Analysis

Drosophila melanogaster G Protein–coupled Receptors

Thomas Brody* and Anibal Cravchik‡

*Neurogenetics Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892; and ‡Celera Genomics, Rockville, Maryland 20850

The G protein–coupled receptors (GPCRs)[1] constitute a large and ancient superfamily of integral cell membrane proteins that play a central role in signal transduction and are activated by an equally diverse array of ligands. GPCRs share a seven hydrophobic α-helical domain structure and transduce signals through coupling to guanine nucleotide–binding regulatory proteins (G proteins). The seven hydrophobic domains are likely to span the membrane and are linked by three extracellular loops that alternate with three intracellular loops. The extracellular NH_2 terminus is usually glycosylated and the cytoplasmic COOH terminus is generally phosphorylated. The presence of a large diversity of GPCR genes may be a characteristic of eukaryotic genomes since >1,000 GPCRs have been identified in the *Caenorhabditis elegans* genome, representing >5% of its total number of genes (Bargmann, 1998).

The completion of the sequencing of the *Drosophila melanogaster* genome allows the analysis of its full repertoire of GPCRs for the first time. Do *Drosophila* GPCRs have counterparts in other phyla, or do they reflect a highly specialized insect biology? The *Drosophila* genome contains ~200 genes coding for GPCRs, including neurotransmitter and hormone receptors, and olfactory and putative taste receptors (Adams et al., 2000; Clyne et al., 2000; Rubin et al., 2000). We have identified 100 genes in the *Drosophila* genome that code for putative neurotransmitter and hormone GPCRs and atypical seven-transmembrane domain (7 TM) proteins, 68 of which are described here for the first time (Fig. 1, red). These genes were manually curated after the use of gene prediction programs Genie and Genscan (Adams et al., 2000), resulting in an enhanced definition of predicted gene structures.

Drosophila GPCRs are classified into four families: rhodopsin-like (Fig. 1 A); secretin-like (Fig. 1 B); metabotropic glutamate–like (Fig. 1 C); and atypical 7 TM proteins (Fig. 1 D). This classification is based on primary and secondary structure predictions, sequence analysis using profile hidden Markov models, and sequence homology searches using BLAST. Despite the greater number and diversity of GPCRs in vertebrates and *C. elegans* as compared with *Drosophila*, the data point to conservation of hormone and neurotransmitter receptors across phyla, suggesting ancient evolutionary origins.

Rhodopsin-like Receptor Family

The rhodopsin-like family encompasses receptors for a large variety of stimuli, such as biogenic amine neurotransmitters, neuropeptides, peptide hormones, light, nucleotides, prostaglandins, leukotrienes, chemotactic peptides, and chemokines. Although their ligands vary considerably in structure, the rhodopsin-like GPCRs show sequence conservation within their seven putative TM domains.

Opsins

The *Drosophila* photopigments form three subgroups: (i) Rh1, Rh2, and Rh6 are related to long wavelength–absorbing invertebrate visual pigments; (ii) Rh3, Rh4, and Rh5 belong to a group of short wavelength–absorbing invertebrate visual pigments (Salcedo et al., 1999); (iii) CG5648, which is a newly identified *Drosophila* opsin (Fig. 1). Subgroups 1 and 2 are more closely related to each other than to CG5648. *Drosophila* opsins are quite distinct from vertebrate opsins and are more closely related to other insect and mollusk opsins and to melanopsin, a dermal opsin from *Xenopus laevis* (Provencio et al., 1998). This level of sequence homology suggests that invertebrate opsins and melanopsin may share a common functional basis and evolutionary origin. Functionally, vertebrate retinal opsins require reisomerization into the 11-cis isomer, whereas invertebrate photopigments retain a covalently linked chromophore (Gärtner and Towner, 1995).

GPCRs for Biogenic Amines, Related Compounds, and Purines

This is a large group of receptors for classical neurotransmitters and neuromodulators that may share a common evolutionary ancestor and is present in vertebrate and invertebrate lineages (Venter et al., 1988). Of the 21 receptors identified in this group, 11 are described here for the first time (Fig. 1). The biogenic amine GPCRs share high levels of sequence similarity within species and across phyla. Therefore, many of the newly described biogenic amine GPCRs cannot easily be classified into subgroups as defined by their putative ligands. Furthermore, it has been suggested that these receptors have changed substrate specificities during evolution (Peroutka and Howell, 1994).

Address correspondence to Anibal Cravchik, Celera Genomics, 45 West Gude Dr., Rockville, MD 20850. Tel.: (240) 453-3353. Fax: (240) 453-4996. E-mail: anibal.cravchik@celera.com

[1]*Abbreviations used in this paper:* GPCR, G protein–coupled receptor; G protein, guanine nucleotide–binding protein; TM, transmembrane.

