by supplying secreted FeLIX (Fig. 3B). Thus, FeLIX may play the more important role in restricting cell specificity of FeLV-T in the infected cat. In this regard, this molecule resembles CD4, which is expressed at highest levels in T lymphocytes and determines the tropism of human immunodeficiency virus–type 1. CD4 may also facilitate infection both as a cell surface molecule and as a soluble protein (*17*). Because FeLIX is shed from the cell and can function as a soluble protein, there may be greater breadth in the cell specificity of T cell–tropic FeLVs in the host than would be predicted by in vitro culture. Indeed, this property of FeLIX may act to augment the severe lymphoid depletion observed in cats infected with FeLV-T (*18*).

Our studies show that FeLV-T has evolved to recognize the same receptor, Pit1, that is used by FeLV-B. This finding may suggest that cells expressing Pit1 are particularly attractive targets during the persistent stages of infection but are not primary cell targets during transmission. Our data also suggest that FeLV-T can infect cells in the cat that have already been infected with FeLV-B, which may allow FeLV-T to maximize its potential cell targets during chronic stages of infection.

Endogenous retrovirus-like sequences are found in many mammals, including mice, cats, and primates (*19*). The origin and function of endogenous proviral sequences have long been a topic of speculation. The studies described here add another layer of intrigue to the mystery of these cellular viral remnants and provide the first example of an infectious, pathogenic retrovirus that has usurped a related endogenous protein as a host cell factor for viral infection. In this manner, T cell–tropic FeLV appears to use a novel strategy to increase its selective advantage in the host.

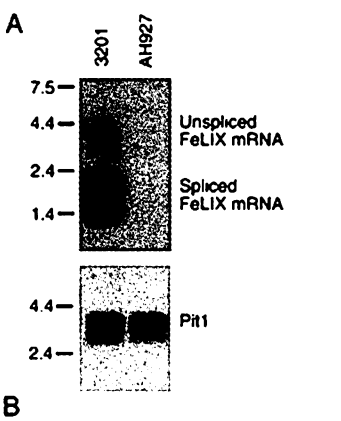

Fig. 3. Expression of FeLIX and Pit1 in feline cells in relation to infectivity. (**A**) Northern blot analyses of feline T (3201) and fibroblast (AH927) cell lines for expression of FeLIX and Pit1. Upper panel: 10 μg of total RNA was loaded in each lane; bands corresponding to the predicted unspliced and spliced FeLIX mRNA are shown. Lower panel: The first blot was stripped and probed with a fragment from feline Pit1. (**B**) Feline AH927 cells were infected in the presence of a 1:2 dilution of the indicated conditioned supernatants, as described in Table 2.

References and Notes

1. J. L. Rohn, M. S. Moser, S. R. Gwynn, D. N. Baldwin, J. Overbaugh, *J. Virol.* **72**, 2686 (1998).
2. P. R. Donahue *et al.*, *J. Virol.* **65**, 4461 (1991).
3. M. Moser, C. Burns, S. Boomer, J. Overbaugh, *Virology* **242**, 366 (1998).
4. M. A. Sommerfelt and R. A. Weiss, *Virology* **176**, 58 (1990).
5. P. S. Sarma and T. Log, *Virology* **44**, 352 (1971).
6. J. L. Rohn and J. Overbaugh, in *Persistent Viral Infections*, I. S. Y. Chen and R. Ahmed, Eds. (Wiley, Sussex, UK, 1999), pp. 379–408.
7. J. Overbaugh, N. Riedel, E. A. Hoover, J. I. Mullins, *Nature* **332**, 731 (1988).
8. Y. Takeuchi *et al.*, *J. Virol.* **66**, 1219 (1992); R. A. Weiss and C. S. Tailor, *Cell* **82**, 531 (1995).
9. T. Kitamura *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9146 (1995).
10. H. K. Deng, D. Unutmaz, V. N. KewalRamani, D. R. Littman, *Nature* **388**, 296 (1997); J. E. J. Rasko, J.-L. Battini, R. J. Gottschalk, I. Mazo, A. D. Miller, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 2129 (1999); C. S. Tailor, A. Nouri, C. G. Lee, C. Kozak, D. Kabat, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 927 (1999).
11. The feline cDNA library was generated using the method and the vector provided by Kitamura (*9*). Viral particles containing the library were generated by cotransfecting 293T cells with the library DNA plus plasmids encoding a packaging-defective FeLV-gag-pol and amphotropic MuLV envelope. Cell-free virus was used to infect D17 cells; after 2 days, cells were challenged with FeLV-ΔΨ EECC virus. FeLV-ΔΨ EECC was engineered to be defective in packaging its own genome but competent to package and transfer a variety of retroviral vectors encoding selectable marker genes (*3*). Two viruses carrying vectors with different drug-selectable markers were applied simultaneously, one encoding neomycin phosphotransferase and the other encoding a histidinol dehydrogenase gene, and infected cells were selected in the presence of G418 and histidinol. When single-cell clones became visible, they were isolated and expanded.
12. J. G. Quigley *et al.*, *Blood* **95**, 1093 (2000).
13. S. Boomer, M. Eiden, C. C. Burns, J. Overbaugh, *J. Virol.* **71**, 8116 (1997).
14. D. V. Kumar, B. T. Berry, P. Roy-Burman, *J. Virol.* **63**, 2379 (1989).
15. A. S. McDougall *et al.*, *J. Virol.* **68**, 2151 (1994).
16. S. V. Johann, J. J. Gibbons, B. O'Hara, *J. Virol.* **66**, 1635 (1992); M. P. Kavanaugh *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7071 (1994).
17. J. S. Allan, J. Strauss, D. W. Buck, *Science* **247**, 1084 (1990).
18. J. Overbaugh, P. R. Donahue, S. L. Quackenbush, E. A. Hoover, J. I. Mullins, *Science* **239**, 906 (1988).
19. R. Weiss, N. Teich, H. Varmus, J. Coffin, *RNA Tumor Viruses* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1985).
20. M. A. Eglitis, M. V. Eiden, C. A. Wilson, *J. Virol.* **67**, 5472 (1993).

