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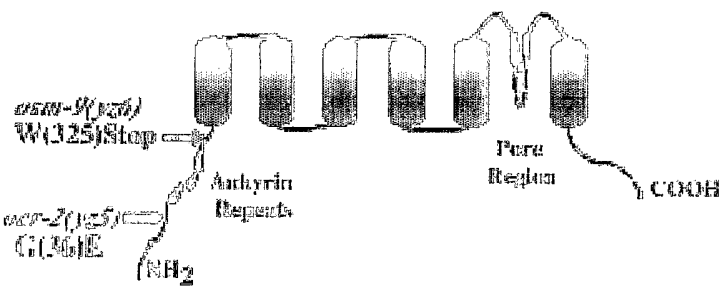
(54) Title: METHODS FOR SCREENING THERAPEUTIC AGENTS FOR SEROTONIN RELATED DISEASES AND OTHER DISORDERS



(57) Abstract: The invention provides methods of screening for compounds which affect the synthesis of serotonin, using the nematode worm C. *elegans*. The invention further provides methods for screening therapeutic agents for serotonin-related diseases.

METHODS FOR SCREENING THERAPEUTIC AGENTS FOR SEROTONIN RELATED DISEASES AND OTHER DISORDERS

BACKGROUND OF THE INVENTION

The monoamine 5HT acts as a neurotransmitter and a hormone to induce behavioral and endocrine responses to changes in environmental and physiologic states. A deficit in 5HT is implicated in a broad spectrum of disorders such as depression, eating disorders and type II diabetes (reviewed by Lucki, 1998; Leibowitz and Alexander, 1998; Davidson et al., 2000). In both vertebrates and invertebrates, 5HT is synthesized in a set of neurons with diverse synaptic properties and connectivities. Activation of serotonergic neurons by sensory stimuli was first clearly demonstrated in 1976 through studies in *Aplysia*, where sensory stimuli increase 5HT signals to induce synaptic facilitation and behavioral sensitization (Brunelli et al., 1976). Long-standing questions are: how do serotonergic neurons integrate sensory signals into 5HT neurotransmission, and are different serotonergic neurons regulated by cell-specific molecular mechanisms?

TRP-related proteins are a superfamily of cation channels that share structural homology to the Drosophila transient receptor potential (TRP) protein (reviewed by Clapham et al., 2001; Montell et al., 2002). TRP channels act as molecular integrators of a wide range of chemical and physical stimuli to regulate behavior and physiological function in both vertebrates and invertebrates (reviewed by Scott and Zuker, 1998; Minke and Cook, 2002). Five genes in the *C. elegans* genome, *ocr-1, ocr-2, ocr-3, ocr-4* and *osm-9*, encode TRPV subfamily proteins characterized by cytoplasmic N-terminal multiple ankyrin repeats, six transmembrane segments and a non-conserved cytoplasmic C terminus (Harteneck et al., 2000; Tobin et al., 2002). These TRPV genes are expressed in the sensory endings of chemosensory neurons and have been shown to regulate sensory functions and social behavior (Colbert et al., 1997; Tobin et al., 2002; de Bono et al., 2002). In mammals, TRPV channels transmit nociceptive stimuli such as pain (Tominaga et al., 1998) and noxious heat (Caterina et al., 1997; Caterina et al., 1999). In addition, mammalian TRPV ion channels also mediate the response to growth factors (Kanzaki et al., 1999). Agonists to TRPV channels induce hypothermia (Meller et al., 1992; Szallasi and Blumberg, 1996) and modulate oxygen consumption (Colquhoun et al., 1995). Mammalian TRPV proteins are expressed in the sensory neurons, as well as in the CNS (Tominaga et al., 1998; Hayes et al., 2000; Mezey et al., 2000; Delany et al., 2001). It has been postulated that TRPV ion channels regulate the release of neural mediators to modulate endocrine activity (Szallasi and Blumberg, 1996), but in vivo evidence has not yet been reported.

Herein, we describe the effect of mutations in two TRPV ion channel genes, *osm-9 and ocr-2*, on biosynthesis of 5HT in *C. elegans*. Previously, we demonstrated that the *tph-1*

gene, which encodes the key 5HT biosynthesis enzyme tryptophan hydroxylase, is essential for 5HT biosynthesis and that *tph-1* expression is regulated by cell-specific mechanisms (Sze et al., 2000; Sze et al., 2002). Here, we show that a signaling pathway involving the *osm-9* and *ocr-2* TRPV channel proteins, CaMKII specifically regulates *tph-1* expression in the serotonergic chemosensory neurons ADF. Results from this study reveal a remarkably neuron-specific mechanism regulating 5HT biosynthesis and provide genetic insights into how a neuron transduces the events at the cell-surface to 5HT signaling.

Thus, in the present invention, the present inventor has used the nematode *C. elegans* as a model animal system to identify and analyze genes and molecular pathways that control the level of serotonin synthesis in specific serotonergic neurons. This research is motivated by two facts: overwhelming evidence that neuronal components and signaling pathways are highly conserved across phyla, and powerful *C. elegans* genetics that permits unbiased search and characterizes genes acting in specific serotonergic neurons. Thus, genes identified in *C. elegans* will generate candidate drug targets for human diseases.

SUMMARY OF THE INVENTION

In one embodiment, the present invention relates to a transgenic model animal useful for screening therapeutic agents for serotonin-related diseases, comprising a mutation in a gene encoding a TRPV channel protein and a stably integrated *tph1::*marker fusion gene, wherein the mutation results in reduced *tph1::*marker expression. A marker may be any gene whose expression gives rise to a detectable phenotype. In a preferred embodiment, the marker is *gfp* or other fluorescent protein-encoding gene. Other suitable markers will be apparent to one of skill in the art.

Another embodiment of the present invention relates to a transgenic model animal useful for screening therapeutic agents for serotonin- related diseases, comprising a mutation in *osm-9* and a stably integrated *tph1::*marker fusion gene, wherein the mutation results in reduced *tph1::*marker expression. A marker may be any gene whose expression gives rise to a detectable phenotype.

Another embodiment of the present invention relates to a transgenic model animal useful for screening therapeutic agents for serotonin- related diseases, comprising a mutation in *ocr-2* and a stably integrated *tph1::*marker fusion gene, wherein the mutation results in reduced tph1::marker expression.

Another embodiment of the present invention relates to a method for screening therapeutic agents for serotonin-related diseases, comprising the steps of: providing a model animal as recited above; dispersing a target drug into agar; culturing the model animal on the agar with drug; and detecting expression of green fluorescent protein (GFP).

Another embodiment of the present invention relates to a method for screening therapeutic agents for serotonin-related diseases, comprising the steps of: providing a model

animal as recited above; dispersing a target drug into agar; culturing the model animal on the agar with drug; and detecting expression of green fluorescent protein (GFP).

Another embodiment of the present invention relates to a method of identifying compounds which enhance or up-regulate the synthesis of serotonin, which method comprises: contacting *C. elegans* which exhibit reduced serotonin synthesis compared to wild type *C. elegans* in one or more cell types or tissues with a compound under test; and detecting a phenotypic, biochemical or behavioral change in the *C. elegans* indicating a reversion towards wild type serotonin synthesis in the one or more cell types or tissues which exhibit reduced serotonin synthesis in the absence of the compound.

Another embodiment of the present invention relates to a method of identifying compounds which enhance or up-regulate the synthesis of serotonin, which method comprises: contacting *C. elegans* which exhibit reduced serotonin synthesis compared to wild type *C. elegans* in one or more cell types or tissues due to a defective TRPV channel protein with a compound under test; and detecting a phenotypic, biochemical or behavioral change in the *C. elegans* indicating a reversion towards wild type serotonin synthesis in the one or more cell types or tissues which exhibit reduced serotonin synthesis in the absence of the compound. As used herein, a "defective TRPV channel" is one that results in reduced serotonin synthesis as compared to normal or "wild type" levels of serotonin synthesis, and may result from any alteration in the gene encoding the TRPV channel protein, including but not limited to, base substitution, missense and nonsense mutations, or any posttranslational modification of the TRPV channel protein, so as to result in reduced serotonin synthesis.

Still another embodiment of the present invention relates to a transgenic model animal useful for screening therapeutic agents for type II diabetes, comprising *ocr-2(yz5)*, *daf-7(e1372)* and a stably integrated *tph1::*marker fusion gene, wherein the mutation results in reduced *tph1::*marker expression. *daf-7* encodes a TGF-β molecule (Schackwitz et al.,Neuron 17, 719-728, 1996; Ren et al., Science 274, 1389-1391, 1996).

Still another embodiment of the present invention relates to a method for screening therapeutic agents for type II diabetes, comprising the steps of providing a model *C. elegans* as recited above; dispersing a target drug into agar; culturing the *C. elegans* on the agar with drug; and detecting a progression of the development of the *C. elegans* past the Dauer larval stage in the presence of the target drug.

Still another embodiment of the present invention relates to isolated nucleic acids and purified polypeptides for a novel mutant *ocr-2(yz5)* having a G to A base change at nucleotide position 107 downstream from the translational start, changing amino acid 36 from the wild-type glycine (Gly) to glutamic acid (Glu). This mutant effects *tph-1* expression specifically in the ADF chemosensory neurons. The nucleotide sequence of wild type *ocr-2* is at NCBI AF047660; the amino acid sequence of the *ocr-2(yz5)* mutant is shown as SEQ ID NO. 1.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Cell-specific effects of *yz5* and *yz6* mutations on 5HT biosynthesis. (A) The position of serotonergic neurons in the head, and the axon from HSN (shown in red). Also shown are the nonserotonergic amphid chemosensory neurons that express both *osm-9* and *ocr-2* (in green) (Tobin et al., 2002). The drawing is adapted, with permission, from Starich et al. (Starich et al., 1995). **(B) GFP expression of an integrated *tph-1::gfp* fusion gene in wild-type and mutant animals.** In wild type, GFP is strongly expressed in the ADF, NSM and HSN neurons. *yz5* and *yz6* mutations specifically downregulate the GFP expression in the ADF chemosensory neurons. Note that neither the mutation alone, nor *yz5;yz6* double mutation affects GFP levels in NSM and HSN. *tph-1::gfp* expression in ADF is restored in the mutant animals carrying a wild-type *osm-9* or *ocr-2* transgene, respectively. **(C) Anti-5HT antibody staining of wild-type and mutant animals.** *yz5* and the *yz6* mutant animals show reduction or absence of 5HT immunoreactivity in the ADF neurons. ADF 5HT immunoreactivity is recovered in the mutants carrying a wild-type *osm-9* or *ocr-2* transgene. All the animals shown are adult hermaphrodites. Anterior is towards the left. Quantification of *tph-1::gfp* expression in various genetic backgrounds is presented in Table 1, below. **(D) A schematic representation of the domain organization of the TRPV subfamily.** The approximate site of the *yz6* and *yz5* mutation in the channel structure is indicated. The drawing is adapted, with permission, from Gunthorpe et al. (Gunthorpe et al., 2002).

Fig. 2. Mutations in the TRPV channel proteins do not cause general cell fate transformation of the ADF neurons. (A) Expression of a *ocr-2::gfp* reporter in a wild-type animal. Both *osm-9* and *ocr-2* are expressed in ADF (Tobin et al., 2002), but not in other serotonergic neurons. **(B-D) Expression of ADF markers in *osm-9(yz6)* and *ocr-2(yz5)* mutants.** The GFP reporters were examined in wild-type, *yz6* and *yz5* mutant animals. The expression patterns in wild-type animals have been published: *lin-11::gfp* (Hobert et al., 1998), and *cat-1::gfp* and *gtpch-1::gfp* (Sze et al., 2002). Representative examples of the reporter expression patterns in mutant animals are shown. **(B) The expression of *lin-11::gfp* overlaps with *osm-9* and *ocr-2* in the ADF and ADL chemosensory neurons.** Shown is a *yz6* mutant animal strongly expressing *lin-11::gfp* in ADF and ADL. The position of the cell body and the morphology of the axon and dendrite are normal (indicated by arrowheads). **(C) A GFP reporter of the CAT-1/vesicular monoamine transporter is localized to the synapses of the serotonergic neurons and dopaminergic neurons** (Sze et al., 2002). Unlike the dramatic reduction of *tph-1::gfp* expression in the ADF neurons (Fig. 1B, Table 1), *cat-1::gfp* can be clearly observed in the ADF neurons of *yz5* and *yz6* mutants. We have repeatedly observed that *cat-1::gfp* expression in the ADF neurons is relatively weaker in *yz5* and *yz6* mutants than in wild-type animals, but the differences were not statistically significant. The GFP is distributed as punctate pattern, presumably the fusion CAT-1 is associated with synaptic components. **(D)**

A GFP reporter of a probable GTP-cyclohydrolase I gene *gtpch-1* is strongly expressed in serotonergic and dopaminergic neurons (Sze et al., 2002). Shown is a *yz6* animal expressing *gtpch-1::gfp* in ADF and in other serotonergic neurons.

Fig. 3. TRPV channel-dependent regulation of *tph-1* expression is mediated by a specific cis-regulatory region of *tph-1* and is modulated by *unc-43* CaMKII. (A) Expression of a GFP reporter under the control of the sequence –132 to –377 of *tph-1* and a *pes-10* minimal promoter in wild-type and *osm-9* mutant animals. In wildtype animals, GFP is strongly expressed in the ADF neurons, but not in any other serotonergic neuron, indicating that this *tph-1* cisregulatory region specifically mediates *tph-1* expression in the ADF neurons. The ADF expression of this GFP reporter is significantly reduced in *yz6* mutant animals; hence, the TRPV signaling stimulates this neuron-specific transcriptional mechanism. In addition to ADF, this GFP reporter is often expressed in two other neurons, which are tentatively identified as the ASI chemosensory neurons based on the relative position of the cell body (White et al., 1986). The GFP intensity in ASI is not significantly affected by the TRPV mutation. **(B) *tph-1::gfp* expression is unaffected by Gα mutations but is modulated by unc-43 CaMKII activity.** All the strains bear the same GFP reporter. The average of ADF GFP intensity in wild-type animals is defined as 1, and the average GFP intensity in other strains is normalized against the wild-type average. The data are the summary of four independent trials, and the total number of animals scored for each strain is indicated next to the bar. Error bars indicate the mean of the standard error (s.e.m.).

