Analysis of osm-6, a Gene That Affects Sensory Cilium Structure and Sensory Neuron Function in Caenorhabditis elegans

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ABSTRACT

Mutation in the Caenorhabditis elegans gene osm-6 was previously shown to result in defects in the ultra-structure of sensory cilia and defects in chemosensory and mechanosensory behaviors. We have cloned osm-6 by transposon tagging and transformation rescue and have identified molecular lesions associated with five osm-6 mutations. The osm-6 gene encodes a protein that is 40% identical in amino acid sequence to a predicted mammalian protein of unknown function. We fused osm-6 with the gene for green fluorescent protein (GFP); the fusion gene rescued the osm-6 mutant phenotype and showed accumulation of GFP in ciliated sensory neurons exclusively. The OSM-6::GFP protein was localized to cytoplasm, including processes and dendritic endings where sensory cilia are situated. Mutations in other genes known to cause ciliary defects led to changes in the appearance of OSM-6::GFP in dendritic endings or, in the case of daf-19, reduced OSM-6::GFP accumulation. We conclude from an analysis of genetic mosaics that osm-6 acts cell autonomously in affecting cilium structure.

CILIA are found in most animal species, many protists and some plants. Many cilia are motile. Motile cilia can sweep fluid over an epithelial surface or propel a swimming cell; the eukaryotic flagellum, which is related to the cilium in structure, is also used for swimming, as in the green alga Chlamydomonas or in the sperm of many species. Nonmotile cilia are found in certain sensory receptors, including phototransducing rods and cones and olfactory neurons.

The nematode Caenorhabditis elegans has nonmotile cilia or flagella—its sperm, for example, are nonflagellated crawling cells (Wolf et al. 1978; Nelson and Ward 1980). Nonmotile cilia, however, are present in 60 of the 302 neurons that make up the nervous system of the C. elegans hermaphrodite (Ward et al. 1975; Warren et al. 1975; Perkins et al. 1986; White et al. 1986; Hall and Russe1 1991). The cilia are situated in dendritic endings of sensory neurons. Most of the ciliated nerve endings are either exposed directly to the external environment or embedded in the animal’s external cuticle and are strongly implicated, by two lines of evidence, in the reception of chemosensory and mechanosensory stimuli.

One line of evidence implicating C. elegans cilia in sensory transduction involves mutants with structurally abnormal cilia (Lewis and Hodgkin 1977; Albert et al. 1981; Perkins et al. 1986). Such mutants are defective in the ability to sense chemical attractants or repellents, both water soluble and volatile (reviewed in Bargmann and Mori 1977). They are also defective in the ability to avoid high osmolarity (Culotti and Ruskell 1978). The ability to form dauer larvae under conditions of limited food and high concentration of a pheromone secreted by a dense population of worms (reviewed in Thomas 1993; Riddle and Albert 1997) is another chemosensory behavior that is defective in mutants with aberrant cilia. Also affected is the ability to recover from the dauer state, which involves the sensing of food and low pheromone concentration. Finally, cilia-defective mutants are mechanosensory defective: they show a reduced ability to respond to taps on their noses (Kaplan and Horvitz 1993). Although it is possible that the cilia-defective mutants are also defective in a nonciliary neuron function, the simplest view is that cilia are directly involved in transducing chemosensory and mechanosensory signals.

The second line of evidence is based on the behavioral consequences of killing cilia-containing neurons with a laser microbeam. These experiments have focused attention on particular ciliated neurons as sensory receptors; the roles of the ciliated endings are inferred. Thus, different subsets of ciliated neurons have been implicated in the ability to chemotax to water-soluble attractants (Bargmann and Horvitz 1991a) or volatile odorants (Bargmann et al. 1993), to avoid repellents (Bargmann et al. 1990), to form dauer larvae or not and to recover from the dauer state (Bargmann et al. 1991b).
Cilia-defective mutants provide a powerful approach to the interesting question of how cilia are assembled. This approach has been fruitful in elucidating the assembly of Chlamydomonas flagella, for example (reviewed in Johnson 1995). Mutations in the C. elegans gene osm-6 result in shortened axonemes of all cilia that have been inspected by serial section electron microscopy (Perkins et al. 1986). The base of each cilium, which corresponds to the transition zone of the Chlamydomonas flagellum, is ultrastructurally normal in the osm-6 mutant, but the region distal to the transition zone is greatly reduced in length, and doublet microtubules, linked to the cell membrane, assemble ectopically at positions proximal to the transition zone. It has been proposed that the ectopic microtubules are misassembled components of the axoneme and that the osm-6 gene product may be required for the assembly of peripheral microtubule doublets in the ciliary template (Perkins et al. 1986). We report here our cloning and molecular characterization of osm-6 and its spatial pattern of expression. We also describe our mosaic analysis of osm-6 function.