21. We thank M. Eiden, D. Miller, M. Emerman, J. Abkowitz, and J. Quigley for helpful discussions and comments on the manuscript; M. Eiden, D. Miller, and T. Kitamura for reagents; and J. Sugai, C. Hankenson, and H. Cheng for technical assistance. Supported by the National Cancer Institute.

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Candidate Taste Receptors in *Drosophila*

Peter J. Clyne,*† Coral G. Warr,* John R. Carlson‡

Little is known about the molecular mechanisms of taste perception in animals, particularly the initial events of taste signaling. A large and diverse family of seven transmembrane domain proteins was identified from the *Drosophila* genome database with a computer algorithm that identifies proteins on the basis of structure. Eighteen of 19 genes examined were expressed in the *Drosophila* labellum, a gustatory organ of the proboscis. Expression was not detected in a variety of other tissues. The genes were not expressed in the labellum of a *Drosophila* mutant, *pox-neuro*^{70}, in which taste neurons are eliminated. Tissue specificity of expression of these genes, along with their structural similarity, supports the possibility that the family encodes a large and divergent family of taste receptors.

Although two putative mammalian taste receptors have recently been described (*1*), remarkably little is understood in general about taste receptors across species. A computer algorithm that seeks proteins with particular structural properties, as opposed to proteins with particular sequences, identified a large family of candidate odorant receptors from the *Drosophila* genomic database (*2*). Here, we report that further analysis of genes identified by this algorithm revealed one gene that defines a distinct large family of membrane proteins (*3*); 43 members of this family have been identified in the first 60% of the *Drosophila* genome that has been sequenced thus far (*3*). If the sequenced part of the genome is

Department of Molecular, Cellular, and Developmental Biology, Yale University, Post Office Box 208103, New Haven, CT 06520–8103, USA.

*These authors contributed equally to this work.
†Present address: Department of Biochemistry and Biophysics, University of California at San Francisco, Parnassus Avenue, Box 0448, San Francisco, CA 94143, USA.
‡To whom correspondence should be addressed. E-mail: john.carlson@yale.edu

representative, then extrapolation suggests that the entire genome would encode on the order of 75 proteins, a figure comparable to our estimate of ~100 candidate odorant receptors (*2*). The previously unidentified family of proteins shows no sequence similarities to any known odorant receptors or to any other known proteins. We have tentatively named this the gustatory receptor (GR) family, with each individual gene named according to its cytogenetic location in the genome. Thus, the GR59D.1 and GR59D.2 genes, which we abbreviate here as 59D.1 and 59D.2, refer to two family members located in cytogenetic region 59D on the second chromosome.

The amino acid sequences of 19 members of the GR family indicate the high degree of sequence divergence (Fig. 1). Sequence alignment revealed only one residue conserved among all members of the family shown and only 24 residues conserved among more than half of the genes shown. Fifteen of these conserved residues lie in the vicinity of the COOH-terminus. Amino acid identity between individual genes ranged from a maximum of 34% to <10%. By contrast, other features of the gene family show substantial conservation. The positions of a number of introns are conserved (Fig. 1), suggesting that the family originated from a common ancestral gene. Overall sequence length, ~380 amino acids, is another common feature. All of the genes encode approximately seven predicted transmembrane domains, a feature characteristic of G protein–coupled receptors (GPCRs) (Fig. 2). All 43 of the predicted GR gene products were identified as GPCRs by an algorithm trained to distinguish between GPCRs and other multitransmembrane proteins (*2, 4*).

