INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

ANALYSIS OF *daf-11*, A TRANSMEMBRANE GUANYLYL CYCLASE THAT MEDIATES CHEMOSENSORY TRANSDUCTION IN *C. elegans*

by Deborah Ann Birnby

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

University of Washington

1998

Approved by _____
Chairperson of Supervisory Committee

_____

_____

_____

Program Authorized to Offer Degree GENETICS

Date 3·20·98

Doctoral Dissertation

In presenting this dissertation in partial fulfillment of the requirements for the Doctoral degree at the University of Washington, I agree that the Library shall make its copies freely available for inspection. I further agree that extensive copying of this dissertation is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for copying or reproduction of this dissertation may be referred to University Microfilms, 1490 Eisenhower Place, P.O. Box 975, Ann Arbor, MI 48106, to whom the author has granted "the right to reproduce and sell (a) copies of the manuscript in microform and/or (b) printed copies of the manuscript made from microform."

Signature Deborah Ann Binley

Date 3.20.98

University of Washington

Abstract

ANALYSIS OF *daf-11*, A TRANSMEMBRANE GUANYLYL CYCLASE THAT MEDIATES CHEMOSENSORY TRANSDUCTION IN *C. elegans*

by Deborah Ann Birnby

Chairperson of the Supervisory Committee:
Associate Professor James H. Thomas
Department of Genetics

C. elegans daf-11 mutants have defects in chemosensory responses to a pheromone and to non-volatile and some volatile attractants. *daf-11* encodes a transmembrane guanylyl cyclase (TM-GC) and provides the first example of a TM-GC that functions in taste and smell transduction. DAF-11 function also provides the first genetic model for this class of proteins. The cyclic GMP analogue 8-bromo-cGMP suppresses the pheromone response defect of *daf-11* mutants, indicating that DAF-11 has a functional guanylyl cyclase activity. In *C. elegans*, specific neurons mediate specific sensory responses. *daf-11::gfp* expression is seen in five identified pairs of sensory neurons (ASJ, AWC, ASI, ASK, and AWB) in a pattern consistent with most *daf-11* mutant phenotypes. *daf-11* mutants also have defects in avoidance of the volatile repellent 2-nonanone. Mosaic analysis suggests that the pheromone response is more complex than previously thought. These

results lead to a model for dauer pheromone response and will enable a greater understanding of smell and taste sensory transduction in *C. elegans*.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AC adenylyl cyclase

ANP atrial natriuretic peptide

cGMP cyclic GMP

cAMP cyclic AMP

Daf-c dauer formation constitutive

Daf-d dauer formation defective

ECD extracellular domain

GC guanylyl cyclase

GFP green fluorescent protein

HSP90 heat shock protein 90

IP_3 inositol 1,4,5-trisphosphate

KHD kinase homology domain

NLS nuclear localization signal

NO nitric oxide

PDE phosphodiesterase

PKA protein kinase A

PLC phospholipase C

TEA tetraethylammonium

TGF-β transforming growth factor β

TM-GC transmembrane guanylyl cyclase

ACKNOWLEDGMENTS

No woman is an island, either, and I have many people to thank. I will begin of course with my advisor and Fearless Leader, Jim Thomas. Jim welcomed me into his lab as a naive first year graduate student and taught me to think, debate, and write. I appreciate his willingness to come through in a pinch, his generally strong opinions, and his short memory for my faults. Next come thanks to the members of my dissertation committee -- Celeste Berg, Lee Hartwell, Jim Hurley, and Colin Manoil, with a double dose to Jim for reading my dissertation and a triple dose to Celeste for reading my dissertation at a difficult time.

The past and present members of the Thomas lab have all been pleasant and constantly entertaining co-workers and great teachers. In particular, I thank Frans Tax for a garden and some perspective in my first year in the lab, Mike Ailion for occasional Scrabble and surreality, and Hong Tian for her helpfulness, friendliness, and patience with my four (poorly accented) Chinese words. I have much appreciated Helen Chamberlin's wisdom and humor in the microscope room and out. Other people in the department have been particularly nice to have around at various times through graduate school as well. Jon Schnorr and Tuyen Vu got me through that first quarter, and Doreen Gillespie, Nick Kosuk, and Alan Fjeld, among others, were friends through several others. Creg Darby has offered his ear at many a lunch, and his family has adopted this Thanksgiving orphan more than once. Linda Martin-Morris and Carol Sibley encouraged my interest in teaching and other possible futures. The office staff has been unfailingly friendly and helpful.

I also owe thanks to a number of people outside of this department: Rick Zucker, who invited me to my first Seattle seder and found me another even after he moved away; Christine Taylor, who gave me carrots when I needed them and has a big ear and a big heart and good advice; Jan Tomenaga, who made a place for music; Bruce, Dianne, Ellie, and Gordon, who made places to dance; Kathleen Dyer, Larry Scott, Bob Goldstein, Jerelyn and Doug Resnick, Kathy McMullen, Gene Gousie, Christie Robertson, Roz Andronescu and her daughters Nicole and Liana, Ronit Katz, Aron Krasnopoler, Anthony Fodor, and the many other folkdancers who became my Seattle family. My little sister, without even trying, helped me keep perspective even when I was in the depths of research hell. John Aaron Smolin wisely told me not to go to L.A., and Michele Oakley always loaned her ear, sometimes for hours at a time.

Two people, Jennifer Vowels and Joy Sabl, deserve special thanks for their friendship, support, entertainment, and wisdom. From Jen, who began the work described in this dissertation, I learned about similarities in difference, and from Joy I learned the converse. Jen also taught me patience, and Joy honed my arguing skills.

Lastly I would like to thank my family. My grandparents, Sam and Ida Levy, in their different ways encouraged me to explore when I was young. My mother, Linda Birnby, has always been a friend and in recent years my father, Martin Birnby, has become a friend as well. My brother Jim grew up to be a mensch, and I am very glad to be part of his life. All of them, along with many other relatives, have supported, taught, and encouraged me in many ways throughout my life. It is

perhaps trite but also true to say that I could not have done this work without them.

DEDICATION

L'ima v'abba sheli

(To my mother and father)

CHAPTER 1: INTRODUCTION

This dissertation concerns the molecular cloning and cellular localization of the *C. elegans* gene *daf-11*. *daf-11* mutants have defects in sensory responses analogous to taste and olfaction as well as a defect in response to a pheromone that induces a specialized larval stage called a dauer. This work shows that *daf-11* encodes a member of the transmembrane guanylyl cyclase (TM-GC) family, a family whose role in sensory transduction is best understood in visual transduction, and that it functions in specific sensory neurons. To introduce the main body of this work, this chapter will briefly review the current state of knowledge of olfaction and taste; introduce TM-GC biology, visual transduction, and the role of cyclic GMP (cGMP) in olfaction; and discuss the sensory responses and dauer formation pathway in *C. elegans*.

SENSORY RESPONSE

All organisms must be able to detect and respond to signals about their environments. Sensation is the detection of a stimulus (such as odor, taste, or light) from outside an organism and the communication of information about that stimulus. In animals, this information is communicated to the central nervous system. Three types of sensations, odor, taste, and light, are all detected by specialized neurons located respectively in the olfactory epithelium of the nose, the taste buds of the tongue, or the rod and cone cells of the retina. These neurons are depicted in Figure 1.1. These neurons all have sensory processes that interact with the environment. In the case of taste and

odor, the sensory process is physically exposed to the environment, while in the retina the cell is protected. Since the signal in visual transduction, light, can penetrate the eyeball, the rod cells are also directly exposed to their signal. The similarities of these cells with the amphid neurons of *C. elegans* (Figure 1.5) are discussed later in this chapter.

OLFACTORY TRANSDUCTION

Odorants are detected in most organisms by sensory organs well-exposed to the outer environment such as antennae in insects and the nasal cavity in mammals. They are detected by odorant receptor cells, which then transduce the signal to cells in the olfactory bulb, where signals are integrated and processed. Here I will focus on the initial events in transduction, those that occur in the receptor cells.

Many odorants are hydrophobic, and it is thought that there are accessory proteins (odorant binding proteins) in extracellular mucus surrounding the sensory cells that concentrate them and bring them in contact with the cell surface. Candidate odorant binding proteins have been identified in both vertebrates and invertebrates (Hildebrand and Shepherd, 1997), but direct evidence for a role in olfaction has not been shown. In actual transduction events, the first step is recognition of an odorant by a receptor. Large families of G-protein-coupled receptors have been identified in olfactory neurons in several vertebrates including humans (e.g. Buck and Axel, 1991; Ben-Arie et al, 1994; Parmentier et al, 1992; Ngai et al, 1993), and several sets of G-protein coupled receptors that are largely expressed in sensory neurons have been identified in *C. elegans* (Troemel et al, 1995). One of these genes,

odr-10, has been shown to encode the diacetyl receptor (Sengupta et al, 1996).

Downstream of receptor binding, there are two known pathways, one IP_3-mediated and one cAMP-mediated (Figure 1.2). These pathways are found in the same cell in lobster (Buck, 1996) but in other organisms one pathway or the other may function (Restrepo et al, 1996). When the pathways occur in the same cell, it appears that a given odorant activates only one or the other (Breer, 1993). Either adenylyl cyclase or phospholipase C is activated by a G protein, G_{olf} in the case of the cAMP pathway and G_q or G_o in the case of the IP_3 pathway (Restrepo et al, 1995). The second messengers cAMP or IP_3 are generated, and they open ion channels. These are both general cation channels and Ca^{++} channels (Restrepo et al, 1995; Buck, 1996). The increased $[Ca^{++}]_i$ causes Cl^- channels to open as well, and possibly additional cation channels (Restrepo et al, 1995). As a result of the opening of these channels, the membranes of the sensory cells can become either depolarized, resulting in activation, or hyperpolarized, resulting in repression of basal signaling (Restrepo et al, 1995). It is unclear exactly how the influx of Ca^{++} would cause hyperpolarization. It is possible that the effect is through inhibition of the Na^+/Ca^{++} cation channel (Buck, 1996), as shown in Figure 1.2.

The downstream processing of the signals sent by sensory cells in response to different odorants is a subject of much interest. Briefly, a model (discussed in Buck, 1996) has been proposed in which receptors recognize epitopes on odorants rather than whole molecules. In this model, each cell expresses one receptor. Each odorant interacts with more than one receptor, and each receptor responds to more than one odorant molecule. Thus the response elicited by any single odorant is a

unique combination of activated receptor cells. Given the large number of receptor and downstream cells in vertebrates, this combination of interactions is quite complex. Vertebrate cells are amenable to many forms of study, including in situ hybridization, molecular biology, and electrophysiology, but a simpler model would be useful. *C. elegans*, with its fewer neurons (302 in the adult hermaphrodite (White et al, 1986)), predicted neuronal connections (White et al, 1986), invariant cell lineage and localization (Sulston et al, 1983), behavioral assays, and easy genetic manipulability provides an excellent model. The recent discovery of an apparent family of olfactory receptors in *C. elegans* (Troemel et al, 1995) and proof that one of these molecules functions as a receptor (Sengupta et al, 1996) suggest that *C. elegans* is indeed a useful model in which to study olfaction (see also reviews in Sengupta et al, 1993 and Mori and Ohshima, 1997).

TASTE TRANSDUCTION

Taste transduction is mediated by taste receptor cells, neurons located in the taste buds (Figure1.1B) on the surface of the tongue. These neurons form synapses with gustatory afferent neurons, but in some cases there appear to be synaptic vesicles on both sides of the synapse, and it is not clear which cell is presynaptic and which postsynaptic (Kinnamon and Getchell, 1991). Taste stimuli fall into four main categories: sweet, bitter, salty, and sour. Though taste has been less well-studied than olfaction, there are some data. Salty, sour, and some bitter tastes are thought to be transmitted directly, while sweet and other bitter tastes are, like olfactory stimuli, transmitted through second messenger systems. The discussion below is based on a combination of evidence from several different organisms and is summary of some of the main pathways. Unless otherwise noted, the evidence given here is

based on Kinnamon and Getchell, 1991, Lindemann, 1996, and references therein.

SALTY AND SOUR TASTE

Response to salt, or detection of Na^+ ions, is thought to be mediated directly through Na^+ channels in the apical membrane of taste cells. The response is quick, suggesting that the effect is direct rather than through a messenger. The response is also blocked by the Na^+-channel blocker amiloride. This interaction has been measured directly in rats (a Na^+-stimulated current is blocked by amiloride) and indirectly in humans, where amiloride causes reduced perception of NaCl flavor. The amiloride-gated channel has been isolated and shown to be different from voltage-gated Na^+ channels in several ways. In fact, it is similar to cyclic nucleotide gated channels, which are thought to function in olfactory sensory transduction, in several ways, including its response to amiloride. Combined, this evidence suggests that these amiloride-blocked Na^+ channels directly transduce the Na^+-response that is taste transduction (Figure 1.3 A).

In contrast, response to a sour taste, or the detection of acid, is thought to be directly caused by blockage of a K^+ channel by a proton. In the mudpuppy, it has been shown that acid-induced action potentials are blocked by the K^+-channel blocker TEA and, in single channel recordings, that protons in fact directly block K^+ channels. There is a constitutive K^+ outward current in the apical membrane, and it seems that block of this current causes the action potential that is ultimately perceived as acid taste (Figure 1.3 A). In contrast, in the dog it is

thought that the proton activates a Cl^- gate, causing Cl^- efflux and cellular depolarization.

SWEET AND BITTER TASTE

Like the response to odorants, the response to sweet and some bitter tastes is primarily mediated by second messengers. However, these pathways are less well-understood than those underlying olfactory transduction. There are three main pathways, all thought to be initially mediated by a G-protein coupled receptor and a heterotrimeric G protein: (1) a phospholipase C/IP_3 pathway; (2) an adenylate cyclase pathway that acts either directly through cAMP or through protein kinase A; and (3) a phosphodiesterase pathway. These pathways are summarized on Figure 1.3, and the evidence for them is briefly discussed below.

1. Phospholipase C-mediated transduction (Figure 1.3 B): In rat cells, the sweeteners saccharin and SC-45647 increase IP_3 but not cAMP levels, and these same cells show an increase in Ca^{++} in response to SC-45647 and sucrose. In mouse and rat tissue, several bitter flavors cause an increase in IP_3. In the mouse tissue, the IP_3 increase induced by one of these bitter stimuli, sucrose octaacetate, is inhibited by pertussis toxin, suggesting a dependence on $G_i\alpha$. Animals with reduced sensitivity to sucrose octaacetate have reduced IP_3 synthesis in response to the stimulus. These data are consistent with a model in which a tastant binds to a G-protein-coupled receptor, causing activation of $G\alpha$. $G\alpha$-act activates PLC, which catalyzes formation of IP_3. IP_3 causes release of Ca^{++} from intracellular stores, and the increased Ca^{++} concentration causes neurotransmitter release and signaling.

2. Adenylyl cyclase-mediated transduction (Figure 1.3 C): Sweet tastes appear to also be transduced through an adenylyl cyclase (AC) mediated pathway. AC activity has been shown in bovine taste receptor cells. In the rabbit, AC has been histochemically localized to the apical portion of the taste receptor cell. In rat and pig, sugar has been shown to increase AC activity. In rats, sucrose-induced AC activity is specific to sensory tongue epithelium and is GTP dependent, suggesting involvement of a G-protein in the process. Inhibitors of sweet response also inhibit cAMP production. Lastly, in the mouse, cAMP and cGMP mimic the depolarization and increased resistance of taste receptor cells induced by sucrose. In the blowfly, cAMP analogues mimic signaling effects as well. In membranes from frog taste tissue, cAMP induces an ATP-dependent depolarization. This effect is blocked by a protein kinase inhibitor. A cAMP-dependent protein kinase causes the same effect in the presence of ATP and also closes a K+ channel, causing depolarization. These data suggest a model in which a tastant binds to a G-protein-coupled receptor, causing release of an activated Gα. Gα-act activates adenylyl cyclase, causing an increase in cAMP concentration. cAMP activates PKA, which closes a K^+ channel, causing depolarization, inflow of Ca^{++}, and finally neurotransmitter release and signaling. It is also possible that in some cases, cAMP acts directly on the channel, rather than activating a protein kinase.

3. Phosphodiesterase-mediated transduction (Figure 1.3 D): Some bitter taste transduction is mediated by phosphodiesterase (PDE) activity. This mechanism appears to be an alternative transduction pathway for sweet taste in the frog as well. In the frog, the artificial sweeteners saccharin and NC-01 cause an inward current that is mimicked by peptides derived from the Gα α-transducin. This current is inhibited by PDE inhibitors, forskolin, and the poorly-hydrolyzed cAMP analogue 8-

bromo-cAMP, suggesting involvement of a PDE. The inhibition of the current is not dependent on ATP or protein kinase activity, suggesting a direct effect of cAMP on a channel. Several bitter agents, including quinine and strychnine, have been shown to affect PDE activity, though it remains unclear whether they activate or inhibit the PDE. In the rat and mouse, it has been shown that cAMP levels are lowered in response to the bitter compound denatonium via a G-protein related to transducin.

The simplest model to account for these observations is one in which cAMP normally closes a cation channel. Sensory input activates, via a receptor and associated G-protein, a PDE. The PDE hydrolyzes cAMP, allowing the channel to open. The open channel causes depolarization, release or inflow of Ca^{++}, and neurotransmitter release and signaling. Cation channels that are closed by cyclic nucleotides have been identified in frogs, insects, and mammals. This model is similar to that for visual transduction (see below) in that high cAMP levels correspond to "no input" and a PDE is involved in signaling. However, in this pathway, cAMP closes the channel, and the default state of the cell in the absence of signal (taste) is not depolarized and not signaling. In contrast, in visual transduction, cGMP opens the channel, and the default state of the cell in the absence of input (light) is depolarized and signaling.

TRANSMEMBRANE GUANYLYL CYCLASES AND VISUAL TRANSDUCTION

Transmembrane guanylyl cyclases (TM-GCs) have been identified in animals from *C. elegans* to humans (reviewed in Yuen and Garbers, 1992; Drewett and Garbers, 1994). They are thought to function in various transduction systems including a chemotactic response in sea

urchin sperm, regulation of blood pressure in humans, and visual signaling in mammals. An enterotoxin receptor has also been found in humans, and a TM-GC (GC-D) has been identified in olfactory tissue in the rat. TM-GCs consist of an extracellular domain, a single transmembrane domain, and an intracellular region. The intracellular region contains a domain with homology to protein kinases (KHD, kinase homology domain) and a C-terminal guanylyl cyclase (GC) domain that catalyzes the conversion of GTP to cGMP. The KHD lacks key residues required for kinase catalytic activity (Yuen and Garbers, 1992).

In the case of the enterotoxin and natriuretic receptors, it has been shown that cyclase activity is induced by ligand binding to the extracellular domain (Chinkers et al, 1989; de Sauvage et al, 1991). ATP has been proposed to have both activating and inhibitory effects on cyclase activity (Chinkers et al, 1991; Parkinson et al, 1994). In the case of the enterotoxin receptor, ATP appears to protect the cyclase from inactivation (Vaandrager et al, 1993a; Vaandrager et al, 1993b). Deletion of the KHD results in increased cyclase activity in the natriuretic receptor GC-A (Chinkers and Garbers, 1989), suggesting that this domain normally represses activity. Since ATP potentiates the effects of the ligand ANP on GC-A (Chinkers and Garbers, 1989), it is possible that ATP negatively interacts with this domain.

cGMP is a widely used second messenger that regulates kinases, other nucleotide cyclases, cyclic nucleotide phosphodiesterases, and cGMP-gated ion channels (reviewed in Goy, 1991). For example, in mammalian retinal rod outer segments (Figure 1.4), high intracellular cGMP levels in unstimulated cells open a cation channel. Cations cause depolarization of the rod cell. Light is detected by the seven-pass

receptor rhodopsin, which activates a cGMP phosphodiesterase via a G protein (transducin). This phosphodiesterase hydrolyzes cGMP, causing closure of the cation channel and hyperpolarization of the rod cell (reviewed in Koutalos and Yau, 1993; Hurley, 1987). This pathway will be discussed in more detail in Chapter 4.

cGMP IN OLFACTION

There is also evidence for a role for cGMP in olfaction. Much of this evidence suggests that this cGMP is dependent on nitric oxide (NO) (e.g. Breer et al, 1992; Hopkins et al, 1996; see also Breer and Shepherd, 1993). NO activates the soluble form of guanylyl cyclase but not the transmembrane form (Garbers, 1992). There is also evidence that cGMP does not appear until at least 500 msec after stimulation (Breer et al, 1992), suggesting that this pathway is not a primary response pathway. Instead, it could serve as an intercellular signaling pathway or have a role in adaptation (Breer and Shepherd, 1993). In the proposed intercellular signaling pathway, odorant activates adenylate cyclase, which opens cyclic nucleotide gated channels. Ca^{++}, let into the cell by these opened channels, activates NO synthase. NO synthase catalyzes formation of NO, which diffuses into neighboring cells, where it activates soluble GC, causing synthesis of cGMP and opening of more cyclic-nucleotide gated channels. It is thought that only high levels of odorant would enable the entry of enough Ca^{++} to activate NO synthase. This recruitment of neighboring cells may serve as a way to measure odorant concentrations. A possible role for cGMP in adaptation is suggested by the fact that the cGMP analogue 8-bromo-cGMP causes reduced response to odorant stimulus in both rat cilia and insect antennae (Breer and Shepherd, 1993).

Despite evidence for a role for cGMP and soluble guanylyl cyclase in olfactory signaling, there has not been evidence of a role for TM-GCs in olfaction, or evidence of a role for cGMP in primary olfactory response, until recently. A TM-GC (GC-D) and a cGMP-stimulated PDE (PDE2) have been identified in a subset of olfactory neurons in the mouse. These neurons do not express adenylyl cyclase or a cAMP-specific PDE (PDE4A) (Juilfs et al, 1997). *In vivo*, it is known that mutation of *the C. elegans* gene *odr-1* causes defects in some olfactory responses (Bargmann et al, 1993). Noelle L'Etiole has cloned *odr-1* (N. L'Etiole and C. Bargmann, pers. comm.) and found that it encodes a TM-GC. Interestingly, *odr-1* has many similarities to *daf-11*, the subject of this work. *odr-1* will be discussed in more detail in Chapter 4.

CHEMOSENSATION IN *C. elegans*

C. elegans has several chemosensory responses (reviewed in Bargmann and Mori, 1997). It is able to respond with attraction or avoidance to many chemicals, volatile (olfaction) and non-volatile (taste). (e.g. Ward 1973; Dusenbery 1974; Bargmann et al, 1993; Troemel et al, 1997). In addition, it avoids solutions of high osmotic strength (Culotti and Russel, 1978) and has a specific developmental response (dauer formation; discussed below) to a constitutively secreted pheromone (Golden and Riddle, 1984a).

All of these chemosensory responses are detected through a set of bilaterally symmetric sensory neurons called the amphids, located in the head of the animal (Figure 1.5). Like the sensory neurons discussed

earlier, the amphid neurons are exposed to the environment. Eight pairs of amphids have single or double cilia for which this exposure is direct; four have branched cilia that are only indirectly exposed to the environment. Each function is primarily mediated by a single pair of amphid neuron, identified by laser cell-killing experiments in which an animal with some cell or cells destroyed by a laser microbeam is tested for response to a specific stimulus (described in Bargmann and Avery, 1995). Relevant to this work are the cells discussed in Table 1.1.