Fig. 4. Effects of the TRPV genes on 5HT-modulated behaviors. (A) Dauer metabolic arrest. Similar to the *tph-1* deletion mutation, the TRPV mutations and another ADF-5HT deficit mutation, *nss-1(yz12)*, enhance Dauer arrest of the *daf-7(e1372)* mutation at the 15°C growth temperature. This enhanced Dauer phenotype is suppressed by a deletion mutation of the *daf-16* gene, which is a negative target of the DAF-2/insulin signaling pathway. We noticed that *daf-7;osm-9;daf-16* animals grow slower and sometimes form Dauer-like larvae but then go on to develop to adults, suggesting that the *daf-2* pathway may not be the only signaling affected in the mutant. The *Plin-11::osm-9(+)* transgene was carried as an extrachromosomal array, only the animals carrying the transgenic marker were scored. The *osm-9(yz6)* (n=956), or *ocr-2(yz5)* (n=346) mutants alone do not form Dauers under the assay condition. **(B) Egg-laying behavior.** Unlike *tph-1* mutant animals, the TRPV mutant adults do not accumulate a large amount of fertilized eggs in the uterus. **(C) Feeding behavior.** *tph-1* mutant animals have no detectable 5HT in any serotonergic neuron and exhibit slower pharyngeal pumping rates, whereas animals bearing a mutation in the TRPV genes pump at rates similar to wild-type animals. The feeding and egg-laying behavior represents the summary of two independent trials, ten animals/strain/trial. The Dauer assay is the summary of three independent trials, each in duplicate. Error bars indicate the s.e.m.

Fig. 5. A. α-5HT staining (ADF); B. Bar graph showing osmotic avoidance (ASH); C. Bar graph showing olfactory attraction; D. Bar graph showing diacetyl sensitivity.

Fig. 6. Photograph showing both OCR-2::FLAG and OCR-2(G36E)::FLAG in the plasma membrane and ciliated endings of sensory neurons.

Fig. 7. Comparison of the amino acid sequences of OCR3, mTRPV-2, hTRPV-2, and OCR4.

Fig. 8. A. Mouse and human TRPV cDNA tagged with a FLAG epitope under the control of the *ocr-2* promoter in *ocr-2* deletion mutants. Similar to OCR-2::FLAG, mouse and human TRPV2::FLAG is distributed in the plasma membrane and ciliated endings; **B.** *ocr-2* mutants expressing either mouse or human TRPV2::FLAT exhibit substantially increased tph-1 expression in ADF; **C, D.** Bar graphs showing that no improvement in the response of the transgenic animals to diacetyl or high osmolarity solutions was detected.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

All publications mentioned herein and in any attached documents are incorporated herein by reference for the purpose of describing and disclosing, for example, the compositions and methodologies that are described in the publications which might be used in connection with the presently described invention. The publications listed or discussed above, below and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

Overview: Regulation of serotonin synthesis in a pair of chemosensory neurons by two TRPV cationic channel proteins. Through a genetic screen for mutations that reduce/eliminate serotonin synthesis in specific serotonergic neurons, the present inventor has isolated a set of five mutant genes that dramatically reduce the transcription of the *tph-1* gene, which encodes a key serotonin synthesis enzyme tryptophan hydroxylase, in a pair of the ADF chemosensory neurons. But, these mutations have no effect on *tph-1* expression in any other serotonergic neurons. Genetic mapping and positional cloning revealed that two mutant genes *nss-2* and *nss-3* are alleles of two TRPV cationic channel proteins previously identified as *osm-9* and *ocr-2*, respectively: *osm-9(yz6)* is a nonsense mutation that results in a deletion of the conserved ankyrin motifs and transmembrane regions of the protein, and *ocr-2(yz5)* is a missence mutation at the front of the ankyrin motifs.

Although the Cori Bargmann research group at UC San Francisco first identified the *osm-9* and *ocr-2* genes encoding TRPV channel proteins, the present inventor has discovered the genetic linkage between the TRPV channel proteins and the regulation of neuron-specific serotonin synthesis. Furthermore, the present inventor has isolated a mutation that specifically affects serotonin synthesis but not other functions of the *nss-3/ocr-2* gene. This observation suggests a specific signaling pathway downstream of the TRPV channels regulating serotonin synthesis. Support for this argument is found in our misexpression experiments showing that the *osm-9* and *ocr-2* TRPV channels act in the ADF serotonergic neurons to regulate the *tph-1* expression.

Another important discovery made by the present inventor is the functional linkage between the *osm-9* and *ocr-2* TRPV channel proteins and neuroendocrine pathways that control metabolic homeostasis. Both the *osm-9(yz6)* and *ocr-2(yz5)* mutations cause animals to arrest at a metabolic inactive larval stage, called dauer. Studies by others have shown that a TGF-beta and an insulin signaling pathway act in parallel to regulate whether an animal enters the reproductive lifecycle or developmentally arrests as a dauer larva; disruption of either pathway is sufficient to cause constitutive dauer arrest. Our genetic analysis indicates that the *osm-9* and *ocr-2* mutations are likely affecting the insulin but not the TGF-beta pathway, indicating the TRPV channels may be a component in the insulin-mediated neuroendocrine signaling pathway.

This work provides the first genetic evidence that serotonin synthesis is regulated by ion channels. This mechanism is very likely conserved in mammals. First, there is a large volume of literature showing that TRPV channel activity and serotonin have similar effects on behavior and physiology. This indicates that TRPV channel and serotonin function in the same physiologic system. Second, the mammalian homologues TRPVl and TRPVLI channel proteins are expressed in the serotonergic neurons. Thus, TRPV channel activity may also regulate serotonin signaling in mammals. Third, selective agonists of the TRPV 1 channel regulate the transcription of signaling components, such as neuropeptides, and enzyme nitric oxide synthase. These observations indicate that regulation of the expression level of neuronal signaling molecules is a common output of TRPV channel activity. The fact that the function of TRPV channels is phyletically conserved indicates that *C. elegans* may serve as a simple animal system to explore physiological function of TRPV ion channels and to identify additional components in the signaling pathway.

The method of the invention, which will be hereinafter referred to as the 'serotonin assay' is performed using a *C. elegans* strain which exhibits reduced serotonin activity in one or more cell types or tissues, as compared to the serotonin activity in wild-type *C. elegans*. In a preferred embodiment of the present invention, the serotonin assay is performed using a *C. elegans* strain have reduced serotonin activity specifically in ADF neurons. In another preferred embodiment of the present invention, the serotonin assay is performed using a *C.*

elegans strain having a defective TRPV channel protein. It has been observed that worms which exhibit reduced serotonin activity compared to wild-type worms manifest a variety of phenotypic and behavioral defects. The basis of the serotonin assay is therefore to take worms which exhibit defects due to reduced serotonin activity, contact these worms with the compound under test and screen for phenotypic, behavioral or biochemical changes indicating a reversion towards wild-type serotonin activity. For example, worms with reduced serotonin activity show a developmental arrest at the Dauer larval stage. In this case, screening for developmental progression past the Dauer larval stage in the presence of a test compound would indicate a reversion towards wild-type serotonin activity due to the ability of the compound to enhance or up-regulate serotonin. For comparison purposes, an example of a C. elegans strain which exhibits 'wild-type' serotonin activity is the N2 strain (this strain can be obtained from CGC, University of Minn., USA). The N2 strain has been particularly well characterized in the literature with respect to properties such as developmental progression (see Methods in Cell Biology, Volume 48, Caenorhabditis elegans: Modern biological analysis of an organism, ed. by Henry F. Epstein and Diane C. Shakes, 1995 Academic Press; The nematode Caenorhabditis elegans, ed. by William Wood and the community of C. elegans researchers., 1988, Cold Spring Harbor Laboratory Press; C. elegans II, ed. by Donald L. Riddle, Thomas Blumenthal, Barbara J. Meyer and James R. Priess, 1997, Cold Spring Harbor Laboratory Press.).

C. elegans which exhibit reduced serotonin activity in one or more cell types or tissues can be obtained in several different ways. In a first embodiment, worms with reduced serotonin activity are obtained by treating a culture of worms with a chemical inhibitor of TRPV channel proteins such as, for example, EGTA.

In still further embodiment, the C elegans exhibiting reduced serotonin activity may be a mutant strain in which TPRV function is SPECIFICALLY disrupted in the serotonergic neurons. For example, our ocr-2(yz5) mutations completely abolishes the ocr-2 function in the serotonergic neurons but not in the olfactory neurons. Such mutation is extremely useful in identification of genes and drugs that specifically function and target to the serotonergic neurons, respectively.

A reduction-of-function mutant or a knock-out mutant can be isolated using a classical non-complementation screen, starting with a heterozygote *C. elegans* strain carrying a mutant TRPV allele on one chromosome and a recessive marker close to the wild-type TRPV allele on the other chromosome. The worms are subjected to mutagenesis using standard techniques (EMS or UV-TMP are suitable for this purpose) and the progeny is screened by eye for defects, especially in tissues which express TRPV. Since the screening is performed in the F1 generation, mutations will only give rise to a phenotype if the mutation occurs in the TRPV gene (due to non-complementation) or if the mutation is dominant, which does not occur frequently. These two possibilities can be distinguished in subsequent

generations. A newly introduced TRPV mutation should be linked to the recessive marker. As a further control, DNA sequencing can be performed to determine the nature of the mutation.

The step of 'detecting a phenotypic, biochemical or behavioral change in the *C. elegans* indicating a reversion towards wild type serotonin synthesis' may be performed in several different ways. The method of choice is generally dependent upon the phenotype/behavioral characteristics of the starting worm strain, which is in turn generally dependent upon the nature of the cell types or tissues in which serotonin synthesis is reduced.

The genes identified from this research are candidates for new therapeutic targets of serotonin-related diseases including but not limited to: respiration and thermoregulation disorders, circadian rhythm entrainment, obesity, high blood pressure, high cholesterol, cardiovascular diseases, type II diabetes, eating disorders such as bulimia nervosa and anorexia nervosa, psychotic disorders such as Schizophrenia, Autism, depression, anxiety disorders, impulsivity, alcoholism, social phobia, chronic neural disorders such as migraine, late-onset Alzheimer's disease, Parkinson's diseases, and defects in a variety of behaviors, for example, circadian rhythm entrainment, sleep-wake cycle, appetite, sexual behavior, sensorimotor reactivity, pain sensitivity, and learning.

Also, the animals carrying these mutant genes may be used for drug screens, and for investigating the mechanisms of drug addictions.

Specific Techniques:

Worm strains. The strains used in this study were wild-type *C. elegans* Bristol strain (N2) and mutants *osm-9(yz6), osm-9(n2743), osm-9(ky10), osm-9(n1516), ocr-2(yz5), ocr-2(ak47), odr-10(ky225), osm-11(n1604),tph-1(mg280), daf-7(e1372), daf-16(mgDf50), nss-1(yz12), odr-3(n2150); gpa-3(pk35); gpa-13(pk330); unc-43(n498)gf, unc-43(e408),* and *osm-3(n1540)*. The worms were grown at 20° C and fed with *E. coli* OP50 as the food.

Isolation and characterization of *osm-9(yz6)* and *ocr-2(yz5)* mutants. The *yz6* and *yz5* mutations were isolated based on reduction/absence of GFP in the ADF neurons after ethylmethane sulfonate mutagenesis of wild-type animals carrying an integrated *tph-1::gfp* transgene. The mutagenesis and mutant screens have been described previously (Sze et al., 2002). We screened about 6500 haploid genomes and isolated 24 mutants. None of the mutations completely eliminates *tph-1::gfp* expression in the ADF neurons, but *yz12* (Sze et al., 2002), *yz5, yz6* and other three mutants showed stronger effects. Genetic mapping and complementation analysis indicated all these six strong mutants as single alleles, thus the screen is probably unsaturated. We mapped *yz6* between the polymorphisms in the clones C09G12 and M02B7, and *yz5* between C49H3 and C01F6. A PCR fragment of the cosmid M57 containing 2.8 kb of the upstream sequence, exons/introns and 1.3 kb downstream sequence of *osm-9* restores *tph-1::gfp* expression in ADF of *yz6* animals. A PCR fragment of the cosmid T09A12 containing 2.5 kb upstream sequence, exons/introns and 1.8 kb

downstream sequence of *ocr-2* restores *tph-1::gfp* expression in *yz5* mutants. The molecular lesion of the mutations was determined by PCR amplification of the exons and exon/intron boundaries from the mutant strains and sequencing the PCR fragments.

Expression of *osm-9* from heterologous promoters. The *osm-9* and *ocr-2* genes are co-expressed in six pairs of neurons: ADF, AWA, ADL, ASH, PHA and PHB (Colbert et al., 1997; Tobin et al., 2002). Chimeric constructs were generated by expressing wildtype *osm-9*- or *ocr-2*-coding regions under the control of heterologous promoters that are expressed in a subset of these six pairs of the neurons. The expression pattern of the heterologous promoters overlaps with *osm-9* and *ocr-2* in the following neurons: odr-7, AWA (Sengupta et al., 1994); *osm-10*, ASH, PHA and PHB (Hart et al., 1999); *tax-2*, PHA and PHB (Coburn and Bargmann, 1996); *cat-1*, ADF (Sze et al., 2002); *lin-11*, ADF, ADL (Hobert et al., 1998); *tph-1*(BC), ADF (this work). In each case, we first constructed a fusion of the promoter region to the GFP and *unc-54* 3'-uncoding sequences in the plasmid pPD97.75 (A. Fire) to confirm the expression pattern, then, we replaced the GFP sequence with a genomic sequence encompassing the entire intron and exon regions of *osm-9* or *ocr-2*. Individual constructs were introduced into *yz6* or *yz5* mutants carrying the integrated *tph-1::gfp* reporter. The *tph-1(BC)* construct was generated by inserting the sequence from –132 to –377 upstream of the *tph-1* translational start [the BC region as described previously (Sze et al., 2002)] to a minimal promoter of the *pes-10* gene in the GFP vector pPD122.53 (A. Fire). The plasmid pRF4 containing the dominant Rol-6 gene was co-injected as a transgenic marker for Promoter::gfp constructs, and a plasmid containing *elt-2::gfp* (a gift from J. McGhee) as the marker for Promoter::*osm-9* and Promoter::*ocr-2* constructs.

GFP expression and Immunoanalysis. The expression pattern of these GFP transgenes in wild-type animals has been published: the *tph-1::gfp*, *lin-11::gfp* and *cat-1::gfp* transgenes were integrated into the chromosomes, and *gtpch-1::gfp* was carried as extra chromosomal arrays (Hobert et al., 1998; Sze et al., 2000; Sze et al., 2002). The individual transgenes were crossed into mutants. Thus, the expression of the same transgene in wild-type and mutant animals was compared.