The genes are widely dispersed in the genome, but at the same time, many are found in clusters. The two largest clusters each contain four genes; there are also several clusters of two or three genes. Genes within these clusters are closely spaced, with intergenic distances ranging from 150 to 450 base pairs (bp) in all cases for which the data are currently available. There is no rule specifying the orientation of genes within clusters, unlike the case of the *Drosophila* odorant receptors, in which genes within a cluster are in the same orientation in all clusters examined (*2*).

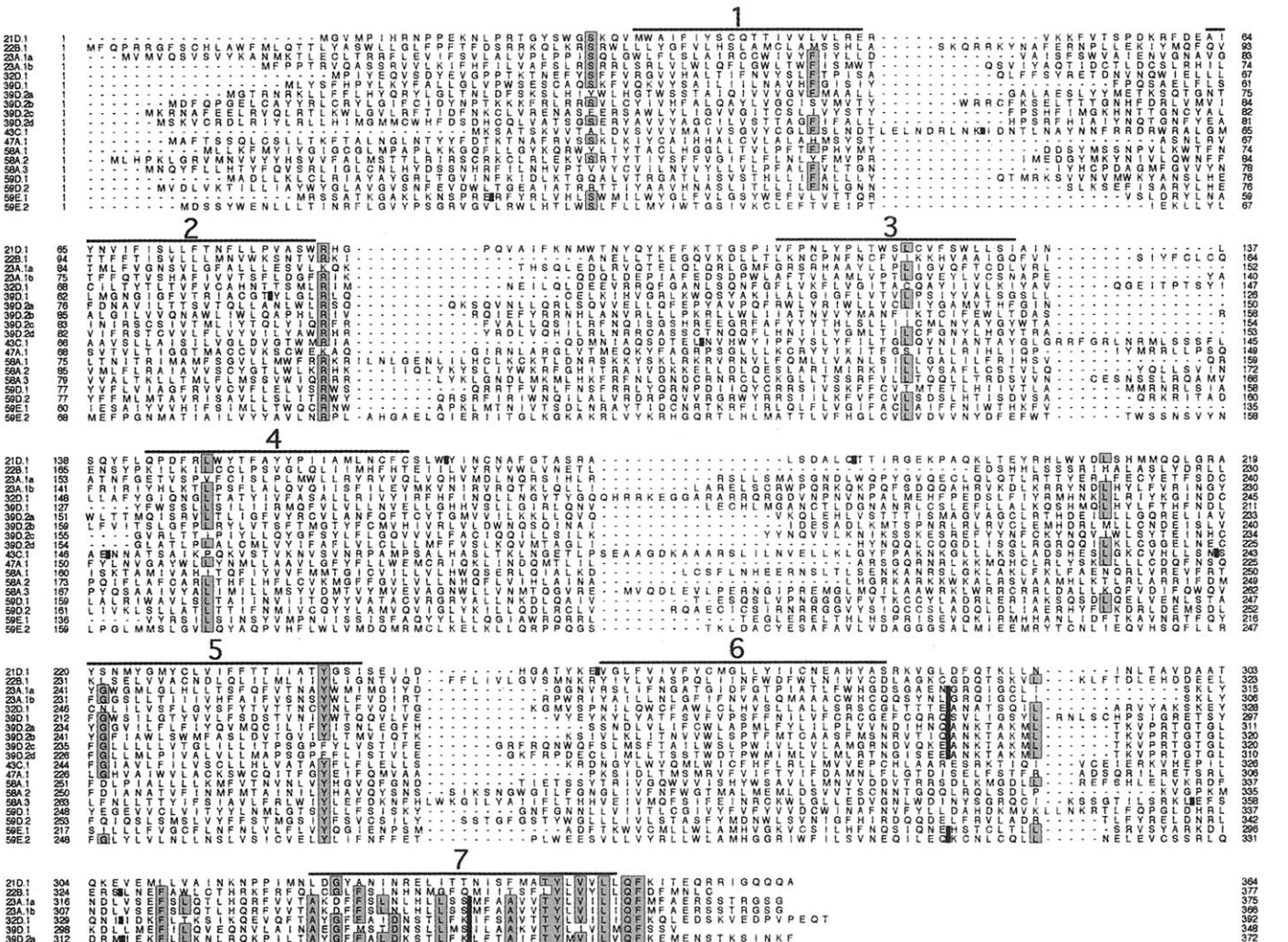

Fig. 1. Amino acid sequence alignment of 19 GR proteins (*25*). Letters following protein designations identify alternative splicing products of individual genes. Residues conserved in >50% of the predicted proteins are shaded. The approximate locations of the seven predicted transmembrane domains are indicated. Intron-exon boundaries are shown with vertical lines. The sequences shown are the first 19 full-length proteins we identified. All DNA sequences are from the BDGP database (*3*).