DAUER FORMATION IN *C. elegans*

Under normal laboratory conditions, *C. elegans* starts life as an egg and undergoes four successive larval stages before becoming a self-fertilizing hermaphroditic adult. This process takes about 3.5 days at 20°C, the standard laboratory temperature. Each larval stage takes approximately the same amount of time in each animal. In contrast, under environmentally harsh conditions an animal can enter an alternate third larval stage called a dauer (Cassada and Russel, 1975). Dauer larvae are non-feeding, desiccation-resistant, and can survive for months, well past the normal lifespan of *C. elegans*. When conditions improve, the animal will re-enter the life-cycle and live out a normal life.

Dauer formation is induced by a constitutively secreted dauer pheromone (Golden and Riddle, 1984a), and favored by conditions of high temperature and low food. Since this decision is influenced by temperature, the process is inherently temperature sensitive (Golden and Riddle, 1984a and 1984b). This pheromone is detected by the

amphid sensilla, sensory organs located in the head of the worm (Albert et al, 1981; Perkins et al, 1986; Shakir et al, 1993; Vowels and Thomas, 1994) (Figure 1.5). At 25°C (the non permissive temperature used in this work) or lower temperatures, pheromone is necessary and sufficient to induce dauer formation in the wild type (Golden and Riddle, 1985; Golden and Riddle, 1984b), but at 27°C some dauers form independent of pheromone. (M. Ailion and J.H. Thomas, pers. comm.). Thus at 25°C and below, we think of dauer formation in wild type animals primarily as a developmental response to pheromone.

Dauer larvae have several morphological modifications that are thought to enable long-term survival (reviewed in Riddle, 1997). The cuticle thickens, the pharynx is remodeled, and granules that are thought to be extra sources of energy appear in the hypodermis and intestine. Dauers can survive without food for up to a month and tend to wave their bodies from points that extend out of the surface of the agar. This behavior, called nictation, may be useful in the wild as a way of being picked up (by a passing larger animal, for example) and carried to a more hospitable new environment.

THE PATHWAY OF DAUER FORMATION GENES

The process of dauer formation may be divided into two distinct phases: one, pheromone sensation and the decision to make a dauer, and two, the execution of this decision by induction of the morphological changes that occur in dauers. Genes involved in both phases have been identified. Most genes, and all genes involved in the decision-making phase, can be divided into two classes: those identified

by mutations that confer a dauer constitutive (Daf-c) phenotype and those identified by mutations that confer a dauer defective (Daf-d) phenotype. Worms that are Daf-c form dauers even in the absence of inducing conditions (low temperature, low pheromone, and abundant food); worms that are Daf-d fail to form dauers even in the presence of inducing conditions (high temperature, high pheromone, low levels of food). The work in this dissertation concerns only genes involved in the decision-making process.

Prior to this work, dauer formation had been understood only in terms of linear pathways (Riddle et al, 1981; Vowels and Thomas, 1992) (Figure 1.6). The cilium structure genes are a set of genes that, when mutant, cause disruption of the ciliated sensory endings of the amphid neurons that are the first cellular step in the response to pheromone. These pathways were generated on the basis of epistasis analysis (Avery and Wasserman, 1992; Hereford and Hartwell, 1974) between Daf-c and Daf-d mutations. Strains carrying one of each type of mutation were generated, and the phenotype of the double mutant was used to determine which mutation lay upstream. Because the pathway was regulatory (vs. biosynthetic) and mutations in the pathway conferred opposite phenotypes, the epistatic phenotype (the phenotype of the double mutant) was interpreted as that caused by the downstream mutation. This led to some confusion and ambiguity, as in many cases epistasis was incomplete. Some genes, most notably *daf-3* and *daf-5*, could not easily be placed in a linear pathway (Vowels and Thomas, 1992).

The observation that the Daf-d phenotype caused by mutation of *daf-3* and *daf-5* fully suppresses the Daf-c mutation of *daf-1,4,7,8*, and *14* and only partially suppresses the Daf-c phenotype caused by mutation of

daf-11 caused us to rethink the previous simple, linear pathway. One possible explanation for the epistasis pattern shown by *daf-3*, *daf-5*, and the above-mentioned Daf-c mutations is that there are two branches to the pathway, one containing *daf-11* and *daf-21* (group 1) and the other containing *daf-1, 4, 7, 8,* and *14* (group 2) (Figure 1.7). In this model, *daf-3* and *daf-5* are downstream of the group 2 genes, which they suppress strongly, but parallel to the group 1 genes, which they suppress only weakly. This weak suppression is presumably due to cross talk between the pathways. The decision whether or not to form a dauer is a major one for the worm, and it incorporates many inputs (at least pheromone, food, and temperature), so it is not surprising that there is some effect of the state of one pathway on the other.

This hypothesis also explained other observations better than a simple linear pathway could. For example, animals carrying any tested mutation in any of these six Daf-c genes (at least 13 alleles; Vowels and Thomas, 1992 and 1994) are incompletely penetrant at 15° C, and each remains responsive to pheromone, indicating that dauer formation itself is not at a maximum level in these mutants. (An exception to this is *daf-11(m84)*, which causes a high percentage of dauer formation even at 15° (Vowels and Thomas, 1994). However, *daf-11(m84)* is not likely to be a null allele since *daf-11(m84)* mutants are wild type for some other *daf-11* phenotypes (Vowels and Thomas, 1994; discussed in Chapter 2)). It seemed unlikely that all of these mutations caused incomplete loss of gene function. In this model, each mutation could cause a complete loss of function, completely eliminating signal transduction through its branch of the pathway, but induction of dauer formation would be incomplete and response to pheromone would remain possible due to the second branch of the pathway.

This hypothesis led to two testable predictions based on the idea that the incomplete penetrance of any single mutation is due to continued function of the other branch of the pathway. The first is that double mutants within each group (e.g. a *daf-4; daf-7* double mutant) should also be incompletely penetrant, since even a double mutant within a single branch should leave the other branch functional. In contrast, double mutants containing one mutation from each branch of the pathway (e.g. a *daf-11; daf-7* double mutant) should be fully penetrant, forming 100% dauers even at the permissive temperature of 15° C. This proved to be true (Thomas et al, 1993).

A second prediction is that double mutants within a group would retain the ability to respond to pheromone. If pheromone response of single mutants is due to transduction through the alternate branch of the pathway, animals mutant for two genes in the same branch of the pathway should retain the ability to respond to pheromone, since the alternate branch is still active. This also proved to be true (Table 1.2).

Neither of these facts can be easily explained by the simple linear pathways previously proposed. Each allele in such a model would have to be incompletely penetrant, and we would predict that a double mutant between any pair of alleles would increase the penetrance, with some double mutants of each type (intra-group and inter-group) having a completely penetrant phenotype. Similarly, we would predict that some intra-group double mutants would cause complete activation of the dauer formation program and therefore have no ability to respond to pheromone. Since the parallel pathway model is more consistent with the data, we now take it as the most accurate model for the processing of the dauer formation input signals at 25° C. Not all genes involved in dauer formation are represented on this

pathway. Some are involved in downstream steps and are included in other models (Gottlieb and Ruvkin, 1994; Larsen et al, 1995). Others have not been definitively placed on the pathway. Some of these will be discussed in Chapter 4.

MOLECULAR IDENTITY OF DAUER FORMATION GENES

Several of the genes shown on this pathway have been cloned and have homology to known proteins. The final gene shown, *daf-12*, has homology to nuclear hormone receptors (P. Larsen, W.-H. Yeh, and D. L. Riddle, pers. comm.). This is consistent with its placement at the most downstream step in the decision-making pathway: it may receive and execute the hormonal signal that is the output of the decision-making process. The hormone itself, however, has not been identified.

Upstream of that signal, the two branches of the pathway correspond to two signal transduction pathways that appear to be mediated by different sets of cells (Bargmann and Horvitz, 1991a; Shackwitz et al, 1996). Several of the Group 2 genes have been cloned. *daf-7* is a TGF-ß homologue (Lim, 1993) and *daf-1* and *daf-4* are the two subunits of the TGF-ß receptor (Georgi et al, 1990; Estevez et al, 1993). *daf-14* and *daf-3* encode members of the SMAD family of proteins, downstream targets of TGF-ß signaling pathways. (T. Inoue and J. H. Thomas, pers. comm., and Patterson et al, 1997).

These genes constitute a well-studied signaling pathway. My intent in undertaking study of *daf-11* was to elucidate the molecular events

underlying the group 1 pathway. I was particularly interested in this pathway for two additional reasons: (1) suppression of the Daf-c phenotype of *daf-11* mutants by cilium structure mutants suggests that DAF-11 function is dependent on intact amphid cilia and therefore that DAF-11 functions early in the transduction process, and (2) *daf-11* mutants have several sensory defects, suggesting that DAF-11 is a multi-purpose signaling molecule.

Table 1.1 Amphid neurons and their functions

Neuron	Function	Reference
ASJ	dauer formation (group 1, promotion)	Shackwitz et al, 1996
AWC	attraction to isoamyl alcohol and benzaldehyde (volatile)	Bargmann et al, 1993
AWA	attraction to pyrazine and diacetyl (volatile)	Bargmann et al, 1993
ASE	attraction to non-volatile attractants	Bargmann and Horvitz, 1991b
ASI	dauer formation (group 2, repression)	Bargmann and Horvitz, 1991a; Schackwitz et al, 1996
ASK	small roles in: chemotaxis to lysine and possibly other non-volatile attractants, dauer formation (group 1, promotion)	Bargmann and Horvitz, 1991b; Schackwitz et al, 1996
AWB	avoidance of 2-nonanone (volatile)	Troemel et al, 1997

Cell assignments are based on cell kill experiments. References as given.

Table 1.2 Dauer formation in response to pheromone.

Genotype	No pheromone	2 units pheromone
N2	0 (1469)	79.1 (1235)
daf-1(m40)	1.2 (328)	97.5 (122)
daf-1(e1287)	0.8 (628)	97.8 (492)
daf-4(e1364)	73.0 (293)	100 (310)
daf-4(m72)	94.8 (310)	100 (225)
daf-7(e13672)	61.4 (606)	99.7 (303)
daf-7(m62)	65.0 (551)	99.7 (391)
daf-8(e1393)	11.7 (643)	98.8 (510)
daf-14(m77)	73.2 (605)	100 (297)
daf-7(e1372); daf-1(m40)	77.7 (256)	100 (177)
daf-7(e1372) daf-4(e1364)	77.4 (366)	100 (353)
daf-8(e1393); daf-7(e1372)	94.6 (427)	99.6 (252)
daf-7(e1372); daf-14(m77)	69.5 (272)	100 (158)
daf-11(m47)	9.2 (437)	79.9 (278)
daf-11(m51)	10.3 (380)	96.8 (526)
daf-11(,87)	16.4 (293)	94.8 (326)
daf-11(m124)	68.2 (277)	99.6 (243)
daf-11(sa195)	36.3 (361)	100 (257)
daf-21(p673)	48.6 (181)	100 (117)
daf-11(m47) daf-21(p673)	26.5 (245)	85.0 (180)
daf-11(m87) daf-21(p673)	1.7 (422)	65.0 (430)
daf-11(m124) daf-21(p673)	6.7 (373)	85.6 (348)

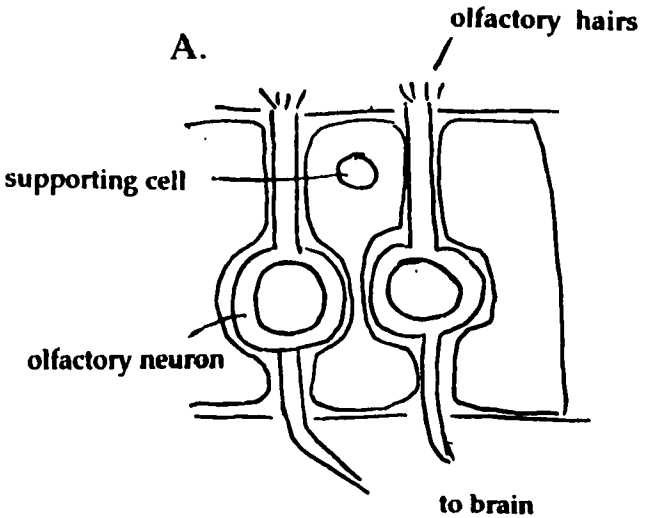
Table 1.2, continued

Percent Dauer formation of single and double Daf-c mutants in response to dauer pheromone at 15°. Pheromone tests were performed using pheromone that was crudely purified as described (Golden and Riddle, 1984a) except that the assay plates contained peptone and the bacteria were not streptomycin treated. These changes were made to reduce dauer formation in order to best assay response to pheromone. Gravid adults were allowed to lay eggs for up to three hours at room temperature and then the plates were incubated at 15° C. Progeny were scored for dauer formation five or six days later. A unit of pheromone was defined as the amount of pheromone per plate required to induce 33% dauer formation in N2 at 25° C using the conditions described in Golden and Riddle 1984a. (data and methods from Thomas et al, 1993)

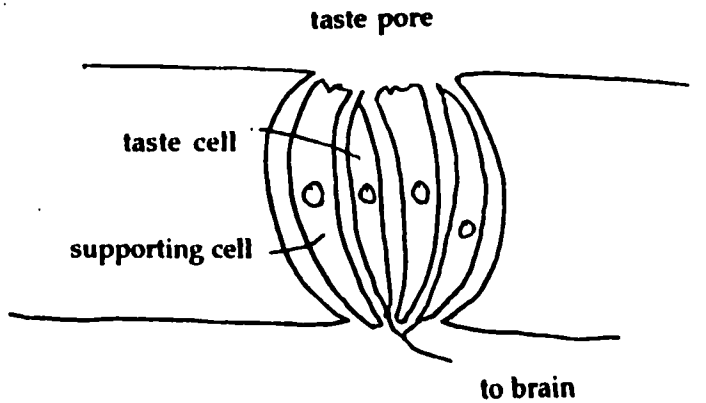
Figure 1.1 The cell types responsible for (A) olfaction, (B) taste, and (C) vision in humans.

After Bossy, 1970.

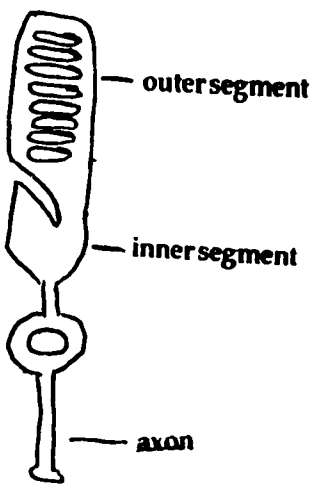
A.


olfactory hairs
supporting cell
olfactory neuron
to brain

B.


taste pore
taste cell
supporting cell
to brain

C.


outer segment
inner segment
axon

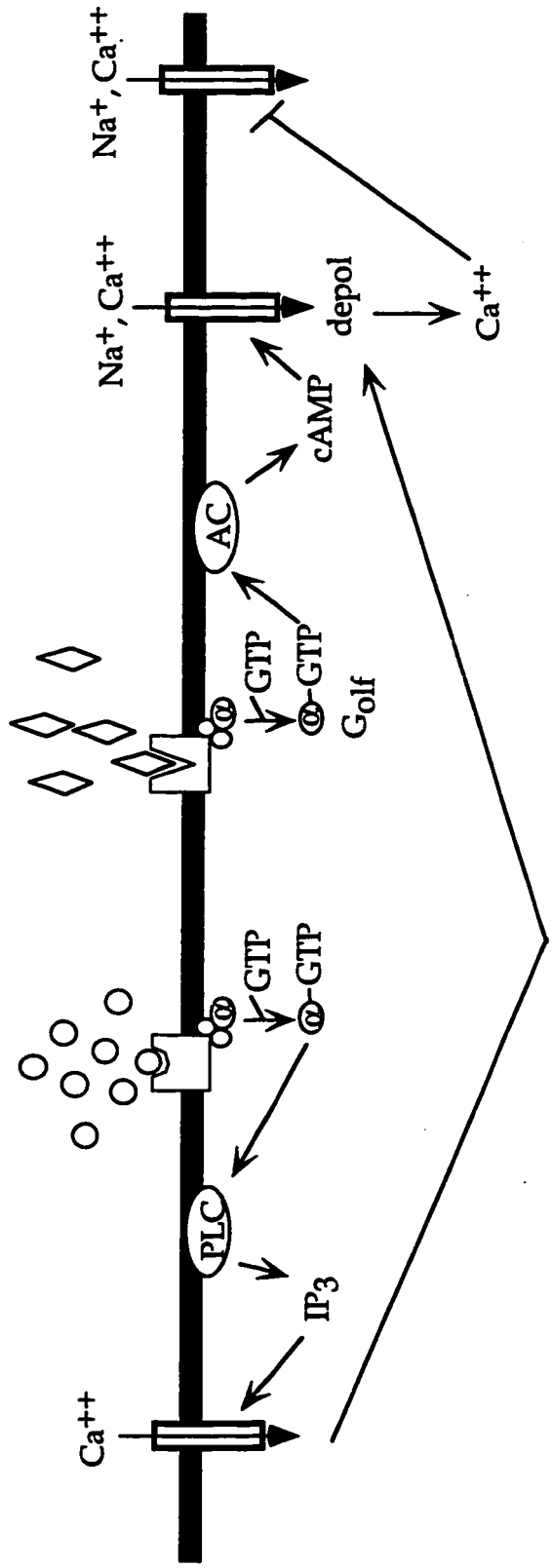


Figure 1.2 Olfactory Transduction

After Buck, 1996. PLC: phospholipase C; AC: adenylyl cyclase. See text for discussion.

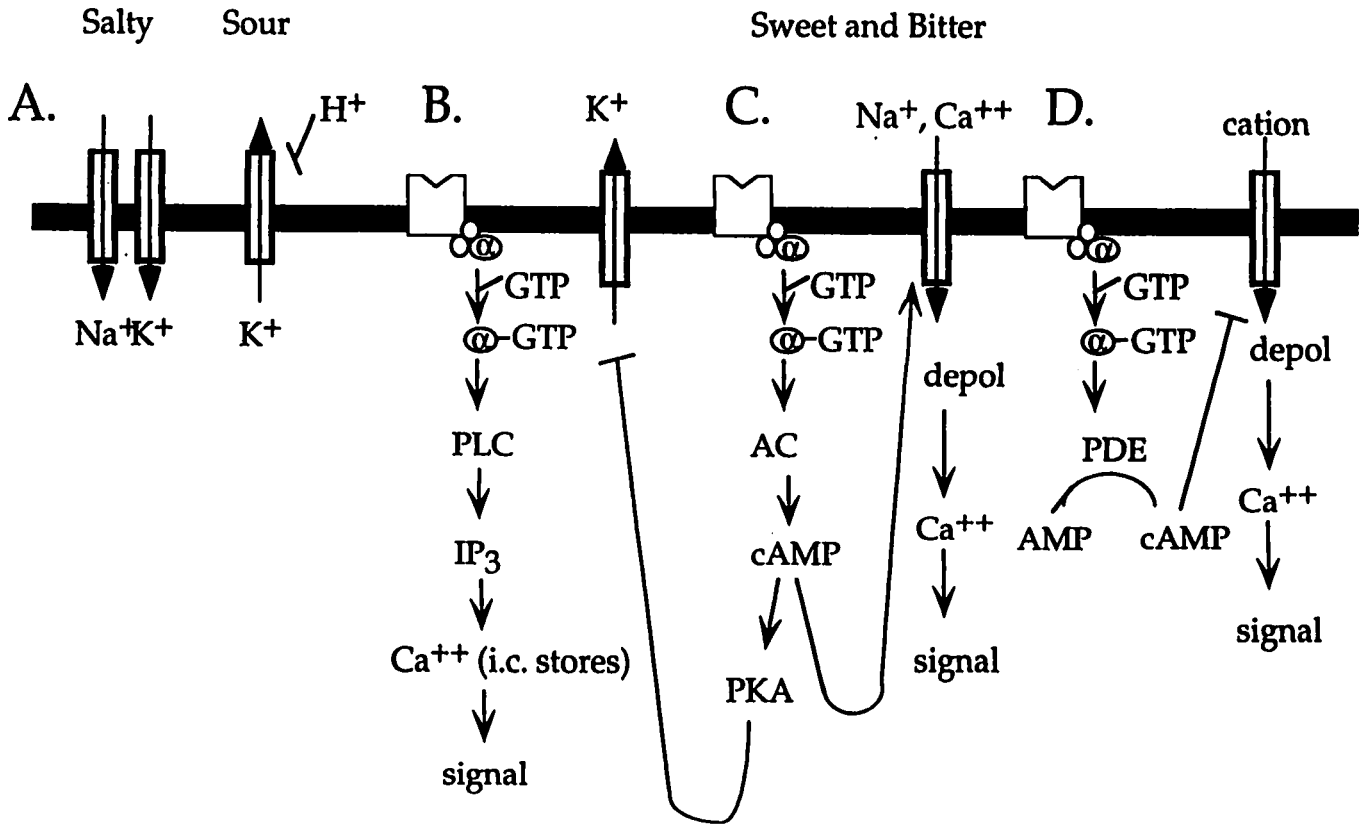


Figure 1.3 Taste Transduction
Main pathways for transduction of the four basic taste categories, based on data combined from several organisms. (A) Salty and sour and some bitter; (B, C, and D): sweet and bitter. (B) phospholipase C mediated (C) adenylyl cyclase mediated, and (D) phosphodiesterase mediated. α: G-α; PLC: phospholipase C; i.c.: intracellular; AC: adenylyl cyclase; PDE: phosphodiesterase. AC and PLC are membrane associated, although they are not drawn as such. See text for discussion.