To quantify GFP intensity of *tph-1::gfp* in the ADF neurons, the images were captured with a Zeiss AxioCam digital camera at a fixed exposure time, and the fluorescence within a 25´25 pixel area of the cell body was scored, hence the same dimension of the ADF neurons in different genetic background was compared.

The staining of anti-5HT antibody was performed using the McIntire-Horvitz whole-mount procedure with modifications as described (McIntire et al., 1992; Sze et al., 2000).

Behavioral assays. Feeding and egg-laying assays were conducted with young adult animals. Well-fed larval stage 4 animals (L4) were picked onto fresh plates seeded with bacteria as the food, and allowed to develop ~20 hours at 20°C. Feeding behavior was assayed by measuring the rate of pharyngeal pumping, which was scored by counting

pharynx terminal bulb contractions (Duerr et al., 1999; Sze et al., 2000). Egglaying behavior was scored by counting the number of fertilized eggs accumulated inside of the uterus of adults, using DIC optics (Sze et al., 2000).

For Dauer assays, 10 young adult animals of a strain were transferred onto a fresh plate and allowed to lay eggs for overnight. The parents were then removed, progeny were allowed to develop at 15°C, and the number of Dauers and L4/adults was scored 5-6 days later. Notice a higher Dauer frequency of *tph-1;daf-7* double mutant animals than we previously reported (Sze et al., 2000). This difference is due to different time points at which we scored Dauers. *Tph-1;daf-7* mutants grow slower, some of the animals were still at pre-Dauer stages at the earlier time point.

Constructs and transgenic lines. All the fusion constructs are generated by PCR. A 2.5 kb sequence upstream from the translational start of the *ocr-2* gene was used to direct the expression of all the transgenes, and every construct has the sequence corresponding to the FLAG® epitope (Sigma) inserted at the end of coding sequence which is fused to *unc-54* 3'-uncoding sequence in the plasmid pPD97.75 (A. Fire). The coding sequences of in the transgenes are: OCR-2::FLAG, introns and exons of the coding regions of wild-type (WT) *ocr-2*; OCR-2(G36E)::FLAG, introns and exons of the cording region from *ocr-2(yz5)* mutants and the sequence corresponding to the red fluorescence protein in the plasmid JY388; OCR-4::FLAG, introns and exons of WT *ocr-4* and the RPF sequence; OCR-4(G33E) is derived from the same WT *ocr-4* sequence using the primers containing the sequence of glutamate substitution for the glyceine at the amino acid 33; OCR-2::OCR-4::FLAG, the genomic sequence from the translational start to the amino acid 186 of *ocr-2* fused to the *ocr-4* genomic sequence spanning from the amino acid 145 to the end of the coding region; mTRPV2::FLAG, the cDNA sequence of mouse TRPV2 in a ATCC clone, IMAGE ID 3487527; Htrpv2::FLAG, the cDNA sequence from human TRPV2 in the ATCC clone, IMAGE ID 4298484.

Purified PCR products were microinjected into *ocr-2(ak47)* mutant worms carrying a *tph-1::gfp* reporter (Sze et al., 2000), and a plasmid containing *elt-2::gfp* (a gift from J. McGhee) was co-injected as a transgenic marker. For each transgenes, 2 – 5 transgenic lines from at least three generations were examined. To test the effect of the transgenes on sensory behaviors, the *tph-1::gfp* was outcrossed from the corresponding transgenic lines, i.e. the same transgenes were compared for the effect on OCR-2-dependent functions.

Germline transformation. To generate transgenic strains, the constructs of interest were injected into *C. elegans* (Mello et al., 1991) at a concentration of 70 ng/ul along with the dominant *elt-2 ::gfp* plasmid as a marker in order to isolate transgenic animals by GFP staining in intestinal nuclei. Multiple independent transgenic lines were established from each injection. Transgenic animals were viewed by fluorescence microscopy. Progeny from the microinjected parents were scored for the presence of enhanced/decreased expression of

the *tph-1::gfp* transgene in the ADF neurons. Cell identifications were made using Nomarski images in well-fed animals raised under uncrowded conditions.

GFP expression and Immunoanalysis in Transgenic Strains. Animals of mixed stages were examined using a 40X objective of a Zeiss Axio plan II fluorescence microscope. The expression pattern of the integrated *tph-1::gfp* transgene in WT, and *ocr-2* and *osm-9* mutant animals has been published (Sze et al, 2000, 2004). The definitions of GFP expression level are: strong, equivalent to GFP in wild-type animals; medium, GFP can be clearly observed at least in the cell body, and very weak/None, GFP was almost visually undetectable. The staining of 5ht antibody was performed using the McIntire-Horvitz whole-mount procedure with modifications as described (McIntire et al., 1992; Sze et al., 2000). To conduct the staining with anti-FLAG® staining, animals were fixed according to the established protocol (Finney et al., 1990), stained with monoclonal anti-FLAG® antibody M2 (Sigma) (1:200), and the staining pattern is detected using Alexa Fluor® 568 rabbit anti-mouse antibody (Molecular Probes) at the dilution 1:2000.

Behavioral Assays in Transgenic Strains. Behavioral assays were conducted with young adult animals. Chemotaxis assays were performed as described (Bargmann et al., 1993). The chemotaxis index (CI) was determined in the following way: CI = (number of animals at attractant)-(number of animals at diluent)/(total number of animals). Unless specified, the dilutions of the odorants in ethanol are: benzaldehyde, 200x, isoamyl alcohol, 200x, diacetyl, 1,000x.

Osmotic avoidance was assayed as described (Vowels and Thomas, 1994). Briefly, 10 young adults animals were placed in the center surrounded by a ring of high osmotic strength solution (7M glycerol), and the number of animals that cross the ring during a period of 15 min were scored.

Caenorhabditis elegans TRPV ion channel regulates 5HT biosynthesis in chemosensory neurons.

yz5 and yz6 mutations specifically affect the production of 5HT in the pair of the chemosensory neurons ADF. Of the 302 neurons present in an adult *C. elegans* hermaphrodite, nine neurons from five distinct classes are detected by antibodies raised against 5HT (Horvitz et al., 1982) (Fig. 1A); four classes exist as left-right symmetric pairs: the ADF chemosensory neurons, the NSM pharyngeal secretory neurons, the HSN motor neurons, and the AIM interneurons and RIH is a single interneuron. These neurons are generated from different lineages during embryogenesis (Sulston et al., 1983). 5HT immunoreactivity can be detected in the ADF, NSM, AIM and RIH neurons shortly after hatching, and in the HSN neurons only in adults. The ability to monitor identified

serotonergic neurons permits the isolation and analysis of mutant genes affecting serotonergic phenotype in specific neurons.

Biosynthesis of 5HT in *C. elegans* requires the *tph-1* gene, which encodes the enzyme tryptophan hydroxylase catalyzing the rate-limiting first step of 5HT biosynthesis. *tph-1* is expressed in serotonergic neurons, and *tph-1* knockout animals have no detectable 5HT (Sze et al., 2000). The present inventor has previously shown that *tph-1* expression in different serotonergic neurons is regulated by distinct transcription programs (Sze et al., 2002). To define genes underlying this neuron-specific regulation of 5HT synthesis, the present inventor conducted a genetic screen for neuron-specific serotonin defective (*nss*) mutants, using a green fluorescent protein (GFP) fusion to *tph-1 (tph-1::gfp)* as a reporter.

yz5 and *yz6* are two of the *nss* mutations that specifically downregulate *tph-1* expression in the ADF neurons. In wildtype animals, *tph-1::gfp* is highly expressed in the ADF, NSM and HSN neurons, but the GFP level in the ADF neurons is dramatically reduced or undetectable in *yz5* and *yz6* mutants (Fig. 1B, Table 1). Consistent with the essential role of *tph-1* in 5HT biosynthesis, staining of the mutant animals with anti-5HT antibody shows reduced/absence of 5HT immunoreactivity in the ADF neurons (Fig. 1C). However, neither the mutation nor *yz5;yz6* double mutation has a detectable effect on *tph-1::gfp* expression or 5HT immunoreactivity in other serotonergic neurons (Table 1; Fig. 1B,C). The ADF neurons are the only serotonergic sensory neurons in hermaphroditic *C. elegans*, the data suggest that *yz5* and *yz6* specifically regulate the serotonergic phenotype of sensory neurons.

Table 1. *osm-9* and *ocr-2* mutations downregulate *tph-1::gfp* expression in the ADF chemosensory neurons

	% of GFP in ADF					
Strains	Strong	Weak	Very weak/ none	NSM	HSN	*n*
Wild type	98	0	2	100	100	122
yz6	0	16	84	100	100	183
osm-9(ky10)	0	27	73	100	100	118
osm-9(n2743)	0	14	86	100	100	138
osm-9(n1516)	0	9	91	100	100	142
yz5	0	22	78	100	100	235
ocr-2(ak47)	0	31	69	100	100	97
yz6; yz5	0	10	90	100	100	205
yz6; Ex[osm-9(+)]	39	47	14	100	100	301
yz5; Ex[ocr-2(+)]	42	50	8	100	100	123
odr-10(ky225)	99	1	0	100	100	104
osm-11(n1604)	98	2	0	100	100	124

All the strains carry the same integrated *tph-1::gfp* transgene.

Strong, equivalent to GFP in wild-type animals shown in Fig. 1B; weak, GFP still detectable in the cell body; very weak/none, GFP almost visually undetectable.

n is the number of animals examined.

Mixed staged animals were observed; GFP in HSN was scored only in adults.

Percentage of animals in each category is shown.

***yz5* and *yz6* are mutations of the *ocr-2* and *osm-9* TRPV channel genes, respectively.** Genetic mapping and transgene rescue of *yz5* and *yz6* mutations revealed two TRPV channel proteins regulating *tph-1* expression in serotonergic chemosensory neurons. The TRPV subfamily is characterized by the cytoplasmic N-terminal multiple ankyrin repeats, six transmembrane segments and a cytoplasmic C terminus (Harteneck et al., 2000) (Fig. 1D). Our genetic mapping and sequencing of the mutant genomic DNA show that *yz6* is a nonsense mutation that results in a stop codon before the transmembrane domain in the *osm-9* TRPV gene, and *yz5* is a missense mutation adjacent to the conserved ankyrin motifs in *ocr-2* (Fig. 1D). To confirm it is the mutation in the TRPV genes that downregulates *tph-1* expression in the ADF neurons, we examined *tph-1::gfp* expression and 5HT immunoreactivity in the *ocr-2* deletion mutant *ak47* and three *osm-9* alleles (*ky10, n2743, n1516*). In every of the mutant strains, *tph-1::gfp* expression in ADF is downregulated and 5HT immunoreactivity in ADF is reduced (Table 1; data not shown). Furthermore, *yz6* and *yz5* mutant animals carrying a transgene containing the wild-type *osm-9* or *ocr-2* gene, respectively, restore *tph-1::gfp* expression and 5HT immunoreactivity (Fig. 1B,C; Table 1). Thus, *yz6* is an allele of the *osm-9* gene, and *yz5* is an allele of *ocr-2*.

TRPV ion channels act cell autonomously to control 5HT biosynthesis. Both *osm-9* and *ocr-2* are expressed in the ADF neurons (Colbert et al., 1997; Tobin et al., 2002), but not in other serotonergic neurons (Fig. 2A). Beside ADF, *osm-9* and *ocr-2* also are co-expressed in five pairs of non-serotonergic chemosensory neurons: the AWA, ADL, ASH neurons in the head, and the PHA and PHB neurons in the tail (Tobin et al., 2002). These head neurons, as well as ADF, are component neurons of the amphid sensory organ, and each class senses distinct signals (Bargmann and Horvitz, 1991a). The cell bodies of these head neurons are clustered together, and their processes run parallel and are interconnected directly or indirectly en passant (White et al., 1986) (Fig. 1A). The TRPV channels could act in the ADF neurons to control *tph-1* expression, or they could function in the other chemosensory neurons that regulate ADF neural activity. The AWA neurons detect the attractive odorant diacetyl, and the ASH and ADL neurons sense aversive signals; *ocr-2(ak47)* and *osm-9* mutant animals are defective in sensing these sensory signals (Colbert et al., 1997; Tobin et al., 2002). We tested whether disruption of these sensory signaling is a cause of reducing *tph-1::gfp* expression. No GFP reduction was observed in animals with defective diacetyl receptor (*odr-10*) (Sengupta et al., 1996) or with defective ASH, ADL (*osm-11*) function (Table 1). Thus, *osm-9/ocr-2* function in these sensory signaling is not required to activate *tph-1* expression in the ADF neurons.

To identify cells in which the TRPV proteins function to upregulate *tph-1* expression, we constructed a series of chimeric genes with the *osm-9*- or *ocr-2*-coding regions under the control of heterologous promoters and generated transgenic animals expressing wild-type *osm-9* or *ocr-2* proteins in a subset of these six neuronal types. When *osm-9(yz6)* and *ocr-*

2(yz5) mutant animals carry a transgene expressed in the ADF neurons, *tph-1::gfp* expression in the ADF neurons is restored, whereas the mutant animals carrying the transgene not expressed in the ADF neurons exhibit reduced *tph-1::gfp* expression similar to their non-transgenic siblings (Table 2). These observations collectively argue that the *osm-9* and *ocr-2* genes act cell-autonomously in the ADF neurons to control the *tph-1* expression.

Table 2. *osm-9* acts in the ADF chemosensory neurons to upregulate the *tph-1* expression

Transgene carried in *osm-9(yz6)* mutants	Transgene expression in ADF	% of *tph-1::gfp* in ADF			
		Strong	Weak	Very weak/ none	*n*
None	–	0	16	84	183
Ex [*Podr-7::osm-9*]	No	0	4	96	31
Ex [*Posm-10::osm-9*]	No	0	14	86	111
Ex [*Ptax-2::osm-9*]	No	0	15	85	100
Ex [*Pcat-1::osm-9*]	Yes	70	28	2	166
Ex [*Plin-11::osm-9*]	Yes	64	35	1	94
Ex [*Ptph-1(BC)::osm-9*]	Weakly	36	23	41	187

The same genomic *osm-9* sequence was fused to individual heterologous promoter fragments. The expression patterns were confirmed by construction of Promoter::gfp fusion. Although Ptph-1(BC) is expressed strongly in ADF of wild-type animals, the ADF expression is much weaker in *osm-9* mutant background (Fig. 3A).

All the transgenes were carried as extrachromosomal arrays, three to five transgenic lines were examined for each construct. For each line, the animals carrying the marker for the fusion construct and their non-transgenic sibling were examined to ensure *tph-1::gfp* expression in the non-transgenic animals was unaffected. The animals carrying the marker for the fusion constructs were scored.