An unusual form of alternative splicing occurs in at least two chromosomal locations. Four large exons in cytogenetic region 39D each contain sequences specifying six predicted transmembrane domains, followed by three small exons that together specify a putative seventh transmembrane domain and the COOH-terminus (Fig. 3). Reverse transcription–polymerase chain reaction (RT-PCR) analysis revealed that each of the four large exons is spliced to the smaller exons, thereby generating four predicted seven transmembrane domain proteins. These four proteins are thus distinct through the first six transmembrane domains and identical in the seventh and in the COOH-terminal sequences. Likewise, in cytogenetic region 23A, there are two large exons, each of which specifies six transmembrane domains and is spliced to two small exons that together encode a seventh transmembrane domain and the COOH-terminus (Fig. 3). Thus, the gene in region 23A encodes two related proteins. This pattern of splicing, in which alternative large 5′ exons encoding most of the protein are joined to common short 3′ exons encoding only a small portion of the protein, is unusual among genes encoding GPCRs and proteins in general. This pattern of splicing provides a mechanism at a single locus for generating products that exhibit a pattern observed for this family in general: extreme diversity among all sequences of the proteins except in a small region in the vicinity of the COOH-terminus.

To assess the tissue specificity of expression, we performed RT-PCR with primers that span introns in the coding regions. Of the 19 transcripts tested, 18 were expressed in the labellum (Fig. 4 and Table 1), the major gustatory organ of the fly (*5*–*8*). Moreover, for most of these genes, expression was labellum-specific in that only 1 of the 19 yielded amplification products from heads depleted of taste organs and only 2 showed expression in the thorax, which contains the thoracic nervous system but no characterized taste sensilla. Likewise, expression in several other tissues, including the abdomen, wings, and legs, was limited to a small fraction of genes (Table 1).

To further analyze gene expression by in situ hybridization, we used 12 GR transcripts as probes. Each probe was used individually and in mixtures of multiple probes. Sequences encompassing all, or nearly all, of each transcript were used, and several diverse methods of signal amplification and detection were used, with a variety of experimental conditions (*9*). None of the genes showed detectable expression in any tissue, including the taste organs. As positive controls, the *pheromone-binding protein-related protein-2* gene (*pbprp-2*), which may encode a carrier of hydrophobic molecules (*10*), showed hybridization in taste sensilla on the labellum, and the *Drosophila* olfactory receptor gene 22A.2 (DOR22A.2) (*2*) hybridized to olfactory sensilla on the antenna. The simplest interpretation of these results is that expression levels of the GR genes are exceedingly low. Consistent with this interpretation is the fact that no expressed sequence tags have been identified for any of the 43 GR transcripts.

To further analyze the tissue specificity of GR expression, we performed a microdissection experiment in which the labral sense organ (LSO) (*7*, *8*), a small taste organ that lines the pharynx, was surgically excised from each of 50 animals. The LSO consists of a very limited number of cells and is highly enriched in taste neurons; it does not, for example, contain muscle cells. By RT-PCR amplification, we detected the expression of seven GR transcripts in this taste organ (Fig. 5). These results indicate that expression of the GR family extends to include at least one additional taste organ besides the labellum. The data are also fully consistent with the notion that the GR genes are expressed in taste neurons.

To confirm the gene expression in taste receptor neurons, we used a *Drosophila* mu-

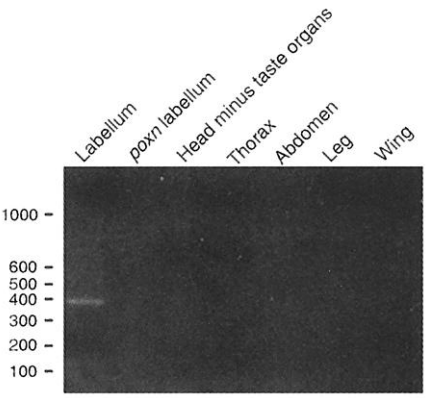

Fig. 4. Tissue specificity of expression of 32D.1 in the labellum. Shown is a gel photograph of an RT-PCR experiment with primers spanning an intron in 32D.1. The size of the predicted PCR product from cDNA is 372 bp; any remaining genomic DNA would generate a product of 559 bp. A cDNA band is observed in the labellum lane only. In addition, 32D.1 is not expressed in the labellum of the $poxn^{70}$ mutant. Positive controls are described in (*26*).