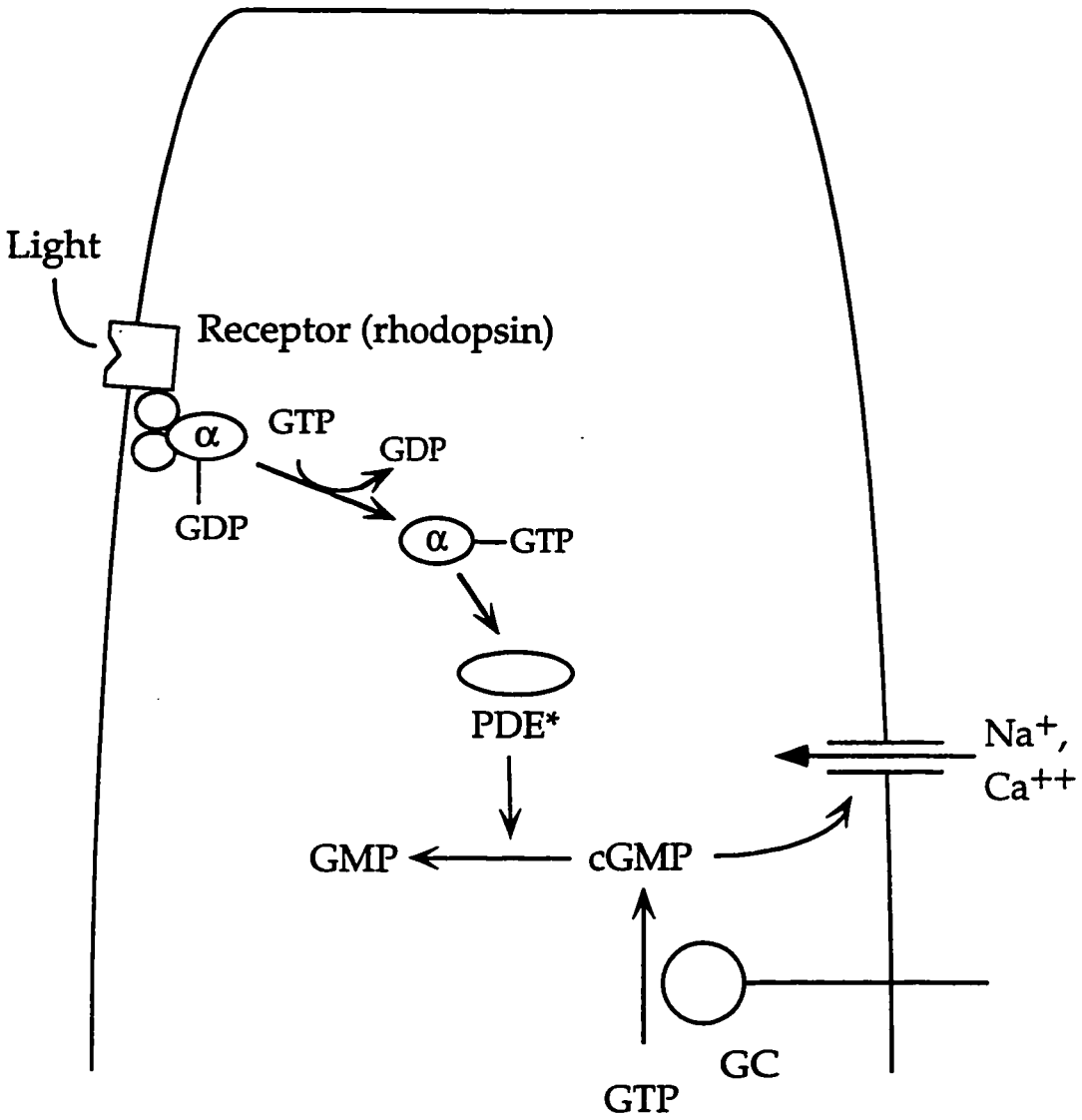


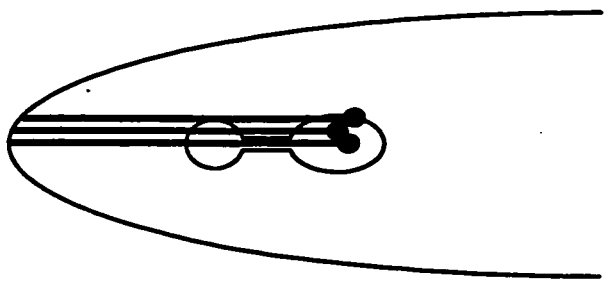
Figure 1.4 Visual Transduction
α: transducin-α; PDE: phosphodiesterase; GC: guanylyl cyclase.
See text for discussion.

Figure 1.5 Amphid sensilla of *C. elegans*

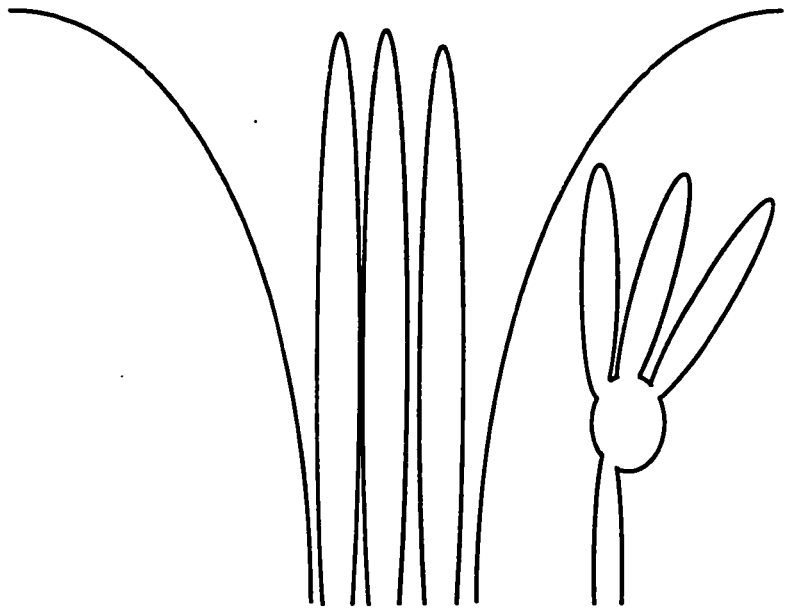
A. Schematic diagram of some sensory amphid neuron cell bodies and dendrites. The pharyngeal bulbs are shown for reference. Anterior is to the left.

B. Diagram of a longitudinal section of the amphid sensillum at the amphidial pore. The sensory endings of the neurons ASJ, ASK, ASI, and ASE are exposed to the environment, while those of AWA are not. AWB and AWC are similar to AWA. After Perkins et al, 1986.

A.



B.



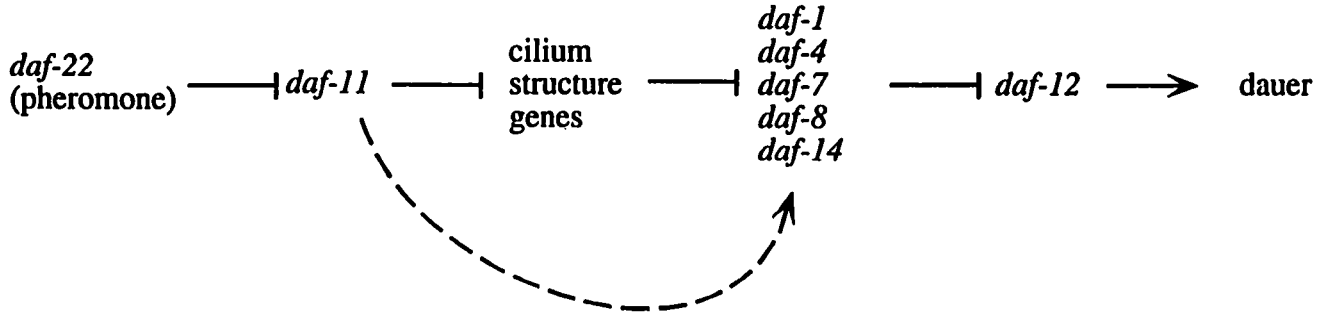


Figure 1.6 Linear Genetic Pathway Controlling Dauer Formation

Pathway is based on epistasis analysis between Daf-c and Daf-d genes (see text). An arrow represents activation and a bar represents repression. After Vowels and Thomas, 1992.

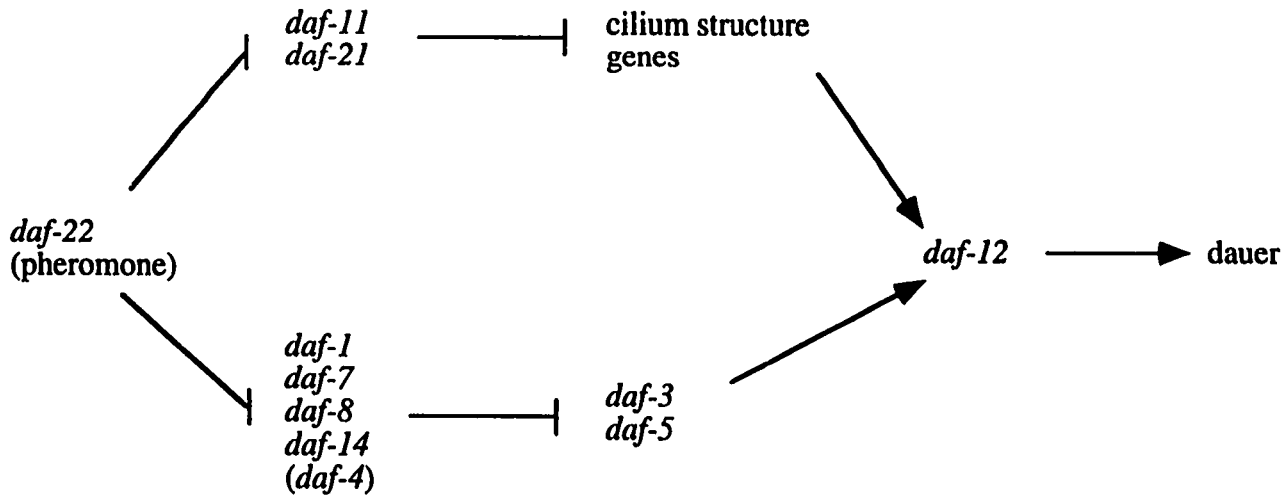


Figure 1.7 Parallel Genetic Pathway Controlling Dauer Formation

Pathway is based on *daf-3* and *daf-5* epistasis results, Daf-c double mutant analysis, and remaining pheromone response in double Daf-c mutants. An arrow represents activation and a bar represents repression. After Thomas et al., 1993.

CHAPTER 2: CLONING OF *daf-11*

INTRODUCTION: *daf-11* AS A MODEL FOR MULTIPLE TYPES OF SENSORY TRANSDUCTION

In addition to the Daf-c phenotype, *daf-11* mutants exhibit defects in several other chemosensory responses mediated by amphid neurons (see Table 1.1) (Vowels and Thomas, 1994). They are defective in response to the volatile attractants isoamyl alcohol and benzaldehyde, sensed by AWC (Bargmann and Horvitz, 1993), and in response to non-volatile attractants sensed by ASE (Vowels and Thomas, 1994; Bargmann and Horvitz, 1991b). Their dauer formation defect is mediated by ASJ (Schackwitz et al, 1996), and in this work I show that they are defective in avoidance of the volatile repellent 2-nonanone, sensed by AWB (Troemel et al, 1997).

These four cells have two different morphologies (Figure 1.5 B). The "S" in ASE and ASJ stands for "single." These neurons have a single ciliated ending that is directly exposed to the environment (Figure 1.5 B). The "W" in AWC and AWB stands for "wing." In contrast to ASE, ASJ, and several other cells, these neurons are not directly exposed to the environment but have large surfaces ("wings") that are ensconced in the amphid sheath cell (Perkins et al, 1986). The fact that DAF-11 appeared to be involved in several transduction processes that occurred in two different types of cells suggested that understanding DAF-11 function could add to our understanding of both olfactory and taste signal transduction in *C. elegans*.

METHODS:

STRAINS:

C. elegans strains were maintained as described by Brenner (1974). The following strains were used in the experiments described in Chapters 2 and 3: N2 (wildtype),

CB1364 *daf-4(e1364ts)*, CB1370 *daf-2(e1370ts)*, CB1372 *daf-7(e1372ts)*, CB1951 *unc-42(e270) sma-1(e30)*, DR20 *daf-12(m20)*, DR40 *daf-1(m40ts,mat)*, DR77 *daf-14(m77ts)*, DR87 *daf-11(m87ts)*, JT191 *daf-28(sa191ts)*, JT195 *daf-11(sa195ts)*, JT5436 *daf-8(e1393ts)*, JT5983 *unc-42(e270)*, JT5985 *sma-1(e30)*, JT5998 *daf-11(m597ts,mut sa103)*, JT6001 *osm-3(p802); daf-11(m597ts,mut)*, JT6023 *daf-11(m597ts,mut sa115)*, JT6051 *daf-11(m597ts,mut) sa116*, JT6100 *unc-42(e270) daf-11(m597ts,mut); osm-1(p808)*, JT6101 *unc-42(e270) daf-11(m597ts,mut); osm-1(p808)*, JT6125 *sma-1(e30)*, JT6130 *daf-21(p673ts,mat)*, JT6134 *daf-11(m597ts,mut) sma-1(e30); osm-1(p808)*, JT6155 *sma-1(e30)*, JT6412 *daf-11(m84ts); daf-12(m20)*, JT6561 *daf-11(sa195ts); daf-12(m20)*, JT6901 *ncl-1(e1865) unc-36(e251); daf-11(sa195ts); ctDp11*, JT6917 *daf-4(e1364ts)*, JT6918 *daf-7(e1372ts)*, JT6919 *daf-14(m77ts)*, JT7672 *tax-4(ks11)*, JT7673 *tax-4(ks28)*, JT7674 *tax-4(p678)*, JT7839 *tax-4(ks11)*, JT7840 *tax-4(ks28)*, JT7841 *tax-4(p678)*, JT8776 *lin-15(n765ts); saEx207*, JT8903 *lin-15(n765ts); saEx237*, JT8904 *lin-15(n765ts); saEx238*, JT9386 *daf11(sa195ts); saEx289*, PR802 *osm-3(p802)*, PR808 *osm-1(p808)*. This work follows the standard *C. elegans* nomenclature (Horvitz et al, 1979).

MOLECULAR BIOLOGY:

Molecular identification of *daf-11*: *daf-11* is located in the cluster of chromosome V. *daf-11(m597)* was isolated by P. Albert and D. Riddle from a strain with active transposition of the *C. elegans* mobile element Tc1. J. Vowels determined by Southern blot analysis of genomic DNA from outcrossed strains that a 1.6 kb Tc1 element inserted into a 0.38 kb NdeI fragment co-segregated with *m597* (Vowels, 1994). Nested inverse PCR was performed on *daf-11(m597)* DNA that had been digested with NdeI and ligated at low concentration to encourage intramolecular ligation. PCR was first performed using OLG34 (GCG TAA CAT TTC GCT TTA TGC ACA CGG) and OLG35 (AGA TTT TGT GAA CAC TGT GGT GAA G), primers oriented out from the ends of Tc1, to amplify a DNA fragment containing sequence flanking the Tc1. This product was re-amplified using OLG23 (CCG GAT CCA CCA AAA GTG GAT ATC TTT TTG GCC AGC), a primer derived from the inverted repeat sequence at the ends of Tc1 and also oriented outward. The resulting PCR product was cloned into pBluescript II KS$^+$ (Stratagene) that had been digested with EcoRI and treated with Klenow to generate blunt ends. This clone is plasmid pTJ277. I verified that pTJ277 contained DNA on the same 4.6 kb HindIII fragment as the Tc1 by performing Southern blot analysis on HindIII-digested genomic DNA from four phenotypic revertants of *daf-11(m597)* and six recombinant strains (Figure 2.1). The recombinants as a group removed each side of chromosome V from the strain in two different recombinants. In each case tested, pTJ277 detected a polymorphism consistent with a Tc1 insertion only in the *daf-11(m597)* strains.

pTJ277 was used as a probe to isolate genomic and cDNA phage. Genomic phage were isolated at a frequency of 1.8×10^{-4} from the

Stratagene λ FIXII genomic *C. elegans* library. Of six isolates fully purified, five overlapped. Sequence analysis was performed on subclones from one of the five overlapping phage to determine part of the *daf-11* genomic sequence. This sequence was also compared to that generated by the *C. elegans* Sequencing Consortium (Watson et al, 1993; Wilson et al, 1994; *C. elegans* Genome Sequencing Consortium, pers. comm.), which sequenced the region containing *daf-11* during the course of this work. *daf-11* corresponds to the Sequencing Consortium gene B0240.3. From approximately 10^6 plaques from one cDNA library (A. Fire, pers. comm.) and $3.2x10^5$ plaques from another (Barstead and Waterston, 1989), one *daf-11* clone was recovered. This cDNA (pTJ342) was subcloned into pBluescript II using the phage KpnI and SacI sites on either side of the insert and was sequenced. This cDNA appeared incomplete on both ends, so an additional $9x10^5$ plaques from the Stratagene mixed-stage *C. elegans* cDNA library (cat # 937006) were probed with a 2.4 kb ClaI-HindIII genomic fragment containing most of the cyclase domain. Four cDNA clones were recovered, and their inserts were excised into pBluescript II using helper phage K07. Restriction enzyme analysis showed that one cDNA of about 1.6 kb was *daf-11*; the other three are presumably other guanylyl cyclases and were not further analyzed. This *daf-11* cDNA (pTJ584) contained a poly-A tail, and its 3' end was sequenced to determine the 3' UTR and exon boundaries at the 3' end of the gene.

RNA sequence at the 5' end of *daf-11* was determined from RT-PCR products generated with the GIBCO-BRL 5' RACE system (Frohman, 1988). Mixed stage RNA for the RT-PCR procedure was isolated either by the method of D. Pilgrim (pers. comm.) or essentially by the method of Miller et al (1988). The 5' end was isolated in two pieces. Primers used were: OLG175 (CTC AGT TAA AAT GAC ACC) for first strand synthesis; OLG193 (AAC TGC AGG TTT AAT TAC CCA AGT TTG AG;

C. elegans splice leader SL1 with a PstI site added) and OLG186 (CCG GAT CCG ACA TGC TTG ATC AGG GC; XhoI site added) for the 5'-most part of the gene, and OLG185 (CCC CTC GAG CTT CAT AAT GCC ACT CGG C) and OLG286 (CGG GAT CCC CGA TCC GCA TAA TTT TTCC) for the internal fragment. The PCR product derived from the 5' end was subcloned into the pBluescript II XhoI and PstI sites and a single clone was sequenced. In addition, the sequence of the 5'-most 360 bp of the gene was obtained from bulk PCR product. For the internal portion of the gene, bulk PCR product was purified from a TAE gel using a NaI/glass bead method (Geneclean, BIO 101) and sequenced directly. In total, sequence of bulk PCR product was performed on exons 1, 8, 9, 10, and parts of 2, 7, and 11. This sequence showed no evidence of a mixed population of cDNA. cDNA sequence from the rest of the gene was based on only one cDNA for each section. Thus alternative splicing cannot be ruled out for these regions.

Sequencing of *daf-11* mutants: Total genomic DNA was isolated from mutants essentially as described (Wood, 1988). DNA to be sequenced was amplified by PCR, and bulk PCR product was sequenced. Mutations were sequenced on both strands.

Sequencing: Sequencing was performed by the ABI Dye Terminator Cycle Sequencing method (Perkin Elmer) using either AmpliTaq DNA Polymerase or AmpliTaq DNA Polymerase FS. The PCR products were analyzed on ABI gels by the University of Washington Biochemistry and Pharmacology DNA Sequencing Facilities.

Construction of GFP fusions: The 4.2 kb upstream of the *daf-11* start codon and the entire coding region were cloned in two pieces from

PCR products generated from the *daf-11*-containing cosmid W04E7. Taq polymerase was mixed with 1% (by units of activity) Pfu polymerase to reduce the mutation rate (after Barnes, 1994). Primers were designed with enzyme sites to facilitate cloning. OLG376 (GTG TGT AAA AAG CCC GGG AAA ATT GAT GC; XmaI site added) and OLG378 (GTC CAT CTC GAG ATT TTT GTG GGA TTC GG; XhoI site added) were used to amplify the 5′ part (fragment A) and OLG377 (CGA TCA ATT TTC CCG GGC TTT TTA CAC AC; XmaI site added) and OLG363 (GCG GAT CCC GTT ATC TGG AAT AGT TGA AGC TTG; BamHI site added) were used to amplify the 3′ part (fragment B). OLG376 and OLG377 each changed two nucleotides in intron 6 to produce the XmaI site used in cloning. Fragment B was digested with XmaI and BamHI and cloned into the XmaI and BamHI sites in pBluescript II KS+ to make pTJ630. Fragment A was digested with XmaI and XhoI and cloned into the XmaI and XhoI sites in pTJ630 to make pTJ631, reconstructing a contiguous genomic structure containing the entire *daf-11* coding region plus 4.2 kb of 5' sequence. This insert was removed from pTJ631 by digestion with XhoI and BamHI and cloned into pPD95.70 (A. Fire, S. Xu, J. Ahnn & G. Seydoux, pers. comm.) that had been digested with SalI and BamHI to make pTJ642, a full-length, in-frame fusion of GFP to the DAF-11 C-terminus. pPD95.70 contains an SV40 nuclear localization signal (NLS). I also generated pTJ643, a full-length, in frame *daf-11::gfp* fusion without an NLS, by inserting the identical insert into pPD95.79, which does not contain an NLS. The shorter fusion (see Results) was pTJ536, a single PCR product (primers OLG311 (CCG CAT GCT GAT TTT TGT GGG ATT CGG) and OLG310 (GCG GAT CCA ATT TGT TAC CAA GAA ACA AGA G)) cloned into the NsiI and BamHI sites in pPD95.67 (A. Fire, S. Xu, J. Ahnn & G. Seydoux, pers. comm.), creating a translational fusion.

GENETIC MAPPING OF *daf-11* WITH RESPECT TO *deg-3*::

deg-3(u662) causes a dominant Unc (uncoordinated) phenotype. The map position of *daf-11* with respect to *deg-3* was tested in two ways. (1) progeny of *unc-42 daf-11/deg-3* heterozygotes were screened for wild type animals; and (2) progeny of *daf-11 sma-1/deg-3* heterozygotes were screened for wild type animals. If *deg-3* maps to the right of *daf-11*, a single recombination event would generate occasional wild-type progeny from the first parent, but it would take a much rarer double recombination event to generate wild-type progeny from the second parent. If *deg-3* maps to the left of *daf-11*, the converse would be true: the second parent would generate occasional wild-type progeny, while the first would not.

The heterozygotes were generated as follows: Wild-type males were crossed to *unc-42 daf-11* (or *daf-11 sma-1*) hermaphrodites, and the resulting males were crossed to deg-3 hermaphrodites. Individual L4 progeny were transferred to new plates. Those that segregated Unc (or Sma) animals were the heterozygotes of interest.

8-BROMO-cGMP ASSAYS:

2 cm plates were filled with 2 ml NGM agar (Wood, 1988) with 8-bromo-cGMP (Sigma) added to a given concentration from a freshly made 250 mM stock. An overnight stock of E. coli OP50 in LB was started, and the next day these bacteria were harvested and resuspended at 5% (w/v) in sterile H_2O. 20 µl of this solution was spotted onto the center of each plate and allowed to dry for a few hours. 8-20 gravid

hermaphrodites were placed on the dried bacterial spot and allowed to lay eggs at room temperature for up to three hours, at which time the adults were removed. The plates, generally containing 50-120 eggs, were placed in a sealed box at 25°. Since 8-bromo-cGMP reduced growth synchrony, plates grown at 25° were scored at various times, generally several times for a single plate, between 36 and 52 hours after egg-laying. Since 8-bromo-cGMP induced dauer recovery in *daf-11(sa195)* dauers (data not shown), one set of experiments was scored particularly rigorously to be sure that I was assaying a block of dauer formation by 8-bromo-cGMP as opposed to an induction of dauer recovery. *daf-11(sa195)* and wild-type animals grown on pheromone were checked every one to three hours from 37 to 47.5 hours post egg-laying. All dauers seen at any point during this time were counted among the total. At the beginning of this time, corresponding animals grown in the absence of 8-bromo-cGMP were pre-dauers (*daf-11(sa195)* animals) or a mix of pre-dauers and L3 larva (wild type animals grown on pheromone plates, since the amount of pheromone used did not induce 100% dauer formation). At the end of this time, the corresponding animals grown in the absence of 8-bromo-cGMP were dauers or well into the dauer molt (*sa195*) or (some animals on pheromone plates only) were L4 larvae or adults. Worms were determined to be in one of three categories: dauers, L3 or older worms, and L2 or L1 worms. Percent dauer formation was calculated as 100*dauers/(dauers+L3 or older animals). For most data points, more than 100 worms were counted and assays were performed on at least two different days. The following were exceptions: the *daf-8* intermediate concentration and *daf-11(m87)* 1.25 mM assays (done only one day each), and *daf-8* 2.5 mM assay (45 worms). 8-bromo-cAMP (Sigma) assays were done in the same manner. The percentage reported is a mean of the percent dauers formed on each plate assayed. Standard error of the mean (SEM) was calculated from data spread among assays for each point.