Gene	Transcript	Similar to	F	W E Value	V
A. Rhodopsin-like receptor family					
Opsin-like					
ninaE	CT14728	Rh2	e-149	e-16	e-52
Rh2	CT33926	ninaE	e-153	e-16	e-53
Rh3	CT14302	Rh4	e-158	e-11	e-44
Rh4	CT27342	Rh3	e-142	e-17	e-47
Rh5	CT16797	Rh4	e-94	e-14	e-50
Rh6	CT16621	ninaE	e-108	e-17	e-49
CG5638	CT17820	Rh3	e-57	e-14	e-44
Receptors for biogenic amines and related compounds					
5-HT receptor-like					
5-HT2	CT1149	5-HT1A	e-44	e-64	e-39
5-HT7	CT4852	CG7078	e-52	e-67	e-64
CG8007	CT24060	5-HT2	e-21	e-35	e-27
5-HT1A	CT34985	5-HT1B	e-117	e-48	e-49
5-HT1B	CT34991	5-HT1A	e-117	e-37	e-29
Dopamine receptors					
DopR	CT27288	CG6919	e-52	e-68	e-68#
DopR2	CT8423	CG7078	e-38	e-62	e-43#
Muscarinic Acetylcholine receptor-like					
mAcR-60C	CT14234	CG7918	e-32	e-69	e-73
CG7918	CT23924	mAcR-60C	e-32	e-49	e-41
Octopamine/ tyramine receptors					
Oamb	CT12841	Ocr	e-28	e-54	e-30
Ocr	CT22999	CG6706	e-96	e-61	e-44
Unclassifiable biogenic amine receptor-like					
CG17004	CT37739	5-HT7	e-18	e-20	e-28
CG7431	CT22855	CG16766	e-70	e-45	e-37
CG16766	CT37292	CG7431	e-53	e-31	e-22
CG12796	CT38338	CG6919	e-23	e-20	e-30
CG6919	CT21432	CG6989	e-82	e-46	e-57
CG6989	CT21650	CG6919	e-82	e-39	e-46
CG7078	CT21843	CG6919	e-73	e-46	e-38
CG18314	CT41076	CG6919	e-21	e-48	e-25
CG7994	CT24036		>e-10	>e-10	e-10
Purine receptors					
Adenosine receptor-like					
CG9753	CT27563	CG6989	e-19	e-29	e-42
Peptide receptors					
Allatostatin receptor-like					
EG:121E7.2	CT9822	CG10001	e-68	e-46	e-46
CG10001	CT28187	EG:121E7.2	e-62	e-35	e-34
FSH/TSH/LH receptor-like					
CG4187	CT13764	CG5042	e-48	e-23	e-24
Fsh	CT23429	CG4187	e-17	e-77	e-103
rk	CT25644	nd	nd	e-21	e-46
CG5042	CT16185	CG4187	e-44	e-25	e-23
Gastrin/CCK receptor-like					
CG6857	CT21155	CG6881	e-96	e-33	e-19
CG6881	CT21314	CG6857	e-96	e-23	e-33
Gonadotropin releasing hormone receptor-like					
CG10698	CT29989	GRHR	e-43	e-34	e-39
GRHR	CT31611	CG10698	e-43	e-48	e-57
Growth hormone secretagoge receptor-like					
CG8784	CT25324	CG8795	e-165	e-38	e-35
CG8795	CT25350	CG8784	e-165	e-40	e-37
CG9918	CT27924	CG8795	e-83	e-53	e-33
Tachykinin receptor-like					
CG1147	CT1960	NepYr	e-31	e-43	e-40
Takr99D	CT6643	Takr86C	e-87	e-54	e-81
NepYr	CT18198	CG10626	e-51	e-36	e-50
Takr86C	CT20223	Takr99D	e-87	e-53	e-75
CG10626	CT29768	Takr99D	e-53	e-41	e-44
Somatostatin receptor-like					
CG7285	CT22465	CG13702	e-96	e-24	e-51
CG13702	CT33159	CG7285	e-96	e-26	e-46
Vasopressin receptor-like					
CG6111	CT19191	GRHR	e-36	e-26	e-45

