Figure S13.**



**Figure S13. The sequence logo of the three GLI-specific and position-specific weight matrixes**. Logos were generated by using the seqLogo package of Bioconductor (Yamagishi and Shimabukuro 2008).

**Figure S14.**

**Figure S14. A predicted GLI2 binding site in the genomic region of *GNAO1****.* [*GNAO1* region](http://www.ensembl.org/Homo_sapiens/Share/42303ad21b81a27e39275442e571aa69164673938115637#_blank) contains one site with very strong scores of all the three motifs (GLI2 consensus: 10.77, GLI2 Jolma2013: 11.99, GLI2-2 Jolma2013 10.64). These scores are close to the theoretical maximum scores with the motifs used. In addition, the site shows 95% identity among mammalian orthologues. The site resides 650 bp before the starting point of the annotated *GNAO1* promoter region. It is a strong candidate for being a real binding site. Red, blue and green peaks show the location of the predicted sites, using the GLI2-2 Jolma2013, GLI2 consensus and GLI2 Jolma2013 motifs, respectively. The conservation of the later site was visualized using the Seaview alignment viewer (Gouy et al. 2010).