In several 8-bromo-cGMP assays, many worms arrested as young larvae or formed larvae with only some characteristics of dauers. I interpret this to mean that 8-bromo-cGMP can affect non-dauer development and execution of the dauer developmental program. These worms were excluded from the data presented. In most cases these arrested or partial-dauer worms constituted <25% of the total worms. The following were exceptions: *daf-11(m87)* 0.6 mM 8-bromo-cGMP (34% not counted); *daf-11(sa195)* 1.25 and 2.5 mM 8-bromo-cGMP (1.25 mM: 42% not counted; 2.5 mM: 39% not counted); *daf-21(p673)* 1.25, 2.5, and 5 mM 8-bromo-cGMP (1.25 mM: 62% not counted; 2.5 mM: 54% not counted; 5 mM: 34% not counted); *daf-4* 5 mM 8-bromo-cGMP (42% not counted); *daf-8* 2.5 and 5 mM 8-bromo-cGMP (2.5 mM: 43% not counted; 5 mM: 64% not counted); *daf-2* 5 mM 8-bromo-cGMP (62% not counted); *tax-4(ks11)* 5 mM 8-bromo-cGMP (67% not counted); *tax-4(p678)* 5 mM 8-bromo-cGMP (43% not counted). Conclusions based on these data were unchanged even if those worms showing partial characteristics of dauers were counted as dauers.

RESULTS:

daf-11 ENCODES A TRANSMEMBRANE GUANYLYL CYCLASE

daf-11(m597) was associated with a Tc1 insertion located on a NdeI fragment by Jennifer Vowels (Vowels, 1994). Southern blot analysis verified that a Tc1 insertion present in strains containing *m597* was absent in recombinants and revertants not containing *m597* (Figure

2.1). Genomic DNA flanking this Tc1 insertion was cloned by inverse PCR, and this flanking DNA was used to localize *daf-11* to a YAC (Y54E7) and a cosmid (W05E7) from the *C. elegans* Genome Project physical map (Wilson et al., 1994).

Our cosmid localization was inconsistent with the published cosmid localization of *deg-3* (Treinin and Chalfie, 1995): *deg-3* had been mapped genetically to the right of *daf-11* but was localized to K30B8 and T21D1, cosmids located to the left of W05E7, the cosmid to which *daf-11* localized. To resolve this contradiction, I re-mapped *daf-11* with respect to *deg-3* and found that *deg-3* mapped genetically to the left of *daf-11*, contrary to the published report (Treinin and Chalfie, 1995) (0/~1950 wild-type progeny from *unc-42 daf-11/deg-3* parents; 2/~2000 wild-type progeny from *daf-11 sma-1/deg-3* parents).

The flanking DNA was also used to isolate phage from cDNA and genomic DNA libraries. Two *daf-11* cDNAs were isolated: pTJ342, which was found to be incomplete at both the 5' and 3' ends; and pTJ584, which was incomplete at the 5' end but contained a poly-adenylation signal and a poly-A tail, indicating a complete 3' end. The region of pTJ584 that extended 3' of pTJ342 was sequenced, and a composite sequence of the last 3 kb of the *daf-11* mRNA was generated. mRNA sequence at the 5' end was determined by sequence of RT-PCR products. These data were combined to generate a composite sequence of the entire *daf-11* mRNA. With Patrick Colacurcio, I also determined genomic sequence for most of the gene from subclones of genomic phage. The Genome Sequencing Consortium (Watson et al, 1993; Wilson et al, 1994; *C. elegans* Genome Sequencing Consortium, pers. comm.) completed the sequence of this region during the course of our work. Comparison of genomic sequence to cDNA sequence indicates

that the *daf-11* mRNA contained 17 exons and spans almost 8 kb of genomic DNA (Figure 2.2). The mRNA contains one long open reading frame predicted to encode a 1077 amino acid protein that is a member of the transmembrane guanylyl cyclase (TM-GC) family (Figure 2.3). As is true of most TM-GCs, DAF-11 homology to the guanylyl cyclases is restricted to the KHD and cyclase domains of the protein. An alignment of the KHD and cyclase domains of DAF-11 to TM-GCs from mouse, sea urchin, rat, and humans is shown in Figure 2.4. There are several *C. elegans* TM-GCs that are very closely related to *daf-11* (J.H. Thomas, pers. comm.; Yu et al, 1997). Outside of *C. elegans*, the sea urchin speract receptor is the closest relative to DAF-11, with 22% identity and 47% similarity in the KHD and 43% identity and 64% similarity in the cyclase domain.

SEQUENCE CHANGES IN *daf-11* MUTANTS:

daf-11 alleles vary in their phenotypic strength. With Hong Tian, I sequenced PCR products generated from mutant genomic DNA in order to determine the specific effects of several daf-11 mutations on protein function. Identified mutations are indicated in Figure 2.4. Most sequenced alleles affect dauer formation, chemotaxis to non-volatile attractants and to volatile attractants sensed by the amphid neuron AWC, and recovery from the dauer state. Generally, dauer formation is more strongly affected at 25° C than at 15° C (Vowels and Thomas, 1994). Animals bearing *m597*, the Tc1 allele with which I cloned *daf-11*, exhibit strong loss of function phenotypes for all of these processes (Vowels and Thomas, 1994). The Tc1 is inserted in the kinase homology domain, at I518. *sa195* and *m87* also cause strong loss-of-function phenotypes (Vowels and Thomas, 1994). *sa195* is a nonsense mutation at Q450 that removes the cyclase domain and most of the

kinase homology domain. *m87* is a missense mutation in the cyclase domain, changing S866 to F. This amino acid is located in a region of strong conservation among TM-GCs and is itself conserved (Figure 1C). *p169* changes G867 to R, and *sa203* causes a nonsense mutation at Q904, late in the cyclase domain. Interestingly, while *p169* mutants formed about 16% (±9.2) dauers at 15° C (N=263), consistent with reported values for several other alleles, *sa203* mutants formed about 88% (±21) dauers at 15° (N=234). It is possible that the truncated protein product interferes with some other function or that a second, undetected mutation is responsible for the unusual phenotype. The location of the Tc1 insertion in *m597* was determined from the sequence of the pTJ277 insert. Only the coding region of the cyclase domain was sequenced for *m87, p169,* and *sa203*. For *sa195,* all coding sequence upstream of the amber mutation was sequenced, and for *m84* (discussed below) the entire coding sequence was determined.

The *daf-11(m84)* allele has several properties that are different from putative null alleles such as *sa195*. *m84* larvae are as strongly Daf-c at 15° C as at 25° C, *m84* adults have weaker defects in response to the volatile attractant isoamyl alcohol, and *m84* dauers recover more quickly at 15° C (Vowels and Thomas, 1994). For each of these phenotypes, *m84* is dominant to *daf-11(m87)* and *daf-11(sa195)*. *m84* is also slightly dominant to the wild-type allele for its dauer formation phenotype (Vowels and Thomas, 1994). These data indicate that *m84* is not a null allele and that the mutation probably affects only some of the functions of DAF-11. *m84* changes G806 to E. G806 lies in a conserved part of the cyclase domain, but the amino acid itself is not conserved (Figure 2.4). It is possible that this amino acid is important in the signaling pathway that induces dauer formation and chemotaxis to non-volatile attractants but not in the other pathways in which DAF-11 is involved. G806E is the only coding sequence change in *m84*, but it is

possible that there is also an undetected effect on gene expression or splicing.

FUNCTIONAL EVIDENCE FOR A ROLE FOR THE *daf-11* TM-GC IN CHEMOSENSATION

Several TM-GCs have been identified in *C. elegans* (Watson et al, 1993; Wilson et al, 1994; *C. elegans* Genome Sequencing Consortium, pers. comm.; J.H. Thomas, pers. comm.; Yu et al, 1997) GFP fusions to several of these genes are expressed in chemosensory neurons (Yu et al, 1997), suggesting that many of them are involved in chemosensory signal transduction processes. To test for biological relevance of the *daf-11* sequence homology to TM-GCs and to test directly for a role for the *daf-11* TM-GC in chemosensation, I assayed the effect of 8-bromo-cGMP on the Daf-c phenotype of *daf-11* mutants. 8-bromo-cGMP is a poorly-hydrolyzed cGMP analogue that can activate many targets of cGMP. If the *daf-11* mutant phenotype is due to a loss of guanylyl cyclase activity, 8-bromo-cGMP might suppress the Daf-c phenotype. When grown at 25° with plentiful food and without 8-bromo-cGMP, over 95% of *daf-11(sa195)* and *daf-11(m87)* larvae formed dauers (Figure 2.5). In contrast, when 5 mM 8-bromo-cGMP was added to the growth medium, less than 1% of the larvae formed dauers. Intermediate concentrations of the drug caused intermediate levels of suppression of the Daf-c phenotype. 8-bromo-cGMP also suppressed pheromone-induced dauer formation in wild-type worms in a dose-dependent manner, directly implicating cGMP in the wild-type dauer formation process.

I hypothesized that 8-bromo-cGMP suppresses *daf-11* mutants by directly substituting for missing cGMP. This hypothesis predicts that 8-

bromo-cGMP would not rescue the Daf-c phenotype of animals mutant for genes that act downstream of or in parallel to *daf-11*. I did not know if it would suppress the Daf-c phenotype of animals mutant for *daf-21*, a gene that lies at the same position as *daf-11* in the genetic pathway regulating dauer formation (Thomas et al, 1993). Suppression of the *daf-21* Daf-c phenotype by 8-bromo-cGMP would indicate that DAF-21 functioned upstream of cGMP, possibly with DAF-11. A lack of suppression would indicate that *daf-21* functioned downstream of cGMP. As expected, 8-bromo-cGMP did not prevent dauer formation in strains mutant for *daf-1*, *daf-4*, *daf-7*, *daf-8*, *daf-14*, *daf-2*, or *daf-28* (Figure 2.6). Of these genes, *daf-2* and *daf-28* are thought to act downstream of or in parallel with *daf-11*, whereas the others act at least partly in parallel (Gottlieb and Ruvkun, 1994; Malone et al, 1996; Thomas et al, 1993). In contrast, 8-bromo-cGMP did rescue the Daf-c phenotype of *daf-21(p673)* (Figure 2.5), suggesting that *daf-21* functions at or upstream of the *daf-11*/cGMP signaling step.

To test whether this suppression is specific to cGMP, I tested the response of *daf-11*, *daf-21*, *daf-1*, *daf-4*, *daf-7*, *daf-14*, *daf-2*, and *daf-28* mutants to 8-bromo-cAMP. At 5 mM, 8-bromo-cAMP caused developmental arrest in the wildtype. Concentrations as low as 1.25 mM caused arrest as well, but 0.5 mM 8-bromo-cAMP allowed normal growth. This concentration of 8-bromo-cAMP did not prevent dauer formation in any of these strains (data not shown). These results implicate cGMP in dauer formation and provide direct evidence that *daf-11* functions *in vivo* as a guanylyl cyclase.

Figure 2.1 *daf-11(m597)* segregates with a Tc-1 insertion on a 4.6 kb HindIII fragment.

Southern hybridization of HindIII-digested *daf-11(m597)* or *daf-11(+)* animals probed with DNA flanking the m597-associated Tc1. Strains shown include recombinants (r) and revertants (R). The recombinants as a group remove each side of chromosome V from the strain in two different recombinants. The polymorphism detected is consistent with Tc1 insertion only in the *daf-11(m597)* strains.

N2 wildtype
PR802
JT6001
JT5998
JT6021
JT6023
JT6051
CB1951
PR808
JT6134
JT6101
JT5983
JT5985
JT6125
JT6155
7
6
5
4
3
daf-11
+ + - + + + + + + - - + + + +
R R R R
r r r r r r

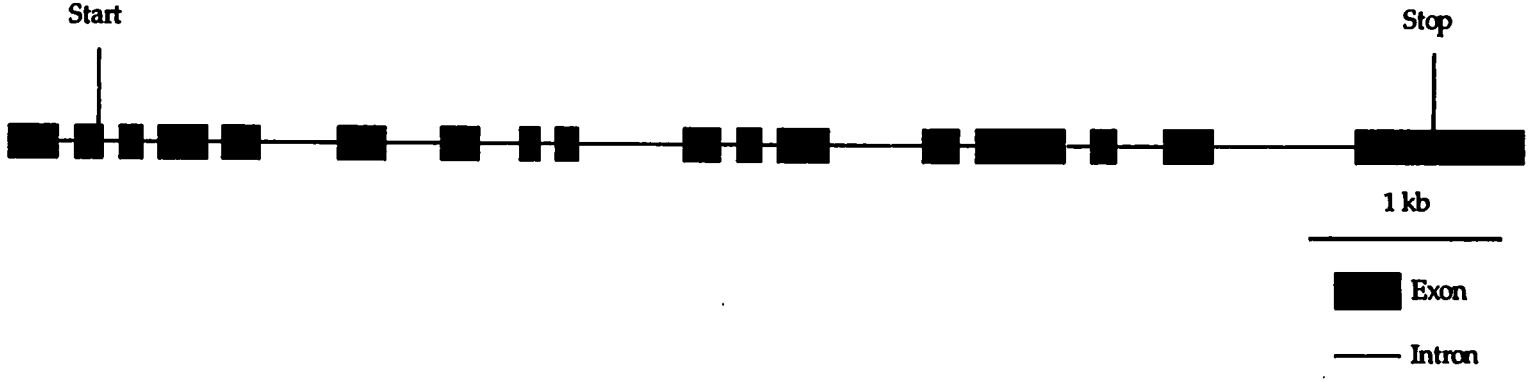


Figure 2.2 Schematic diagram of *daf-11* exons and introns.

Structure was determined by comparison of genomic DNA to cDNA. Start and stop codons are indicated.

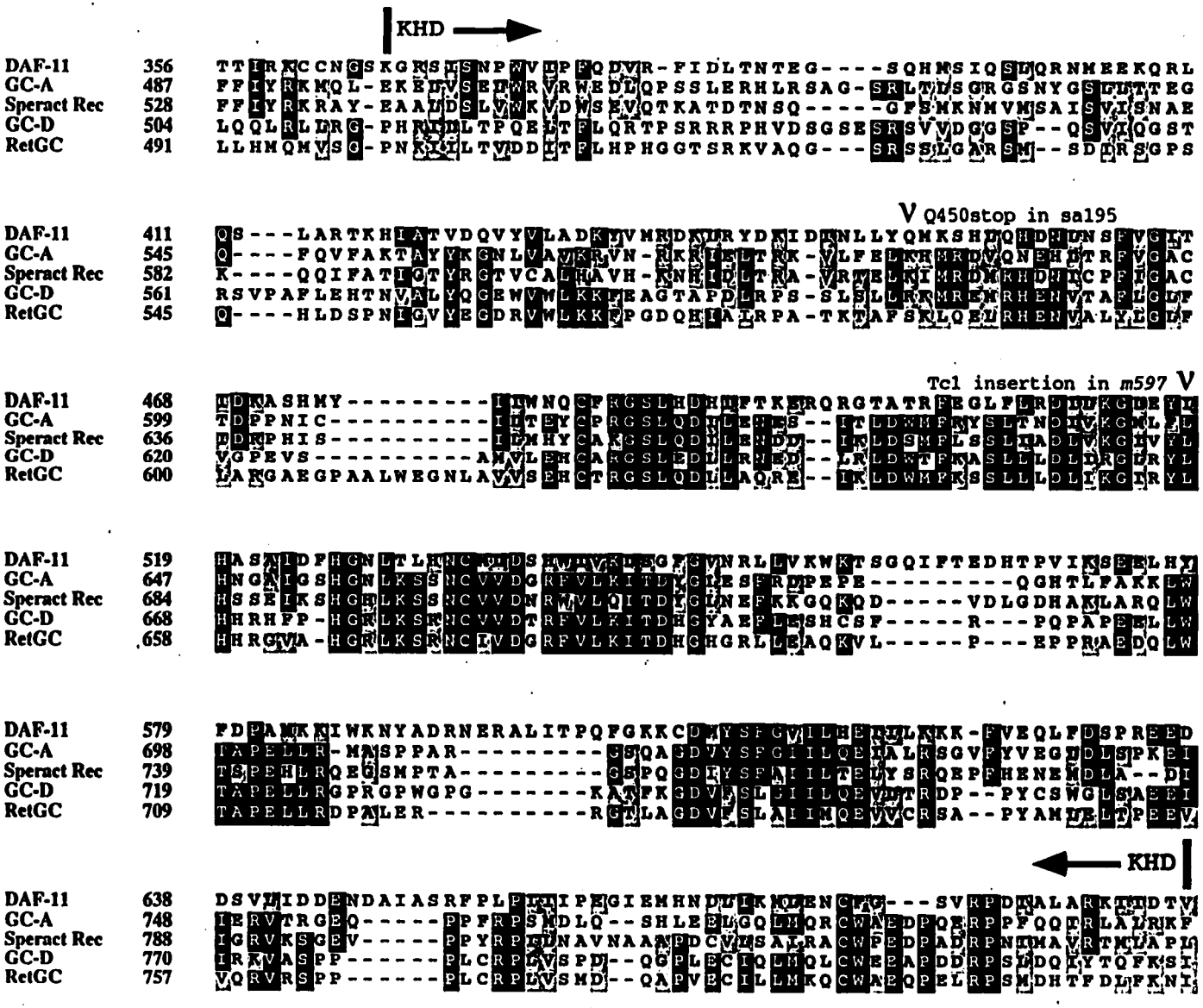
```
1    MGPTCSSNFLRFANLTAEMQSLEINILKGYPYEHPTMIDMVTRSPQNLAQNLVSLLRGFE  60
61   WGQVGAVLCEECYEGDELASEIYFSTIEDIFENNNIALKETVRIGKRENSVNISDAITIF 120
121  EPSARVILLFLGNKLNDYTEFMTAMSMNNYTTEEYTPVIVISKNSLELTFPWKENDAIAE 180
181  LFDKAIIVYNNCYDKSKISSFLSSYSFSTIEETIISLQMYEGYHLLGYYLYTAITNTTLF 240
241  NYVQPEKAISSMSIPGPFGEIFINSNGQRIAGYDVLVVDKSLNSNNFIMPLGTISTDKKC 300
301  PDQACLNFVLNSTSSFEPLKDVPLCGFHGEICDQTGVIIAIAVIMGVLLMFIIILTTIRK 360
361  CCNGSKGRSISNPWVIPFQDVRFIDLTNTEGSQHMSIQSLQRNMEEKQRLQSLARTKHIA 420
421  TVDQVYVLADKYVMRDKLRYDKIDINLLYQMKSHLQHDNLNSFVGITIDKASHMYIIWNQ 480
481  CFRGSLHDHIFTKERQRGTATRFEGLFLRDILKGLEYIHASAIDFHGNLTLHNCMLDSHW 540
541  IVKLSGFGVNRLLVKWKTSGQIFTEDHTPVIKSEELHYFDPAMKKIWKNYADRNERALIT 600
601  PQFGKKCDMYSFGVILHEIILKKKFVEQLFDSPREEDDSVLIDDENDAIASRFPLPIIIP 660
661  EGIEMHNDLIKMLENCFGSVRPDIALARKIIDTVLKMSGSLVDLMIKNLTAYTQGLNETV 720
721  KNRTAELEKEQEKGDQLLMELLPKSVANDLKNGIAVDPKVYENATILYSDIVGFTSLCSQ 780
781  SQPMEVVTLLSGMYQRFDLIISQQGGYKMETIGDAYCVAAGLPVVMEKDHVKSICMIALL 840
841  QRDCLHHFEIPHRPGTFLNCRWGFNSGPVFAGVIGQKAPRYACFGEAVILASKMESSGVE 900
901  DRIQMTLASQQLLEENFPQFVCSNRGGRTIEGIGRILTYWLEGVNAGEQVKVVEFQNDLN 960
961  DELSRIMKKDGELLAAATALKPKDKMTLAKEKVIAERKNEEERLQRQQTLQEALEEHEEE 1020
1021 IEMNEVLVDEDEGEGKPKEVDLTSIVSTQMEELEDEPAGRTIGHGRLDSQASTIPDN   1077
```

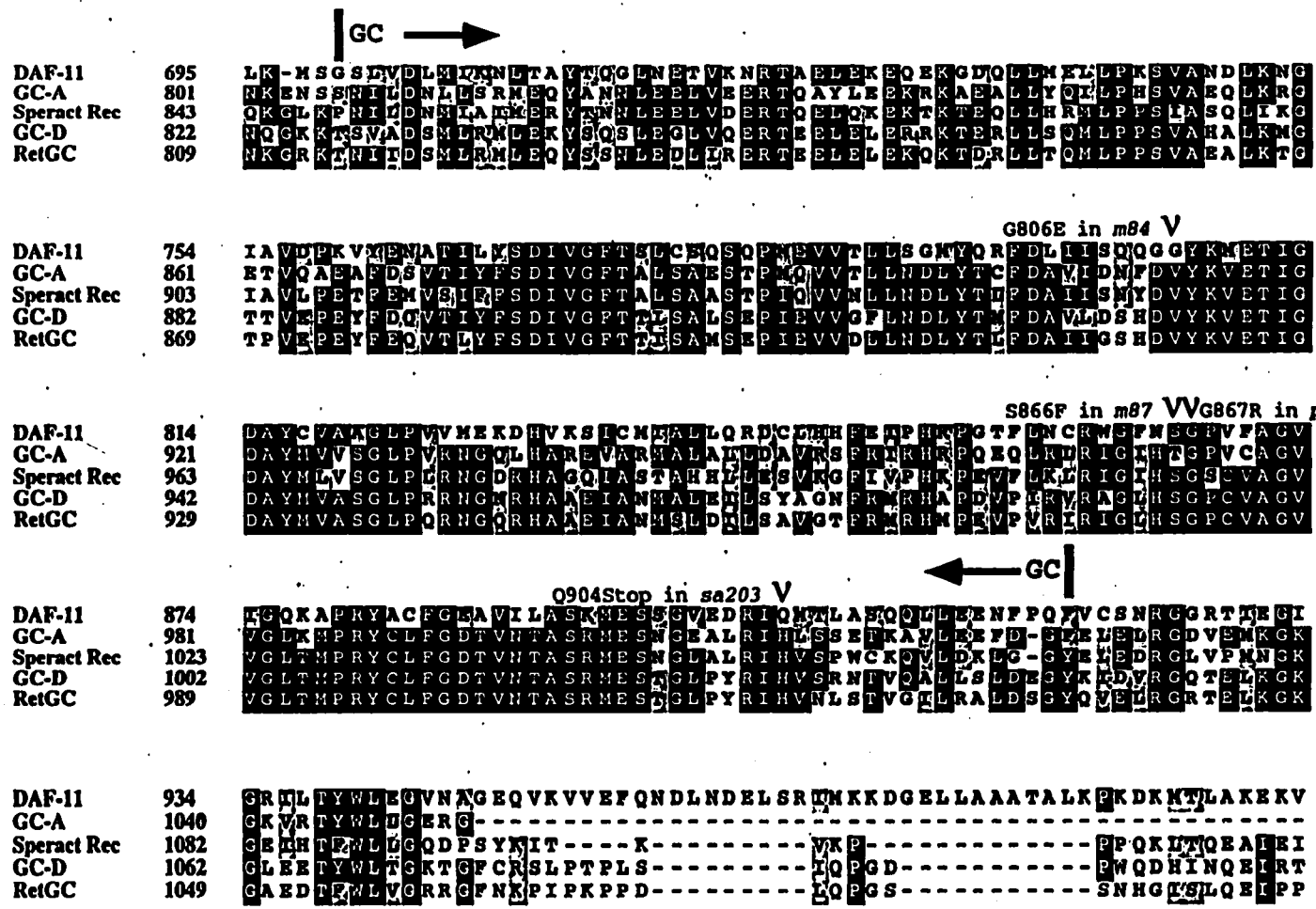
Figure 2.3 Protein sequence of DAF-11 as inferred from cDNA sequence.