Gene	Transcript	Similar to	F	W E Value	V
Unclassifiable peptide receptors					
CG13575	CT32957	CG10626	e-08	e-08	e-8#
CG14003	CT33559	Takr99D	e-14	e-12	e-14#
CG5911	CT18539	Takr86C	e-18	e-19	e-26#
CG10823	CT18916	CG6857	e-10	e-10	e-12
Orphan receptors					
Related to *C. elegans* orphan receptors					
CG2114	CT2366		>e-10	e-27	e-10
CG3171	CT10621	EG:22E5.10	e-50	e-16	e-26
EG:22E5.10	CT14076	EG:22E5.11	e-42	e-16	e-15
EG:22E5.11	CT14137	CG3171	e-58	e-22	e-23
CG5936	CT18637		>e-10	e-18	>e-10
CG8985	CT25824	CG13803	e-162	e-31	>e-10
CG13803	CT33298	CG8985	e-162	e-36	>e-10
CG13229	CT32473	CG13803	e-58	e-35	e-10
Other orphan receptors					
CG9569	CT17758		>e-10	>e-10	>e-10
CG12290	CT19320		>e-10	>e-10	>e-10
CG6986	CT21642	CG16726	e-10	>e-10	>e-10
CG13579	CT32961		>e-10	e-18	>e-10
CG13995	CT33551		>e-10	e-13	e-11
CG7497	CT23019		>e-10	>e-10	>e-10
B. Secretin-like receptor family					
Calcitonin receptor-like					
CG4395	CT4121	CG17415	e-46	e-16	e-41
CG17415	CT38445	CG4395	e-42	e-20	e-61
CG13758	CT33238	CG8422	e-40#	e-65	e-66
Diuretic hormone receptor-like					
CG8422	CT24513	CG12370	e-122	e-25	e-65
CG12370	CT24959	CG8422	e-122	e-30	e-101
HE6 receptor-like					
CG11318	CT31591	CG15556	e-104	e-11	e-20
CG15556	CT35672	CG11318	e-103	>e-11	e-6
Latrophilin-like					
CG8639	CT8755		>e-10	e-47	e-45
Methuselah-like					
mth	CT21390	CG17795		>e-10	>e-10
CG4521	CT14539	CG6965	e-30	e-13	
CG17795	CT16507	mth	e-132	>e-10	>e-10
CG6530	CT20339	CG6536	e-104	>e-10	>e-10
CG6536	CT20351	CG6530	e-115	>e-10	
CG6965	CT21585	CG17795	e-12	>e-10	e-10
CG7476	CT22963	mth	e-66	>e-10	>e-10
CG13406	CT32762	CG17084	e-47	>e-10	>e-10
CG17084	CT33414	CG13406	e-47	>e-10	>e-10
CG17061	CT33415	mth	e-80	>e-10	>e-10
CG16992	CT37715	mth	e-32	>e-10	>e-10
C. Metabotropic glutamate receptor family					
GABA-B receptor-like					
CG3022	CT9836	CG6706	e-96	e-56	e-85
CG6706	CT20836	CG3022	e-120	e-61	e-169
CG15274	CT35221	CG3022	e-49	e-156	e-129
Metabotropic glutamate receptor-like					
CG8692	CT5032	Glu-RA	e-152	e-115	e-157
Glu-RA	CT31153	CG8692	e-144		e-142
Other					
CG7155	CT22117		>e-10	>e-10	>e-10
CG11923	CT35779	CG17215	e-17	>e-10	e-17
D. Atypical 7 TM proteins					
Frizzled-like					
fz	CT12089	fz3	e-37	e-76	e-99
fz2	CT27528	fz	e-76	e-106	e-175
fz3	CT32689	fz	e-37	e-33	e-52
CG4626	CT9057	fz	e-36	e-54	e-76
smo	CT9095	fz2	e-41	e-28	e-141
Others					
stan	CT20776	fat	e-144	e-176	
boss	CT24515		>e-10	>e-10	>e-10

Figure 1. Drosophila GPCR gene families. The newly identified GPCR genes are depicted in red. Left to right: gene name, defined transcript, closest *Drosophila* homologue, BLAST search e-values for the closest *Drosophila* (F), and *C. elegans* (W) and vertebrate (V) GPCRs. The # symbol indicates that the closest mammalian matches are from several different GPCR families. Previously identified *Drosophila* GPCRs (in black): (A) Rhodopsin-like: rhodopsins (Salcedo et al., 1999); mAcR-60C (Onai et al., 1989; Shapiro et al., 1989); dopamine receptors DopR and DopR2 (Gotzes et al., 1994; Feng et al., 1996); octopamine receptors: Ocr (Arakawa et al., 1990; Saudou et al., 1990) and Oamb (Han et al., 1998); serotonin receptors: 5-HT1A and 5-HT1B (Witz et al., 1990; Saudou et al., 1992); 5-HT2 (Colas et al., 1995) and 5-HT7 (Saudou et al., 1990); AlstR (Birgul et al., 1999); *rickets* (Ashburner et al., 1999); GRHR (Hauser et al., 1998); Takr99D (Li et al., 1991); Takr86C (Monnier et al., 1992); and NepYr (Li et al., 1992). (B) Secretin-like receptor: Methuselah (Lin et al., 1998). (C) Metabotropic glutamate receptor: Glu-RA (Parmentier et al., 1996).

Insects, and *Drosophila* in particular, have proven to be ideal experimental organisms for the study of the roles of biogenic amine signaling in development, learning, and addiction. Serotonin (5-HT) is involved in circadian rhythms, locomotion, feeding, learning, and memory in invertebrates. The 5-HT_2 receptor is known to play an early role in coordinating cell movements during gastrulation in *Drosophila* (Colas et al., 1999). Dopamine plays a role in the responses of *Drosophila* to nicotine and ethanol (Bainton et al., 2000). Targeted expression of either stimulatory or inhibitory G-α subunits in dopaminergic and serotoninergic neurons blocks behavioral sensitization to repeated cocaine exposures (Li et al., 2000). Octopamine and tyramine are monoamines thus far identified in arthropods and mollusks. Octopamine has been implicated in the establishment of associative learning in the honeybee (Hammer and Menzel, 1998) and tyramine is essential for sensitization to cocaine in *Drosophila* (McClung and Hirsh, 1999). We identified *Drosophila* receptors for most biogenic amines, with the exception of histamine. In fact, no histamine receptors have been cloned from invertebrates. However, histamine is thought to be the neurotransmitter for *Drosophila* photoreceptors (Hardie, 1987). Therefore, one or more of the unclassifiable biogenic amine receptors may serve the function of histamine receptor (Fig. 1). There is a large amount of evidence supporting the existence of purinergic transmission in invertebrates, but their receptors have never been cloned. The newly identified gene CG9753 encodes a receptor that shares homology with vertebrate adenosine receptors and may constitute the first invertebrate purinergic GPCR.

