Evolutionary Signature of Information Transfer Complexity in Cellular Membrane Proteomes

Jong-Min Kim¹, Byung-Gee Kim¹ and S. June Oh^{2*}

¹School of Chemical and Biological Engineering, College of Engineering, Seoul National University, Seoul 151-742, Korea, ²Department of Pharmacology, College of Medicine and Ubiquitous Healthcare Research Center, Inje University, Busan 614-735, Korea

Abstract

Cell membrane proteins play crucial roles in the cell's molecular interaction with its environment and within itself. They consist of membrane-bound proteins and many types of transmembrane (TM) proteins such as receptors, transporters, channel proteins, and enzymes. Membrane proteomes of cellular organisms reveal some characteristics in their global topological distribution according to their evolutionary positions, and show their own information transfer complexity. Predicted transmembrane segments (TMSs) in membrane proteomes with HMMTOP showed near power-law distribution and frequency characteristics in 6-TMS and 7-TMS proteins in prokaryotes and eukaryotes, respectively. This reaffirms the important roles of membrane receptors in cellular communication and biological evolutionary history.

Keywords: chemoreceptor, G protein-coupled receptor, information transfer complexity, membrane protein topology, seven-transmembrane region, signal transduction, transmembrane protein

Introduction

With the availability of high-throughput biological data, great efforts have been expended to find universal features of DNA sequences in untranslated region (UTR) introns (Hong *et al.*, 2006), 5'UTR (Lynch *et al.*, 2005), 3'UTR, and coding exons (Martignetti and Caselle, 2007) to show a power-law distribution since long-range correlations in noncoding DNA sequence were first observed (Li and Kaneko, 1992; Peng *et al.*, 1992; Voss, 1992). Gene family size in single genomes from archaea,

bacteria, and eukaryota (Huynen & van Nimwegen, 1998; Harrison & Gerstein, 2002) also obey power-law distributions. Such distributions have been observed in many different types of networks such as the World Wide Web (Albert *et al.*, 1999) and biological metabolic networks (Jeong *et al.*, 2000). It seems that all of these observations are found to be consistent with the power law.

The evolutionary history of an organism involves the reactions of the organism to its environments over generations. In biological systems, the composition of proteins located in the cellular membrane determines the interaction of the cell with its environment and consequently how effectively it can communicate with its neighbors and adapt itself to the circumstances. To survive a changing environment, a species adds genes that have effects on beneficial phenotypes over generations. These effects strongly depend on other genes in the genome with which they cooperate.

Membrane proteins are the most important members of inter- and intracellular transduction of materials and information. They are predicted to constitute 20-30% of all the proteins in an animal's genome sequence (Wallin & von Heijne, 1998; Krogh *et al.*, 2001) and are essential for the organism's interaction with its environment. Proteins such as channel proteins and transporters drive the transmembrane movement of ions, solutes, and small molecules. Other proteins such as receptors receive signals by interacting with a large variety of ligands, including hormones, neurotransmitters, autacoids, chemokines, odorants, even light, and transduce them to various kinds of information. Membrane proteins with seven transmembrane segments (7-TMSs) play major roles in signal transformation and information transport in cellular organisms.

A wide variety of genomic and proteomic studies have been carried out since tens of whole genome sequences were determined. In 2003, a database of experimentally characterized transmembrane topologies was constructed and reported as TMPDB; it contained a total of 302 TM protein sequences (Ikeda *et al.*, 2003). Recently, the distributions of membrane protein topologies in both *E. coli* and *S. cerevisiae* membrane proteomes were reported (Daley *et al.*, 2005; Kim *et al.*, 2006). There were a very small number of 7-TMS proteins compared with the numbers of proteins with other TMS numbers in *E. coli*, whereas a larger number of 7-TMS proteins were found in *S. cerevisiae*. Instead, *E.*

*Corresponding author: E-mail o@biophilos.org
Tel +82-51-893-3569, Fax +82-51-797-8569
Accepted 27 April 2009

coli has shown more 6-TMS proteins than 5- and 7-TMS proteins in its proteome.

Polytopic proteins consist of various characteristic structures and functions according to their trans-membrane domain. In proportion as the transfer complexity of cellular information increases, the number of membrane proteins involved in signal transduction may increase.

As a first step toward the exploration of topology distributions in the cellular membrane proteome, we now report the TMS distributions in the membrane proteomes from prokaryotic and eukaryotic organisms.

Methods

Many species genomes have been fully sequenced and annotated. In this study, 114 proteomes consisting of protein-coding genes from 46 prokaryotes (38 Eubacteria, 8 Archaea) and 72 eukaryotes were examined (Table S1 in supplementary material). Amino acid sequence data of protein-coding genes from 114 species were retrieved from public biological databases (Table S2 in supplementary material) and analyzed for the topology prediction of polytopic proteins. We used HMMTOP 2.1 (Tusnády & Simon, 1998; Tusnády & Simon, 2001) for the topology prediction of polytopic proteins. HMMTOP was based on parameters obtained by statistical analy-

sis of protein sequence databases rather than those based on hydrophobicity measures. The method was implemented with hidden Markov models and is considered to show the best performance among TM topology-prediction methods (Ikeda *et al.*, 2002; Inoue *et al.*, 2005; Tusnády & Simon, 2001). It is also suitable for the large-scale analysis of membrane protein topology.