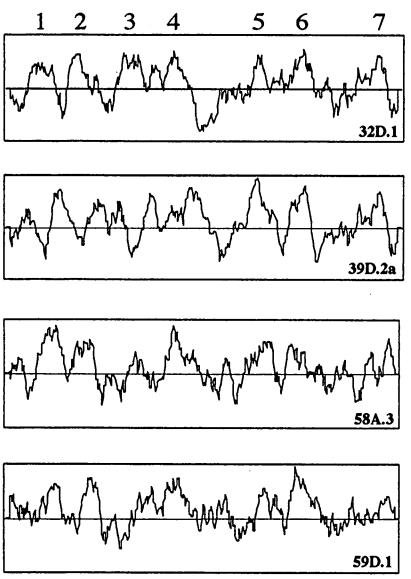

Fig. 2. Representative hydropathy plots of GR proteins. Hydrophobic peaks predicted by Kyte-Doolittle analysis appear above the center lines. The approximate positions of the seven putative transmembrane domains are indicated above the first hydropathy plot. Similar plots were obtained for all of the GR proteins.

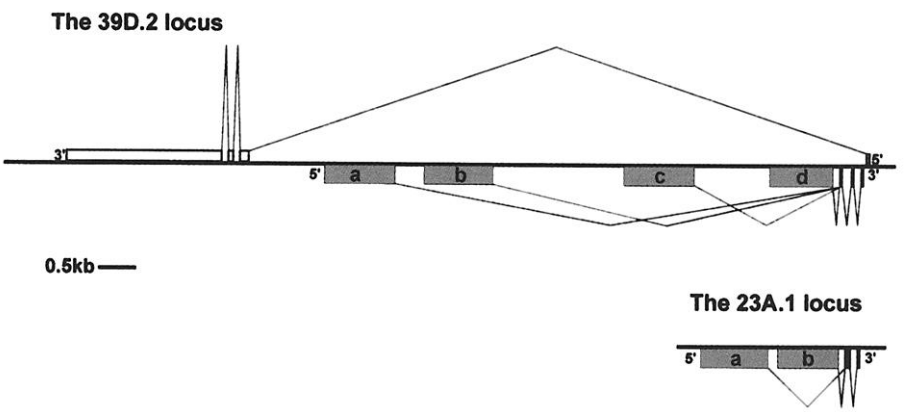

Fig. 3. Genomic organization of the 39D.2 and 23A.1 loci. In the 39D.2 locus, the gray boxes labeled a through d represent four large 5′ exons, each of which can be spliced individually to the three 3′ exons (indicated in black) to produce alternative transcripts encoding four different proteins. All the exons of the 39D.2 locus are located in an intron of another gene, which is in the opposite orientation and whose exons are represented by white boxes. This other gene appears to encode a basic helix-loop-helix transcription factor expressed during embryogenesis. In the 23A.1 locus, the gray boxes labeled a and b represent two alternative large 5′ exons, either of which can be spliced to the two small 3′ exons (indicated in black) to produce transcripts encoding two different proteins.

tant, *pox-neuro*^{70} (*poxn*^{70}), in which chemosensory bristles are transformed into mechanosensory bristles (*11–14*). Specifically, in *poxn*^{70}, which behaves as a null mutation with respect to adult chemosensory organs, chemosensory bristles are transformed into mechanosensory bristles with respect to various morphological and developmental criteria. In particular, most chemosensory bristles in wild-type *Drosophila* are innervated by five neurons: four chemosensory neurons and one mechanosensory neuron. In contrast, wild-type mechanosensory bristles contain a single mechanosensory neuron. In chemosensory bristles transformed to mechanosensory bristles by *poxn*^{70} (*11*), the number of neurons is reduced from five to one. We predicted that if the GR family is in fact expressed in the chemosensory neurons of taste sensilla, their expression would likely be eliminated in the *poxn*^{70} mutant. Consistent with this prediction, 18 of 19 GR transcripts examined were not expressed in the labellum of the *poxn*^{70} mutant (Table 1 and Fig. 4). These results indicate that the GR gene family is expressed in labellar chemosensory neurons.

The large size of this protein family likely reflects the diversity of compounds that flies can detect. The labellar hairs of larger flies are not only sensitive to a variety of simple and compound sugars (*15*), but also to a wide variety of other molecules, such as amino acids (*16*). Behavioral studies have shown that *Drosophila* are sensitive to quinine (*17*), which is perceived by humans as bitter, and other insects have been shown to be sensitive to an array of structurally diverse bitter compounds. Moreover, an individual insect taste receptor cell can respond to a broad range of structurally heterogeneous alkaloids and other bitter molecules (*18, 19*). The extreme diversity of these receptors may not only reflect diversity among the ligands that they bind, but also diversity in the signal transduction components with which they interact. For example, the lack of conserved intracellular regions suggests the possibility that, during the evolution of this sensory modality, multiple G proteins arose, each interacting with a different subset of receptors. Finally, it seems likely that the *Drosophila* genome encodes taste receptors in addition to those of the GR family. Although we have detected expression in the labellum and the LSO, few if any family members are expressed in the leg or wing chemosensory hairs (Table 1), some of which are morphologically similar to labellar taste hairs (*7*). The *Drosophila* olfactory system also contains more than one organ, the antenna and maxillary palp, which respond to all, or nearly all, of the same odorants and which derive from the same imaginal discs (*20*). However, most individual members of the DOR gene family are expressed in one or the other but not in both olfactory organs (*2, 21*). Perhaps the distinction among taste receptor genes is even more extreme in the gustatory system, whose organs derive from different imaginal discs. For example, the legs may express a completely distinct family of genes or a subfamily whose similarities to the present family are sufficiently tenuous as to place it slightly beyond the boundaries that define the GR family.