Peptide GPCRs

We identified 25 putative peptide GPCRs (Fig. 1), 18 of which represent newly discovered genes. The *Drosophila* peptide GPCRs were assigned to nine different ligand types. Approximately 30 different types of peptide GPCRs have been identified in vertebrates. Thus, there appears to be a paucity of peptide receptor types in *Drosophila*, suggesting that there will be fewer cognate peptide hormones in *Drosophila* than in vertebrates. *Drosophila* peptide GPCRs also appear to be more closely related to vertebrate than to *C. elegans* peptide GPCRs. This finding is

surprising given the extensive differences between insects and vertebrates in growth and hormonal regulation.

Sequence analyses of the novel putative *Drosophila* peptide GPCRs suggest roles for them in regulation of growth, fluid balance, visceral functions, and sexual development. Allatostatin is a 15–amino acid insect neuropeptide that inhibits juvenile hormone synthesis (Bendena et al., 1999). The receptors for LH, FSH, and TSH belong to a family of GPCRs characterized by large NH_2-terminal extracellular domains containing leucine repeats, which are important for interaction with glycoprotein ligands (Hsu et al., 1998). A mutant phenotype is known for only one *Drosophila* peptide GPCR: the *rickets* mutation, which leads to developmental defects suggesting a role for this receptor in limb development (Ashburner et al., 1999). The gene *rickets* (*rk*) bears homology to vertebrate leucine-rich repeat containing GPCRs. Another putative hormone receptor gene, CG6111, encodes a protein related to mammalian vasopressin receptors. Three novel *Drosophila* genes code for putative growth hormone secretagogue (GHS) receptors: CG8784, CG8795 (two closely related genes located in tandem on opposite strands of chromosome 3R), and CG9918. The vertebrate GHS receptors are involved in regulation of growth hormone release and their endogenous ligand is unknown. The presence of GHS-like receptors in *Drosophila* is provocative and should help to elucidate the identity of their ligands and the functions of their vertebrate homologues.

Orphan GPCRs

14 *Drosophila* GPCRs, 12 of which are newly described here, did not show significant sequence homology to functionally characterized receptors and were included in the orphan receptor group (Fig. 1). Most of these orphan GPCRs showed higher degrees of sequence identity to *C. elegans* than to vertebrate GPCRs. This could be explained because their vertebrate homologues have not yet been identified. Alternatively, these orphan GPCRs may play developmental or physiological roles common between *C. elegans* and *Drosophila.*

Secretin-like Receptor Family

The secretin-like family includes receptors for many hormones such as secretin, calcitonin, vasoactive intestinal peptide, and parathyroid hormone and related peptides. The secretin-like receptors are characterized by long NH_2-terminal domains containing five conserved cysteine residues that may form disulfide bonds and by short third cytoplasmic domains. We identified three novel GPCRs related to vertebrate calcitonin receptors (Fig. 1). Calcitonin receptors are involved in the regulation of Ca^{2+} homeostasis in vertebrates. Two receptors, encoded by CG8422 and CG12370, are related to insect diuretic hormone receptors (Fig. 1). Insect diuretic hormones are a group of peptides involved in the regulation of fluid and ion secretion (Reagan, 1994). The newly identified *Drosophila* diuretic hormone receptors share 57% sequence identity, suggestive of a gene duplication. One novel latrophilin-like receptor gene was also identified (CG8639). Latrophilins are a heterogeneous group of Ca^{2+}-independent receptors for α-latrotoxin, a potent presynaptic neurotoxin that stimulates massive neurotransmitter exocytosis leading to nerve terminal degeneration (Holz and Habener, 1998). The endogenous ligands for latrophilins are unknown and may be involved in control of synaptic exocytosis. Genes CG11318 and CG15556 define another subgroup in the secretin-like receptor family, coding for two novel receptors that share 41% sequence identity. These GPCRs are distantly related to the HE6 receptor, a human receptor of unknown function specifically expressed in the epididymis (Osterhoff et al., 1997).

Methuselah-like Receptor Family

Methuselah is a *Drosophila* GPCR involved in modulation of life span and stress response. The mutant line *methuselah*, with a heterozygous mutation in the *mth* gene, showed increased average life span and enhanced resistance to various forms of stress (Lin et al., 1998). The Methuselah receptor is also essential for normal development since flies homozygous for the *mth* mutation displayed pre-adult lethality. No counterparts for *mth* have been identified in vertebrates or *C. elegans.* We have identified 10 novel genes related to *mth* in the *Drosophila* genome (Figs. 2 and 3). Methuselah is most closely related to Mth-like 2 (CG17795; 60% sequence identity). Two gene clusters were identified in this family. The genes CG17084, CG17061, and *mth* form a cluster on chromosome 3L. CG6530 and CG6536 are located in tandem on chromosome 2R and share 76% sequence identity at the protein level, indicating a fairly recent duplication. CG16992 and CG7674 predict truncated receptors but their classification as potential pseudogenes needs experimental confirmation. Identification of the ligands for the Methuselah-like receptors should be of major biological interest.