The putative transmembrane domain is marked with a dotted underline. The KHD is indicated by a single underline, and the guanylyl cyclase domain is indicated by a double underline.

Figure 2.4 Alignment of the intracellular portion of *daf-11* with other guanylyl cyclases.

Alignment was determined by Clustal W 1.4. The C-terminal 84 amino acids of DAF-11 show no homology to the other proteins and are not shown. The C-termini of the other proteins were truncated as follows: 5 AA from GC-A, 10 AA from the speract receptor, 9 AA from GC-D, and 15 AA from RetGC. Amino acids that are identical in at least three of the sequences are boxed in black, and those similar in at least three sequences are boxed in gray. Domains and identified *daf-11* mutations are indicated. GC-A is the ANP receptor from *M. musculus* (accession no. p18293); speract receptor is from *S. pupuratus* (accession no. p16065); GC-D is expressed specifically in the olfactory neurons of *R. norvegicus* (accession no. l37203); and RetGC is human RetGC-1, expressed in the retina (accession no. 623415).


KHD
DAF-11 356
GC-A 487
Speract Rec 528
GC-D 504
RetGC 491
Q450stop in sa195
DAF-11 411
GC-A 545
Speract Rec 582
GC-D 561
RetGC 545
Tc1 insertion in m597
DAF-11 468
GC-A 599
Speract Rec 636
GC-D 620
RetGC 600
DAF-11 519
GC-A 647
Speract Rec 684
GC-D 668
RetGC 658
DAF-11 579
GC-A 698
Speract Rec 739
GC-D 719
RetGC 709
KHD
DAF-11 638
GC-A 748
Speract Rec 788
GC-D 770
RetGC 757


GC
DAF-11 695
GC-A 801
Speract Rec 843
GC-D 822
RetGC 809
G806E in m84
DAF-11 754
GC-A 861
Speract Rec 903
GC-D 882
RetGC 869
S866F in m87
G867R in p169
DAF-11 814
GC-A 921
Speract Rec 963
GC-D 942
RetGC 929
GC
Q904Stop in sa203
DAF-11 874
GC-A 981
Speract Rec 1023
GC-D 1002
RetGC 989
DAF-11 934
GC-A 1040
Speract Rec 1082
GC-D 1062
RetGC 1049

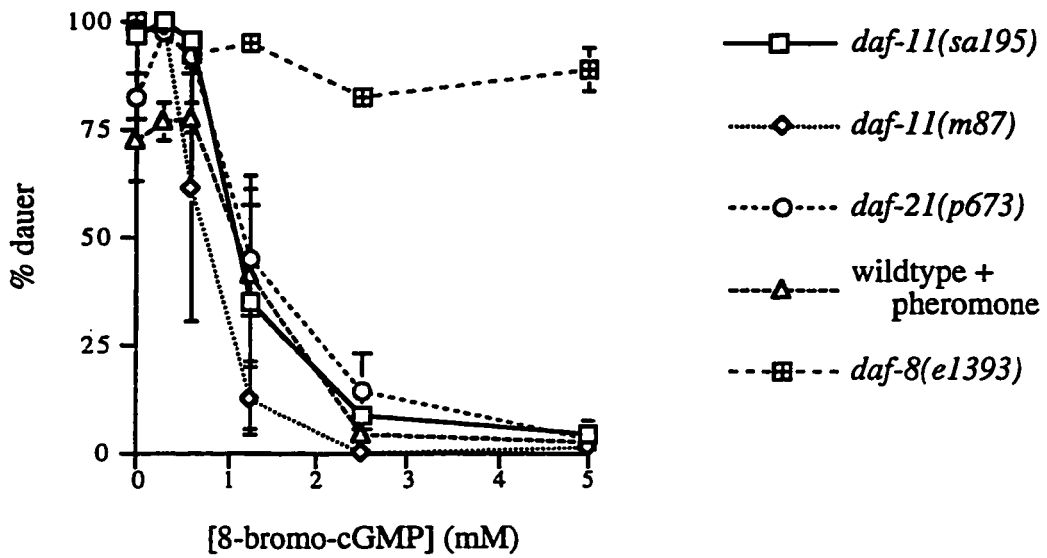


Figure 2.5 Effect of 8-bromo-cGMP on the wild type grown in the presence of pheromone and on *daf-11*, *daf-21*, and *daf-8* mutants.

Standard errors of the mean (SEMs) are indicated by bars.

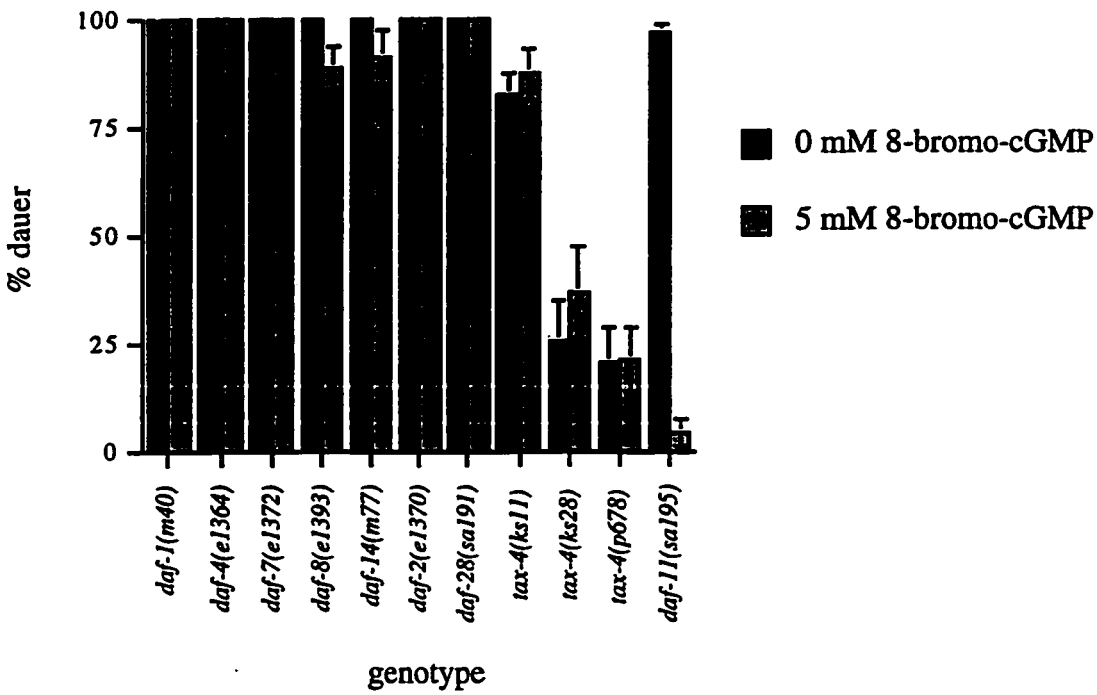


Figure 2.6 Effect of 8-bromo-cGMP on other Daf-c mutants.

For each strain, the percent dauers formed in the absence (solid bar) and presence (striped bar) of 5 mM 8-bromo-cGMP is shown. Data from *daf-11(sa195)* animals are shown for comparison. SEMs are indicated by bars.

CHAPTER 3: LOCALIZATION OF *daf-11* FUNCTION:

INTRODUCTION:

Dauer pheromone is sensed through the amphid neurons, a pair of bilaterally symmetric sensory organs located in the head of the animal (Vowels and Thomas, 1994). Cell kill studies have identified two sets of sensory neurons involved in dauer formation. When the neurons ADF and ASI are killed in wild-type larvae, dauers form in the absence of pheromone (Bargmann and Horvitz, 1991a; Schackwitz et al, 1996), indicating that these neurons normally repress dauer formation in the absence of pheromone. Cell kill experiments suggest that the TGF-ß (group 2) branch of the dauer pathway acts in ADF and ASI (Bargmann and Horvitz, 1991a; Thomas et al, 1993; Schackwitz et al, 1996). Killing the neuron ASJ in wild-type larvae grown on pheromone reduces dauer formation, suggesting that ASJ promotes dauer formation in response to pheromone. Killing ASJ also suppresses the Daf-c phenotype of *daf-11* mutants, suggesting that this *daf-11* phenotype results from activation of ASJ (Schackwitz et al, 1996). Wild-type worms recover quickly from the dauer state when removed from inducing conditions. ASJ is also required for recovery from the dauer state (Bargmann and Horvitz, 1991a), and most *daf-11* mutants are also defective in this recovery (Vowels and Thomas, 1994).

daf-11 is also required for chemotaxis to some attractants. *daf-11* mutants are defective in response to the non-volatile attractants Cl^-, cAMP, and biotin (Vowels and Thomas, 1994), which are primarily

sensed by the exposed amphid neuron ASE (Bargmann and Horvitz, 1991b). They also have defects in response to the volatile attractants isoamyl alcohol and benzaldehyde and slight defects in response to 2,4,5-trimethylthiazole, but not to pyrazine and diacetyl (Vowels and Thomas, 1994). Response to isoamyl alcohol and benzaldehyde requires the amphid neuron AWC, and response to pyrazine and diacetyl requires the amphid neuron AWA. Both AWC and AWA can mediate response to 2,4,5-trimethylthiazole (Bargmann et al, 1993). This pattern of defects suggests that *daf-11* mutants have a functional deficit in the sensory neurons ASE and AWC. Thus I expected *daf-11::gfp* expression in ASJ, AWC, and ASE.

METHODS:

STRAINS:

see Chapter 2 methods.

CONSTRUCTION AND ANALYSIS OF TRANSGENIC WORMS:

Young adult worms were injected as described (Mello et al, 1991). To generate worms with the full-length, NLS-bearing fusion or the exon 5 fusion, *lin-15(n765)* worms were injected with a mixture of 200 ng/µl pTJ642 and 60 ng/µl pbLH98 (*lin-15(+)*) (full-length fusion) or 50 ng/µl pTJ536 and 60 ng/µl pbLH98 (exon 5 fusion). *lin-15(n765)* causes a

multi-vulva (Muv) phenotype when animals are grown at 25° C, and rescue of the Muv phenotype was used to identify transgenic animals (Huang et al, 1994). An extrachromosomal array that was generated in a *lin-15(n765)* animal and contained pTJ642 and pbLH98 was crossed into *daf-11(sa195)* worms and rescued the *daf-11(sa195)* dauer formation phenotype. Thus I predicted that pTJ643 (identical to pTJ642 except without an NLS) would also rescue this phenotype. JT9386 (pictured in Figure 3.1) was generated by injecting JT195 *daf-11(sa195)* worms with 200 ng/µl pTJ643 and screening progeny for non-dauers. In all cases, injected animals were grown at 20° for one day and then transferred to 25° C. Progeny of injected animals were screened two and three days after injection. Non-Muv or non-dauer worms were picked and their progeny were screened for inheritance of a transgenic array.

Worms were grown at 25° C to enable identification of transgenic animals, except for assay of GFP expression in ASE in adults (see Results below), in which worms were grown at 20° C. In that assay, expression in ASE was only assessed in sides of animals in which GFP was expressed in at least ASJ and AWC. In most cases, expression was also seen in ASI and ASK, and occasionally in a cell that could be AWB, but positive identification was not possible. In other assays, expression was assessed only in sides in which all relevant cells could be identified and in which at least one cell expressed GFP. GFP expression was observed using epifluorescence microscopy, and fluorescent cells were identified by Nomarski microscopy on the same animal. Photographs were taken on Kodak Elite II slide film (ISO 400) and manipulated with Adobe Photoshop. Brightness and contrast were varied, annotation was added, and color was converted to grayscale.

Pheromone conditions for GFP expression: JT8904 *lin-15(n765); saEx238* or JT9386 *daf-11(sa195); saEx289* animals were grown on pheromone at 25° as described (Thomas et al, 1993). Worms were grown on 50 or 80 ul pheromone, an amount sufficient to induce over 90% dauer formation in the wildtype grown at 25° C. GFP expression in L1 larvae was observed one day after eggs were laid, and dauers were counted and assayed for GFP expression two days after eggs were laid.

RESCUE OF *daf-11* DAUER FORMATION AND RECOVERY DEFECTS BY *saEx289*:

Plates were 2 cm NGM plates with 80 ul M9, pheromone, or a mix (0, 10, or 20 U pheromone) added. Plates were prepared and food was added as for 8-bromo-cGMP assays (Chapter 2). Eggs were laid at room temperature for approximately three hours and then plates were placed in a sealed box with a wet paper towel (to prevent drying of the agar) at 25°. Dauers were counted after two days, and on relevant plates non-dauers were removed. At this time, some animals were pre-dauers, dauers, or in the dauer molt. Counts were performed at this time to be sure that no dauers were missed due to quick recovery. Non-dauer animals were all clearly L4s or adults. Virtually all of the pre-dauers formed dauers. If any did not form dauers, the error would be in the direction of underestimating dauer formation and overestimating recovery. At 72 and 96 hours (and on some plates also at 51 and 68 hours), non-dauers were counted and removed again. At 96 hours the number of dauers remaining on the plate was counted. The percent dauer formation was calculated as 100*(# dauers)/(total # worms) at 48 hours, and the percent recovery was calculated as 100*(total # of recovered animals)/(total # recovered animals + dauers left on plate at 96 hours). Dauers that crawled up the side of the plate were not

counted, as they did not have a chance to recover. Each data point was repeated on at least two days and on four to nine plates.

CHEMOTAXIS ASSAYS:

Assays of response to non-volatile attractant (NaCl) were performed essentially as described (Bargmann and Horvitz, 1991b) except that worms were allowed to swim for 15 to 30 minutes on a chemotaxis plate without attractant between the last wash and the assay itself. This step allowed the worms to better acclimate to the assay conditions and led to more reproducible results (E. Troemel and C. Bargmann, pers. comm.)

Volatile avoidance assays were performed as described (Troemel et al, 1997) except that worms were washed three times with S basal and once with water. Worms were grown and assays performed at 20° C. Because a large number of *daf-11* mutant animals form dauers at 20°C, worms were grown in a *daf-12* background to block dauer formation and allow synchronous growth of a large population of animals (as in Vowels and Thomas, 1994).

MOSAIC ANALYSIS:

Four gravid JT6901 animals were placed on a plate at 25° C. Three days later, mosaics were identified either visually or (in most cases) by flooding the plate with 1% SDS and picking those animals that

thrashed. 1% SDS kills non-dauers, and although Unc dauers survive treatment, they do not thrash. Mosaics were inspected by Nomarski microscopy, and cells were assayed for presence or absence of the extrachromosomal array by analyzing the Ncl (enlarged nucleolus) phenotype of several amphid cells. It was not possible to analyze all amphids in each animal. In most cases, ASI, ADL, ASK, ASE, ASH, AWC, AUA, and ASJ on each side were analyzed, and often several other cells were also analyzed.

RESULTS:

Based on the various phenotypes of *daf-11* mutants and the neurons implicated in these processes, I expected *daf-11* expression in the sensory neuron pairs ASJ, AWC, and ASE. To identify cells in which *daf-11* is expressed, I constructed a fusion gene containing 4.2 kb upstream of *daf-11* and its entire coding region fused to *gfp* (Chalfie et al, 1994) at the C-terminus. This transgenic array rescued the Daf-c phenotype of and induced recovery from the dauer state in *daf-11(sa195)* animals (Table 3.1). *gfp*-expressing cells were identified (Table 3.2) by comparison to known positions of amphid neurons (White et al, 1986). A similar expression pattern was seen in JT8903 *lin-15(n765); saEx237* and JT8904 *lin-15(n765); saEx238*. The *daf-11::gfp* construct injected to create *saEx238* and *saEx239* is the same as that used to generate *saEx289* (in JT9386), except that they also contain a nuclear localization signal (NLS). GFP expression in JT9386, containing the non-NLS construct, was visible in the ciliated dendrite sensory endings, as expected, as well as in the cell bodies and dendrites (Figure 3.1 A and B). In some adults, expression was only seen in the ciliated endings (Figure 3.1 C-F) and the cell bodies, suggesting that within the dendrites the protein is primarily localized to the ciliated endings.

Expression was not seen in the phasmid neurons or other cells. The expression pattern I observed is consistent with phenotypic evidence for DAF-11 function in sensory transduction, as discussed below and summarized in Table 3.3.

EXPRESSION OF C-TERMINAL *daf-11::gfp* IN ASJ, ASI, AWC, AND ASK:

The fusion protein was reproducibly expressed in the amphid neurons ASJ and ASI (Table 3.2), the main cells implicated in the response to dauer pheromone. Dauer formation both in *daf-11* mutants and in response to pheromone is dependent on ASJ activity; killing ASJ suppresses *daf-11* or pheromone-induced dauer formation (Schackwitz et al, 1996). Expression of *daf-11::gfp* in ASJ supports a model in which the DAF-11 guanylyl cyclase acts in ASJ to regulate dauer pheromone response. One possibility is that the cGMP produced by DAF-11 blocks ASJ activity and thus prevents dauer formation. In this model, dauer pheromone or *daf-11* mutation reduces the cGMP level, activating ASJ and inducing dauer formation.

ASI is also involved in dauer formation. Cell kill experiments suggest that ASI acts in conjunction with ADF to repress dauer formation in the absence of dauer inducing conditions (Bargmann and Horvitz, 1991a), and it is thought that ADF and ASI mediate the function of the TGF-ß (Group 2) branch of the sensory transduction pathway that acts in parallel to *daf-11*. (Bargmann and Horvitz, 1991a; Thomas et al, 1993; Schackwitz et al, 1996). A *gfp* fusion to *daf-7*, one of the genes in this branch, is expressed in ASI (Ren et al, 1996; Schackwitz et al, 1996), supporting this model. Thus expression of *daf-11::gfp* in ASI was unexpected. Various explanations can reconcile these findings. First,

one of the many other TM-GCs that have been identified in *C. elegans* (Yu et al, 1997; J.H. Thomas, personal communication) may function redundantly with DAF-11 in ASI to regulate dauer formation, masking a role for DAF-11 in dauer pheromone response in ASI. Second, it is possible that DAF-11 function in ASI is unrelated to dauer formation. Third, expression of *daf-11::gfp* in ASI could be an artifact of our method of assaying expression. Indeed, an unexpectedly large fraction of gfp fusions to neuronally expressed genes show expression in ASI (Troemel et al, 1995; D. Weinshenker and J.H. Thomas, unpublished observations).

daf-11::gfp expression was also seen in AWC and ASK neurons (Table 3.2). AWC is required for chemotaxis toward the volatile attractants isoamyl alcohol and benzaldehyde. Since *daf-11* mutants are defective in response to these attractants, expression in AWC was predicted. There is evidence that ASK plays a minor role in chemotaxis to lysine and possibly other non-volatile attractants (Bargmann and Horvitz, 1991b). ASK may also play a small part in promoting dauer formation in response to pheromone (Schackwitz et al, 1996). DAF-11 may function in ASK in one of these responses.

daf-11::gfp EXPRESSION IN AWB SUGGESTS AN AVOIDANCE DEFECT FOR *daf-11* MUTANTS:

A shorter fusion construct, containing the same promoter region and the first 136 codons of *daf-11* (up to the fifth exon) fused to *gfp*, was expressed in AWC, AWB, and occasionally in ASI and ASK (JT9552, Table 3.2). A similar extrachromosomal array, generated by injecting a solution containing 195 ng/ul of the same construct, also caused *gfp*

expression in ASI, but the strain stopped expressing *gfp* before quantitative data were collected. The lack of expression in ASJ and the rare expression in ASI and ASK by the shorter construct suggests that important regulatory sequences are located downstream of the fifth exon. I do not know why the longer fusion was only rarely expressed in AWB. Since AWB is required for avoidance of the repulsive compound 2-nonanone (Troemel et al, 1997), I tested the response of *daf-11* mutants to this compound. Because worms bearing *daf-11(m84)* have some phenotypic differences from those bearing other *daf-11* alleles, I tested both *daf-11(sa195)* and *daf-11(m84)* worms for this response. The strains tested also contained a *daf-12* mutation to prevent dauer formation (see Experimental Procedures), so *daf-12* mutants were also tested. Mutation of *daf-12* did not impair response. I found that both *daf-11(sa195)* and *daf-11(m84)* mutants were defective in response to either undiluted or a 10^{-1} dilution of 2-nonanone (Figure 3.2). This finding is consistent with the expression of *daf-11::gfp* in AWB and implicates DAF-11 in a fourth sensory transduction pathway.

daf-11::gfp EXPRESSION IS NOT SEEN IN ASE:

In addition to their other phenotypes, *daf-11* mutants are defective in chemotaxis toward non-volatile chemicals, a process mediated primarily by ASE. However, *daf-11::gfp* expression was only rarely seen in ASE in JT9386 larvae (Table 3.2) and was not seen in ASE in JT9386 adults grown at 20° C (the temperature at which chemotaxis assays are performed) (0/21 cells; only the "up" side was scored for each animal since in the thick adult animal it is difficult to see the "down" side clearly). This lack of expression could be due to missing regulatory sequences in the construct, or the effect of *daf-11* mutation on

chemotaxis may not be mediated through ASE. To differentiate between these possibilities, I tested whether *saEx289*, which carries the non-NLS full-length GFP fusion, could rescue the chemotaxis defect. I found partial rescue (Table 3.1), suggesting that at least part of the effect of *daf-11* mutation on chemotaxis is indirect. However, *gfp* expression is weak and variable in some cells, and our observations on strains containing the exon 5 construct suggest that a given line might not represent the complete complement of cells in which the construct can be expressed. Also, *daf-11::gfp* could be expressed in ASE at a level sufficient for *daf-11* function but too low to be visible. Thus it remains possible that *daf-11::gfp* is expressed in ASE although I am unable to detect it in JT9386.