The data are the summary of at least three independent trials from multiple generations of the animals. *n* is the number of animals carrying the fusion transgene examined. The definition of the GFP strength is described in Table 1. Percentage of animals in each category is shown.

TRPV ion channels regulate specific aspects of serotonergic phenotype. Mutations in OSM-9 and OCR-2 channels may disrupt the regulatory pathway of *tph-1* transcription, or they may induce ADF neural degeneration due to ion imbalances. To address these possibilities, we assessed if the *yz6* and *yz5* mutations result in a general morphological transformation of the ADF neurons. The LIM-homeodomain transcription factor *lin-11* is co-expressed with *osm-9* and *ocr-2* in the ADF and ADL chemosensory neurons (Freyd et al., 1990; Hobert et al., 1998). We crossed an integrated *lin-11::gfp* transgene into *osm-9(yz6)* and *ocr-2(yz5)* mutants; no reduction of *lin-11::gfp* expression levels can be detected in the mutant backgrounds (Fig. 2B). Judged by fluorescence microscopy, the morphology of the cell body and the processes of the ADF neurons are indistinguishable in the mutant and wild-type animals at any developmental stage. Thus, these mutations do not cause the ADF neurons to die prematurely. Rather, these results point to a signaling pathway downstream of the OSM-9 and OCR-2 TRPV channels regulating the transcription of *tph-1* in the ADF neurons.

Does this TRPV channel signaling specifically regulate *tph-1* expression, or does it regulate the expression of all genes involved in 5HT synthesis and neurotransmission? We have explored this question by examining GFP reporters of marker genes. In each case, the GFP reporter construct was first introduced into wild-type animals, and the resulting transgene was then crossed into mutants. Thus, the expression of the same transgene in different genetic backgrounds was compared. CAT-1/vesicular monoamine transporter is required for 5HT neurotransmission (Duerr et al., 1999; Nurish et al., 1999). *cat-1::gfp* is a functional fusion of GFP to the entire protein coding segment of the *cat-1* gene and is localized to the synapses of the serotonergic neurons (Sze et al., 2002). The gene F32G8.6 encodes a probable GTP-cyclohydrolase I (*gtpch-1*), a co-factor of tryptophan hydroxylase for 5HT biosynthesis, and a *gtpch-1::gfp* fusion gene also is expressed in the serotonergic neurons (Sze et al., 2002). Unlike the dramatic reduction of *tph-1::gfp* in the ADF neurons, there is no significant reduction of *cat-1::gfp* or *gtpch-1::gfp* in *yz6* and *yz5* mutant backgrounds (Fig. 2C,D). These observations are consistent with our previous results that the expression of *tph-1*, *cat-1* and *gtpch-1* is differentially regulated in serotonergic neurons (Sze et al., 2002). These data demonstrate a great specificity of the TRPV channel signaling within the ADF neurons and suggest the transcriptional regulation of the *tph-1* gene as a major target.

The TRPV channel signaling modulates a neuron-specific transcription program. Analysis of the *tph-1* promoter has revealed a discrete cisregulatory region essential for *tph-1* expression in the ADF neurons (Sze et al., 2002). We investigated whether the TRPV ion channel signaling acts through this neuron-specific transcriptional regulatory mechanism. A GFP reporter under the control of the sequence –132 bp to –377 bp of *tph-1* and a minimal promoter from the *pes-10* gene is specifically expressed in the ADF neurons of wild-type animals, but the ADF GFP intensity is significantly reduced in *yz6* mutant background (Fig. 3A). Thus, the 246 bp cis-regulatory region is sufficient to activate *tph-1* expression in the ADF neurons, and signaling from the OSM-9 and OCR-2 ion channels regulates the activity of this neuron-specific transcriptional regulatory program.

Unlike mutations in the POU-transcription factor UNC-86 that completely abolish *tph-1::gfp* expression in the NSM and HSN neurons (Sze et al., 2002), none of the mutations in *osm-9* or *ocr-2*, nor the mutation of both eliminates *tph-1::gfp* expression in the ADF neurons (Table 1; Fig. 3B), indicating that the TRPV activity modulates *tph-1* expression levels but is not essential for the transcription. In *yz6* background, the GFP reporter under the control of the 246-bp *tph-1* cisregulatory sequence fused to a *pes-10* minimal promoter is expressed in the ADF neurons at slightly higher levels than the *tph-1::gfp* reporter under the control of the 3.1 kb of the *tph-1* promoter (Fig. 1B, Fig. 3A). This could reflect the difference in the basal expression level of the *tph-1* and *pes-10* promoter in the reporter constructs. Alternatively, it could be an indication of additional cis-regulatory elements

mediating inhibition of *tph-1* expression but the elements are not present in the 246 bp *tph-1* sequence.

***unc-43* CaMKII acts downstream or in parallel with the TRPV ion channels to modulate 5HT biosynthesis.** The *odr-3* Gα protein is essential for *osm-9* and *ocr-2* function in olfactory, osmosensory and mechanosensory behaviors mediated by the AWA and ASH neurons (Roayaie et al., 1998; Colbert et al., 1997; Tobin et al., 2002). It has been proposed that ODR-3 activity modulates the outputs of the TRPV channel signaling (Roayaie et al., 1998). *odr-3* also is expressed in the ADF neurons; however, the *odr-3(n2150)* deletion mutation does not cause a significant reduction of *tph-1::gfp* expression (Fig. 3B). Thus, OSM-9 and OCR-2 can activate *tph-1* expression in the absence of *odr-3* activity. Beside *odr-3*, the ADF neurons express two other Gα proteins, *gpa-3* and *gpa-13* (Jansen et al., 1999). *tph-1::gfp* expression is unaffected in *gpa-3* or *gpa-13* deletion mutants (Fig. 3B). However, animals bearing a double mutation of TRPV and Gα still exhibit reduced *tph-1::gfp* expression in the ADF neurons similar to the TRPV mutants. These data indicate that unlike the role of ODR-3 in the sensory behaviors, the Gα proteins do not play an essential role in OSM-9/OCR-2-dependent regulation of *tph-1* expression. Because the sensory behaviors and 5HT production are mediated by different neurons, our results suggest that OSM-9 and OCR-2 TRPV channels in different neurons may be regulated by different mechanisms.

CaMKII is a critical mediator of Ca^{2+} signaling. The *unc-43* gene encodes the only *C. elegans* CaMKII (Reiner et al., 1999; Rongo and Kaplan, 1999). An *unc-43* loss-of-function mutation causes a twofold reduction of *tph-1::gfp* expression in the ADF neurons (Fig. 3B), but has no effect on other serotonergic neurons (not shown). Conversely, the *unc-43(n498)* gain-of-function mutation partially blocks the downregulation of *tph-1* expression in *yz6* (Fig. 3b) and *yz5* mutants (data not shown). One simple model to explain these data would be that activation of OSM-9 and OCR-2 channels increases ADF intracellular Ca^{2+} which stimulates UNC-43 to induce *tph-1* transcription, whereas the *unc43(n498)* mutation, which causes constitutive Ca^{2+}-independent activity (Reiner et al., 1999), bypasses the need of the channel function. However, these results do not exclude the possibility that UNC-43 acts less directly in the TRPV channel signaling pathway. These results suggest that CaMKII acts downstream of or in parallel with the OSM-9 and OCR-2 TRPV channels to control 5HT production in the ADF chemosensory neurons.

Dauer phenotype of *osm-9* and *ocr-2* mutants. We find that both *osm-9* and *ocr-2* mutants show developmental defects reminiscent of *tph-1* deletion mutants (Fig. 4A). The DAF-2/insulin receptor and DAF-7/TGFβ signaling act in parallel to control whether an animal enters the reproductive lifecycle or developmentally arrests at the metabolically inactive Dauer larval stage. Disruption of either pathway causes conditional abnormal arrest at the Dauer stage, but disruption of both the pathways causes constitutive Dauer arrest (Ogg et al., 1997) (reviewed by Riddle, 1997). Similar to the *tph-1* deletion mutation, the *osm-*

9(yz6); *osm-9(ky10)* and *ocr-2(yz5)* mutations enhance the Dauer phenotype of *daf-7(e1372)* mutants (Fig. 4A). At the 15°C growth temperature, about 10% of *daf-7* mutant animals arrest as Dauers, but more than 70% form Dauers when *daf-7* mutants carry a mutation in *osm-9* or *ocr-2*. Ninety-eight percent of *daf-7;tph-1* double mutant animals form Dauers, whereas 10-15% *tph-1* mutants form Dauers (Sze et al., 2000). None of the TRPV mutants on their own formed Dauers when assayed under the same condition, although they grow slower than wild-type animals. Because *e1372* is a *daf-7*-null mutation, this enhanced Dauer phenotype of the double mutants implies that the TRPV mutations affect a pathway parallel to *daf-7*.

Enhancement and suppression genetics and other molecular experiments implicate that the TRPV mutations affect 5HT inputs to the insulin pathway. The DAF-16/forkhead transcription factor is a negative target of DAF-2/insulin signaling, and the *daf-16(mgDf50)* mutation bypasses the need of DAF-2 (Ogg et al., 1997). The Dauer phenotype of *daf-7*; *tph-1* and *daf-7;osm-9* can be suppressed by the *daf-16(mgDf50)* mutation (Fig. 4A), indicating a reduction of DAF-2/insulin signaling in the double mutants that promotes the Dauer formation. Dauer arrest is also enhanced in *daf-7(e1372)* mutants carrying a mutation in the *nss-1* gene, which also specifically affects *tph-1* expression in ADF (Sze et al., 2002). This raises the possibility that it is the reduction of ADF 5HT signals that downregulates the DAF-2/insulin pathway. However, expression of the wild-type *osm-9*-coding sequence under a *lin-11* promoter only mildly suppresses the Dauer phenotype of *osm-9(yz6);daf-7(e1372)* mutants (Fig. 4A), indicating that other *osm-9*-expressing cells also contribute to the normal development.

osm-9 and *ocr-2* mutant animals do not display every deficit observed in mutants with all the serotonergic neurons defective. 5HT regulates several *C. elegans* behaviors. For example, applying exogenous 5HT to *C. elegans* stimulates pharyngeal pumping and egg-laying (Avery and Horvitz, 1990; Weinshenker et al., 1995), whereas 5HT-deficient mutants *tph-1* and *cat-1* exhibit a slower pumping rate and accumulate a large number of fertilized eggs in the uterus (Duerr et al., 1999; Sze et al., 2000). However, *osm-9* and *ocr-2* mutant animals do not accumulate excess eggs in the uterus and their pharyngeal pumping rates are equivalent to wild-type animals (Fig. 4B,C). Thus, 5HT signals from the other neurons are sufficient for these behaviors. But, we cannot exclude subtle behavioral changes that are difficult to detect visually.

The genetic evidence presented here has two major implications. The phenotype of the TRPV mutants represents exquisite specificity in the control of 5HT production, as exemplified by our demonstration that mutations of the *osm-9* and *ocr-2* TRPV genes specifically downregulate 5HT biosynthesis in the ADF neurons, and this TRPV ion channel regulation of 5HT production is mediated by a neuron-specific transcription program. Our finding that the *osm-9* and *ocr-2* TRPV channel genes act in the ADF neurons, function

upstream of CaMKII to control the key 5HT biosynthesis gene *tph-1* provides insights in elucidating the genetic pathway by which a serotonergic neuron couples the activity at the cell surface and 5HT signaling.

A TRPV channel-dependent transcription program controls 5HT signaling. It has been demonstrated in many experimental systems that sensory stimuli induce 5HT signals to produce changes in behavior and physiology (e.g. Barzilai et al., 1989; Boadle-Biber, 1993; Milner et al., 1998). Until this study, no endogenous membrane protein has been shown to act in a serotonergic neuron to regulate 5HT signaling. One important finding from this study is the pronounced effect of *osm-9* and *ocr-2* mutations on the expression of the 5HT synthesis gene *tph-1* (Fig. 1). This indicates that the production of 5HT is a site where sensory information is integrated to 5HT signaling. 5HT can be released by controlled exocytosis at the synapses as well as via paracrine 'volume transmission', and even during the controlled exocytosis it is newly synthesized 5HT preferentially released to induce changes in the postsynaptic targets (Attwell et al., 1993; Sanders-Bush, 1982). Hence, the level of 5HT production is one mechanism controlling both forms of 5HT neurotransmission.

Transcriptional regulation may represent a general principle of regulation of hormones and neuromodulators. For example, *C. elegans'* favorite growth environment upregulates the expression of the *daf-7*/TGFβ and *daf-28*/insulin genes to induce *C. elegans* proceeding reproductive development (Schackwitz et al., 1998; Li et al., 2003); in rats, noxious sound stimuli may alter the transcription of their tryptophan hydroxylase gene in a neuron-specific manner (reviewed by Boadle-Biber, 1993); and the expression of tyrosine hydroxylase can be modulated by hormones in mice (Kumer and Vrana, 1996). Transcriptional regulation of these signaling molecules is likely a mechanism to exert a relatively slow but profound effect in the signaling pathways.

Mechanisms of TRPV channel action in the ADF neurons. Our genetic results indicate that the *osm-9* and *ocr-2* channel proteins interact with different signaling transduction pathways to induce different behavioral outputs. *osm-9* and *ocr-2* are co-expressed in four pairs of the amphid sensory neurons, and are required for a normal response to attractive and aversive odorants mediated respectively by AWA, and ASH and ADL, as well as for ASH-mediated mechanosensory and osmosensory function (Colbert et al., 1997). There are genetic evidences indicating that *osm-9* and *ocr-2* function in these sensory behaviors requires the *odr-3* Gα protein (Roayaie et al., 1998). However, *tph-1* expression is unaffected by mutations in *odr-3* or in other two Gα proteins expressed in the ADF neurons (Fig. 3B). Although the exact mode of activation of OSM-9 and OCR-2 in any neuron has not yet been defined, our data indicate that the activity of the channels in the ADF neurons is regulated by different signaling molecules. It is interesting to note that *osm-9* and *ocr-2* also act in the ASH and ADL neurons to regulate social behavior independent of *odr-3* activity

(de Mono et al., 2002); hence, the OSM-9 and OCR-2 can induce specific behaviors by coupling distinct signaling systems.