Metabotropic Glutamate Receptor–like Family

The ligands for the metabotropic glutamate–like GPCRs include calcium ions and amino acid neurotransmitters glutamate and γ-amino butyric acid (GABA). Glutamate is a major excitatory neurotransmitter in invertebrates, whereas GABA is generally released from inhibitory

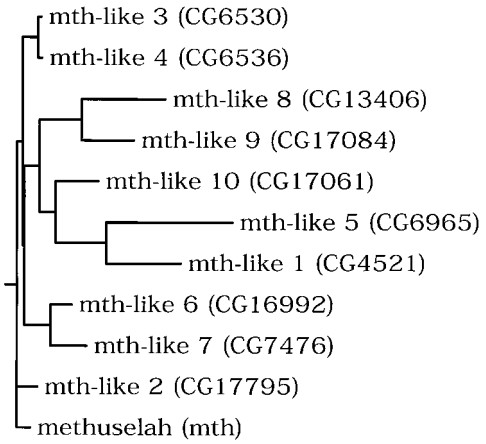

Figure 2. Phylogeny tree of the Methuselah-like subfamily performed using the neighbor-joining method.

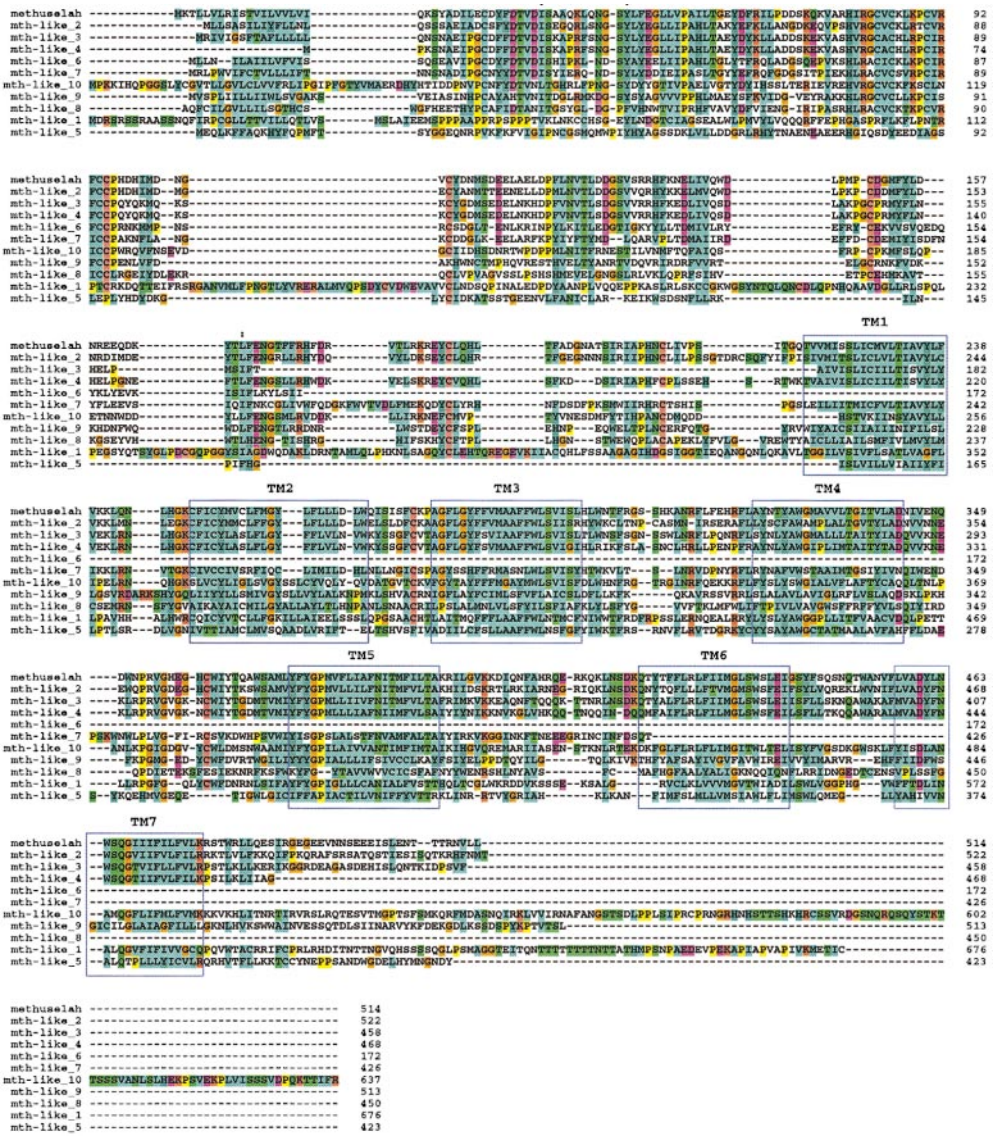

Figure 3. Multiple sequence alignment of the mth-like receptors performed using Clustal X. The topology of the seven putative TM domains is indicated.

synaptic terminals. The metabotropic glutamate–like GPCRs are characterized by very long NH_2-terminal extracellular domains containing ~17 conserved cysteine residues that may form disulfide bonds. Eight members of the metabotropic glutamate receptor–like family were identified in the *Drosophila* genome; seven of them are described here for the first time (Fig. 1). The novel metabotropic glutamate and GABA-B receptor–like genes show very high degrees of sequence conservation with their vertebrate homologues, suggesting similar roles in synaptic function.