REGULATION OF *daf-11::gfp* BY PHEROMONE:

Exposure to dauer pheromone did not alter expression of *daf-11::gfp* in L1 larvae (Table 3.2), suggesting that the effect of pheromone on dauer formation is not mediated by reducing *daf-11* gene expression. In contrast, pheromone does reduce expression a *daf-7::gfp* fusion protein in ASI. (Ren et al, 1996; Schackwitz et al, 1996). Since 8-bromo-cGMP rescues the Daf-c phenotype of *daf-11* mutants and blocks the dauer-inducing effects of pheromone in wild-type larvae, pheromone presumably reduces cGMP levels in ASJ. It is possible that pheromone directly inactivates DAF-11 guanylyl cyclase activity, perhaps by binding to the extracellular domain. However, since *daf-11* mutants have defects in several sensory processes in addition to pheromone response, this is an unlikely general model for DAF-11 function. I favor a model in which pheromone regulates cGMP levels in ASJ either by indirectly inactivating the *daf-11* TM-GC or stimulating a cGMP phosphodiesterase. A phosphodiesterase is involved in cGMP

mediated visual transduction in mammals, where it is activated by light to lower cGMP levels in rod outer segments (Miki et al, 1975).

daf-11 expression in ASI is also unaffected by pheromone. This suggests that if *daf-11* does have a role in the ASI-mediated response to pheromone, it is regulated post-transcriptionally, in contrast to the model for *daf-7* function.

daf-11::gfp EXPRESSION IN ASJ IN DAUERS:

Under appropriate environmental conditions, dauers recover and resume their normal life cycle (Cassada and Russell, 1975). Cell-kill experiments show that ASJ is the only amphid neuron essential for this recovery (Bargmann and Horvitz, 1991a). Strong loss-of-function *daf-11* mutants are severely defective in recovery from the dauer state (Vowels and Thomas, 1994). Since *daf-11::gfp* is expressed in ASJ prior to the dauer state, I hypothesized that DAF-11 might function in ASJ in dauers to promote recovery. To test this hypothesis, I assayed expression of *daf-11::gfp* in dauers that were induced by dauer pheromone at 25°. For this experiment I used JT8904 *lin-15(n765); saEx238*. The *daf-11::gfp* construct used to generate *saEx238* is identical to that used to generate *saEx289* except that it has a nuclear localization signal. *gfp* expression in ASJ was analyzed in both sides of 13 dauers (26 cells total). I saw definite expression in ASJ in six cells and expression in 12 more cells whose positions were consistent with ASJ. The arrangement of neurons in the dauer larva is somewhat different from that in the well-described L1, and positive identification was not always possible. These results, combined with the *saEx289* induction of recovery in *daf-11* mutants (Table 3.1), suggest that *daf-11* is expressed

in ASJ in most dauers and that DAF-11 functions in that cell to mediate recovery from the dauer state.

MOSAIC ANALYSIS OF *daf-11:*

Mosaic analysis was undertaken with the hope of addressing two questions. First, does DAF-11 function in ASJ to prevent dauer formation? I hoped that in answering this question I could also determine whether DAF-11 has a dauer-formation role in ASI. This seemed unlikely, since cell-kill experiments suggest that ASI and ADF mediate the TGF-ß group 2 pathway and ASJ mediates the cGMP group 1 pathway (Bargmann and Horvitz, 1991a; Schackwitz et al, 1996), but expression of *daf-11* in ASI raised the issue. The second question I hoped to address with mosaic analysis arose because the amphid neurons are bilaterally symmetric. There are two of each type of cell (e.g. ASJ, AWC) in each animal, one on the left and one on the right. When an organ or cell type is bilaterally symmetric, the question of domineering versus submissive (Hotta and Benzer, 1972) arises. In the case of *daf-11*, the question is whether mutation in one side of the animal is sufficient to induce dauer formation (in which case *daf-11* mutation is domineering to wild type) or whether both sides have to be mutant in order to induce dauer formation (in which case *daf-11* mutation is submissive to wild type).

Mosaic analysis in *C. elegans* takes advantage of two aspects of *C. elegans* biology. The first is that cell division follows the same pattern in every animal, and that pattern is known (Sulston et al, 1983). The second is that *C. elegans* chromosomes are holocentric (Albertson and Thomson, 1982). Since there is no single centromeric region, any piece

of DNA can be carried as a chromosome. Small extrachromosomal fragments, however, are lost at mitosis with some frequency. This process generates animals that are mosaic for the lost fragment. Generally, the fragment contains wild-type copies of a gene or genes for which the chromosomal copies carry a recessive mutation. Thus cells in which the fragment are present are wild type, and cells in which the fragment are lost are mutant.

In the case of *daf-11*, the strain used was JT6901 *ncl-1(e1865) unc-36(e251); daf-11(sa195ts); ctDp11*. The duplication *ctDp11* is a fusion of *sDp3* and *ctDp8* that covers part of chromosome III and part of chromosome V (Hunter and Wood, 1992). *ncl-1* and *unc-36* are on III and *daf-11* is on V; *ctDp11* carries wild type copies of each of these genes. *ncl-1* is cell-autonomous, and *ncl-1(e1865)* causes an enlarged nucleolus (Hedgecock and Herman, 1995). Thus it serves as a marker in each cell for presence (wild-type nucleolus) or absence (enlarged nucleolus) of *ctDp11*. The allele used for this experiment, *daf-11(sa195)*, contains an early nonsense mutation (Figure 2.4) and is a putative null allele. Since *ctDp11* contains *daf-11(+)*, a wild-type nucleolus indicates a cell is wild-type for *daf-11*, and an enlarged nucleolus indicates that a cell is missing DAF-11 function. I started with the premise that DAF-11(+) function is required only in amphid neurons to prevent dauer formation (Vowels and Thomas, 1994). Because few amphids have been implicated in dauer formation (Bargmann and Horvitz, 1991a; Schackwitz, 1996), I started with the further assumption that few amphids would need to be mutant for *daf-11* to induce dauer formation. I did not start with an assumption that *daf-11* acted in ASJ.

UNC-36 function is required in the AB.p lineage (Kenyon, 1986). All amphid neurons are derived from the AB lineage. Animals completely

lacking the duplication, or those that lost the duplication at the divisions generating AB or AB.p, would be Unc. By picking non-Unc dauers, I was able to screen for animals that lost the duplication either in AB.a (which gives rise to seven amphids on the left) or at a later division in AB.p. This approach increases the chance of getting an animal with a small number of mutant amphids and therefore makes each animal more informative.

I screened approximately 283,000 animals with 1% SDS and found 25 mosaics (1 mosaic / ~11,300 animals). I also obtained some mosaics by screening visually for non-Unc dauers. In several cases, I was not able to identify cells reliably enough to collect data. In 19 animals, I could identify and score enough cells to make a reasonable estimate of the division at which the duplication was lost. Nine of these animals were found by visual inspection and ten by 1% SDS screening. In analyzing each animal, the most conservative estimation of loss was made. These data are summarized in Figure 3.3. As a control, amphid neurons of non-Unc non-dauers were inspected for presence or absence of *ctDp11* (Table 3.4). I was not able to score the Ncl phenotype in these animals until the L4 stage. Since cells in older animals are located in more variable positions, it was not always possible to identify the neurons correctly, so the cell identifications are not certain in these control data.

The data shown in Figure 3.3 is divided into three classes: Animals for which a single loss of the duplication could account for the data (Figure 3.3 A), animals for which loss of the duplication at two different cell divisions could account for the data (Figure 3.3 B), and animals in which only a more complex pattern of loss could account for the data (Figure 3.3 C). In addition to these animals, in four animals all amphid neurons were wild type.

Two conclusions can be drawn from these data. One, given the premise that dauer formation is mediated through amphid neurons, the four non-Unc dauers in which all amphids are wild type tell us that *daf-11(sa195)* is very slightly dominant. Since *sa195* is a putative null allele (Figure 2.4), this result suggests that *daf-11(+)* is very slightly haplo-insufficient. The slight dominance led me to question the significance of the remaining data. Perhaps the non-Unc dauers I found resulted from dominance, and the losses I observed were coincidental. Comparison to the control data (17/24 non-Unc non-dauers had all wild type amphid neurons, versus 4/19 in the non-Unc dauers), suggested that the mutant amphids in the non-Unc dauers were not merely coincidental. Loss of DAF-11 in any of several amphid neurons seemed to increase the likelihood of a worm becoming a dauer. However, no clear pattern of necessary cells emerged. This suggests that while DAF-11 function may be mainly mediated through ASJ (Schackwitz et al, 1996), it also acts in other cells.

Cell kill experiments indicate that if ASJ is killed in one side of a *daf-11* mutant, dauer formation is partly suppressed (Schackwitz et al, 1996). This result suggests that *daf-11* functions in ASJ and that in ASJ, the dauer-inducing effect of *daf-11(sa195)* is partly domineering with respect to the dauer-preventing effect of *daf-11(+)*. The mutant cells seen in non-Unc dauers are generally only on one side of the animal, consistent with a model in which *daf-11(sa195)*-induced dauer induction is domineering over *daf-11(+)* dauer prevention. However, since in these animals *daf-11* is mutant in several cells other than ASJ, this experiment cannot tell us whether *daf-11(sa195)* is domineering or submissive in ASJ.

Table 3.1 Rescue of *daf-11* dauer formation, recovery, and chemotaxis defects by *saEx289*.

	% dauer ± SD (N)	% dauer ± SD (N)	% recovery ± SD (N)	NaCl Chemotaxis index ± SD
µl Phe	0	40	40	NA
wildtype	0 ± 0 (494)	91.7 ± 4.2 (627)	6.3 ± 4.6 (440)	0.74 ± 0.097
JT195 *daf-11(sa195)*	98.8 ± 1.5 (367)	100 ± 0 (329)	3.5 ± 4.4 (108)	0.20 ± 0.094
JT9386 *daf-11(sa195); saEx289*	21.4 ± 5.2 (999)	78.6 ± 4.8 (643)	39.2 ± 15.3 (333)	0.5 ± 0.13

Table 3.2 GFP expression in lines bearing *daf-11::gfp* fusions.

	amphid neuron					
strain	ASJ	AWC	ASE	ASI	ASK	AWB
JT9386	37/43	38/43	3/43	40/43	37/43	1/43[a]
JT9552	0/34	32/34	0/34	1/34	2/34	28/34
JT8903 and JT8904	36/36	22/36	0/36	36/36	34/36	0/36
JT9386, pheromone	19/21	16/21	1/21	14/21	13/21	0/21
JT9386, no pheromone	17/20	19/20	0/20	14/20	18/20	0/20[a]

JT9386 bears the full-length, non-NLS fusion. JT9552 bears the exon 5, NLS fusion. JT8903 and JT8904 bear the full-length, NLS fusion.

Data is presented as number of cells (not number of worms). Only worms in which there was expression in at least one cell were counted. Only sides in which all relevant cells could be identified were included, except for [a] : In two more sides in each case, expression was seen in a cell that was probably AWB, but positive identification was not possible. Data from JT8903 and JT8904 are combined.

Table 3.3 A summary of the involvement of sensory neurons and *daf-11* in various chemosensory processes.

Neuron	Function	DAF-11 required	*daf-11::gfp* expression	*saEx289* rescue
ASJ	dauer formation (group 1, promotion)[a]; dauer recovery[d]	yes[a,f]	yes	yes (formation and recovery)
AWC	attraction to isoamyl alcohol and benzaldehyde (volatile)[b]	yes[f]	yes	N.T.
AWA	attraction to pyrazine and diacetyl (volatile)[b]	no	no	N.A.
ASE	attraction to non-volatile attractants[c]	yes[f]	no	yes
ASI	dauer formation (group 2, repression)[a,d]	no[a]	yes	N.A.
ASK	small roles in: chemotaxis to lysine and possibly other non-volatile attractants, dauer formation (group 1, promotion)[a,c]	unknown	yes	N.A.
AWB	avoidance of 2-nonanone (volatile)[e]	yes	yes	N.T.

Table 3.3, continued

References: [a]Schackwitz et al, 1996; [b]Bargmann et al, 1993; [c]Bargmann and Horvitz, 1991b; [d]Bargmann and Horvitz, 1991a; [e]Troemel et al, 1997; and [f]Vowels and Thomas, 1994. N.T. Not tested, N.A. Not applicable.

Table 3.4 Mutant cells in non-Unc non-dauers.

Mutant cells in non-Unc non-dauers

number of animals	mutant amphid neurons
17	none
1	ASKL
1	ADLL
1	ASKL and ASIL
1	possibly AWBR
1	possibly ASKL
1	possibly AWCL
1	one of ASF, AWA, ASG, or AWB

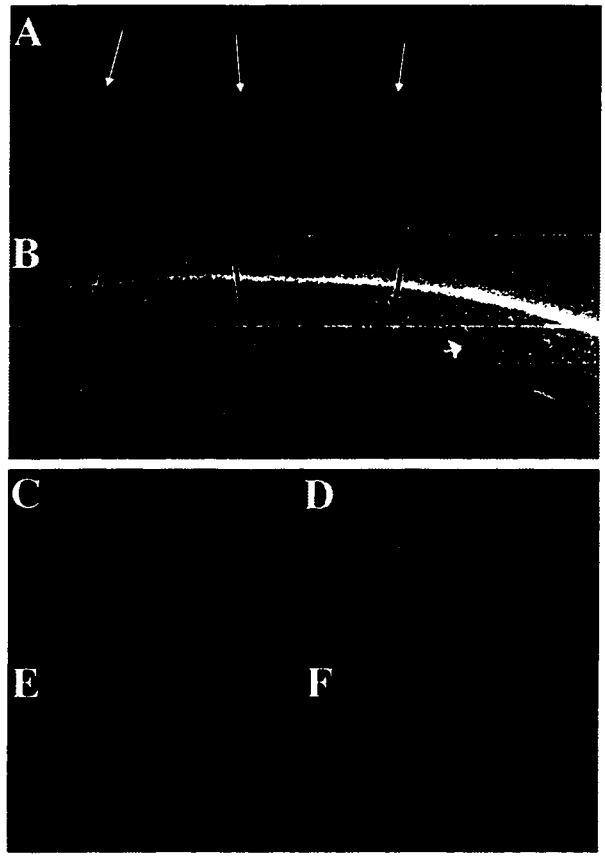
Figure 3.1 GFP expression in JT9386, bearing C-terminal *daf-11:: gfp* fusion

A. Fluorescence in JT9386 L1. Arrows indicate dendrite of amphid neurons.

B. Nomarski image of the same worm. Long black arrows correspond to arrows in A. Short white arrow indicates AWC. Short black arrow indicates ASJ. ASI and ASK, seen in A, are out of the plane of focus in B.

C and D. Fluorescence and Nomarski images of an adult JT9386, showing expression in the cilia. Arrows in D correspond to fluorescence in C. Expression is also seen in the cell body (not shown in figure).

E and F. Fluorescence and Nomarski images of adult JT195.


A
B
C
D
E
F

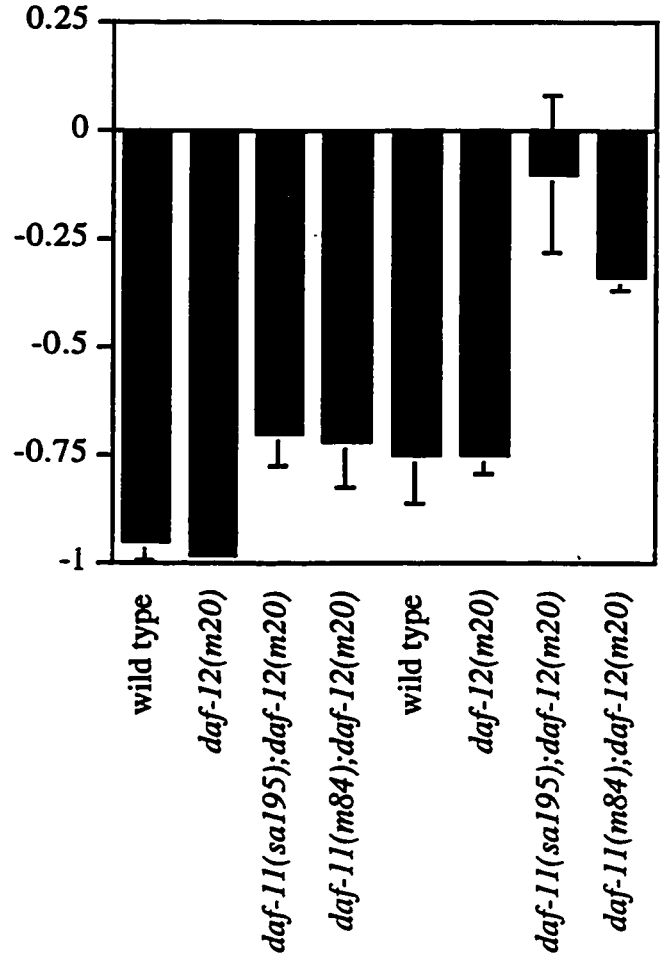


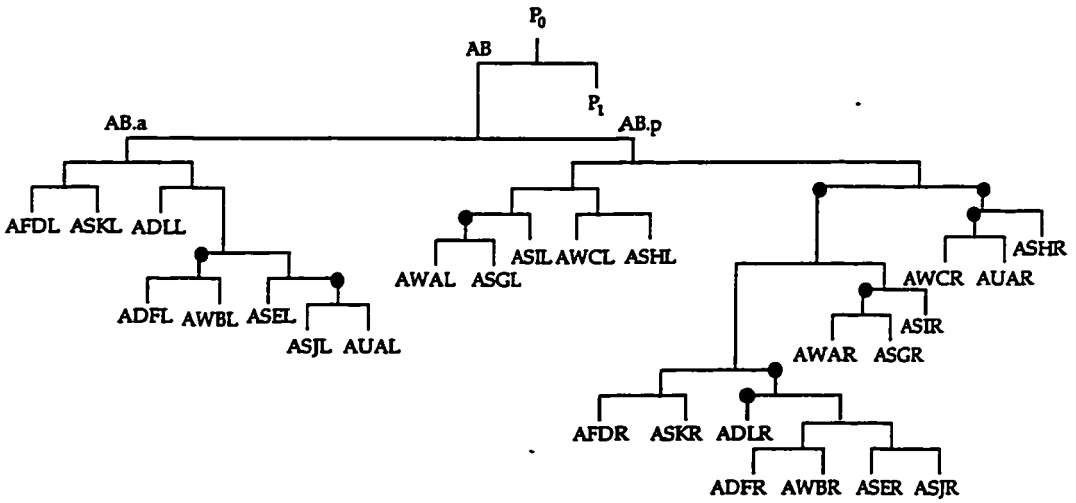
Figure 3.2 Avoidance of 2-nonanone

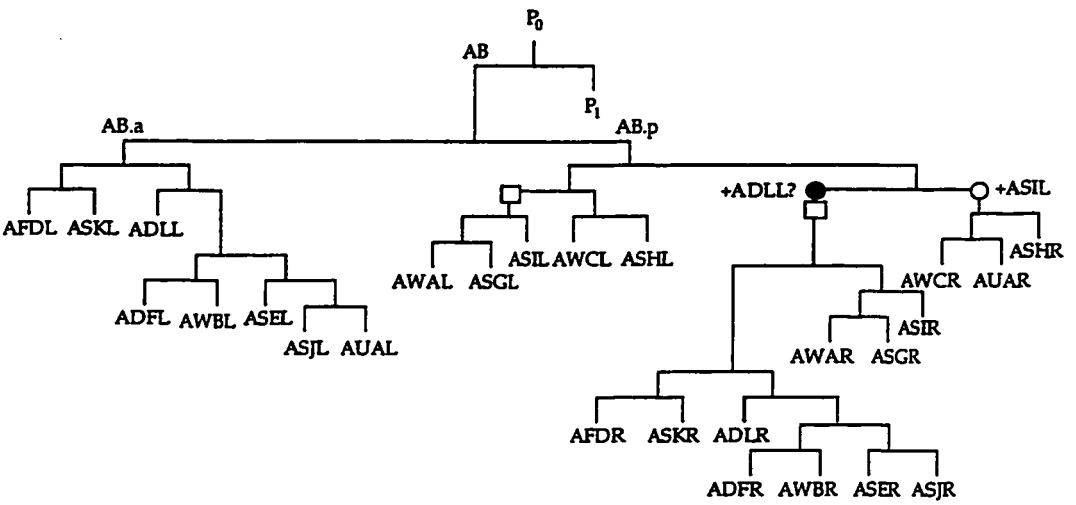
Response of wild-type and *daf-12(m20)*, *daf-11(sa195);daf-12(m20)* and *daf-11(m84);daf-12(m20)* animals to the repulsive volatile compound 2-nonanone. Response is calculated as ((#worms in the third of the plate containing 2-nonanone) - (#worms in the third of the plate opposite to the 2-nonanone))/(total # worms). A strong avoidance response is -1. Responses to undiluted (black bars) and a 1:10 dilution (gray bars) of 2-nonanone are shown. Each data point is the average of 2-4 assays with >300 worms total. SD is shown for each data point.

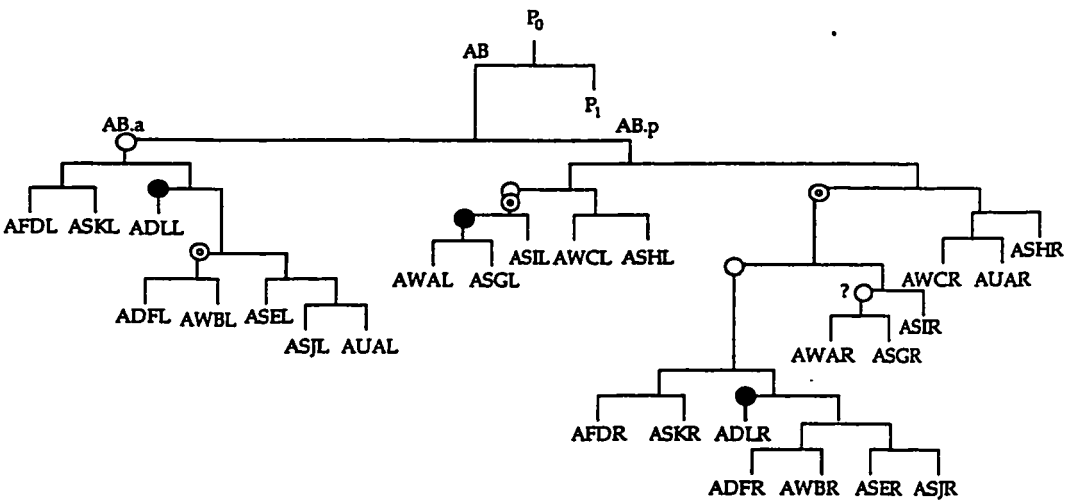
Figure 3.3 *daf-11* mosaics

Diagram (modified from Herman, 1984) is a tree of relationship among *C. elegans* amphid neurons. (A): Animals in which a single loss accounted for the data. Loss of the free duplication is indicated by a dot. Each dot corresponds to a different animal. (B) Animals in which a double loss accounted for the data. Loss of the free duplication is indicated by a mark. Each type of mark corresponds to one animal. In two of the three animals, the second loss is indicated by "+ [a cell]" noted next to the mark for the first loss. (C) Animals in which three or four losses accounted for the data. Loss of the free duplication is indicated by a mark. Each type of mark corresponds to one animal. One uncertain loss is marked with ?