Each protein-coding FASTA entry out of a species' whole proteome was analyzed by HMMTOP to produce an output file in the long format to count the TMS number of each entry. The HMMTOP results of protein-coding genes in each proteome were sorted and grouped into bundles according to the TMS number of each gene, using custom Python scripts. To assess the global relationship between TMS number and the corresponding protein number in the proteomes from all 114 species, we constructed a scatter plot. We also investigated the trends in the populations of TMS numbers from each species.

Results and Discussion

TMS distribution in membrane proteomes

The TMS number of each protein-coding gene was plotted logarithmically against the corresponding protein number in the proteome of each species to produce a

Fig. 1. Global TMS distribution in predicted cellular membrane proteomes. Each proteome from 114 species was plotted logarithmically with its TMS number and corresponding protein number.

global topology distribution (Fig. 1). The overall TMS distribution in the proteomes of 114 species shows a power-law pattern similar to those of the other biological features mentioned previously.

Table 1 shows the list of biological classes for which protein-coding genes were analyzed and their tendency between the numbers of 7-TMS proteins and 6-TMS proteins.

We can see that there are more 7-TMS proteins than 6-TMS proteins in the higher eukaryotes, particularly with the exception of plants. The tendency was confused among some species of fungi (represented as

Table 1. Biological classes of 114 species included in the membrane proteome analysis and their tendency between the numbers of 7-TMSPs (7-transmembrane segment proteins) and 6-TMSPs

Biological classes	No. Species	7>6*	Biological classes	No. Species	7>6
Mammalia	18	o	Metazoa	1	o
Aves	1	o	Fungi	18	+**
Fish	3	o	Plants	6	x
Amphibia	1	o	Protists	10	x
Insects	8	o	Red/green algae	3	x
Worm	1	o	Eubacteria	38	x
Deuterostome	2	o	Archaea	8	x

*7>6: number of 7-TMSPs>number of 6-TMSPs.

**'+ ' represents the mixture of 'o' and 'x'. (The list of species was provided as supplementary material).

'+' in Table 1). Prokaryotes showed a different tendency in the distribution of TMS numbers.

To find characteristics in the plot, we examined the tendencies of the TMS distribution and found that a peculiar pattern of 7-TMS proteins appeared in the topological distribution of eukaryotic membrane proteomes. In Fig. 2, the numbers of proteins corresponding to each TMS number decreased rapidly until the TMS number reached six, and gave an asymptotic pattern to zero after eight, except at seven. In particular, in multicellular eukaryotic organisms, the number of 7-TMS proteins is much higher than that of 6-TMS proteins. Seven-TMS proteins in eukaryotic organisms consist mainly of membrane receptors functioning as key members in cellular signal transduction.

The ratio of 7-TMS protein number to that of whole proteins in an organism reveals a cross section of cellular information transfer complexity. To approximate information transfer complexity in an organism, we set an arbitrary number, namely the seven (zayin)-TMSP part score (zps), and it was calculated as:

$$zps = N_{(7-TMSP)} / N_{(TPG)}$$

where $N_{(7-TMSP)}$ is the number of 7-TMS proteins and $N_{(TPG)}$ is the total number of protein-coding genes in an organism. Similarly, we can also compute another arbitrary number, namely the six (vav)-TMSP part score (vps), which was calculated as:

$$vps = N_{(6-TMSP)} / N_{(TPG)}$$

where $N_{(6-TMSP)}$ is the number of 6-TMS proteins and

Fig. 2. TMS distribution in eukaryotic membrane proteomes from 35 species (Mammalia to Metazoa in Table 1). The numbers of 7-TMS proteins are indicated by red arrows. The small graph in dark blue shows the distribution pattern for the average value of each TMS number.

Table 2. *zps*s and *rzvs* from selected species

Species	<i>zps</i> *	<i>rzv</i> **	Species	<i>zps</i>	<i>rzv</i>
<i>E. caballus</i> (horse)	0.072	5.290	<i>C. intestinalis</i> (vase tunicate)	0.020	1.804
<i>B. taurus</i> (cow)	0.064	4.774	<i>D. pulex</i> (water flea)	0.009	1.555
<i>R. norvegicus</i> (rat)	0.062	5.201	<i>P. trichocarpa</i> (poplar tree)	0.007	0.728
<i>C. elegans</i> (nematode)	0.061	4.214	<i>V. carteri</i> (Volvox, green alga)	0.007	0.758
<i>X. tropicalis</i> (Xenopus)	0.059	3.724	<i>O. sativa</i> (rice)	0.007	0.763
<i>M. musculus</i> (mouse)	0.055	5.168	<i>G. max</i> (soybean)	0.006	0.780
<i>D. rerio</i> (zebrafish)	0.035	2.994	<i>R. communis</i> (castor oil plant)	0.006	0.747
<i>H. sapiens</i> (human)	0.027	3.257	<i>M. truncatula</i> (medick)	0.004	0.873
<i>F. catus</i> (cat)	0.024	1.695	<i>S. cerevisiae</i> (budding yeast)	0.008	0.930
<i>G. gallus</i> (chicken)	0.024	1.802	<i>S. pombe</i> (ssion yeast)	0.009	1.158
<i>A. gambiae</i> (mosquito)	0.020	1.665	<i>E. coli</i> (prokaryote)	0.008	0.388
<i>D. melanogaster</i> (fruit fly)	0.018	1.545	<i>X. campestris</i> (prokaryote)	0.005	0.273

zps*: 7 (zayin)-TMS part score, *rzv*: ratio of $z(\text{ayin})ps/v(\text{av})ps$.