The reciprocal effects of the *unc-43* CaMKII loss- and gain-of-function mutations on *tph-1* expression indicate that the amount of Ca^{2+} modulates 5HT biosynthesis (Fig. 3B). The TRP superfamily is Ca^{2+}-permeable channels, and CaMKII is known as an important mediator of Ca^{2+} signaling (reviewed by Hanson and Schulman, 1992). Our genetic study shows that the constitutively active, Ca^{2+}-independent *unc-43(n498)* CaMKII (Reiner et al., 1999) can partially activate *tph-1* expression in *osm-9* deletion mutant animals (Fig. 3B). These results support the model that UNC-43 is a downstream effector of the OSM-9 and OCR-2 channels: Ca^{2+} influx through OSM-9 and OCR-2 activates UNC-43 CaMKII, which induces phosphorylation cascades to activate *tph-1* expression. However, *unc-43(lf)* mutants still express a substantial amount of *tph-1*, and the *unc-43(gf)* mutation does not completely bypass OSM-9 activity, indicating that other OSM-9/OCR-2 downstream signaling molecules may act in parallel with UNC-43 to regulate *tph-1* expression.

The effect of OSM-9 and OCR-2 TRPV ion channels in the ADF neurons is strikingly specific, given the involvement of Ca^{2+} and CaMKII. The general architecture of the ADF neurons is unaffected, we could not detect a significant change in the expression levels of ADF marker genes or genes directly involved in the serotonergic phenotype, nor we could detect an effect of *unc-43(lf)* or (*gf*) mutations on *tph-1* expression in other serotonergic neurons. This specificity demonstrates that 'multi-functional, widespread' signaling molecules may play a refined role in a particular native cellular setting. It is conceivable that such differential regulation of the serotonergic phenotype genes would allow the ADF neurons to adjust 5HT neurotransmission in response to multiple sensory signals. As TRPV channels are expressed in the serotonergic locus in mammals (Tominaga et al., 1998; Mezey et al., 2000), it would be interesting to determine whether there is a link between TRPV mutations and 5HT deficiency in humans.

The role of TRPV channels in a sensoryneuroendocrine signaling pathway. The Dauer phenotype of the *osm-9* and *ocr-2* mutants demonstrates a genetic link between the TRPV ion channels and endocrine signaling (Fig. 4). *C. elegans* Dauer/non-Dauer development reflects two alternative metabolic states controlled by sensory inputs to neuroendocrine signaling pathways. The pathways from DAF-7/TGFb and DAF-2/insulin receptor converge to stimulate reproductive growth; harsh environmental conditions transduced by the amphid chemosensory neurons suppress the endocrine signaling to induce Dauer arrest (reviewed by Riddle, 1997). Our enhancement and suppression genetics implies that *osm-9* and *ocr-2* regulate the DAF-2/insulin-receptor signaling pathway (Fig. 4). The TRPV channels are probably acting in the ADF neurons to modulate endocrine activity: worms bearing defective ADF neurons tend to form Dauers (Shakir et al., 1993), and laser ablation experiments implicate ADF but not the other *osm-9* and *ocr-2* co-expressing

chemosensory neurons in Dauer formation (Bargmann and Horvitz, 1991b). However, expression of the wild-type *osm-9*-coding sequence under a *lin-11* promoter only partially suppresses *daf-7;osm-9* Dauer phenotype, indicating that *osm-9* activity in other cells also modulates Dauer phenotype. Alternatively, the *lin-11* promoter may not be able to express sufficient amount of *osm-9* to induce a wild-type level of ADF 5HT signals (Table 2), or ectopic expression of *osm-9* in other *lin-11*-expressing cells may interfere with the neuroendocrine signaling cascades for normal development. In mammals, 5HT regulates insulin synthesis, release and response (Breum et al., 1995; Peschke et al., 1997). It has been proposed that a feedback regulatory loop between hypothalamus 5HT and circulating hormones such as insulin, leptin and adipose tissue-derived hormone modulates the satiety and maintains metabolic and energy homeostasis (Leibowitz and Alexander, 1998). Interestingly, mouse TRPV-like channels can be activated by insulin-like growth factors (Kanzaki et al., 1999). We propose that 5HT is one mediator of TRPV channels and endocrine activity.

OCR-2 TRPV channel activity in C elegans serotonergic neurons is mediated by a single nucleotide and can be substituted by human TRPV2

OCR-2 channel protein differentiates sensory functions. In addition to the control of 5HT biosynthesis in the chemosensory neurons ADF, OCR-2 and OSM-9 also act in several non-serotonergic sensory neurons: in the AWA neurons to induce attractive response to the odorant diacetyl, and in the ASH neurons to elicit avoidance to noxious environmental stimuli such as high osmolarity, odor-repellent and nose touch, and animals bearing a deletion mutation of either *ocr-2* or *osm-9* do not respond to these sensory stimuli (Fig. 5b,c). Both OCR-2 and OSM-9 proteins are localized to plasma membrane and the ciliated sensory endings of ADF as well as of these non-seroteonergic sensory neurons. Although the exact stoichiometric composition of the channel has not yet been determined, OCR-2 and OSM-9 require each other for routing to the sensory cilia. Consistent with the idea that OCR-2 and OSM-9 assemble to a heteromeric channel, mutant animals with either *ocr-2* or *osm-9* mutations exhibit as strong a phenotype as those of the double mutation. The essential function of OCR-2/OSM-9 channel in these sensory neuron functions provides a tractable system to investigate the channel molecular mechanisms in different cellular settings within an organism.

A first distinction of OCR-2/OSM-9 signaling cascade in the serotonergic neurons is revealed from the study of the Gα protein ODR-3. ODR-3 is expressed in AWA, ASH, as well as in ADF, and is required for OCR-2/OSM-9 function in the AWA and ASH neurons. However, ODR-3 and two other Gα proteins that are expressed in the ADF do not have a

detectable effect on OCR-2/OSM-9-dependent activation of *tph-1* expression. Thus, OCR-2/OSM-9 may induce specific outputs by coupling distinct signaling systems.

Like OSM-9, OCR-2 is predicted to embody an architecture characteristic of the mammalian vanilliod receptor-related channels of the TRP superfamily - six transmembrane segments, a cytoplasmic N-terminus with three ankyrin motifs, and a cytoplasmic C-terminus. As discussed above, the *ocr-2(yz5)* allele was identified from a genetic screen for mutations that down regulate *tph-1::gfp* expression in the ADF neurons and contains a single nucleotide change that results in a substitution of glutamate for glycine in the N-terminal region (G36E) (Fig. 7). Staining of anti-5HT antibody indicates that ADF 5HT immunoreactivity is nearly eliminated in *ocr*-2G36E mutants (Fig. 5a). To explore if this glycine underscores an essential element for the general function of OCR-2/OSM-9 channel or if it is a site of integration of the signaling cascade specifically regulating 5HT biosynthesis, we tested the effect of *ocr*-2G36E on AWA and ASH function. Similar to the *ocr-2* deletion allele *ocr-2(ak47)*, *ocr*-2G36E worms are defective in ASH-mediated avoidance to high osmotic solutions (Fig. 5b). By contrast, whereas *ocr-2(ak47)* mutants are defective in response to AWA-mediated attraction to the odorant diacetyl, *ocr*-2G36E mutants responded to diacetyl as well as wild-type animals (Fig. 5c). To determine if the OCR-2(G36E) substitution causes a subtle effect in the sensitivity to diacetyl, we assayed the response to diacetyl in a series of dilutions. There is no significant difference between wild-type and *ocr*-2G36E worms observed (Fig. 5d). Thus, the G36E substitution disrupts OCR-2/OSM-9 channel signaling pathways in the ADF and ASH neurons but does not perturb the olfactory sensory transduction. This result indicates that the primary structure at the N-terminus of OCR-2 is a part of determinants of cellular specificity of the channel function.

To ascertain the G36E substitution does not alter the expression pattern of OCR-2, we tagged FLAG® epitope to the C-terminus of the wild-type OCR-2 and OCR-2(G36E), introduced the fusion constructs individually into *ocr-2(ak47)* mutants, and stained the transgenic lines with anti-FLAG antibody. Both OCR-2::FLAG and OCR-2(G36E)::FLAG were detected in the plasma membrane and ciliated endings of these sensory neurons (Fig. 6). OCR-2::FLAG but not OCR-2(G36E) transgene can restore ADF to express *tph-1* in *ocr-2(ak47)* background: 68% *ocr-2(ak47)* worms carrying OCR-2::FLAG showed strong *tph-1::gfp* in ADF (n=79), compared to 0% of that carrying OCR-2(G36E) (n = 150) and 0% of non-transgenic *ocr-2(ak47)* (n = 235). These data indicate that FLAG-tagged OCR-2 is localized to the cellular sites where OCR-2 acts and that G36E substitution does not prevent the subcellular translocation. One possible explanation for this result is that OCR-2 exerts a fundamental role in the assembly of transduction complexes by selectively binding to different signaling components; the *yz5* G36E substitution might result in altered affinity for a common component in both ADF and ASH neurons. The immuno-fluorescent straining of OCR-2(G36E)::FLAG is slightly lower than that of OCR-2::FLAG (Fig. 6). This could be a

reflection of the mosaicism of the transgenic arrays. Alternatively it could be an indication of less-stability of OCR-2(G36E) resulted from the failure of binding to the partner or altered structure.

Functional determinants in the N-terminal region of OCR-2. The cytoplasmic N-terminal region is crucial for activation of TRPV channels. All TRPV channels contain multiple ankyrin motifs adjacent to the transmembrane domain; changes in this region often eliminate the channel activity. For example, a splicing variant of rat TRPV1 that is lacking of most of the N-terminal region is expressed properly but does not respond to any identified stimulus, and point mutations in ankryin motifs of OSM-9 completely abolish the function. However, our *yz5* G36E is located about 220 amino acids upstream from the first ankryin motif and affects a subset of OCR-2 function (Fig. 7). To understand the molecular mechanisms that specify OCR-2 functions, we explored the potential structure from the predicted translational start to upstream of the ankryin motifs of OCR-2. Structural alignments predict that the G36 residue is located in a coil region between two helices and is accessible to the surface, and G36E substitution shifts the start of the C-terminal helical fold and thus alters intra- and inter-molecular interactions in the region.

The C elegans genome encodes 5 TRPV genes, *ocr-1, ocr-2, ocr-3, ocr-4* and *osm-9*. The sequence of before the ankryin repeats of *ocr-4* shows the least similarity to that of *ocr-2* but the region corresponding to the *ocr-2 (yz5)* point mutation is highly conserved in *ocr-4* (Fig. 7). *ocr-4* is expressed in the OLQ neurons and its function has yet to be determined. If this region and this glycine is particularly involved in the channel function in the ADF and ASH neurons, *ocr-4* might substitute these *ocr-2* functions. To test this possibility, we expressed the *ocr-4* coding sequence tagged with a FLAG epitope under the control of the *ocr-2* promoter and introduced the construct into *ocr-2* deletion mutants. *ocr-2* deletion mutants expressing OCR-4::FLAG in *ocr-2*-expressing cells show substantially increased *tph-1::gfp* expression and improved odor and osmo sensory behavior.

To test the importance of the glycine residue for the channel function, we introduced the corresponding G-to-E substitution (G33E) to *ocr-4*. This substitution completely eliminates the ability of *ocr-4* to upregulate *tph-1* expression in the ADF neurons. This result confirms that the G-to-E substitution causes the *yz5* phenotype, and indicates that the glycine residue is likely to underscore a functional moiety conserved among the family members.

ocr-4::flag transgene only partially rescues *tph-1::gfp* expression in *ocr-2* deletion mutants. To test if this lower activity is ascribed to the N-terminal region, we replaced the sequence from the translational start to the predicted first ankyrin motif of *ocr-4* with the corresponding region of *ocr-2*. The OCR-2-OCR-4::FLAG chimera gene rescues *tph-1::gfp* expression significantly better than OCR-4::FLAG, indicating sequence elements within this region of OCR-2 are necessary for an optimal function of channel in the pathway that regulate 5HT biosynthesis. The sequence of this region is quite divergent between *ocr-2* and

ocr-4, making impossible to guess the critical residues. We constructed a series of small internal deletions within this region of *ocr-2* based on the predicted structure. However, all these mini-deletions affect the function as well as the distribution pattern (data not shown). These results suggest that the structure of the N-terminal region of the TRPV channel proteins can determine the specificity as well as the activity of the channel.

Although OCR-4::FLAG can be detected in the plasma membrane and the ciliated endings in OCR-2-expressing cells, OCR-4::FLAG often aggregate to punctuates on the plasma membrane and is frequently detected in the dendrites, whereas OCR-2::FLAG exhibits a more uniform distribution on the membrane and is rarely detected in the dendrites, indicating that the interaction between OCR-4 and subcellular localization components are not optimal.

Mammalian TRPV2 genes can upregulate *tph-1* expression in the ADF neurons. 5HT signaling controls development, metabolism and endocrine activity in disparate phyla. As shown above, worms bearing a mutation in OCR-2/OSM-9 channel are more vulnerable to arrest as metabolically inactive Dauer larvae and this abnormal metabolic arrest is in part due to reduced insulin neuroendocrine signaling. TRPV2 is a mammalian member of TPRV subfamily of ion channels. TRPV2 is expressed in sensory ganglion as well as in most areas of the CNS and can be regulated by insulin growth factors. To explore an ortholog relationship between *ocr-2* and TRPV2, we sought to test the ability of TRPV2 to regulate *tph-1* expression in the ADF neurons.

We expressed mouse and human TRPV cDNA tagged with a FLAG epitope under the control of the *ocr-2* promoter in *ocr-2* deletion mutants. Similar to OCR-2::FLAG, mouse and human TRPV2::FLAG is distributed in the plasma membrane and ciliated endings (Fig. 8a). Whereas *tph-1::gfp* expression in the ADF neurons is nearly undetectable in *ocr-2* mutants, *ocr-2* mutants expressing either mouse or human TRPV2::FLAT exhibit substantially increased tph-1 expression in ADF (Fig. 8b). The GFP intensity in TRPV-2::FLAG animals is not as strong as in *ocr-2*;OCR-2::FLAG animals: more than 99% of *ocr-2* deletion mutants show very weak/nondetectable *tph-1::gfp* expression in ADF, 59% (n=82) and 79% (n=95) of *ocr-2* deletion mutants bearing respectively mouse and human TRPV2 show medium level of ADF GFP, but 42% (n=273) of *ocr-2* deletion mutants bearing OCR-2::FLAG showed strong and medium GFP, indicating mammalian TRPV2 and OCR-2 are not completely equivalent.

Thus far, there is no evidence that another TRPV protein co-express with TRPV2, suggesting that TRPV2 can function as monomeric channel. We have tested this possibility by asking whether TRPV2 genes can restore ADF to express *tph-1::gfp* in *osm-9* and *osm-9;ocr-2* double mutants. 62% (n= 77) of *osm-9 (yz6)*; mouse TRPV2 and 69% of *osm-9(yz6)*; human TRPV2 animals showed medium level of *tph-1::gfp* in the ADF neurons.