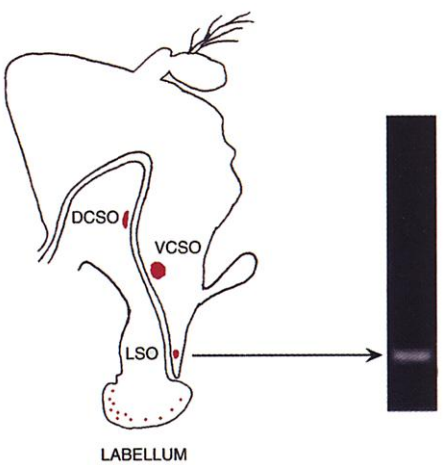

Fig. 5. GR gene expression in microdissected labral sense organs (LSOs). The red areas show the four major taste organs of the *Drosophila* head: the LSO, the dorsal cibarial sense organ (DCSO), the ventral cibarial sense organ (VCSO), and the labellum. The gel track shows an amplification product from RNA extracted from 50 LSOs, amplified with primers N23A.3J and N23A.2D from two exons of gene 23A.1. Specifically, one primer is from the large exon 23A.1a (Fig. 3), and the other is from the first common exon at the 3′ end. The amplification product is 430 bp, which is the expected length for a cDNA product; any remaining genomic DNA would generate a product of 1598 bp. The primer pair did not amplify a product from nongustatory tissue (Table 1). The following transcripts were detected in the LSO: 22B.1, 23A.1a, 23A.1b, 32D.1, 39D.2c, 43C.1, and 58A.2.

Table 1. Tissue-specific expression of GR genes. RT-PCR was performed from RNA extracted from the indicated tissues (*26*). All primer pairs spanned introns. Positive controls are described in (*26*).

Gene	Labellum	*poxn* labellum	Head minus taste organs	Thorax	Abdomen	Leg	Wing
21D.1	+	−	−	−	−	−	−
22B.1	+	−	−	−	+	+	+
23A.1a	+	−	−	−	−	−	−
23A.1b	+	−	−	−	−	−	−
32D.1	+	−	−	−	−	−	−
39D.1	+	−	−	−	−	−	−
39D.2a	+	−	−	−	−	−	−
39D.2b	+	−	−	−	−	−	−
39D.2c	+	+	−	+	+	−	−
39D.2d	+	−	−	+	−	−	+
43C.1	+	−	+	−	+	+	+
47A.1	+	−	−	−	−	−	−
58A.1	+	−	−	−	−	−	−
58A.2	+	−	−	+	−	−	−
58A.3	+	−	−	−	−	−	−
59D.1	+	−	−	−	−	−	−
59D.2	+	−	−	−	−	−	−
59E.1	−	−	−	−	+	−	+
59E.2	+	−	−	−	−	−	−

References and Notes

1. M. A. Hoon *et al.*, *Cell* **96**, 541 (1999).
2. P. J. Clyne *et al.*, *Neuron* **22**, 327 (1999). The algorithm examines the physicochemical properties of the amino acids in an open reading frame (ORF) and then uses a nonparametric discriminant function to identify ORFs likely to encode multitransmembrane domain proteins.
3. The first exon of 23A.1b (Fig. 1) was identified by the computer algorithm described in (*2*). Examination of the genomic DNA surrounding the first exon of 23A.1b identified other exons, and the genomic structure of this gene was determined with RT-PCR. Using the sequence of this gene, we performed an extensive series of tBLASTn searches of the Berkeley *Drosophila* Genome Project (BDGP) sequence database (available at http://www.fruitfly.org), which identified ORFs of 38 other genes of the GR family. The full sequences of these genes were identified by an analysis of the genomic DNA flanking these ORFs as described in (*2*), using the *Drosophila* intron-exon consensus splice sequences and RT-PCR analysis. The 39 genes encode a total of 43 proteins. The National Center for Biotechnology Information (NCBI) accession number of the BDGP genomic clone on which each transcript is found and the sequence range in the genomic clone for the predicted coding region are given as follows for each GR transcript shown in Fig. 1 (NCBI and BDGP data are as of 16 October 1999): transcript GR21D.1, accession number AC004420, range 34784–33509; GR22B.1, AC003945, 31740–30551; GR23A.1a, AC005558, 108490–106118; GR23A.1b, AC005558, 107351–106118; GR32D.1, AC005115, 19779–21141; GR39D.1, AC007208, 62553–64348; GR39D.2a, AC005130, 9170–16119; GR39D.2b, AC005130, 10410–16119; GR39D.2c, AC005130, 12989–16119; GR39D.2d, AC005130, 14750–16119; GR43C.1, AC005452, 50105–51583; GR47A.1, AC007352, 114644–115920; GR58A.1, AC004368, 62323–61087; GR58A.2, AC004368, 62511–63791; GR58A.3, AC004368, 65521–64229; GR59D.1, AC006245, 68825–70050; GR59D.2, AC006245, 70261–71505; GR59E.1, AC005639, 30167–31539; and GR59E.2,