$N_{(TPG)}$ is the total number of protein-coding genes in an organism. We then calculated the ratio of *zps* to *vps* (*rzv*) as follows:

$$rzv = zps/vps.$$

rzv values for motile multicellular organisms are all greater than 1.5 and most *zps*s of eukaryotes are higher than 0.010, except for those of plants and fungi (Table 2). In all six tested plants, *zps* values were less than 0.007. This may have resulted from the difference of connective systems between plants and animals.

In the taxonomic tree of 114 species, all 35 species in the branch of Bilateria in Metazoa group were shown to have more 7-TMS proteins than 6-TMS ones as shown in Fig. 2. Ten species among Fungi group except *Saccharomycetaceae* also showed *rzv* values just over 1.0 (Fig. 3). Out of consideration for the taxonomic position of the clade in the lineage, fungi may be regarded as on the border between highly communicative organisms and less complex ones.

Functional evolution of sensory receptors

Most physicochemical sensory receptors such as neurotransmitter receptors (Catapano & Manji, 2007), photoreceptors (rhodopsin) (Hargrave *et al.*, 1983), olfactory receptors (Buck & Axel, 1991), gustatory receptors (Abe *et al.*, 1993), vomeronasal receptors (Dulac & Axel, 1995), and even mechanoreceptors (Makino *et al.*, 2006) are 7-TMS proteins coupled with G protein for their molecular functions. Receptors with other signal-transducing mechanisms, including PDZ or EVH domain-containing partner, are also considered to be 7-TMS proteins (Bockaert & Pin, 1999). Recently, the crystal structure of rhodopsin has been determined to show a seven-helix transmembrane structure (Palczewski *et al.*,

2000). On the other hand, the three-dimensional structures of membrane (cation) channels, such as the voltage-sensitive sodium channel, IP2R1, and canonical transient receptor potential-3 (TRPC3), have been also reconstructed by single-particle analysis from electron microscopic images and determined to have six transmembrane segments (Mio *et al.*, 2008). Six-TMS proteins in prokaryotic membrane proteomes showed a characteristic pattern in membrane topology distribution (Fig. 4).

To scrutinize the functional distribution of animal membrane proteome GO functions in 7-TMS and 6-TMS proteins from *C. familiaris*, membrane proteome was examined with gene ontology using BLAST2GO (Conesa *et al.*, 2005). Among the 6-TMS protein sequences annotated by the GO biological process, the proportion of protein members participating in molecular transportation was larger than 17%, whereas there were about 1.8% in annotated 7-TMS protein sequences. The proteins involved in sensory perception, in contrast, constituted most of the annotated 7-TMS proteins (Figs. S1-S2 in supplementary material).

The superfamily of 7-TMS proteins is one of the largest such families in invertebrates and vertebrates including mammals (Bockaert & Pin, 1999), and includes various receptors that recognize a great diversity of ligands, as mentioned above.

In vertebrates, the 7-TMS receptor gene family consists of hundreds of members, over 1% of the genome. For the olfactory receptor (OR), as an example of an in-bound signal mediator, insect OR genes have been reported in fruit flies (Robertson *et al.*, 2003), mosquitoes (Hill *et al.*, 2002), and honey bees (Robertson and Wanner, 2006). Among the 62 *Drosophila* OR genes, atypical receptor OR83b is known to be highly conserved in amino acid sequence among different orders of insects (Krieger *et al.*, 2003), and is coexpressed with

Fig. 3. Conventional taxonomic tree of 114 species according to the NCBI taxonomy. Groups of *rv* values greater than 1.0 were enclosed with boxes (A) and (B). (A) Bilateria in metazoa group. (B) Part of fungi group (excluding Saccharomycetaceae).

another OR gene in most olfactory receptor neurons (Larsson *et al.*, 2004; Benton *et al.*, 2006). It was thought to be conserved functionally across 250 million years of evolution in insects (Jones *et al.*, 2005). However, chemosensory receptors such as olfactory and gustatory receptors in insects are not topologically close to GPCRs and are possibly more closely related to 6-TMS ion channels. They are known to possess inverted orientation to those of vertebrates and have the function of ligand-activated or cyclic-nucleotide-activated

nonselective cation channels (Bargmann, 2006; Sato *et al.*, 2008; Wicher *et al.*, 2008). This could be a case of transient phenomenon in the differentiation and development of membrane proteins that have moved their main surmounting target from molecular barrier to information transmission. With the OR gene repertoire, we can see that motile multicellular organisms have evolved in accordance with their ecological circumstances (Table 3).