Do mammalian TRPV2 function in all OCR-2/OSM-9 signaling cascades, or they only retain the function in 5HT signaling? We tested ability of TRPV2::FLAG to rescue the olfactory and osmotic sensation of *ocr-2* deletion mutants. We failed to detect any improvement in the response of the transgenic animals to diacetyl or high osmolarity solutions (Fig. 8c,d). These results suggest that the mammalian TRPV2 cannot be integrated into these sensory signaling transduction pathways but can interact with the molecular machinery that controls *tph-1* expression in the ADF neurons.

Genetics and behavioral analysis of ocr-2/osm-9 mutants congruent with pharmacological and physiological study of mammalian TRPV channels indicating that TRPV channels can mediate the response to multiple sensory modalities, yet, specificity must preserve in vivo. Several lines of evidence suggest that one layer of the specificity is conferred by binding of ligands and intracellular signaling components to specific residues in the intracellular side of TRPV channels. The binding site of vanilloid has been mapped to a segment of TRPV1 in the cytoplasmic leaflet between transmembrane segments 2 and 3; a mutation in this site causes TRPV1 insensitive to vanilloids but the response to acid, heat retained. Activation of TRPV4 by heat and phorbol derivates requires a tyrosine residue at the predicted N-terminus of the transmembrane segment 3 and is independent of the regulation of the channel by osmotic strength.

The results from this study reveal the cytoplasmic N-terminal region upstream of the ankryin repeats of TRPV channels as a part of determinants of cell-specific function of OCR-2 channels in *C. elegans*: the *yz5* G36E substitution completely disrupts the function of OCR-2 in osmosensation mediated by ASH and the regulation of *tph-1* expression in ADF, but leaves the diacetyl sensation mediated by AWA intact.

All Molecular biology work was performed as described by Sambrook et al. Molecular cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, or using minor modifications of the methods described therein.

All manipulations of *C. elegans* worms were performed using techniques described in Methods in Cell Biology, vol 84; *Caenorhabditis elegans*: modern biological analysis of an organism, ed. Epstein and Shakes, academic press, 1995, or using minor modifications of the methods described therein.

Transgenic *C. elegans* strains were constructed by injection of plasmid DNA into worms using standard techniques known in the art (see Methods in Cell Biology, vol 84 as mentioned above).

Although described with reference to a *C. elegans* model system, it will be appreciated by the skilled artisan that any animal model may be used having a TRPV-encoding gene homologous to those described for *C. elegans*, wherein a mutation in that gene results in reduced serotonin synthesis.

While this invention is described in detail with reference to a certain preferred embodiments, it should be appreciated that the present invention is not limited to those precise embodiments. Rather, in view of the present disclosure which describes the current best mode for practicing the invention, many modifications and variations would present themselves to those of skill in the art without departing from the scope and spirit of this invention. In particular, it is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, constructs, and reagents described as such may vary, as will be appreciated by one of skill in the art.

WHAT IS CLAIMED IS:

1. A transgenic model animal useful for screening therapeutic agents for serotonin-related diseases, comprising a mutation in *osm-9* and a stably integrated *tph1::*marker fusion gene, wherein the mutation results in reduced *tph1::*marker expression.

2. The animal of claim 1, wherein the mutation is *osm-9(yz6)*.

3. The animal of claim 1, wherein the animal is *C. elegans.*

4. The animal of claim 3, wherein in the marker is a gene for green fluorescent protein (*gfp*).

5. A transgenic model animal useful for screening therapeutic agents for serotonin-related diseases, comprising a mutation in *ocr-2* and a stably integrated *tph1::*marker fusion gene, wherein the mutation results in reduced tph1::marker expression.

6. The animal of claim 5, wherein the mutation is *ocr-2(yz5)*.

7. The animal of claim 5, wherein the animal is *C. elegans.*

8. The animal of claim 7, wherein in the marker is *gfp*.

9. A method for screening therapeutic agents for serotonin-related diseases, comprising the steps of:

providing a model animal as recited in claim 4;

dispersing a target drug into agar;

culturing the model animal on the agar with drug; and

detecting expression of green fluorescent protein (GFP).

10. A method for screening therapeutic agents for serotonin-related diseases, comprising the steps of:

providing a model animal as recited in claim 8;

dispersing a target drug into agar;

culturing the model animal on the agar with drug; and

detecting expression of green fluorescent protein. (GFP)

11. A method of identifying compounds which enhance or up-regulate the synthesis of serotonin, which method comprises:

contacting *C. elegans* which exhibit reduced serotonin synthesis compared to wild type *C. elegans* in one or more cell types or tissues with a compound under test; and

detecting a phenotypic, biochemical or behavioral change in the *C. elegans* indicating a reversion towards wild type serotonin synthesis in the one or more cell types or tissues which exhibit reduced serotonin synthesis in the absence of the compound.

12. A method as claimed in claim 11 wherein the *C. elegans* contains a defective TRPV channel protein.

13. A method as claimed in claim 12 wherein the *C. elegans* contains a missence mutation in *osm-9*.

14. A method as claimed in claim 12 wherein the *C. elegans* contains a missence mutation in *ocr-2*.

15. A method as claimed in claim 12 wherein the *C. elegans* contains a nonsense mutation in *osm-9*.

16. A method as claimed in claim 12 wherein the *C. elegans* contains a nonsense mutation in *ocr-2*.

17. The method as claimed in claim 12 wherein the C. elegans exhibits reduced synthesis of serotonin specifically in ADF neurons.

18. A method as claimed in claim 11 wherein the *C. elegans* is a mutant *C. elegans* which exhibits reduced serotonin activity in one or more cell types or tissues.

19. A method as claimed in claim 18 wherein the *C. elegans* is a mutant *C. elegans* which exhibits reduced activity of TRPV channel protein in one or more cell types or tissues.

20. The method as claimed in claim 19 wherein the *C. elegans* exhibits reduced synthesis of serotonin specifically in ADF neurons.

21. A method as claimed in claim 11 wherein the *C. elegans* exhibit reduced serotonin synthesis in the ADF neurons, as compared to wild type *C. elegans* and the step of

detecting a phenotypic, biochemical or behavioral change in the *C. elegans* indicating a reversion towards wild type serotonin synthesis comprises detecting a progression of the development of the *C. elegans* past the Dauer larval stage in the presence of the compound under test.

22. A method as claimed in claim 20 wherein the *C. elegans* further contain a transgene comprising a stably integrated *tph1::*marker fusion gene.

23. A method as claimed in claim 22 wherein the marker is a nucleic acid encoding a fluorescent protein.

24. A method as claimed in claim 23 wherein the step of detecting a phenotypic, biochemical or behavioral change in the *C. elegans* indicating a reversion towards wild type serotonin synthesis comprises comparing the level of fluorescence in the absence of the compound under test and the level of fluorescence in the presence of the compound under test.

25. A model animal useful for screening therapeutic agents for serotonin-related diseases, comprising a mutation in a gene encoding a TRPV channel protein and a stably integrated *tph1::*marker fusion gene, wherein the mutation results in reduced *tph1::*marker expression.

26. The animal of claim 25, wherein the animal is *C. elegans.*

27. The animal of claim 25, wherein the gene encoding TRPV channel protein is *osm9* or a homolog thereof.

28. The animal of claim 27, wherein the mutation is *osm-9(yz6).*

29. The animal of claim 25, wherein the gene encoding TRPV channel protein is *ocr2* or a homolog thereof.

30. The animal of claim 29, wherein the mutation is *ocr-2(yz5).*

31. The animal of claim 25, wherein in the marker is a gene for green fluorescent protein (*gfp*).

32. A transgenic model animal useful for screening therapeutic agents for type II diabetes, comprising *ocr-2(yz5)*, *daf-7(e1372)* and a stably integrated *tph1::*marker fusion gene, wherein the mutation results in reduced *tph1::*marker expression.

33. The animal of claim 32, wherein the animal is *C. elegans.*

34. A method for screening therapeutic agents for type II diabetes, comprising the steps of:

providing a model *C. elegans* as recited in claim 33;

dispersing a target drug into agar;

culturing the *C. elegans* on the agar with the drug; and

detecting a progression of the development of the *C. elegans* past the Dauer larval stage in the presence of the target drug.

35. A transgenic model animal useful for identifying compounds which enhance insulin signaling in serotonin deficient mutants, comprising *ocr-2(yz5)*, *daf-7(e1372)*.

36. The animal of claim 35, wherein the animal is *C. elegans.*

37. A method for identifying compounds which enhance insulin signaling in serotonin deficient mutants, comprising the steps of:

providing a model *C. elegans* as recited in claim 36;

mutagenizing the *C. elegans* with EMS; and

detecting a progression of the development of the *C. elegans* past the Dauer larval stage.

38. An isolated nucleic acid comprising a sequence that encodes a polypeptide having the sequence of SEQ. ID NO. 1.

39. An isolated nucleic acid comprising a sequence that encodes a polypeptide having the sequence of SEQ. ID NO. 1 with conservative amino acid substitutions.

40. An isolated nucleic acid comprising a sequence that encodes a polypeptide comprising a fragment of SEQ ID NO. 1 at least 8 residues in length and containing amino acid 36.

41. A purified polypeptide, the amino acid sequence of which consists of SEQ ID NO. 1.

42. A purified polypeptide, the amino acid sequence of which comprises at least ten consecutive residues of SEQ ID NO. 1 and which includes amino acid 36.

43. A purified polypeptide, the amino acid sequence of which comprises residues 1 - 200 of SEQ ID NO. 1.

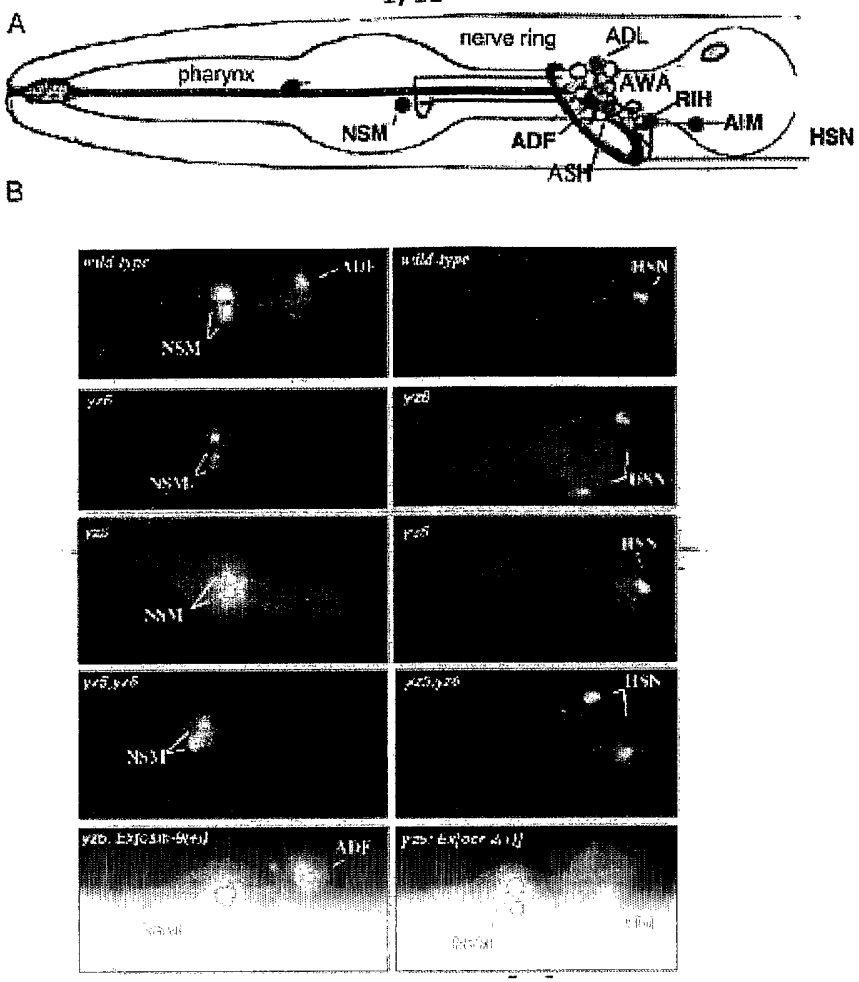
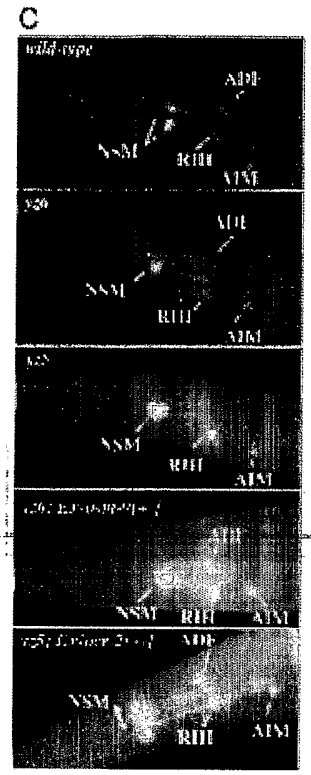

A
nerve ring
ADL
pharynx
AWA
RIH
AIM
HSN
NSM
ADF
ASH
B
wild type
ADF
NSM
wild type
HSN
yz6
NSM
yz8
HSN
yz5
NSM
yz5
HSN
yz5;yz6
NSM
yz5;yz6
HSN
yz6::Ex[odr-9(+)]
ADF
yz8::Ex[odr-9(+)]