All cells that descend from the marked point are presumed mutant for *daf-11;* other cells are wildtype. Not all cells were scored in each animal. The duplication was presumed to be lost at the earliest point that was consistent with all cells that were scored.


P0
AB
P1
AB.a
AB.p
AFDL ASKL ADLL
ADFL AWBL ASEL
ASJL AUAL
AWAL ASGL
ASIL AWCL ASHL
ASHR
AWCR AUAR
ASIR
AWAR ASGR
AFDR ASKR ADLR
ADFR AWBR ASER ASJR


P0
AB
P1
AB.a
AB.p
AFDL ASKL ADLL
ADFL AWBL ASEL
ASJL AUAL
AWAL ASGL
ASIL AWCL ASHL
+ADLL?
+ASIL
ASHR
AWCR AUAR
ASIR
AWAR ASGR
AFDR ASKR ADLR
ADFR AWBR ASER ASJR


P0
AB
P1
AB.a
AB.p
AFDL ASKL ADLL
ADFL AWBL ASEL
ASJL AUAL
AWAL ASGL
ASIL AWCL ASHL
ASHR
AWCR AUAR
?
ASIR
AWAR ASGR
AFDR ASKR ADLR
ADFR AWBR ASER ASJR

CHAPTER 4: DISCUSSION

A PATHWAY CONTROLLING *C. elegans* DAUER FORMATION

In addition to *daf-11*, several other genes are candidates for functioning in sensory transduction events controlling dauer formation. A model for this transduction process is provided by mammalian visual transduction, in which a TM-GC (RetGC) is known to function (reviewed in Koutalos and Yao, 1993; Hurley, 1987). This model is shown in Figure 4.1. In this pathway, the heterotrimeric G-protein transducin mediates activation of a cGMP phosphodiesterase in response to light (Baehr et al, 1982). Two G-protein alpha subunits, *gpa-2* and *gpa-3*, are implicated in dauer formation. Loss of function mutations in these genes cause a Daf-d (dauer formation defective) phenotype, and animals carrying activated *gpa-2* or *gpa-3* transgenes have a Daf-c phenotype (Zwaal et al, 1997). *gpa-3::lacZ* is expressed in amphid neurons implicated in regulating dauer formation, and the Daf-c phenotype of animals carrying an activated *gpa-3* transgene is suppressed by defects in the ciliated endings of the amphid neurons (Zwaal et al, 1997). Loss of *gpa-3* function weakly suppresses the Daf-c phenotype caused by *daf-11(m47)* (Zwaal et al, 1997). This result could suggest that *gpa-3* functions either downstream of or in parallel to *daf-11*. However, it is also consistent with the fact that *daf-11(m47)* is a weak allele (Vowels and Thomas, 1992) (see Figure 4.1 B); the *daf-11(m47)* mutation causes slightly lowered cGMP levels, enhancing dauer formation slightly. In this model, loss of *gpa-3* function would result in reduced PDE activity, allowing more cGMP to remain non-hydrolyzed and suppressing the Daf-c phenotype. In stronger alleles, cGMP levels would be too low for lowered PDE activity caused by

absence of GPA-3 to have an effect. These findings are consistent with a role for *gpa-3* analogous to that of transducin in visual transduction. A putative cyclic nucleotide phosphodiesterase (PDE) (F26A1.14) has been identified by the Sequencing Consortium (Watson et al, 1993; Wilson et al, 1994; *C. elegans* Genome Sequencing Consortium, pers. comm.), but it is not yet known whether it is involved in dauer formation. No other cyclic nucleotide PDEs have been identified in *C. elegans* to date.

In visual transduction, cGMP acts directly on an excitatory cyclic nucleotide gated ion channel (Fesenko et al, 1985). In *C. elegans*, *tax-4* and *tax-2* have recently been shown to encode α and β subunits of a cyclic nucleotide gated ion channel (Komatsu et al, 1996; Coburn and Bargmann, 1996). A *tax-4::gfp* fusion is expressed in the amphid neurons ASK, ASI, ASG, ASE, AFD, AWC, and ASJ (Komatsu et al, 1996), a set that includes four of the five neurons in which *daf-11::gfp* fusions are expressed, and *tax-4* mutants are weakly Daf-c (Komatsu et al, 1996; Chapter 2). To test whether TAX-4 is a target of cGMP synthesized by DAF-11, I measured the effect of the cGMP analogue 8-bromo-cGMP on dauer formation in *tax-4* mutants. If TAX-4 is a target of cGMP synthesized by DAF-11, the Daf-c phenotype of *tax-4* mutants should not be suppressed by growth on the analogue. As predicted, I found that 8-bromo-cGMP had no effect on dauer formation in *tax-4* mutants (Figure 2.6).

One question raised by this model concerns the interaction between cGMP and the channel. Absence of ASJ suppresses dauer formation; therefore signal from ASJ induces dauer formation (Schackwitz et al, 1997). Since *daf-11* mutation causes dauer formation, DAF-11(+) must repress dauer formation and therefore repress signaling from ASJ. Since influx of cations causes depolarization and signaling, this suggest

that the TAX-4/TAX-2 channel is closed by cGMP. A conductance that is prevented by cyclic nucleotides has been identified in mammalian taste tissue, though it is thought to be a Ca^{++} conductance (Kolesnikov and Margolskee, 1995). *tax-4* expressed in HEK293 cells forms channels that can be gated by cyclic nucleotides (Komatsu et al, 1996). However, it is thought that *in vivo* TAX-2 and TAX-4 function together, and electrophysiology has not been done on a channel containing both subunits. Thus it is possible that the TAX-2/TAX-4 channel is closed by cGMP. This part of the model awaits further study.

Another gene, *daf-21*, is known by genetic interactions to act at the same point in the genetic pathway as *daf-11* (Thomas et al, 1993). *daf-21* encodes an HSP90 (heat shock protein 90) (E.A. Malone and J.H. Thomas, pers. comm.). While *daf-21* differs from *daf-11* in several respects (Vowels and Thomas, 1994; J.J. Vowels, 1994, Mori and Ohshima, 1997), it does have relatively specific sensory defects. This observation implicates a previously unidentified player in TM-GC signal transduction. HSP90 is known to interact with several kinds of molecules, including kinases (reviewed in Pratt, 1997). It is possible that DAF-21 interacts with and regulates the kinase homology domain of *daf-11*. The function of this domain is not well understood (see below).

This genetic model system can be used to determine *in vivo* the relationships among known and novel components of a cGMP transduction pathway. Mutations already exist in many of these genes and gene disruptions could be generated in the phosphodiesterase and in other potential components discovered by sequence analysis (Zwaal et al, 1993). These mutations can be used to analyze the functional relationships among these genes in the control of dauer formation. In addition, study of the role of DAF-21 in dauer formation and its

interaction with DAF-11 will help elucidate the role of a previously unknown component of TM-GC signal transduction.

OLFACTION AND TASTE RESPONSE IN *C. elegans*

The response of *C. elegans* to volatile compounds (olfaction) and non-volatile compounds (taste) is a subject of much interest. Several genes have been implicated in response to both volatile and non-volatile compounds, among them *daf-11* and *daf-21* (Vowels and Thomas, 1994), *tax-2* (Coburn and Bargmann, 1996), and *tax-4* (Komatsu et al, 1996). Animals mutant for some genes, including *odr-1* and several others, are able to respond normally to non-volatile attractants but are defective in response to volatile attractants (Bargmann et al, 1993). *che-1*, *che-6* (Bargmann et al, 1993) and *che-16* (E. Troemel and C. Bargmann, pers. comm.) mutants are able to respond to volatile but not non-volatile attractants. Searches are underway for additional mutants defective in response to non-volatile attractants (S. Wicks, C. de Vries, and R. Plasterk, pers. comm.).

This catalogue of genes and defects suggests that part of the machinery for taste and olfactory transduction is shared but parts are unique to each pathway. S. Wicks, C. de Vries, and R. Plasterk have identified about 40 alleles of genes specifically involved in taste transduction (pers. comm.). One of these genes, *che-3*, has been cloned and shown to encode a dynein heavy chain. It is required for intact ciliated endings of the amphid neurons. The identity of and interactions among the remaining genes are a subject of further study (S. Wicks, C. de Vries, and R. Plasterk, pers. comm.)

The molecular identities of several of the olfaction-specific genes are known. Among these are *odr-10*, a 7-pass, G-protein-coupled receptor that is the diacetyl receptor (Sengupta et al, 1996) and *odr-3*, an alpha subunit of a G protein (Roayaie et al, 1998). The involvement of these genes in olfaction provides clear evidence for G-protein mediated signal transduction of olfactory signals. The identification of a large family of 7-pass receptors in *C. elegans* (Troemel et al, 1995) suggests that G-protein-mediated transduction is prominent in the nematode. Other genes required for olfaction have molecular identities that suggest a less direct role in signal transduction. *odr-7* encodes a transcription factor required for *odr-10* expression (Mori and Ohshima, 1997), and *odr-4* encodes a novel gene product required for localization of *odr-10* (N. Dwyer and C. Bargmann, pers. comm.). *odr-2* encodes a small GP-1 linked protein (J. Chou, P. Sengupta, and C. Bargmann, pers. comm.).

daf-11 and *odr-1* encode transmembrane guanylyl cyclases (TM-GCs). These genes provide the first evidence that TM-GCs play a role in olfactory transduction. The taste defect in *daf-11* mutants (Vowels and Thomas, 1994) is the first evidence of a role for TM-GCs in taste response. Many other TM-GCs have been identified in *C. elegans* (Yu et al, 1997 and J.H. Thomas, pers. comm.), and GFP fusions to several of these genes are expressed in sensory neurons (Yu et al, 1997), suggesting that this family of proteins comprises a significant transduction mechanism in *C. elegans*.

Interestingly, both *daf-11* and *odr-1* mutants are defective in response to odorants sensed by the amphid neuron AWC but are able to respond to those sensed by AWA (Vowels and Thomas, 1994; Bargmann et al,

1993), suggesting that these genes may function together. TM-GCs are thought to dimerize (Garbers and Lowe, 1994). Perhaps DAF-11 and ODR-1 act together to transduce AWC-mediated olfactory signals.

odr-1 mutants are not Daf-c and are able to respond to non-volatile attractants (N. L'Etiole and C. Bargmann, pers. comm.; Bargmann et al, 1993), so in these two pathways DAF-11 either forms homodimers or forms heterodimers with an as-yet unidentified TM-GC. Several TM-GCs have been identified by the C. elegans Sequencing Consortium (Watson et al, 1993; Wilson et al, 1994; *C. elegans* Genome Sequencing Consortium, pers. comm.; Yu et al, 1997; J.H. Thomas, pers. comm.), so this is a plausible model. Exhaustive screens for Daf-c mutants have been executed (e.g. Swanson and Riddle, 1981; Riddle et al, 1981; Malone and Thomas, 1994). The only identified gene that acts at the same place as *daf-11* in the genetic pathway is *daf-21*, an HSP90. Thus DAF-11 appears not to require a heterodimerization partner to transduce the dauer pheromone signal. It is possible, however, that in a wild type animal DAF-11 acts as a heterodimer with another TM-GC but that gene has not been identified in mutant screens because DAF-11 is capable of functioning as a homodimer in its absence. Since it is likely that many genes mediating non-volatile attractive response remain unidentified, it is not possible to predict whether DAF-11 functions as a hetero- or homodimer in taste response. Searches for taste-defective mutants are underway (S. Wicks, C. de Vries, and R. Plasterk, pers. comm.). The identification of genes involved in taste transduction will help address this question.

TRANSMEMBRANE GUANYLYL CYCLASES IN SENSORY TRANSDUCTION: POSSIBLE FUTURE DIRECTIONS IN STUDY OF *daf-11*

The role of DAF-11 in olfactory and taste transduction mechanisms provides evidence that TM-GCs are involved in these transduction pathways. To date, TM-GCs have been primarily identified in vertebrates and studied *in vitro* or in cell culture. The ease of experimental manipulation of *C. elegans* provides an ideal system in which to study *in vivo* the role of TM-GCs in sensory transduction.

1. Analysis of TM-GC domains: The function of the various domains of TM-GC molecules is a major question. The protein consists of an N-terminal extracellular domain (ECD), a single transmembrane domain, a domain that has been termed a "kinase homology domain" (KHD), and a cyclase domain. The catalytic role of the cyclase domain, forming cGMP from GTP, is known, but the role of the ECD and KHD remains unclear.

Jim Thomas has analyzed the KHD and ECD of several of the *C. elegans* TM-GCs identified by the Sequencing Consortium (J.H. Thomas, pers. comm.). He found three significant facts about the KHD. First, the KHD is missing key kinase residues (Hanks et al, 1988; see also Yuen and Garbers, 1992), so it is unlikely to have kinase activity. Second, there are regions of significant conservation of KHD sequence that are not conserved among true kinases, suggesting a function distinct from phosphorylation. Third, key ATP-binding residues (Hanks et al, 1988) are not completely conserved in TM-GCs. This observation creates difficulty in interpreting already contradictory reports of both positive and negative effects of ATP on cyclase activity (e.g. Chinkers et al, 1991; Parkinson et al, 1994; Vaandrager et al, 1993a and 1993b; Chinkers and Garbers, 1989).

Function of the ECD is also unknown in many cases. Ligands have been identified for several TM-GCs (Garbers, 1992), but for others, including DAF-11 and the RetGC involved in visual transduction, no ligand is known. However, the size of the ECD is conserved, and homology (28%) has been observed in at least one case, between a *C. elegans* TM-GC (*gcy-12*) of unknown function and DrGC-1, a *Drosophila* TM-GC that is expressed in the early embryo and may therefore have a developmental role (J. H. Thomas, pers. comm.; McNeil et al, 1995). This homology across large evolutionary distance indicates that the ECD has a significant role even in cases where the ligand is not yet identified.

daf-11 null animals transgenic for *daf-11* alleles bearing specific deletions would enable us to assess *in vivo* the functions of the domains. The deletions and the predicted phenotypes of the transgenic animals are pictured in Figure 4.2. The Daf-c mutation *daf-11(sa195)* encodes an early stop codon (Figure 2.4) and is a putative null. A cyclase domain deletion would be predicted to have the same phenotype, and an overexpression transgene would cause the opposite phenotype, a Daf-d animal. The phenotype of transgenic animals with deletions of the KHD would depend on the function of the KHD. If it is required for cyclase activity, the deletion would be Daf-c; if it represses cyclase activity, the deletion would be Daf-d. Since there are no predictions for function of the ECD, I can make no predictions for the phenotype of the deletion. Similar experiments can be used to elucidate function of specific regions within the domain, for example to isolate positive or negative regulatory sequences within the KHD.

2. Intergenic interactions: Double mutant analysis among *daf-11* and the other identified TM-GCs (*odr-1* and the *gcy* genes) can be used to

study TM-GC dimerization. For example, if mutation of a second TM-GC enhances one of the *daf-11* defects, it would suggest that these two TM-GCs either interact or function redundantly in vivo. Two-hybrid analysis could be used to study interactions among TM-GCs and between TM-GCs and other proteins. If two proteins are found to interact genetically, analysis of deletions (transgenic animals or two-hybrid experiments) could determine if the interaction is physical and, if it is, identify the interacting domains of the proteins. This approach could be especially useful in understanding the role of *daf-21* (Vowels and Thomas, 1994; see above) in dauer formation and other sensory processes. E. Malone is studying this question (E. Malone, pers. comm.)

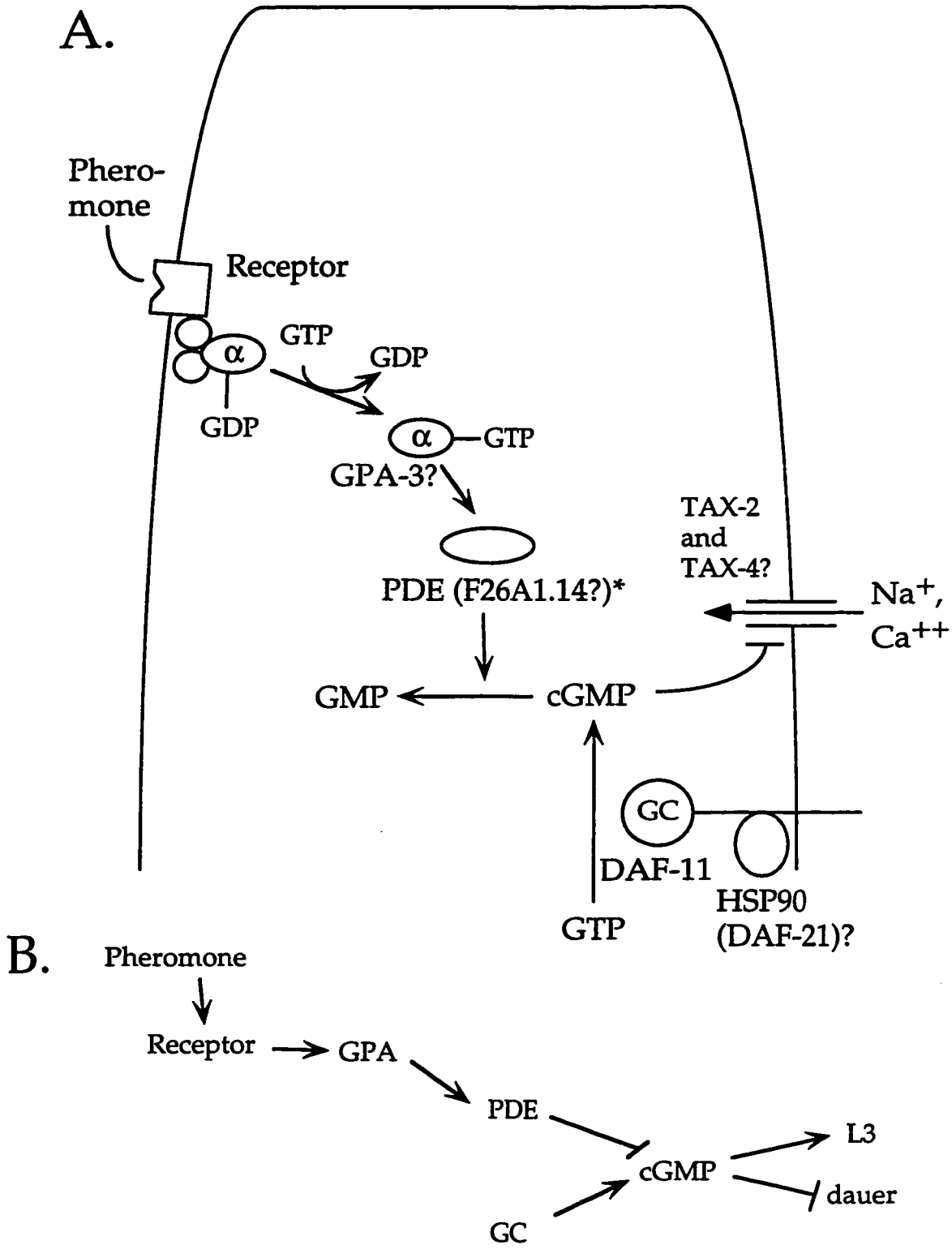
CONCLUSION

The *C. elegans* gene *daf-11* encodes a member of the transmembrane guanylyl cyclase (TM-GC) family, a family known to function in a number of transduction pathways, including the sensory pathway for visual transduction in mammals. 8-bromo-cGMP rescues the *daf-11* Daf-c phenotype, providing direct *in vivo* evidence for cGMP function in this transduction pathway. *daf-11* mutants exhibit defects in several sensory processes, providing direct evidence that TM-GCs function in other sensory processes as well. *daf-11::gfp* is expressed in a number of sensory neurons that are consistent with its various defects. Further study of *daf-11* and other genes with which it interacts genetically and physically will enable us to better understand the in vivo mechanism of this important signaling pathway.

Figure 4.1 A Model for Dauer Formation via the Group I genes.

A. In the sensory neuron cilia. The possible target channel is pictured.

B. A formal genetic model, eliminating the possible target channel. PDE= phosphodiesterase F26A1.14. There is no direct evidence for a role of GPA or PDE in the process, and the receptor has not been identified.


A.
Phero-
mone
Receptor
α
GTP
GDP
GDP
α
GTP
GPA-3?
PDE (F26A1.14?)*
TAX-2
and
TAX-4?
Na+,
Ca++
GMP
cGMP
GC
DAF-11
HSP90
(DAF-21)?
GTP
B.
Pheromone
Receptor
GPA
PDE
cGMP
L3
dauer
GC

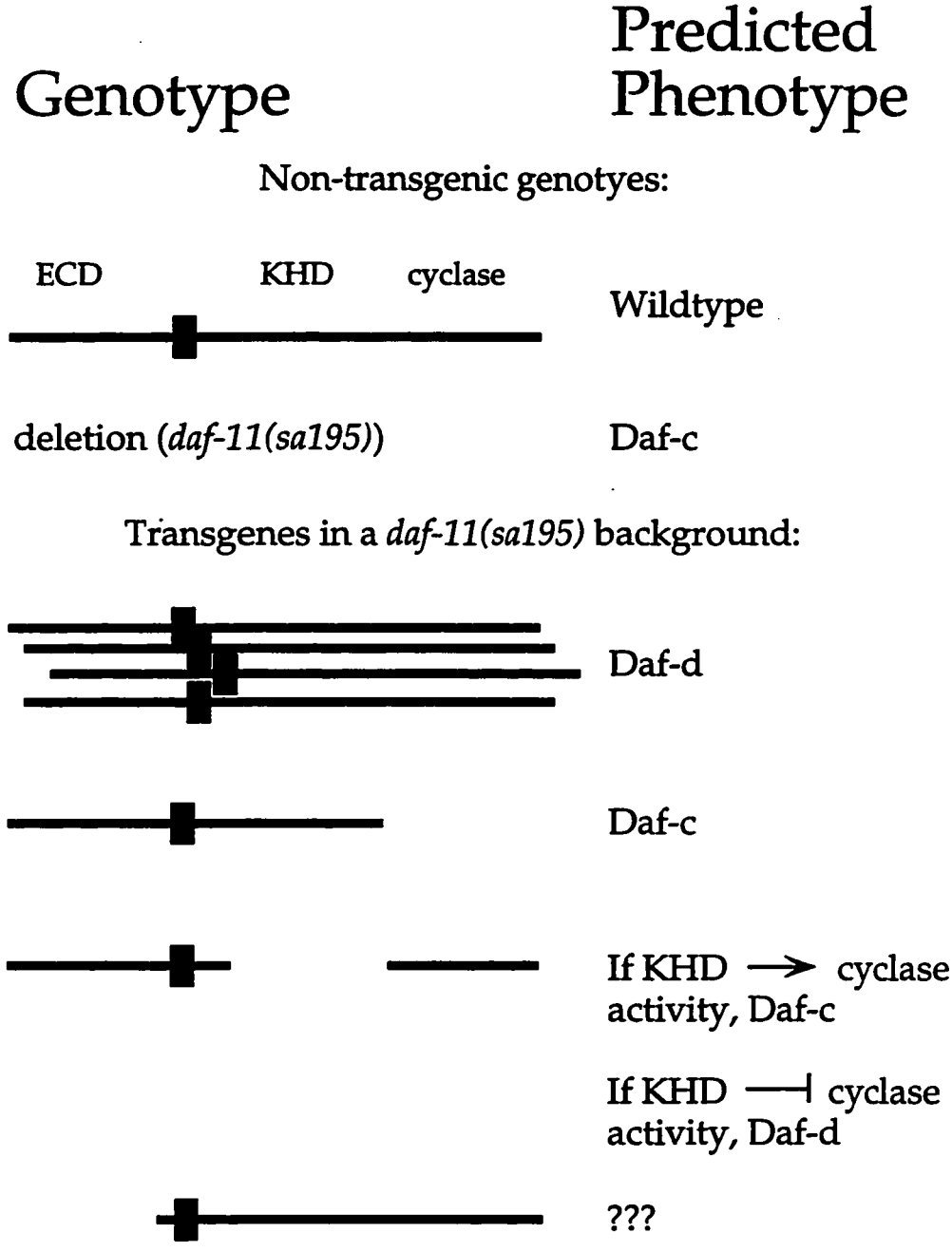


Figure 4.2 Predicted phenotypes of transgenics bearing various *daf-11* deletions.