One possible explanation of this phenomenon is a combination of positive selection and purifying selection

Fig. 4. TMS distribution in prokaryotic membrane proteomes. The numbers of 6-TMS proteins are indicated by green arrows. The small graph in dark blue shows the distribution pattern for the average values of the TMS numbers.

Table 3. Number of functional olfactory receptors in model organisms

Species	No. of OR (func/total)*	References
<i>D. melanogaster</i>	62	Robertson <i>et al.</i> , (2003)
<i>A. gambiae</i>	79	Hill <i>et al.</i> , (2002)
<i>T. rubripes</i>	44/57	Alioto and Ngai, (2005)
<i>D. rerio</i>	143/160	Alioto and Ngai, (2005)
<i>G. gallus</i>	141/229	Lagerström <i>et al.</i> , (2006)
<i>C. familiaris</i>	872/1,094	Quignon <i>et al.</i> , (2005)
<i>M. musculus</i>	1,037/1,391	Niimura and Nei, (2005)
<i>R. norvegicus</i>	1,201/1,493	Quignon <i>et al.</i> , (2005)
<i>M. mulatta</i>	309/606	Go and Niimura, (2008)
<i>H. sapiens</i>	388/802	Niimura and Nei, (2005)

*Total means the sum of functional gene number and pseudogene number.

of membrane-signaling proteins. As organisms evolve, cellular information transfer becomes more complex and crucial than material transportation. In other words, as the signal-transducing system of an organism becomes more complex, the organism demands more participants such as various membrane receptors to operate its cellular signaling system to increase the organismal complexity. Simultaneously, when another function in an organism such as visionary ability improves, its olfactory ability retrogresses. An interesting fact is that almost all protein receptors participating and acting mainly in sensory systems are 7-TMS receptor proteins.

Acknowledgments

This work was supported by the 2006 Inje University research grant.

Supplementary material

Supplementary documents are attached to the manuscript.

References

- Abe, K., Kusakabe, Y., Tanemura, K., Emori, Y., and Arai, S. (1993). Primary structure and cell-type specific expression of a gustatory G protein-coupled receptor related to olfactory receptors. *J. Biol. Chem.* 268, 12033-12039.
- Albert, R., Jeong, H., and Barabasi, A.-L. (1999). Diameter of the world-wide web. *Nature* 401, 130-131.
- Bargmann, C. I. (2006). Comparative chemosensation from receptors to ecology. *Nature* 444, 295-301.
- Benton, R., Sachse, S., Michnick, S. W., and Vosshall, L. B. (2006). Atypical membrane topology and heteromeric function of Drosophila odorant receptors *in vivo*. *PLoS Biol.* 4, e20.
- Bockaert, J., and Pin, J. P. (1999). Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J.* 18, 1723-1729.
- Buck, L., and Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65, 175-187.
- Catapano, L. A., and Manji, H. K. (2007). G protein-coupled receptors in major psychiatric disorders. *Biochim. Biophys. Acta*, 1768, 976-993.