Atypical 7 TM Proteins

The Frizzled-like proteins, Starry night (Flamingo) and Bride of sevenless, are defined here as atypical 7 TM proteins, a group of receptors that share the typical topology of GPCRs but show no sequence conservation with members of the other GPCR families (Fig. 1). These receptors are involved in tissue polarity and cell–cell signaling but their signal transduction pathways are unclear. However, there is evidence that a rat homologue of the Frizzled-like group couples to G proteins (Slusarski et al., 1997). We identified a novel atypical 7 TM protein gene, CG4626, which encodes a Frizzled-like protein that is more closely related to mammalian Frizzled 4 than to other *Drosophila* Frizzled-like proteins.

Starry night (Stan) is a complex protein containing 7 TM domains and several cadherin, EGF-like, and laminin G domains. The *stan* gene may have evolved from the combination of ancestral genes coding for a secretin-like GPCR and a cell adhesion molecule. In *Drosophila*, Stan is implicated in establishment of tissue polarity (Taylor et al., 1998). A novel atypical 7 TM protein that may be distantly related to secretin-like GPCRs is encoded by CG20776, which contains multiple TM domains and several leucine-rich repeats thought to be involved in protein–protein interactions. Bride of sevenless (Boss) is another atypical 7 TM protein that might be distantly related to the metabotropic glutamate–like GPCRs.

In conclusion, GPCRs constitute a very large superfamily of proteins that play a central role in eukaryotic signal transduction. The families of typical GPCRs include the

rhodopsin-like, secretin-like, and metabotropic glutamate–like receptors, fungal mating pheromone, Dictyostelium cAMP receptors, and *C. elegans* chemoreceptors. Additionally, there are three putative (or atypical) GPCR families: the Frizzled-like receptors and *Drosophila* olfactory and putative taste receptors (Clyne et al., 2000; Rubin et al., 2000). All the different GPCR families share the same seven membrane–spanning domain topology. The evolutionary relationship between the different families is uncertain since there are no significant degrees of sequence similarity between them. It is likely that they have evolved independently and convergently adopted the G protein signal transduction pathway.

Our analysis focused on the typical *Drosophila* GPCRs, particularly the neurotransmitter and hormone GPCRs, and how they compare with those found in vertebrates and *C. elegans*. Most of the 100 *Drosophila* GPCRs described in Fig. 1 show a high degree of sequence conservation with vertebrate GPCRs. Only eight *Drosophila* GPCRs appear to be more closely related to *C. elegans* that to vertebrate receptors. We have identified 68 novel *Drosophila* GPCRs including the *mth*-like receptors, a unique subfamily of GPCRs that appears to have no counterpart in vertebrates or *C. elegans*. There is evidence indicating that the *mth*-like receptor subfamily plays an important role in *Drosophila* development, stress response, and regulation of life span (Lin et al., 1998).

There has been a large expansion and diversification of chemoreceptors in *C. elegans*. There is also evidence of an expansion of the peptide receptors in vertebrates and odorant receptors in mammals. *Drosophila* GPCRs have not expanded to a similar degree: in particular there appears to be a lower number of peptide receptors than expected. This is somewhat surprising, since it has been suggested that peptide transmitters predate biogenic amines in evolution (Walker et al., 1996). In *C. elegans*, the expansion of GPCR genes is mirrored by an expansion in G protein subunits: 20 α-, 2 β-, and 2 γ-subunit genes have been identified in the *C. elegans* genome (Bargmann, 1998). In contrast, the *Drosophila* genome contains only 6 α-, 3 β-, and 2 γ-subunit genes.

The organization of the GPCR genes in *Drosophila* genome shows several differences with other eukaryotic genomes analyzed to date. GPCR genes form large clusters in the genomes of *C. elegans* and mammals. In contrast, only small clusters of GPCR genes were identified in the *Drosophila* genome: six consisting of two genes and one of three genes. Substantial proportions of the vertebrate GPCR genes are thought to be intronless, but only 5 out of the 100 *Drosophila* GPCR genes described here were predicted to be intronless. The *C. elegans* and mammalian genomes contain a large number of GPCR pseudogenes. We identified only eight genes in the *Drosophila* genome that appear to code for incomplete GPCRs, but their identity as pseudogenes will require further experimental investigation.

Now that the full repertoire of *Drosophila* GPCRs is known, the next step is to match the newly identified receptors with their cognate ligands and biological functions. Systematic mutation of the *Drosophila* GPCRs will help determine their roles in development, neural function, and behavior and may also yield insights into the functions and mutational pathologies of their vertebrate homologues. For example, it is becoming clear that substantial overlap exists in the biological components of addiction in vertebrates and flies; consequently *Drosophila* should prove invaluable as a model for the study of addiction. Although it has served as a model organism for nearly a century, *Drosophila* has now been cast in a new role, which should further the investigation of the mechanisms of development, neural function, and disease, for which the analyses of GPCRs will prove crucial.