Fig. 1A, B


C
wild-type
ADF
NSM
RHI
AIM
ADF
NSM
RHI
AIM
NSM
RHI
AIM
NSM
RHI
AIM

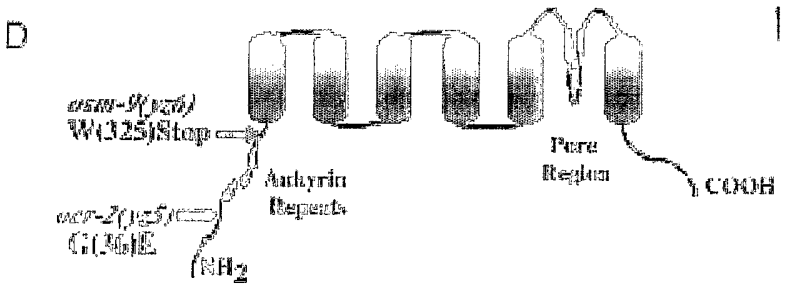

D
W(325)Stop
Ankyrin Repeats
Pore Region
COOH
NH2

FIG. 1C, D

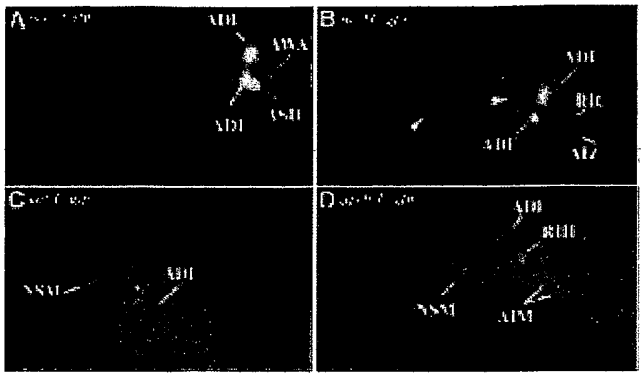
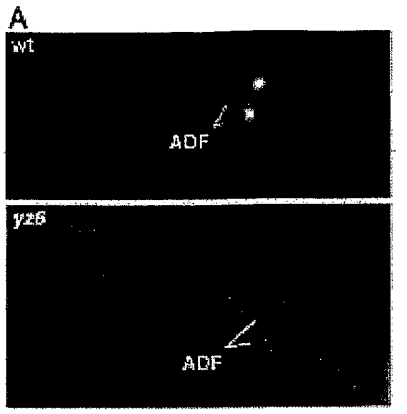


Fig. 2


A
wt
ADF
yz6
ADF

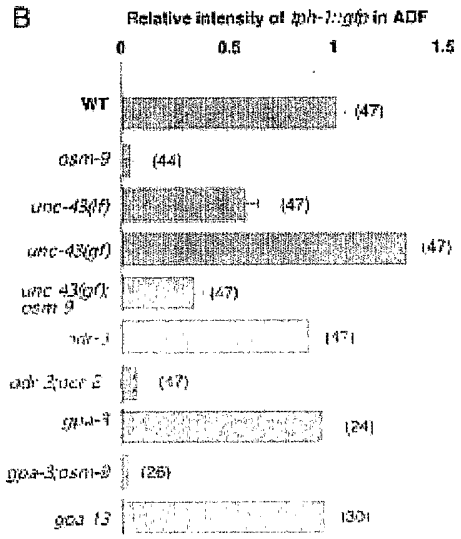

B
Relative intensity of tph-1::gfp in ADF
0
0.5
1
1.5
WT
(47)
osm-9
(44)
unc-43(lf)
(47)
unc-43(gf)
(47)
unc-43(gf); osm-9
(47)
odr-3
(47)
odr-2(ucr-2
(47)
gpa-9
(24)
gpa-3;osm-9
(26)
gpa-13
(30)

Fig. 3

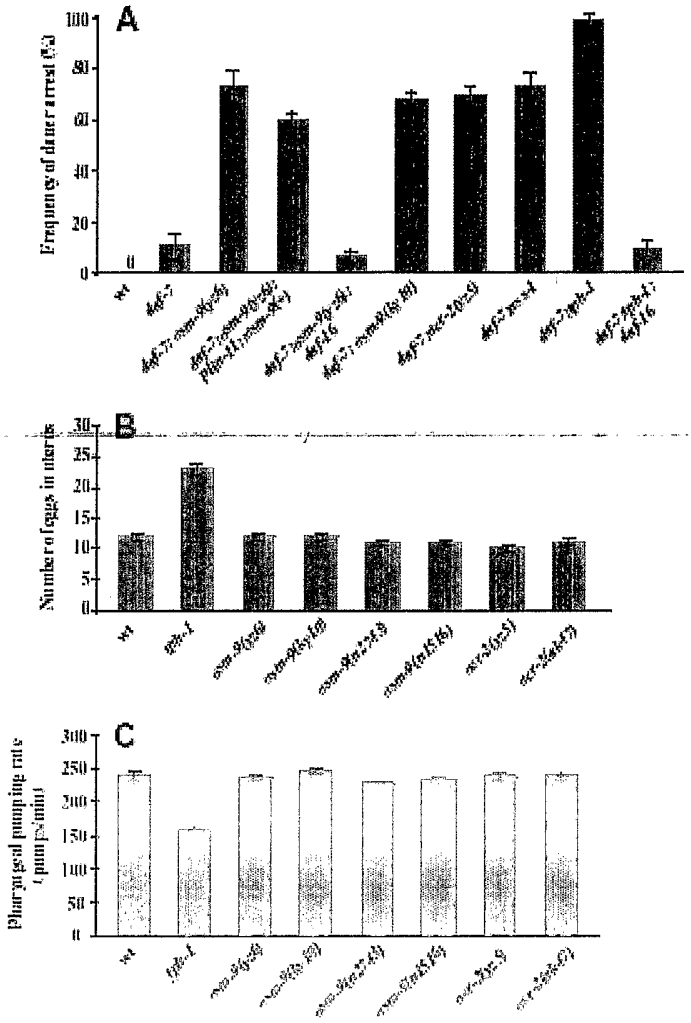

A
Frequency of dauer arrest (%)
100
80
60
40
20
0
B
Number of eggs in uterus
30
25
20
15
10
5
0
C
Pharyngeal pumping rate
(pumps/min)
300
250
200
150
100
50
0

Fig. 4

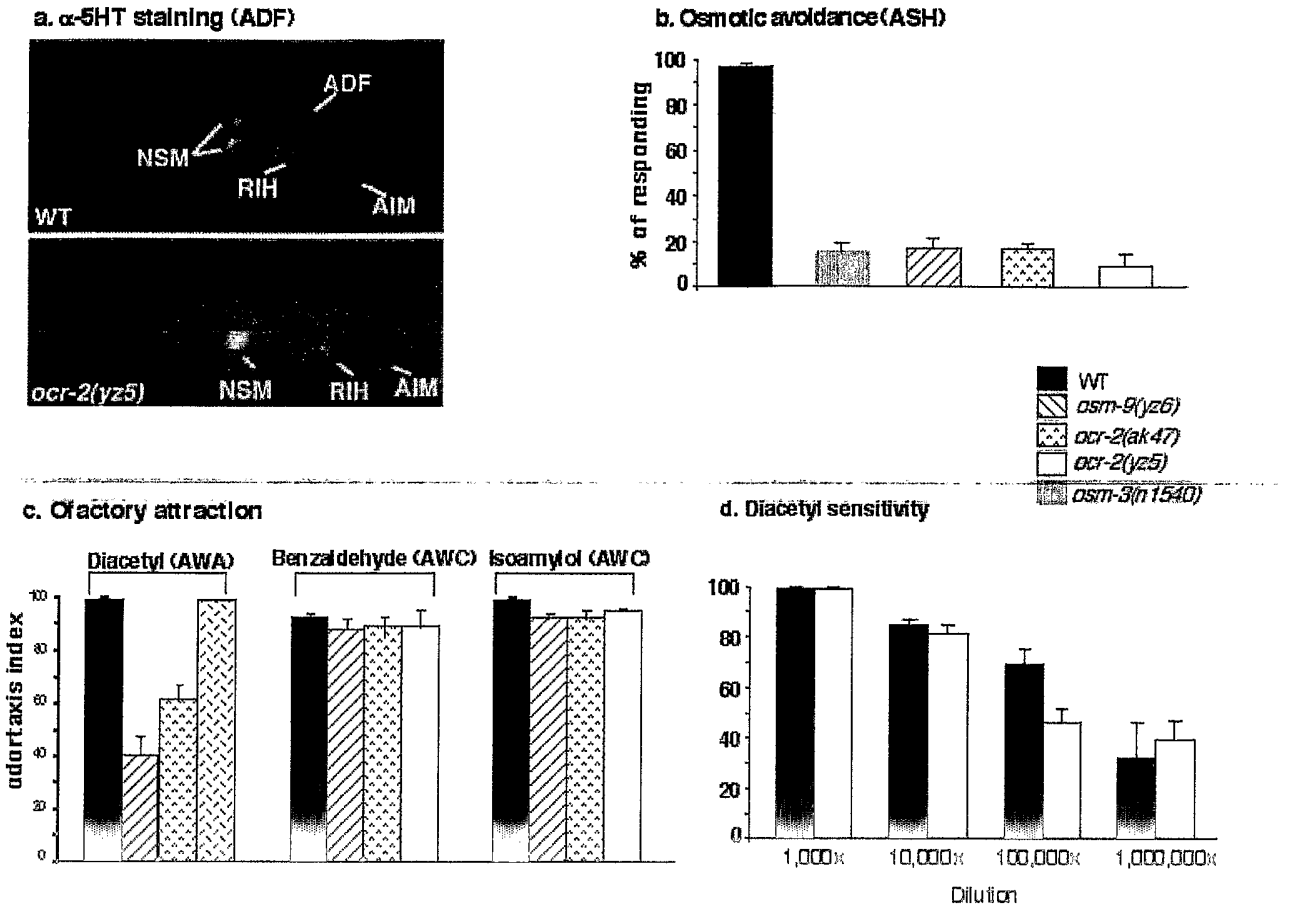


Figure 1.

FIG. 5

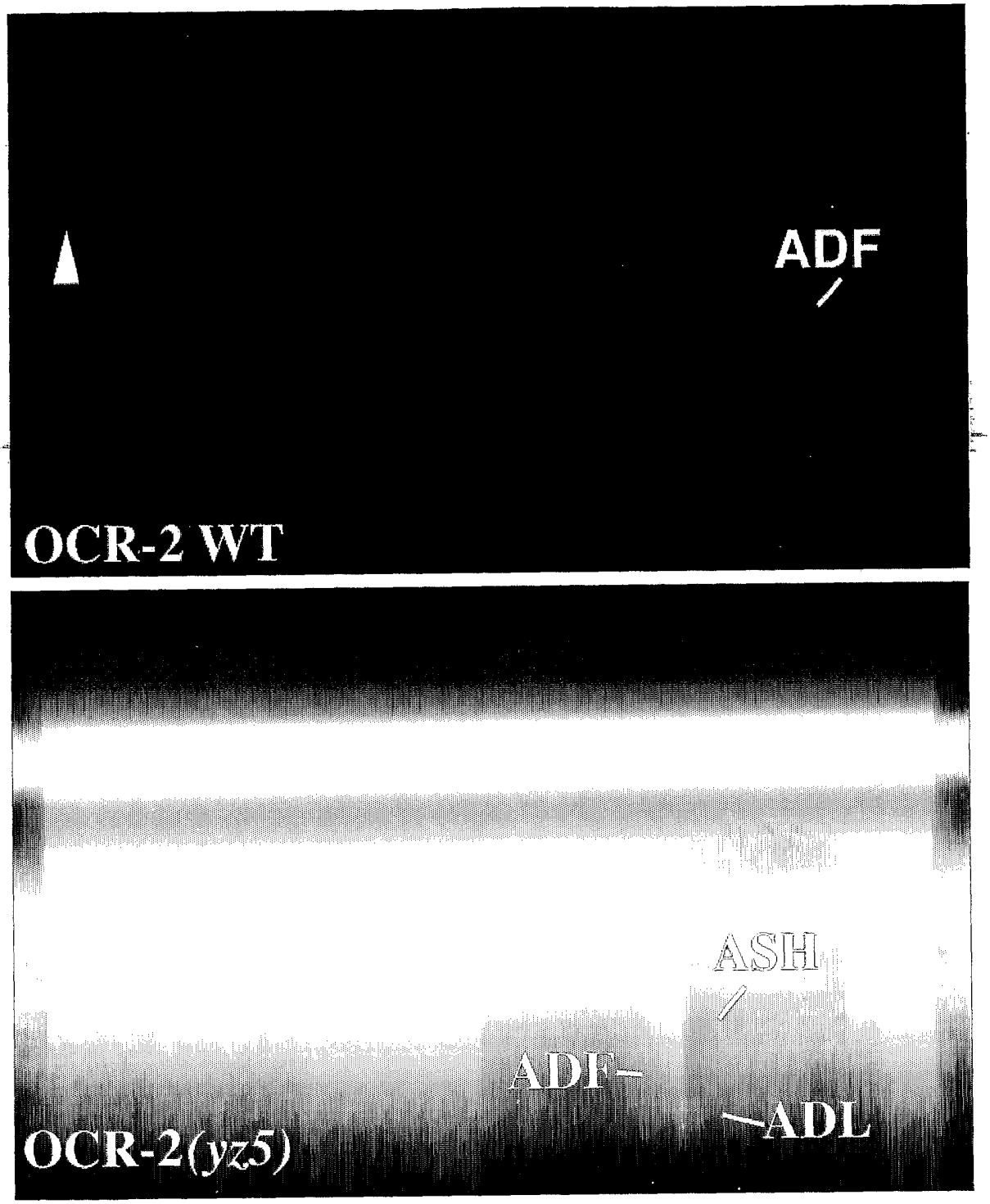


Fig. 6

```
OCR2        1 MG--SSSSTQSSMNNFVGMGRLREDMKIYQLVDMHGGGELLP----
WLRYAIISSDTSFIDAYLETKVKDFLYNGGKGKLVTVTELVKLRNKERNERLGAFSRKKGKGKSGPNVL
DDFNQEGENVGDLKKALKLLDGGGKGGRNESKYRE 144
mTRPV-2     1 M---TSASNPPAF-RLETSDG-DEEG----SAEVN-KGKNEP----
PPMESPFQGEDRNFSPQI--KVN-LNYRKGLGPSQQ--DPN-RFDRDR--LFSVVSR--G----
VPEELTGLLEYL------RRTSKYLT--------DSAYTE 108
hTRPV-2     1 M---TSPSSSPVF-RLETLDGGQEDG-----SEAD-
RGKLDFGSGLPPMESQFQGEDRKFAPQI--RVN-LNYRKGTGASQP--DPN-RFDRDR--LFNAVSR--
G----VPEDLAGLPEYL------SKTSKYLT--------DSEYTE 112
OCR4        1 MGNASSAVTAGIKNQVNASNN-AINK----LADMSGRGFLAD----
LAMDAVRTGNHEELDKQILEKVQPMLYNNGQGEMIPLSDLIAQRHKER--TGNTFAV--P----
VPSTECRFICW----------KLNS--------RGAVGE 114
                *   :*                          :    *            :
:  :*    *  * *      :      ::*       :          *
*                *

OCR2      145 ISWKLEERGSMGETIIGCCLLHASDIHNALVLKILDY-----YP-
KLLNDIHISEDFY-GLSPLHQAIINTDCKLVYKFLKLGADVNSR-
CYGAFFCADDQKASRTDSLEHEYVELSLKTN--YTGNMYLGE----------YPLSFAA 273
mTRPV-2   109 G--------STGKTCLMKAVLNLQDGVNACILPLLQIDRDSGNPQPLVNA-
QCTDEFYRGHSALHIAIEKRSLWCVKLLVENGANVHIRAC-GRFFQ-KHQ-GTCFYFGELPLSLAAC-
TK-QWDVVTYLLENP---H-----QPASLEA 236
hTRPV-2   113 G--------STGKTCLMKAVLNLKDGVNACILPLLQIDRDSGNPQPLVNA-
QCTDDYYRGHSALHIAIEKRSLQCVKLLVENGANVHARAC-GRFFQ-KGQ-GTCFYFGELPLSLAAC-
TK-QWDVVSYLLENP---H-----QPASLQA 240
OCR4      115 ---------TLLHTCFLAGLPNHMK---LLAERLIAI-----FP-
KIINDFYLSDEYY-GETVLHMGIVSENAEFVRYLLKSGADVHAR-
CSGNFFTCDDQKGSRTDHPEVERAILSKHTKYPGNPTMSSKKNPIFSHIYWGEYFLSFAA 244
                          :   *     : :          ::        *   ::*
::::* * : **   *        *    :   ** *: * * * **    *  :     *       :  *
* *  *

OCR2      274
CLNQPESFRLLLAFKANPNAQDTNGNSVLHMCVIHENMAMFKLALECGASLRTVNKQSLSPLTLAAKLA
KKEMFDELLELEGD----------
SVWAYGDASSCAYPLAKIDTINETTGELNEASALSLVVYGQTVER---LELLDGLLD 410
mTRPV-2   237 TDSLGNTVLHALVMIAD-NSPE-NSALVIHM--
YDSLLQMGARLCPTVQLEDICNHQGLTPLKLAAKEGKIEIFRHILQREFSGLYQPLSRKFTEWCYGPVR
VSLYDLSSVDSWEK-----N--SVLEIIAFHCKSPHRHRMVVLEPLNK 375
```