- Conesa, A., Gtz, S., Garca-Gmez, J. M., Terol, J., Taln, M., and Robles, M. (2005). Blast2go: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21, 3674-3676.
- Daley, D. O., Rapp, M., Granseth, E., Meln, K., Drew, D., and von Heijne, G. (2005). Global topology analysis of the *Escherichia coli* inner membrane proteome. *Science* 308, 1321-1323.
- Dulac, C., and Axel, R. (1995). A novel family of genes encoding putative pheromone receptors in mammals. *Cell* 83, 195-206.
- Hargrave, P. A., McDowell, J. H., Curtis, D. R., Wang, J. K., Juszczak, E., Fong, S. L., Rao, J. K., and Argos, P. (1983). The structure of bovine rhodopsin. *Biophys Struct Mech* 9, 235-244.
- Harrison, P. M., and Gerstein, M. (2002). Studying genomes through the aeons: protein families, pseudogenes and proteome evolution. *J. Mol. Biol.* 318, 155-1174.
- Hill, C. A., Fox, A. N., Pitts, R. J., Kent, L. B., Tan, P. L., Chrystal, M. A., Cravchik, A., Collins, F. H., Robertson, H. M., and Zwiebel, L. J. (2002). G protein-coupled receptors in *Anopheles gambiae*. *Science* 298, 176-178.
- Hong, X., Scofield, D. G., and Lynch, M. (2006). Intron size, abundance, and distribution within untranslated regions of genes. *Mol. Biol. Evol.* 23, 2392-2404.
- Huynen, M. A., and van Nimwegen, E. (1998). The frequency distribution of gene family sizes in complete genomes. *Mol. Biol. Evol.* 15, 583-589.
- Ikeda, M., Arai, M., Lao, D. M., and Shimizu, T. (2002). Transmembrane topology prediction methods: a re-assessment and improvement by a consensus method using a dataset of experimentally-characterized transmembrane topologies. *In Silico Biol.* 2, 19-33.
- Ikeda, M., Arai, M., Okuno, T., and Shimizu, T. (2003). Tmpdb: a database of experimentally-characterized transmembrane topologies. *Nucleic Acids Res.* 31, 406-409.
- Inoue, Y., Yamazaki, Y., and Shimizu, T. (2005). How accurately can we discriminate G protein-coupled receptors as 7-TMS TM protein sequences from other sequences? *Biochem. Biophys. Res. Commun.* 338, 1542-1546.
- Jeong, H., Tombor, B., Albert, R., Oltvai, Z. N., and Barabási, A. L. (2000). The large-scale organization of metabolic networks. *Nature* 407, 651-654.
- Jones, W. D., Nguyen, T.-A. T., Kloss, B., Lee, K. J., and Vossell, L. B. (2005). Functional conservation of an insect odorant receptor gene across 250 million years of evolution. *Curr. Biol.* 15, R119-R121.
- Kim, H., Meln, K., Osterberg, M., and von Heijne, G. (2006). A global topology map of the *Saccharomyces cerevisiae* membrane proteome. *Proc. Natl. Acad. Sci. U.S.A.* 103, 11142-11147.
- Krieger, J., Klink, O., Mohl, C., Raming, K., and Breer, H. (2003). A candidate olfactory receptor subtype highly conserved across different insect orders. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* 189, 519-526.
- Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E. L. (2001). Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J. Mol. Biol.* 305, 567-580.
- Larsson, M. C., Domingos, A. I., Jones, W. D., Chiappe, M. E., Amrein, H., and Vossell, L. B. (2004). Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron* 43, 703-714.
- Li, W., and Kaneko, K. (1992). Long-range correlation and partial spectrum in a noncoding DNA sequence. *Europhys. Lett.* 17, 655-660.
- Lynch, M., Scofield, D. G., and Hong, X. (2005). The evolution of transcription-initiation sites. *Mol. Biol. Evol.* 22, 1137-1146.
- Makino, A., Prossnitz, E. R., Bnemann, M., Wang, J. M., Yao, W., and Schmid-Schnbein, G. W. (2006). G protein-coupled receptors serve as mechanosensors for fluid shear stress in neutrophils. *Am J. Physiol. Cell. Physiol.* 290, C1633-C1639.
- Martignetti, L., and Caselle, M. (2007). Universal power law behaviors in genomic sequences and evolutionary models. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* 76, 021902.
- Mio, K., Ogura, T., and Sato, C. (2008). Structure of six-transmembrane cation channels revealed by single-particle analysis from electron microscopic images. *J. Synchrotron Radiat.* 15, 211-214.
- Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Trong, I. L., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., and Miyano, M. (2000). Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* 289, 739-745.
- Peng, C. K., Buldyrev, S. V., Goldberger, A. L., Havlin, S., Sciortino, F., Simons, M., and Stanley, H. E. (1992). Long-range correlations in nucleotide sequences. *Nature* 356, 168-170.
- Robertson, H. M., and Wanner, K. W. (2006). The chemoreceptor superfamily in the honey bee, *Apis mellifera*: expansion of the odorant, but not gustatory, receptor family. *Genome Res.* 16, 1395-1403.
- Robertson, H. M., Warr, C. G., and Carlson, J. R. (2003). Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 100(Suppl 2), 14537-14542.
- Sato, K., Pellegrino, M., Nakagawa, T., Nakagawa, T., Vossell, L. B., and Touhara, K. (2008). Insect olfactory receptors are heteromeric ligand-gated ion channels. *Nature* 452, 1002-1006.
- Tusnády, G. E., and Simon, I. (1998). Principles governing amino acid composition of integral membrane proteins: application to topology prediction. *J. Mol. Biol.* 283, 489-506.
- Tusnády, G. E., and Simon, I. (2001). The HMMTOP transmembrane topology prediction server. *Bioinformatics* 17, 849-850.
- Tusnády, G. E., and Simon, I. (2001). Topology of membrane proteins. *J. Chem. Inf. Comput. Sci.* 41, 364-368.
- Voss, R. F. (1992). Evolution of long-range fractal correlations and 1/f noise in DNA base sequences. *Phys. Rev. Lett.* 68, 3805-3808.
- Wallin, E., and von Heijne, G. (1998). Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms. *Protein Sci.* 7, 1029-1038.
- Wicher, D., Schfer, R., Bauernfeind, R., Stensmyr, M. C., Heller, R., Heinemann, S. H., and Hansson, B. S. (2008). *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. *Nature* 452, 1007-1011.