The authors would like to thank Kristin Scott for sharing information on *Drosophila* GPCRs; and Judith Brody, Leslie Vosshall, Harold Gainer, and Joseph Campbell for their helpful comments about the manuscript.

Submitted: 7 April 2000
Revised: 23 June 2000
Accepted: 23 June 2000

References

Adams, M.D., S.E. Celniker, R.A. Holt, C.A. Evans, J.D. Gocayne, P.G. Amanatides, S.E. Scherer, P.W. Li, R.A. Hoskins, R.F. Galle, et al. 2000. The genome sequence of *Drosophila melanogaster*. *Science*. 287:2185–2195.

Arakawa, S., J.D. Gocayne, W.R. McCombie, D.A. Urquhart, L.M. Hall, C.M. Fraser, and J.C. Venter. 1990. Cloning, localization, and permanent expression of a *Drosophila* octopamine receptor. *Neuron*. 4:343–354.

Ashburner, M., S. Misra, J. Roote, S.E. Lewis, R. Blazej, T. Davis, C. Doyle, R. Galle, R. George, N. Harris, et al. 1999. An exploration of the sequence of a 2.9-Mb region of the genome of *Drosophila melanogaster*: the Adh region. *Genetics*. 153:179–219.

Bainton, R.J., L.T. Tsai, C.M. Singh, M.S. Moore, W.S. Neckameyer, and U. Heberlein. 2000. Dopamine modulates acute responses to cocaine, nicotine and ethanol in *Drosophila*. *Curr. Biol*. 10:187–194.

Bargmann, C.I. 1998. Neurobiology of the *Caenorhabditis elegans* genome. *Science*. 396:2028–2033.

Bendena, W.G., B.C. Donly, and S.S. Tobe. 1999. Allatostatins: a growing family of neuropeptides with structural and functional diversity. *Ann. NY Acad. Sci*. 897:311–329.

Birgul, N., C. Weise, H.J. Kreienkamp, and D. Richter. 1999. Reverse physiology in *Drosophila*: identification of a novel allatostatin-like neuropeptide and its cognate receptor structurally related to the mammalian somatostatin/galanin/opioid receptor family. *EMBO (Eur. Mol. Biol. Organ.) J*. 18:5892–5900.

Clyne, P.J., C.G. Warr, and J.R. Carlson. 2000. Candidate taste receptors in *Drosophila*. *Science*. 287:1830–1834.

Colas, J.F., J.M. Launay, O. Kellermann, P. Rosay, and L. Maroteaux. 1995. *Drosophila* 5-HT2 serotonin receptor: coexpression with fushi-tarazu during segmentation. *Proc. Natl. Acad. Sci. USA*. 92:5441–5445.

Colas, J.F., J.M. Launay, J.L. Vonesch, P. Hickel, and L. Maroteaux. 1999. Serotonin synchronises convergent extension of ectoderm with morphogenetic gastrulation movements in *Drosophila*. *Mech. Dev*. 87:77–91.

Feng, G., F. Hannan, V. Reale, Y.Y. Hon, C.T. Kousky, P.D. Evans, and L.M. Hall. 1996. Cloning and functional characterization of a novel dopamine receptor from *Drosophila melanogaster*. *J. Neurosci*. 16:3925–3933.

Gärtner, W., and P. Towner. 1995. Invertebrate visual pigments. *Photochem. Photobiol*. 62:1–16.

Gotzes, F., S. Balfanz, and A. Baumann. 1994. Primary structure and functional characterization of a *Drosophila* dopamine receptor with high homology to human D1/5 receptors. *Receptors Channels*. 2:131–141.

Hammer, M., and R. Menzel. 1998. Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. *Learn. Mem*. 1998 5:146–156.

Han, K.A., N.S. Millar, and R.L. Davis. 1998. A novel octopamine receptor with preferential expression in *Drosophila* mushroom bodies. *J. Neurosci*. 18:3650–3658.

Hardie, R.C. 1987. Is histamine a neurotransmitter in insect photoreceptors? *J. Comp. Physiol. [A]*. 161:201–213.

Hauser, F., L. Sondergaard, and C.J. Grimmelikhuijzen. 1998. Molecular cloning, genomic organization and developmental regulation of a novel receptor from *Drosophila melanogaster* structurally related to gonadotropin-releasing hormone receptors for vertebrates. *Biochem. Biophys. Res. Commun*. 249: 822–828.

Holz, G.G., and J.F. Habener. 1998. Black widow spider alpha-latrotoxin: a presynaptic neurotoxin that shares structural homology with the glucagon-like peptide-1 family of insulin secretagogic hormones. *Comp. Biochem. Physiol. B Biochem. Mol. Biol*. 121:177–184.