AC005639, 30036–28714. Accession numbers for the other genes are as follows (data are as of 5 January 2000) (complete sequences are available for the first four and only partial sequences are available for the remaining genes; LU, location unknown): transcript GR1F.1, accession number AL035632, range 7301–8711; GR47F.1, AC005653, 42838–44204; GR68D.1, AC006492, 46040–44916; GR77E.1, AC006490, 104929–103117; GR28A.1, AC008354, 66711–66973; GR57B.1, AC007837, 102661–103185; GR65C.1, AC004251, 23136–24215; GR93F.1, AC012873, 35043–35228; GR93F.2, AC012892, 2781–2650; GR93F.3, AC012892, 4271–4143; GR93F.4, AC012892, 6482–5559; GR94E.1, AC008200, 72472–72308; GR97D.1, AC007984, 121300–121977; GR98B.1, AC007817, 45506–46916; GR98B.2, AC007817, 10695–10784; GR98B.3, AC007817, 45189–45284; GR98B.4, AC007817, 39658–39765; GRLU.1, AC017438, 22141–21398; GRLU.2, AC017138, 10997–11122; GRLU.3, AC015395, 43210–43612; GRLU.4, BACR28P1-T7, 28–129; GRLU.5, BACR28P1-T7, 388–734; GRLU.6, BACR06I03-T7, 1028–48; and GRLU.7, AC012799, 8212–8123.

4. All of the GR proteins were identified as GPCRs when the algorithm was modified to distinguish previously described GPCRs from ion channels. The algorithm was set to positively identify 95% of previously described GPCRs, with 4.3% false positives. Most ion channels have six transmembrane domains.
5. R. Falk, N. Bleiser-Avivi, J. Atidia, *J. Morphol.* **150**, 327 (1976).
6. V. Dethier, *The Hungry Fly* (Harvard Univ. Press, Cambridge, MA, 1976).
7. R. Stocker, *Cell Tissue Res.* **275**, 3 (1994).
8. S. Nayak and R. Singh, *Int. J. Insect Morphol. Embryol.* **12**, 273 (1983).
9. For in situ hybridization to RNA, between 800 bp and 1 kbp of the coding regions of 12 GR transcripts were subcloned into the pGEM-T Easy vector (Promega). Digoxygenin-labeled RNA probes were generated and hydrolyzed according to the manufacturer's instructions (Boehringer Mannheim). Initially, hybridization and detection of probes were performed as was previously described for the *Drosophila* odorant receptors (*2*), with standard chromogenic detection. Subsequently, an alternative set of hybridization and washing conditions was used (*21*). Both methods successfully detected expression of the DOR22A.2 gene (*2*) in the antenna and the *pbprp-2* gene (*10*) in the labellum, but they did not detect expression of any of the GR genes, even when many other experimental conditions were varied. Among the variations tested were the use of increased probe concentrations, nonhydrolyzed probes, combinations of probes, alternative fixation conditions, and less stringent hybridization and washing conditions. We then tried to detect expression by adapting an alternative signal detection method for use on *Drosophila* cryosections: tyramide signal amplification in combination with alkaline-phosphatase–based visualization, described in (*22*). This method successfully detected expression of DOR22A.2 in the antenna but also failed to detect expression of GR genes.
10. C. Pikielny, G. Hasan, F. Rouyer, M. Rosbash, *Neuron* **12**, 35 (1994).
11. T. Awasaki and K. Kimura, *J. Neurobiol.* **32**, 707 (1997).
12. C. Dambly-Chaudiere *et al.*, *Cell* **69**, 159 (1992).
13. E. Nottebohm *et al.*, *Neuron* **12**, 25 (1994).
14. E. Nottebohm, C. Dambly-Chaudiere, A. Ghysen, *Nature* **359**, 829 (1992).
15. V. Dethier, *Q. Rev. Biol.* **30**, 348 (1955).
16. A. Shiraishi and A. Kuwabara, *J. Gen. Physiol.* **56**, 768 (1970).
17. L. Tompkins, M. Cardosa, F. White, T. Sanders, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 884 (1979).
18. J. Glendinning and T. Hills, *J. Neurophysiol.* **78**, 734 (1997).
19. R. Chapman, A. Ascoli-Christensen, P. White, *J. Exp. Biol.* **158**, 241 (1991).
20. J. Carlson, *Trends Genet.* **12**, 175 (1996).
21. L. B. Vosshall, H. Amrein, P. S. Morozov, A. Rzhetsky, R. Axel, *Cell* **96**, 725 (1999).
22. H. Yang, I. Wanner, S. Roper, N. Chaudhari, *J. Histochem. Cytochem.* **47**, 431 (1999).
23. M. Perin *et al.*, *J. Biol. Chem.* **266**, 615 (1991).
24. Available as supplementary Web material at www.sciencemag.org/feature/data/1046815.shl
25. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
26. The amount of each tissue used to prepare cDNA was that determined to give approximately the same signal with a pair of positive control primers, CGGATCCCTATGTCAAGGTG and GAAGAGCTTCGTGCTGGTCT, representing the *Drosophila synaptotagmin* gene (*23*). Specifically, the amount of tissue used in each cDNA preparation was as follows: 50 labella, 5 heads from which taste organs (the labellum, the LSO, the dorsal cibarial sense organ, and the ventral cibarial sense organ) had been surgically removed, 20 thoraces, 20 abdomens, 200 legs, and 20 anterior wing margins (the portion of the wing containing chemosensory sensilla). Complementary DNA preparation and PCR were performed as in (*2*). For all genes, primer pairs (*24*) that span introns were used to distinguish bands amplified from cDNA from those amplified from any remaining genomic DNA. All negative results were confirmed by testing at least one additional primer pair.
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Correlates of Sleep and Waking in *Drosophila melanogaster*