Table S1. List of selected species

Species	Abbr.	Database	Domain	Species	Abbr.	Database	Domain
<i>Acyrtosiphon pisum</i>	Acy.pisu	NCBI	Euk.	<i>Takifugu rubripes</i>	Tak.rubr	Ensembl	Euk.
<i>Aedes aegypti</i>	Aed.aegy	Ensembl	Euk.	<i>Tetraodon nigroviridis</i>	Tet.nigr	Ensembl	Euk.
<i>Anopheles gambiae</i>	Ano.gamb	Ensembl	Euk.	<i>Tetrahymena thermophila</i>	Tet.ther	JCVI	Euk.
<i>Apis mellifera</i>	Api.mell	NCBI	Euk.	<i>Thalassiosira pseudonana</i>	Tha.pseu	JGI	Euk.
<i>Arabidopsis thaliana</i>	Ara.thal	NCBI	Euk.	<i>Theileria parva</i>	parv	NCBI	Euk.
<i>Aspergillus nidulans</i>	Asp.nidu	NCBI	Euk.	<i>Tribolium castaneum</i>	Tri.cast	NCBI	Euk.
<i>Bombyx mori</i>	Bom.mori	SilkDB	Euk.	<i>Trichoderma virens</i>	Tri.vire	JGI	Euk.
<i>Bos taurus</i>	Bos.taur	NCBI	Euk.	<i>Trypanosoma brucei</i>	Try.bruc	NCBI	Euk.
<i>Caenorhabditis elegans</i>	Cae.eleg	Ensembl	Euk.	<i>Ustilago maydis</i>	Ust.mayd	NCBI	Euk.
<i>Canis familiaris</i>	Can.fami	Ensembl	Euk.	<i>Volvox carteri</i>	Vol.cart	JGI	Euk.
<i>Cavia porcellus</i>	Cav.porc	Ensembl	Euk.	<i>Xenopus tropicalis</i>	Xen.trop	Ensembl	Euk.
<i>Chlamydomonas reinhardtii</i>	Chl.rein	JGI	Euk.	<i>Agrobacterium tumefaciens</i>	Agr.tume	NCBI	Prok.
<i>Ciona intestinalis</i>	Cio.inte	NCBI	Euk.	<i>Anabaena variabilis</i>	Ana.vari	NCBI	Prok.
<i>Cryptococcus neoformans</i>	Cry.neof	NCBI	Euk.	<i>Aquifex aeolicus</i>	Aqu.aeol	NCBI	Prok.
<i>Cryptosporidium parvum</i>	Cry.parv	NCBI	Euk.	<i>Bacillus subtilis</i>	Bac.subt	NCBI	Prok.
<i>Cyanidioschyzon merolae</i>	Cya.mero	U-Tokyo	Euk.	<i>Brucella melitensis</i>	Bru.meli	NCBI	Prok.
<i>Danio rerio</i>	Dan.reri	NCBI	Euk.	<i>Campylobacter jejuni</i>	Cam.jezu	NCBI	Prok.
<i>Daphnia pulex</i>	Dap.pule	JGI	Euk.	<i>Caulobacter crescentus</i>	Cau.cres	NCBI	Prok.
<i>Dasyatis novemcinctus</i>	Das.nove	Ensembl	Euk.	<i>Chlamydia muridarum</i>	Chl.muri	NCBI	Prok.
<i>Debaryomyces hansenii</i>	Deb.hans	NCBI	Euk.	<i>Chlamydomonas pneumoniae</i>	Chl.pneu	NCBI	Prok.
<i>Dictyostelium discoideum</i>	Dic.dics	NCBI	Euk.	<i>Clostridium perfringens</i>	Clo.perf	NCBI	Prok.
<i>Drosophila melanogaster</i>	Dro.mela	Ensembl	Euk.	<i>Deinococcus radiodurans</i>	Dei.radi	NCBI	Prok.
<i>Encephalitozoon cuniculi</i>	Enc.cuni	NCBI	Euk.	<i>Escherichia coli</i>	Esc.coli	NCBI	Prok.
<i>Equus caballus</i>	Equ.caba	NCBI	Euk.	<i>Fusobacterium nucleatum</i>	Fus.nucl	NCBI	Prok.
<i>Erinaceus europaeus</i>	Eri.euro	Ensembl	Euk.	<i>Haemophilus influenzae</i>	Hae.infl	NCBI	Prok.
<i>Felis catus</i>	Fel.catu	Ensembl	Euk.	<i>Halobacterium salinarum</i>	Hal.sali	NCBI	Prok.
<i>Gallus gallus</i>	Gal.gall	NCBI	Euk.	<i>Helicobacter pylori</i>	Hel.pylo	NCBI	Prok.
<i>Giardia lamblia</i>	Gia.lamb	GiardiaDB	Euk.	<i>Lactococcus lactis</i>	Lac.lact	NCBI	Prok.
<i>Gibberella zeae</i>	Gib.zeae	NCBI	Euk.	<i>Listeria monocytogenes</i>	Lis.mono	NCBI	Prok.