Hsu, S.Y., S.G. Liang, and A.J. Hsueh. 1998. Characterization of two LGR genes homologous to gonadotropin and thyrotropin receptors with extracellular leucine-rich repeats and a G protein-coupled, seven-transmembrane

region. *Mol. Endocrinol.* 12:1830–1845.
Li, X.J., W. Wolfgang, Y.N. Wu, R.A. North, and M. Forte. 1991. Cloning, heterologous expression and developmental regulation of a *Drosophila* receptor for tachykinin-like peptides. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3221–3229.
Li, X.J., Y.N. Wu, R.A. North, and M. Forte. 1992. Cloning, functional expression, and developmental regulation of a neuropeptide Y receptor from *Drosophila melanogaster*. *J. Biol. Chem*. 267:9–12.
Li, H., S. Chaney, M. Forte, and J. Hirsh. 2000. Ectopic G-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in *Drosophila melanogaster*. *Curr. Biol.* 10:211–214.
Lin, Y.J., L. Seroude, and S. Benzer. 1998. Extended life-span and stress resistance in the *Drosophila* mutant *methuselah*. *Science.* 282:943–946.
McClung, C., and J. Hirsh. 1999. The trace amine tyramine is essential for sensitization to cocaine in *Drosophila*. *Curr. Biol.* 9:853–860.
Monnier, D., J.F. Colas, P. Rosay, R. Hen, E. Borrelli, and L. Maroteaux. 1992. NKD, a developmentally regulated tachykinin receptor in *Drosophila*. *J. Biol. Chem*. 267:1298–1302.
Onai, T., M.G. Fitzgerald, S. Arakawa, J.D. Gocayne, D.A. Urquhart, L.M. Hall, C.M. Fraser, W.R. McCombie, and J.C. Venter. 1989. Cloning, sequence analysis and chromosome localization of a *Drosophila* muscarinic acetylcholine receptor. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 255:219–225.
Osterhoff, C., R. Ivell, and C. Kirchhoff. 1997. Cloning of a human epididymis-specific mRNA, HE6, encoding a novel member of the seven transmembrane-domain receptor superfamily. *DNA Cell Biol.* 16:379–389.
Parmentier, M.L., J.P. Pin, J. Bockaert, and Y. Grau. 1996. Cloning and functional expression of a *Drosophila* metabotropic glutamate receptor expressed in the embryonic CNS. *J. Neurosci.* 16:6687–6694.
Peroutka, S.J., and T.A. Howell. 1994. The molecular evolution of G protein-coupled receptors: focus on 5-hydroxytryptamine receptors. *Neuropharmacology*. 33:319–324.
Provencio, I., G. Jiang, W.J. De Grip, W.P. Hayes, and M.D. Rollag. 1998. Melanopsin: an opsin in melanophores, brain, and eye. *Proc. Natl. Acad. Sci. USA*. 95:340–345.
Reagan, J.D. 1994. Expression cloning of an insect diuretic hormone receptor. A member of the calcitonin/secretin receptor family. *J. Biol. Chem*. 269:9–12.
Rubin, G.M., M.D. Yandell, J.R. Wortman, G.L. Gabor Miklos, C.R. Nelson, I.K. Hariharan, M.E. Fortini, P.W. Li, R. Apweiler, W. Fleischmann, et al. 2000. Comparative genomics of the eukaryotes. *Science.* 287:2204–2216.
Salcedo, E., A. Huber, S. Henrich, L.V. Chadwell, W.H. Chou, R. Paulsen, and S.G. Britt. 1999. Blue- and green-absorbing visual pigments of *Drosophila*: ectopic expression and physiological characterization of the R8 photoreceptor cell-specific Rh5 and Rh6 rhodopsins. *J. Neurosci.* 19:10716–10726.
Saudou, F., N. Amlaiky, J.L. Plassat, E. Borrelli, and R. Hen. 1990. Cloning and characterization of a *Drosophila* tyramine receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 91:3611–3617.
Saudou, F., U. Boschert, N. Amlaiky, J.L. Plassat, and R. Hen. 1992. A family of *Drosophila* serotonin receptors with distinct intracellular signalling properties and expression patterns. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:7–17.
Shapiro, R.A., B.T. Wakimoto, E.M. Subers, and N.M. Nathanson. 1989. Characterization and functional expression in mammalian cells of genomic and cDNA clones encoding a *Drosophila* muscarinic acetylcholine receptor. *Proc. Natl. Acad. Sci. USA*. 86:9039–9043.
Slusarski, D.C., V.G. Corces, and R.T. Moon. 1997. Interaction of Wnt and a Frizzled homologue triggers G-protein-linked phosphatidylinositol signalling. *Nature.* 390:410–413.
Taylor, J., N. Abramova, J. Charlton, and P.N. Adler. 1998. Van gogh. A new *Drosophila* tissue polarity gene. *Genetics.* 150:199–210.
Venter, J.C., U. di Porzio, D.A. Robinson, S.M. Shreeve, J. Lai, A.R. Kerlavage, S.P. Fracek, Jr., K.U. Lentes, and C.M. Fraser. 1988. Evolution of neurotransmitter receptor systems. *Prog. Neurobiol.* 30:105–169.
Walker, R.J., H.L. Brooks, and L. Holden-Dye. 1996. Evolution and overview of classical transmitter molecules and their receptors. *Parasitology*. 113(Suppl):3–33.
Witz, P., N. Amlaiky, J.-L. Plassat, L. Maroteaux, E. Borrelli, and R. Hen. 1990. Cloning and characterization of a *Drosophila* serotonin receptor that activates adenylate cyclase. *Proc. Natl. Acad. Sci. USA*. 87:8940–8944.