BIBLIOGRAPHY

Albert, P.S., Brown, S.J., and Riddle, D.L. (1981). Sensory control of dauer larva formation in *Caenorhabditis elegans*. J Comp Neurol 198:435-451.

Albertson, D.G. and Thomson, J.N. (1982). The kinetochores of *Caenorhabditis elegans*. Chromosoma 86:409-428.

Avery, L. and Wasserman, S. (1992). Ordering gene function: the interpretation of epistasis in regulatory hierarchies. Trends Genet 8:312-316.

Baehr, W., Morita, E.A., Swanson, R.J., and Applebury, M.L. (1982). Characterization of bovine rod outer segment G-protein. J Biol Chem 257:6452-6460.

Bargmann, C.I. and Avery, L. (1997). Laser Killing of Cells in Caenorhabditis elegans. In *Caenorhabditis elegans*: Modern Biological Analysis of an Organism. Ed. Epstein, H.F. and Shakes, D.C. Acadmic Press, San Diego.

Bargmann, C.I. and Horvitz, H.R. (1991a). Control of larval development by chemosensory neurons in *Caenorhabditis elegans*. Science 251:1243-1246.

Bargmann, C.I. and Horvitz, H.R. (1991b). Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. Neuron 7:729-742.

Bargmann, C.I., Hartwieg, E., and H.R. Horvitz (1993). Odorant-selective genes and neurons mediate olfaction in *C. elegans*. Cell 74:515-527.

Bargmann, C.I. and Mori, I. (1997). Chemotaxis and Thermotaxis. In *C. elegans* II. Ed. Riddle, D.L. et al. Cold Spring Harbor Laboratory Press, NY.

Barnes, W.M. (1994). PCR amplification of up to 35-kb DNA with high fidelity and high yield from lambda bacteriophage templates. Proc Natl Acad Sci 91:2216-2220.

Barstead, R.J., and Waterston, R.H. (1989). The basal component of the nematode dense-body is vinculin. J Biol Chem 264:10177-10185.

Ben-Arie, N., Lancet, D., Taylor, C., Khen, M., Walker, N., Ledbetter, D.H., Carrozzo, R., Patel, K., Sheer, D., Lehrach, H., et al (1994). Olfactory

receptor gene cluster on human chromosome 17: possible duplication of an ancestral receptor repertoire. Hum Mol Genet 3:229-235.

Bossy, J. (1970). Atlas of neuroanatomy and special sense organs. Philadelphia: Saunders.

Breer, H. (1993).Second messenger signalling in olfaction. Ciba Found Symp 179:97-109.

Breer, H., Klemm, T., and Boekhoff, I. (1992). Nitric oxide mediated formation of cyclic GMP in the olfactory system. Neuroreport 3:1030-1032.

Breer, H., and Shepherd, G.M. (1993). Implications of the NO/cGMP system for olfaction. Trends Neurosci 16:5-9

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. Genetics 77:71-94.

Buck, L.B. (1996). Information coding in the vertebrate olfactory system. Annu Rev Neurosci 19:517-544.

Buck, L. and Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65:175-187.

Cassada, R. and Russel, R.L. (1975). The dauer larva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev Biol 46:326-342.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W., and Prasher, D.C. (1994). Green fluorescent protein as a marker for gene expression. Science 263:802-5.

Chinkers, M., and Garbers, D.L. (1989). The protein kinase domain of the ANP receptor is required for signalling. Science 245:1392-1394.

Chinkers M., Garbers D.L., Chang M.S., Lowe D.G., Chin H.M., Goeddel D.V., Schulz S. (1989). A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor. Nature 338:78-83.

Chinkers, M., Singh, S., and Garbers, D.L. (1991). Adenine nucleotides are required for activation of rat atrial natriuretic peptide receptor/guanylyl cyclase expressed in a baculovirus system. J Biol Chem 266:4088-4093.

Coburn, C.M., and Bargmann, C.I. (1996). A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. Neuron 17:695-706.

Culotti J.G. and Russell R.L. (1978). Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. Genetics 90:243-256.

de Sauvage F.J., Camerato T.R., and Goeddel D.V. (1991). Primary structure and functional expression of the human receptor for *Escherichia coli* heat-stable enterotoxin. J Biol Chem 266:17912-17918.

Drewett, J.G. and Garbers, D.L. (1994). The family of guanylyl cyclase receptors and their ligands. Endocrine Rev 15:135-162.

Dusenbery, D.B. (1974). Analysis of chemotaxis in the nematode *Caenorhabditis elegans* by countercurrent separation. J Exp Zool 188:41-48.

Estevez, M., Attisano, L., Wrana, J.L., Albert, P.S., Massague, J., and Riddle, D.L. (1993). The *daf-4* gene encodes a bone morphogenetic protein receptor controlling *C. elegans* dauer larva development. Nature 365:644-649.

Fesenko, E.E., Kolesnikov, S.S., and Lyubarsky, A.L. (1985). Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. Nature 313:310-313.

Frohman, M.A., Dush, M.K., and Martin, G.R. (1988). Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. Proc Natl Acad Sci 85:8998-9002.

Garbers, D. L. (1992). Guanylyl cyclase receptors and their endocrine, paracrine, and autocrine ligands. Cell 71:1-4.

Garbers, D.L. and Lowe, D.G. (1994). Guanylyl cyclase receptors. J Biol Chem 269:30741-30744.

Georgi, L.L., Albert, P.S., and Riddle, D.L. (1990). *daf-1*, a *C. elegans* gene controlling dauer larva development, encodes a novel receptor protein kinase. Cell 61:635-645.

Golden, J.W., and Riddle, D.L. (1984a). A pheromone-induced developmental switch in *Caenorhabditis elegans*: Temperature-sensitive mutants reveal a wild-type temperature-dependent process. Proc Natl Acad Sci 81:819-823.

Golden, J.W., and Riddle, D.L. (1984b). The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. Devl Biol 102:368-378.

Golden, J.W., and Riddle., D. L. (1985). A gene affecting production of the *Caenorhabditis elegans* dauer-inducing pheromone. Mol Gen Genet 198: 534-536.

Gottlieb, S. and Ruvkun, G. (1994). *daf-2, daf-16* and *daf-23*: Genetically interacting genes controlling dauer formation in *Caenorhabditis elegans*. Genetics 137, 107-120.

Goy, M.F. (1991). cGMP: the wayward child of the cyclic nucleotide family. Trends Neurosci 14:293-299.

Hanks, S.K., Quinn, A.M., and Hunter, T. (1988). The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241:42-52.

Hedgecock EM and Herman RK (1995). The *ncl-1* gene and genetic mosaics of *Caenorhabditis elegans*. Genetics 141:989-1006.

Herman, R.K. (1984) Analysis of genetic mosaics of the nematode *C. elegans*. Genetics 108: 165-180.

Hereford, L.M. and Hartwell, L.H. (1974) Sequential gene function in the initiation of *Saccharomyces cerevisiae* DNA synthesis. J Mol Biol 84:445-461.

Hildebrand, J.G. and Shepherd, G.M. (1997). Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. Annu Rev Neurosci 20:595-631.

Hopkins, D.A., Steinbusch, H.W., Markerink-van Ittersum, M., and De Vente, J. (1996). Nitric oxide synthase, cGMP, and NO-mediated cGMP production in the olfactory bulb of the rat. J Comp Neurol 375:641-658.

Hotta, Y. and Benzer, S. (1972).Mapping of behaviour in *Drosophila* mosaics. Nature 240:527-535.

Horvitz, H. R., Brenner, S., Hodgkin, J., and Herman, R. K. (1979). A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol Gen Genet 175:129-133.

Huang, L.S., Tzou, P., and Sternberg, P.W. (1994). The *lin-15* locus encodes two negative regulators of *Caenorhabditis elegans* vulval development. Mol Biol Cell 5:395-412.

Hunter, C. P. and Wood, W.B. (1992). Evidence from mosaic analysys of the masculinizing gene *her-1* for cell interactions in *C. elegans* sex determination. Nature 533:551-555.

Hurley, J.B. (1987). Molecular properties of the cGMP cascade of vertebrate photoreceptors. Ann Rev Physiol 49:793-812.

Juilfs, D.M., Fulle, H.J., Zhao, A.Z., Houslay, M.D., Garbers, D.L., and Beavo, J.A. (1997). A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. Proc Natl Acad Sci 94:3388-3395.

Kenyon, C. (1986). A gene involved in the development of the posterior body region of *C. elegans*. Cell 46:477-487

Kinnamon, S. C., and Getchell, T. V. (1991). Sensory transduction in olfactory receptor neurons and gustatory receptor cells. In Smell and Taste in Health and Disease, Ed. by T. V. Getchell., R. L. Doty, L. M. Bartoshuk, and J. B. Snow, Jr., Raven Press, NY.

Kolesnikov, S. and Margolskee, R. (1995). A cyclic-nucleotide-suppressbile conductance activated by transducin in taste cells. Nature 376: 85-88.

Komatsu, H., Mori, I., Rhee, J.S., Akaike, N., and Ohshima, Y. (1996). Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans*. Neuron 17:707-718.

Koutalos, Y., and Yau, K.W. (1993). A rich complexity emerges in phototransduction. Curr Opin Neurobiol 3:513-519.

Larsen P.L., Albert P.S., and Riddle D.L. (1995). Genes that regulate both development and longevity in *Caenorhabditis elegans*. Genetics 139:1567-1583.

Lim, C.-S. (1993). The Caenorhabidits elegans *daf-7* gene encodes a novel member of the transforming growth factor-β superfamily. Ph.D. Dissertation, University of Missouri.

Lindemann, B. (1996). Taste reception. Physiol Rev 76:718-766.

Malone E.A., and Thomas J.H. (1994). A screen for nonconditional dauer-constitutive mutations in *Caenorhabditis elegans*. Genetics 136:879-886.

Malone, E.A., Inoue, T., and Thomas, J.H. (1996). Genetic analysis of the roles of *daf-28* and *age-1* in regulating *Caenorhabditis elegans* dauer formation. Genetics 143, 1193-1205.

McNeil, L., Chinkers, M., Forte, M. (1995). Identification, characterization, and developmental regulation of a receptor guanylyl cyclase expressed during early stages of *Drosophila* development. J Biol Chem 270:7189-7196.

Mello, C.C., Kramer, J.M., Stinchcomb, D., and Ambros, V. (1991). Efficient gene transfer in *C. elegans*: Extrachromosomal maintenance and integration of transforming sequences. EMBO J 10:3959-3970.

Miki, N., Baraban, J.M., Keirns, J.J., Boyce J.J., and Bitensky M.W. (1975). Purification and properties of the light-activated cyclic nucleotide phosphodiesterase of rod outer segments. J Biol Chem 250:6320-6327.

Miller, L.M., Plenefisch, J.D., Casson, L.P., and Meyer, B.J. (1988). *xol-1*: a gene that controls the male modes of sex determination and X chromosome dosage compensation in *C. elegans*. Cell 55:167-183.

Mori, I. and Ohshima, Y. (1997). Molecular neurogenetics of chemotaxis and thermotaxis in the nematode *Caenorhabditis elegans*. Bioessays 19:1055-1064.

Ngai, J., Chess, A., Dowling, M.M., Necles, N., Macagno, E.R., Axel, R. (1993). Coding of olfactory information: topography of odorant receptor expression in the catfish olfactory epithelium. Cell 72:667-680.

Parkinson S.J., Carrithers S.L., Waldman S.A. (1994). Opposing adenine nucleotide-dependent pathways regulate guanylyl cyclase C in rat intestine. J Biol Chem 269:22683-22690.

Parmentier, M., Libert, F., Schurmans, S., Schiffmann, S., Lefort, A., Eggerickx, D., Ledent, C., Mollereau, C., Gerard, C., Perret, J., et al (1992). Expression of members of the putative olfactory receptor gene family in mammalian germ cells. Nature 355:453-455.

Patterson, G.I., Koweek, A., Wong, A., Liu, Y., and Ruvkun, G. (1997). The DAF-3 Smad protein antagonizes TGF-beta-related receptor signaling in the *Caenorhabditis elegans* dauer pathway. Genes Dev 11:2679-2690.

Perkins, L.A., Hedgecock, E.M., Thomson, J.N., and Culotti, J.G. (1986). Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev Biol 117:456-487.

Pratt, W.B. (1997). The role of the hsp90-based chaperone system in signal transduction by nuclear receptors and receptors signaling via MAP kinase. Annu Rev Pharmacol Toxicol 37:297-326.

Ren P., Lim C.S., Johnsen R., Albert P.S., Pilgrim D., Riddle D.L. (1996). Control of *C. elegans* larval development by neuronal expression of a TGF-beta homolog. Science 274:1389-1391.

Restrepo, D., Teeter, J.H., and Schild, D. (1996). Second messenger signaling in olfactory transduction. J Neurobiol 30:37-48.

Riddle, D.L (1997). Genetic and Environmental Regulation of Dauer Larva Development. In C. elegans II. Ed. Riddle, D.L. et al. Cold Spring Harbor Laboratory Press, NY.

Riddle, D.L., Swanson, M.M., and Albert, P.S. (1981). Interacting genes in nematode dauer formation. Nature 270:668-671.

Roayaie K., Crump J.G., Sagasti A., Bargmann CI (1998). The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. Neuron 20:55-67.

Schackwitz W.S., Inoue, T., and Thomas, J.H. (1996). Chemosensory neurons function in parallel to mediate a pheromone response in *C. elegans*. Neuron 17:719-728.

Sengupta, P., Chou, J.H., Bargmann, C.I. (1996). *odr-10* encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. Cell 1996 84:899-909.

Sengupta, P., Colbert, H.A., Kimmel, B.E., Dwyer, N., Bargmann, C.I. (1993). The cellular and genetic basis of olfactory responses in *Caenorhabditis elegans*. Ciba Found Symp 179:235-244.

Shakir, M.A., Miwa, A.J., and Saddiqui, S.S. (1993). A role of ADF chemosensory neurones in dauer formation behavior in C. *elegans*. NeuroReport 4:1151-1154.

Sulston J.E., Schierenberg E., White J.G., and Thomson J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev Biol 100:64-119.

Swanson, M.M., Riddle, D.L. (1981). Critical periods in the development of the *Caenorhabditis elegans* dauer larva. Dev Biol 84:27-40.

Thomas, J.H., Birnby, D.A., and Vowels, J.J. (1993). Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. Genetics 134:1105-1117.

Treinin, M., and Chalfie, M. (1995). A Mutated Acetylcholine Receptor Subunit Causes Neuronal Degeneratio in *C. elegans*. Neuron 14:871-877.

Troemel E.R., Chou J.H., Dwyer N.D., Colbert H.A., Bargmann C.I. (1995). Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. Cell 83:207-218.

Troemel, E.R., Kimmel, B.E., and Bargmann, C.I. (1997). Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. Cell 91:161-169.

Vaandrager A.B., van der Wiel E., and de Jonge H.R. (1993a). Heat-stable enterotoxin activation of immunopurified guanylyl cyclase C: Modulation by adenine nucleotides. J Biol Chem 268:19598-19603.

Vaandrager A.B., Schulz S., De Jonge H.R., and Garbers D.L. (1993b). Guanylyl cyclase C is an N-linked glycoprotein receptor that accounts for multiple heat-stable enterotoxin-binding proteins in the intestine. J Biol Chem 268:2174-2179.

Vowels, J.J. (1994). Genes mediating chemosensory responses in *Caenorhabditis elegans*. Ph.D. Dissertation, University of Washington.

Vowels, J.J., and Thomas, J.H. (1992). Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. Genetics 130:105-123.

Vowels, J.J., and Thomas, J.H. (1994). Multiple chemosensory defects in *daf-11* and *daf-21* mutants of *Caenorhabditis elegans*. Genetics 138:303-316.

Watson, A., Smaldon, N., Lucke, R., and Hawkins, T. (1993). The *Caenorhabditis elegans* genome sequencing project: first steps in automation. Nature 362:569-570.

Ward, S. (1973). Chemotaxis by the nematode *Caenorhabditis elegans*: identification of attractants and analysys of the response by use of mutantts. Proc Natl Acad Sci 70:817-821.

White, J.G., Southgate, E., Thomson, J.N, and Brenner, S. (1986). The structure of the nervous system of the nematode . Phil Trans of the Royal Soc of London 314B:1-340.

Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Burton, J., Connell, M., Bonfield, J., Copsey, T., Cooper, J. (1994). 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. Nature 368:32-38.

Wood, W.B., ed. (1988). The nematode *Caenorhabditis elegans*. Cold Spring Harbor Press, NY.

Yu, S., Avery, L., Baude, E., and Garbers, D.L. (1997). Guanylyl cyclase expression in specific sensory neurons: A new family of chemosensory receptors. Proc Natl Acad Sci 94:3384-3387.

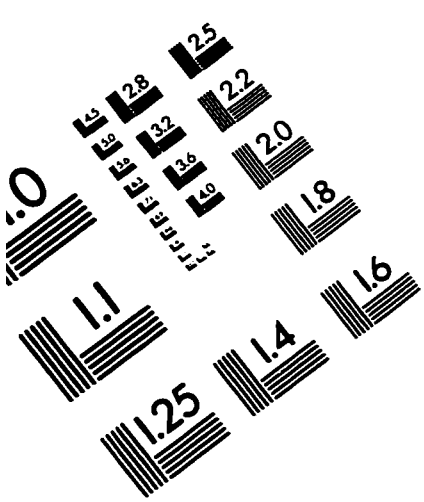
Yuen, P.S.T. and Garbers, D.L. (1992). Guanylyl cyclase-linked receptors. Ann Rev Neurosci 15:193-225.

Zwaal, R.R., Broeks, A., van Meurs, J., Groenen, J.T., and Plasterk, R.H. (1993). Target-selected gene inactivation in *Caenorhabditis elegans* by using a frozen transposon insertion mutant bank. Proc Natl Acad Sci 90:7431-7435.

Zwaal, R.R., Mendel, J.E., Sternberg, P.W., and Plasterk, R.H.A. (1997). Two neuronal G proteins are involved in chemosensation of the C. *elegans* dauer inducing pheromone. Genetics 145:715-27.

VITA

Deborah Ann Birnby began life at Albany Medical Center before moving to an Albany, NY, apartment with her parents, a biologist and a metallurgist. At three, she and her family moved to the nearby town of Schenectady, where she acquired a brother; got the chicken pox; took many classes; learned algebra, calculus, titration, and a bit of history; made friends with some odd birds; and read a lot. She left Schenectady for the quiet, clean town of Cambridge, MA, where she attended the Massachusetts Institute of Technology, a school that, despite its best efforts, remains distinct from Harvard. There she made friends with several more odd birds. One of them, Andrew Marc Greene (who is not a biologist), helped her see that biology was more interesting than mechanical engineering. Her studies at Random Tech included biology, life in the laboratory, fireproofing stage furniture, emacs, and human behavior. Deciding that she needed to move further from home, she attended graduate school at the University of Washington in Seattle, WA, where she learned about worms and took several more semesters of her "life in the laboratory" course; learned to dance, to cook, and to teach; made more friends; and spent a lot of time communicating with people "back east." Time will tell where she will live next, but she is certain that she will continue learning.



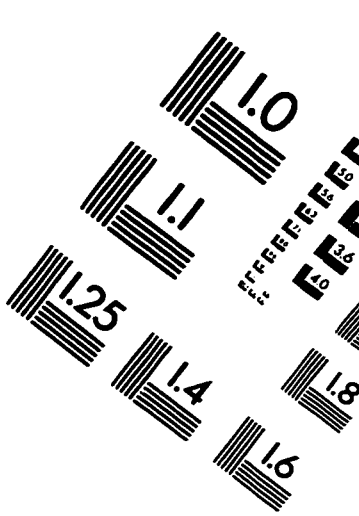
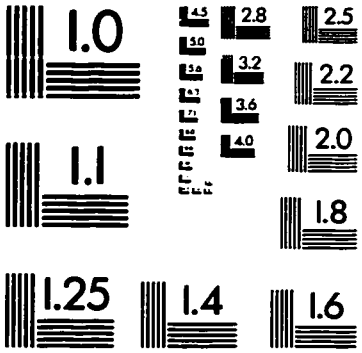
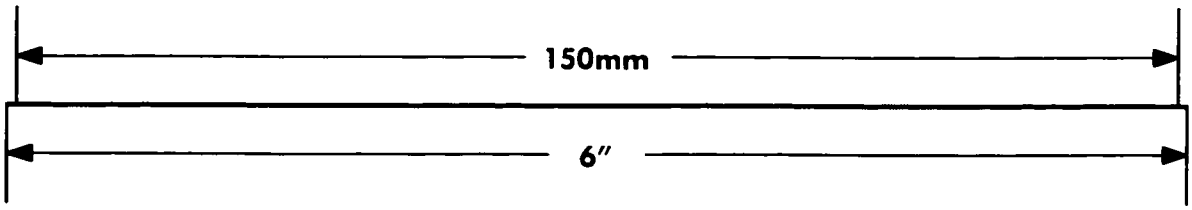
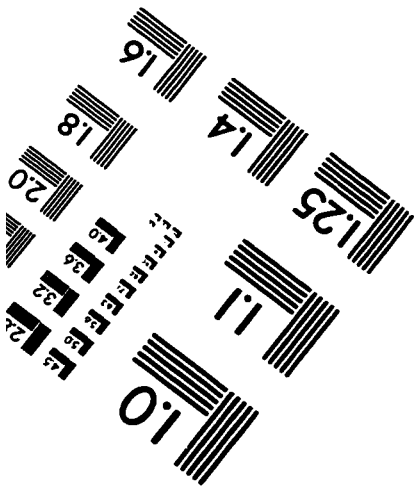
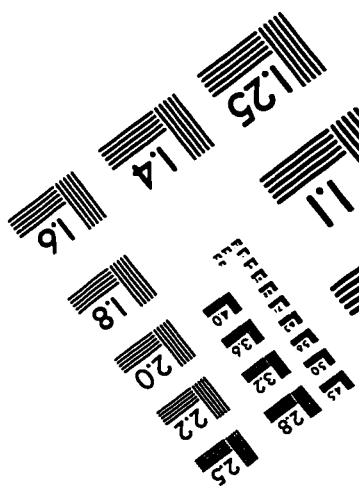


IMAGE EVALUATION
TEST TARGET (QA-3)









APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989