<i>Glycine max</i>	Gly.max	JGI	Euk.	<i>Mesorhizobium loti</i>	Mes.loti	NCBI	Prok.
<i>Homo sapiens</i>	Hom.sapi	NCBI	Euk.	<i>Methanosarcina acetivorans</i>	Met.acet	NCBI	Prok.
<i>Laccaria bicolor</i>	Lac.bico	JGI	Euk.	<i>Methanococcus jannaschii</i>	Met.jann	NCBI	Prok.
<i>Leishmania infantum</i>	Lei.infa	NCBI	Euk.	<i>Methanothermobacter</i>	Met.ther	NCBI	Prok.
				<i>thermautotrophicus</i>			
<i>Loxodonta africana</i>	Lox.afri	Ensembl	Euk.	<i>Mycobacterium leprae</i>	Myc.lepr	NCBI	Prok.
<i>Macaca mulatta</i>	Mac.mula	Ensembl	Euk.	<i>Mycobacterium tuberculosis</i>	Myc.tube	NCBI	Prok.
<i>Medicago truncatula</i>	Med.trun	Medicago.org	Euk.	<i>Neisseria meningitidis</i>	Nei.meni	NCBI	Prok.
<i>Monodelphis domestica</i>	Mon.dome	NCBI	Euk.	<i>Oceanobacillus iheyensis</i>	Oce.ihey	NCBI	Prok.
<i>Mus musculus</i>	Mus.musc	Ensembl	Euk.	<i>Pasteurella multocida</i>	Pas.mult	NCBI	Prok.
<i>Myotis lucifugus</i>	Myo.luci	NCBI	Euk.	<i>Pseudomonas aeruginosa</i>	Pse.aeru	NCBI	Prok.
<i>Nasonia vitripennis</i>	Nas.vitr	NCBI	Euk.	<i>Pyrococcus abyssi</i>	Pyr.abys	NCBI	Prok.
<i>Neurospora crassa</i>	Neu.cras	NCBI	Euk.	<i>Pyrobaculum aerophilum</i>	Pyr.aero	NCBI	Prok.
<i>Ornithorhynchus anatinus</i>	Orn.anat	NCBI	Euk.	<i>Ralstonia solanacearum</i>	Ral.sola	NCBI	Prok.
<i>Oryctolagus cuniculus</i>	Ory.cuni	Ensembl	Euk.	<i>Serratia proteamaculans</i>	Ser.prot	NCBI	Prok.
<i>Oryza sativa</i>	Ory.sati	NCBI	Euk.	<i>Sinorhizobium meliloti</i>	Sin.meli	NCBI	Prok.
<i>Pan troglodytes</i>	Pan.trog	Ensembl	Euk.	<i>Staphylococcus aureus</i>	Sta.aure	NCBI	Prok.
<i>Paramecium tetraurelia</i>	Par.tetr	ParameciumDB	Euk.	<i>Streptococcus pyogenes</i>	Str.pyog	NCBI	Prok.
<i>Phanerochaete chrysosporium</i>	Pha.chry	JGI	Euk.	<i>Streptomyces coelicolor</i>	Str.coel	NCBI	Prok.
<i>Phytophthora ramorum</i>	Phy.ramo	JGI	Euk.	<i>Sulfolobus solfataricus</i>	Sul.solf	NCBI	Prok.
<i>Pichia stipitis</i>	Pic.stip	NCBI	Euk.	<i>Thermoplasma acidophilum</i>	The.acid	NCBI	Prok.
<i>Plasmodium falciparum</i>	Pla.falc	NCBI	Euk.	<i>Thermosynechococcus elongatus</i>	The.elon	NCBI	Prok.
<i>Populus trichocarpa</i>	Pop.tric	JGI	Euk.	<i>Thermotoga maritima</i>	The.mari	NCBI	Prok.
<i>Rattus norvegicus</i>	Rat.norv	Ensembl	Euk.	<i>Thermoanaerobacter tengcongensis</i>	The.teng	NCBI	Prok.
<i>Ricinus communis</i>	Ric.comm	JCVI	Euk.	<i>Treponema pallidum</i>	Tre.pall	NCBI	Prok.
<i>Saccharomyces cerevisiae</i>	Sac.cere	Ensembl	Euk.	<i>Vibrio cholerae</i>	Vib.chol	NCBI	Prok.
<i>Schizosaccharomyces pombe</i>	Sch.pomb	NCBI	Euk.	<i>Xanthomonas campestris</i>	Xan.camp	NCBI	Prok.
<i>Strongylocentrotus purpuratus</i>	Str.purp	NCBI	Euk.	<i>Xylella fastidiosa</i>	Xyl.fast	NCBI	Prok.
<i>Sus scrofa</i>	Sus.scro	NCBI	Euk.	<i>Yersinia pestis</i>	Yer.pest	NCBI	Prok.