Fig. 7 (1-3)

```
hTRPV-2  241 TDSQGNTVLHALVMISD-NSAE-NIALVTSM--
YDGLLQAGARLCPTVQLEDIRNLQDLTPLKLAAKEGKIEIFRHILQREFSGLSH-
LSRKFTEWCYGPVRVSLYDLASVDSCEE-----N--SVLEIIAFHCKSPHRHRMVVLEPLNK 378
OCR4     245
CLSQPECIRMLVAHGADVNAADINGNTILHICTIHENYEMFKMALSLGADLHIQNRQNLTPLTLAAFLA
KKEMMQRIVEEERT----------
VNWTYGRTQSAAYPLEHVDSIEPTSGSINQKSVLTIAVYGEKVEH---LSLLPHLLE 381
                :     :    :   :  *: : *   :  :                          *
* *:** ***   * *:   *:: *               *:**    : * *  :*:         *  *
* :  :     *   : :*  *

OCR2     411
TLLEAKWEAFAKRNMIVSFTAFTLYYICFVTAFTLRPIGFSTEMLTEGWINRYSEPFPGRYGKNSTLQQ
VKPVINATSRGLVEWSEPLSQCHLRNYWDFDIPFANSYIRLVFELFVVIGICVQVFLDFRDIKRIGRKK
NWNVLTAFPAKI 560
mTRPV-2  376 -LLQEKWDRLIPR-FFFNFACYLVYMIIFTIVAYHQPS-LEQP----------
----------AIPSS-------------------------KATFGDSML-
LLGHILILLGGIYLLLGQLWYFWRRRLFIWISFMDSY-FEI 469
hTRPV-2  379 -LLQAKWDLLIPK-FFLNFLCNLIYMFIFTAVAYHQPT-LKKQ----------
----------AAPHL-------------------------KAEVGNSML-
LTGHILILLGGIYLLVGQLWYFWRRHVFIWISFIDSY-FEI 472
OCR4     382 QLVHHKWIAYGRKTLPAQLSMFCIYFICVTSCFLLRPSPFERQQH----------
----------IANDLICFYD--------------FKTRRFNESAVSTIIY-
HLLHLICVTGAILYLVQALLHIKNVGYHLYVLGLSGFFAKA 493
               *:  **     :          :*          :*
:  : *    :                   :  :

OCR2     561
TFKLTYFLVLAMIFCFLACDLSPVLLVVDNVLITVTMIFTTVHYLYYCRVIRFVGPFVLMVYTIIATDI
FRFMLIYGIFLMGFSQSFSLIFLSCIREANVIKKLITDQSEASEGSDNKFNLTRQISAYDTAIVKNAEV
FENVMQSFIEAF 710
mTRPV-2  470 LFLVQALLCVLS---QVLRFVETEWYLP---
LLVSSLVLGWLNLLYYTRGFQHTGIYSVMIQKVILRDLLRFLLVYLVFLFGFAVALVSLSREARSP---
---------KAPEDS----NTT---------VCEKP-TLGQEEEP--VPY 585
hTRPV-2  473 LFLFQALLCVVS---QVLCFLAIEWYLP---
LLVSALVLGWLNLLYYTRGFQHTGIYSVMIQKVILRDLLRFLLIYLVFLFGFAVALVSLSQEAWKP---
---------EAPTGP----NAT---------ESVQP-MEGQEDEGNGAQY 590
OCR4     494 IFLFSCILMVITFFLRIFCLDEAEDIVW---IVIVLLTS--
LKFLFFCRGFKSVGFFVLMLYKIIIRDLMRFFLIYLVIVVGFSQAFYVIF-LGYK----------
RSDSG-----N----------REPSIMSNVAES----Y 602
               *     * :     ::          :    ::   :    :  *:::*  :  *
: :*:  :*  *: ** *:* : : **: :   :                    :        *
:  :     :

OCR2     711 VRTFILT--IGEFTVLYRNLALCPANTMVWIGKVV--
FILFELFVSIMQFNMLIAMMTRTYETIFQTQLE-
YKRQRAQVILMLELSLSPKERHQYLLKYSRPTGTN-KKTRSLVVSKKSAFSRDSKKGQK-
VLEEKMKKMIEEKKAVLKR 853
```

Fig. 7 (2-3)

```
mTRPV-2  586 GGILDASLELFKFTIGMGELAFQEQLR--
FRGVVLLLLLAYVLLTYVLLLNMLIALMSETVNSVATDSWSIWKLQKAISVLEMENG----------
YWWCRRKRHR--AGRLLKVGTKGDGIPDERWCFR--VEEVNWAAWEKTLPTLSE 719
hTRPV-2  591 RGILEASLELFKFTIGMGELAFQEQLH--
FRGMVLLLLLAYVLLTYILLLNMLIALMSETVNSVATDSWSIWKLQKAISVLEMENG----------
YWWCRKK-QR--AGVMLTVGTKPDGSPDERWCFR--VEEVNWASWEQTLPTLCE 723
OCR4     603 VRMFIMS--LTEFTVFFEQLEECEHT---VIGKIT--
FVVYMLLVTLLLINMLIAMMTNTYTEVSGNSLE-WLRQWSAIILMMEQSFDPAT----
RLRYQRRYGIRFDGGEKLVLLLKDKMTEEEFECQRKKMHEERRKFREELRTNQRR 740
```

```
OCR2     854 KMKDMEIKEGIRFVTGYSRTPRPHTQYMNRGAGGGANGVQNGNGAAH 900
mTRPV-2  720 DPSGAGITGYKK---NPTSK--PGK---N--SASEEDHLPLQVLQSH 756
hTRPV-2  724 DPSGAGVPRTLE---NPVLAS-PPKEDED--GASEENYVPVQLLQSN 764
OCR4     741 RP--VFICKQGA---NYFKS--KHR------HTNNKLIIPTG----- 769
```

Fig. 7 (3-3)

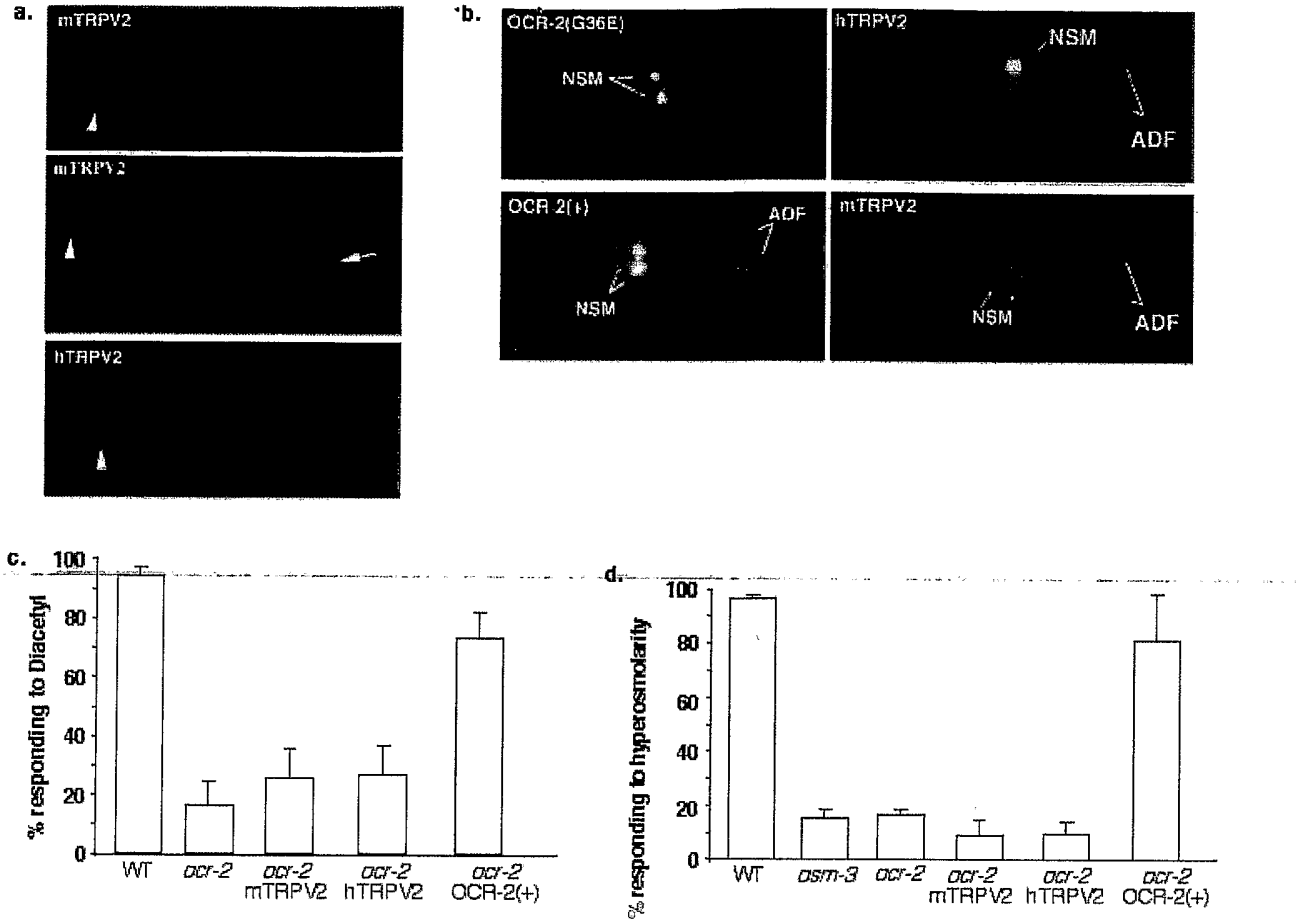

a.
mTRPV2
mTRPV2
hTRPV2
b.
OCR-2(G36E)
NSM
hTRPV2
NSM
ADF
OCR-2(+)
ADF
NSM
mTRPV2
NSM
ADF
c.
% responding to Diacetyl
100
80
60
40
20
0
WT
ocr-2
ocr-2 mTRPV2
ocr-2 hTRPV2
ocr-2 OCR-2(+)
d.
% responding to hyperosmolarity
100
80
60
40
20
0
WT
osm-3
ocr-2
ocr-2 mTRPV2
ocr-2 hTRPV2
ocr-2 OCR-2(+)

Fig. 8

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 3

(2) INFORMATION FOR SEQ ID NO. 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 900 amino acids

(B) TYPE: amino acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

```
  1 MGSSSSTQSS MNNFVGMGRL REDMKIYQLV DMHGGEELLP WLRYAIISSD TSFIDAYLET
 61 KVKDFLYNGG KGKLVTVTEL VKLRNKERNE RLGAFSRKKG KGKSGPNVLD DFNQEGENVG
121 DLKKALKLLD GGGKGGRNES KYREISWKLE ERGSMGETII GCCLLHASDI HNALVLKILD
181 YYPKLLNDIH ISEDFYGLSP LHQAIINTDC KLVYKFLKLG ADVNSRCYGA FFCADDQKAS
241 RTDSLEHEYV ELSLKTNYTG NMYLGEYPLS FAACLNQPES FRLLLAFKAN PNAQDTNGNS
301 VLHMCVIHEN MAMFKLALEC GASLRTVNKQ SLSPLTLAAK LAKKEMFDEI LELEGDSVWA
361 YGDASSTAYP LAKIDTINET TGELNEASAL SLVVYGQTVE HLELLDGLLD TLLEAKWEAF
421 AKRNMIVSFT AFTLYYICFV TAFTLRPIGF STEMLTEGWI NRYSEPFPGR YGKNSTLQQV
481 KPVINATSRG LVEWSEPLSQ CHLRNYWDPD IPFANSYIRL VFELFVVIGI CVQVFLDFRD
541 IKRIGRKKWW NVLTAFPAKI TFKLTYFLVL AMIPTRLACD LSPVLLVVDN VLITVTMIFT
601 TVHYLYYCRV IRFVGPFVLM VYTIIATDIF RFMLIYGIFL MGFSQSFSLI FLSCEREANV
661 IKKLITDQSE ASEGSDNKFN LTRQISAYDT AIVKNAEVFE NVMQSPIEAF VRTFILTIGE
721 FTVLYRNLAL CPANTMVWIG KVVFILFELF VSIMQFNMLI AMMTRTYETI FQTQLEYKRQ
781 RAQVILMLEL SLSPKERHQY LLKYSRPTGT NKKTRSLVVS KKSAFSRDSK KGQKVLEEKM
841 KKMIEEKKAV LKRKMKDMEI KEGIRPVTGY SRTPRPHTQY MNRGAGGGAN GVQNGNGAAH
```