Paul J. Shaw, Chiara Cirelli, Ralph J. Greenspan, Giulio Tononi*

Drosophila **exhibits a circadian rest-activity cycle, but it is not known whether fly rest constitutes sleep or is mere inactivity. It is shown here that, like mammalian sleep, rest in *Drosophila* is characterized by an increased arousal threshold and is homeostatically regulated independently of the circadian clock. As in mammals, rest is abundant in young flies, is reduced in older flies, and is modulated by stimulants and hypnotics. Several molecular markers modulated by sleep and waking in mammals are modulated by rest and activity in *Drosophila*, including cytochrome oxidase C, the endoplasmic reticulum chaperone protein BiP, and enzymes implicated in the catabolism of monoamines. Flies lacking one such enzyme, arylalkylamine *N*-acetyltransferase, show increased rest after rest deprivation. These results implicate the catabolism of monoamines in the regulation of sleep and waking in the fly and suggest that *Drosophila* may serve as a model system for the genetic dissection of sleep.**

Sleep is ubiquitous in mammals and birds and must serve a fundamental biological function that is as yet unknown (*1*). Both vertebrates and invertebrates often display a prominent circadian organization of rest and activity. But do invertebrates, such as *Drosophila*, sleep? If this were known, powerful genetic tools could be used to investigate sleep mechanisms and functions.

In mammals, sleep is distinguished from inactivity both behaviorally and electrophysiologically. In invertebrates, the identification of sleep-like states depends primarily on the behavioral analysis of quiescence, increased arousal threshold, and increased rest after prolonged waking (a criterion that indicates that rest is under homeostatic control) (*2*). Recently, molecular screening has revealed that sleep and waking also differ in the expression of several neural genes (*3*). We therefore evaluated whether *Drosophila* has sleep-like states by investigating both behavioral and molecular characteristics of its rest-activity cycle.

Continuous, high-resolution measurement of fly behavior (5-day-old virgin females, Canton-S) was achieved with an ultrasound activity monitoring system (*4*). This system detects fine movements of the fly's head, wings, and limbs, in good agreement with visual observation (*5*). Flies subjected to 12 hour:12 hour light/dark cycles exhibited sustained periods of activity and quiescence, with >90% of quiescence (henceforth referred to as rest) occurring during the dark period (Fig. 1A) (*6*). To monitor rest-activity patterns in large numbers of flies, we used an infrared activity monitoring system, which confirmed a robust circadian organization of activity and showed good correspondence with the ultrasound system (*7*).

To determine whether periods of rest are associated with increased arousal thresholds, we subjected flies to vibratory stimuli of increasing intensity [0.05*g* (acceleration), $n = 12$; 0.1*g*, $n = 10$; and 6.0*g*, $n = 8$] (*8*). Flies that had been behaviorally awake readily responded to intensities of 0.05*g* and 0.1*g* (90% of trials). Flies that had been behaviorally quiescent for 5 min or longer rarely showed a behavioral response to these stimuli (<20% of trials; $P <$

The Neurosciences Institute, 10640 John Jay Hopkins Drive, San Diego, CA 92121, USA.

*To whom correspondence should be addressed. E-mail: tononi@nsi.edu