Table S2. List of selected public biological databases

DB name	URL	remark
Ensembl	ftp://ftp.ensembl.org/	Ensembl 51 ^[1]
GiardiaDB	http://giardiadb.org/	CRITICA ^[2] and GLIMMER ^[3]
JCVI	ftp://ftp.jcvi.org/	GLIMMER, AAT ^[4] , and PASA ^[5]
JGI	ftp://ftp.jgi-psf.org/	DOE Joint Genome Institute
Medicago.org	http://www.medicago.org/	<i>M. truncatula</i> , IMGAG*
NCBI	ftp://ftp.ncbi.nih.gov/	including GNOMON ^[6]
ParameciumDB	http://paramecium.cgm.cnrs-gif.fr/	<i>P. tetraurelia</i> , Genoscope ^[7]
SilkDB	ftp://silkworm.swu.edu.cn/	<i>B. mori</i> , BGI Gene Finder ^[8]
U-Tokyo	http://merolae.biol.s.u-tokyo.ac.jp/	<i>C. merolae</i>

*The International Medicago Genome Annotation Group

- [1] P. Flicek, B. L. Aken, K. Beal, B. Ballester, M. Caccamo, Y. Chen, L. Clarke, G. Coates, F. Cunningham, T. Cutts, T. Down, S. C. Dyer, T. Eyre, S. Fitzgerald, J. Fernandez-Banet, S. Grf, S. Haider, M. Hammond, R. Holland, K. L. Howe, K. Howe, N. Johnson, A. Jenkinson, A. Khri, D. Keefe, F. Kokocinski, E. Kulesha, D. Lawson, I. Longden, K. Megy, P. Meidl, B. Overduin, A. Parker, B. Pritchard, A. Prlic, S. Rice, D. Rios, M. Schuster, I. Sealy, G. Slater, D. Smedley, G. Spudich, S. Trevanion, A. J. Vilella, J. Vogel, S. White, M. Wood, E. Birney, T. Cox, V. Curwen, R. Durbin, X. M. Fernandez-Suarez, J. Herrero, T. J. P. Hubbard, A. Kasprzyk, G. Proctor, J. Smith, A. Ureta-Vidal, S. Searle, Ensembl 2008., Nucleic Acids Res 36 (Database issue) (2008) D707-D714.
- [2] J. H. Badger, G. J. Olsen, Critica: coding region identification tool invoking comparative analysis., Mol Biol Evol 16 (4) (1999) 512-524.
- [3] A. L. Delcher, D. Harmon, S. Kasif, O. White, S. L. Salzberg, Improved microbial gene identification with glimmer., Nucleic Acids Res 27 (23) (1999) 4636-4641.
- [4] X. Huang, M. D. Adams, H. Zhou, A. R. Kerlavage, A tool for analyzing and annotating genomic sequences., Genomics 46 (1) (1997) 37-45.
- [5] B. Haas, S. Salzberg, W. Zhu, M. Pertea, J. Allen, J. Orvis, O. White, C. R. Buell, J. Wortman, Automated eukaryotic gene structure annotation using evidencemodeler and the program to assemble spliced alignments., Genome Biol 9 (1) (2008) R7.
- [6] A. Nagy, H. Hegyi, K. Farkas, H. Tordai, E. Kozma, L. Bnyai, L. Patthy, Identification and correction of abnormal, incomplete and mispredicted proteins in public databases., BMC Bioinformatics 9 (2008) 353.
- [7] J.-M. Aury, O. Jaillon, L. Duret, B. Noel, C. Jubin, B. M. Porcel, B. Sgurens, V. Daubin, V. Anthouard, N. Aiach, O. Arnaiz, A. Billaut, J. Beisson, I. Blanc, K. Bouhouche, F. Cmara, S. Dharcourt, R. Guigo, D. Gogendeau, M. Katinka, A.-M. Keller, R. Kissmehl, C. Klotz, F. Koll, A. L. Moul, G. Lepre, S. Malinsky, M. Nowacki, J. K. Nowak, H. Plattner, J. Poulain, F. Ruiz, V. Serrano, M. Zagulski, P. Dessen, M. Btermier, J. Weissenbach, C. Scarpelli, V. Schchter, L. Sperling, E. Meyer, J. Cohen, P. Wincker, Global trends of whole-genome duplications revealed by the ciliate paramecium tetraurelia., Nature 444 (7116) (2006) 171-178.
- [8] Q. Xia, Z. Zhou, C. Lu, D. Cheng, F. Dai, B. Li, P. Zhao, X. Zha, T. Cheng, C. Chai, G. Pan, J. Xu, C. Liu, Y. Lin, J. Qian, Y. Hou, Z. Wu, G. Li, M. Pan, C. Li, Y. Shen, X. Lan, L. Yuan, T. Li, H. Xu, G. Yang, Y. Wan, Y. Zhu, M. Yu, W. Shen, D. Wu, Z. Xiang, J. Yu, J. Wang, R. Li, J. Shi, H. Li, G. Li, J. Su, X. Wang, G. Li, Z. Zhang, Q. Wu, J. Li, Q. Zhang, N. Wei, J. Xu, H. Sun, L. Dong, D. Liu, S. Zhao, X. Zhao, Q. Meng, F. Lan, X. Huang, Y. Li, L. Fang, C. Li, D. Li, Y. Sun, Z. Zhang, Z. Yang, Y. Huang, Y. Xi, Q. Qi, D. He, H. Huang, X. Zhang, Z. Wang, W. Li, Y. Cao, Y. Yu, H. Yu, J. Li, J. Ye, H. Chen, Y. Zhou, B. Liu, J. Wang, J. Ye, H. Ji, S. Li, P. Ni, J. Zhang, Y. Zhang, H. Zheng, B. Mao, W. Wang, C. Ye, S. Li, J. Wang, G. K.-S. Wong, H. Yang, B. A. Group, A draft sequence for the genome of the domesticated silkworm (bombyx mori), Science 306 (5703) (2004) 1937-1940.

Fig. S1. Functional distributions of (A) 6-TMS and (B) 7-TMS proteins in *C. familiaris* produced by BLAST2GO molecular function at GO level 5.

(A) Sequence distribution: biological_process (Filtered by #Seqs: cutoff=5.0)

(B) Sequence distribution: biological_process (Filtered by #Seqs: cutoff=5.0)

Fig. S2. Sequence distributions of (A) 6-TMS and (B) 7-TMS proteins in *C. familiaris* produced by BLAST2GO biological process.