**MATERIALS AND METHODS**

**General genetic methods, genes and alleles:** Growth media and culture and mating techniques were as described by Brenner (1974) and Sulston and Hodgkin (1988). Nematode strains were grown and mated at 20°C unless otherwise noted. Nomenclature is standard (Horvitz et al. 1979). Genes and mutations used in this work were the following (where no citation is given, see Hodgkin et al. 1988): LG (linkage group) I: che3(e1124), che3(e1805). LGII: daf-19(m86). LGIII: daf-1(e1865), unc-36(e251), sup-5(e1464), dpy-18(e364). LGIV: osm-3(p802), daf-10(e387), him-8(e489). LGV: unc-42(e270), daf-11(m47), m78ts, osm-6(p811), m511, m533, m201, sa119 (Starich et al. 1995), smal-1(e30), che11(e810), vab-8(e1017). LGX: che2(e1033), osm-5(p813), osm-1(p808). Except for the strain in which the spontaneous mutations osm-6(m533) arose, which was RJ7097, a derivative of RJ7096 (Morii et al. 1988), all strains descended from the wild-type strain N2 (Brenner 1974).

Mutations in osm-6 abolish the ability of D cell anterior and four posterior sensory neurons in living animals to fill with the fluorescent dyes 5-fluorescein isothiocyanate (FITC) or 3′-dioctadecyloxacarbocyanine perchlorate (DiO) (Hedgecock et al. 1985; Perkins et al. 1986; Starich et al. 1995). We used this defect in dye filling, the Dyf phenotype, to follow osm-6 mutations in genetic crosses. To reduce Tc1 copy number, the original strains carrying osm-6(m511) and osm-6(m533) were crossed to N2 males. Heterozygous hermaphrodite progeny were allowed to give self-progeny, among which Dyf (homozygous osm-6) animals were identified to initiate new lines. This procedure was carried out at least 10 times for each mutation, after which a him-8; unc-42 osm-6 strain was generated for each mutation. In addition, unc-42 osm-6 vab-8 stocks were generated for each of the two Tc1-insert mutations, and crosses over each side of osm-6 were selected to reduce the number of closely linked Tc1 elements.

Assays for neuronal filling of FITC or DiO and behavioral assays for chemotaxis and ability to form dauer larvae were performed as described by Starich et al. (1995).

**Identification and outcrossing of spontaneous osm-6 revertants:** Each of the mutant strains bearing one of the spontaneous mutations osm-6(m511) or osm-6(m533) was screened for spontaneous reversion of the defect in dauer larva formation, the Daf-d phenotype, conferred by the osm-6 mutation. Dauer larvae formed in starved cultures were selected, as described by Starich et al. (1995), by virtue of their resistance to 1% sodium dodecyl sulfate (SDS) (Cassada and Russell 1975). Selected dauer larvae were allowed to recover in the presence of food, and the resulting stocks were scored for dye filling. Several independent revertants from each mutant, all both non-Daf-d and non-Dyf, were identified. The following procedure, described for a specific example, was used to reduce the Tc1 copy number of several revertants. Hermaphrodites of genotype him-8; unc-42 osm-6(m533), which had been outcrossed to N2 or N2-derived strains at least 12 times, as described in the preceding section, were mated with him-8 males, and the male cross-progeny were mated to osm-6(m533mn374) (revertant) hermaphrodites. F1 hermaphrodites were picked and allowed to self. From broods containing Unc-42 Dyf animals, generated by him-8; unc-42 osm-6(m533)/osm-6(m533mn374) hermaphrodites, non-Unc animals were picked. Those that segregated no Unc self-progeny were picked, from which a him-8; osm-6(m533mn374) line was established. Males of this line were mated with him-8; unc-42 osm-6(m533) hermaphrodites. Non-Unc male progeny were backcrossed to him-8; unc-42 osm-6(m533) hermaphrodites. The last step was repeated at least 10 times. At each step, it was determined that Unc segregants were Dyf and non-Unc segregants were non-Dyf. After the final cross, homozygous him-8; unc-42 osm-6(m533) and him-8; unc-42 osm-6(m533mn374) stocks were established.

**Molecular biology:** Standard molecular biology techniques (Sambrook et al. 1989) were used. Two C. elegans cDNA libraries were used: a kgt10 mixed-stage library provided by S. Kim and a λZAP mixed-stage library provided by R. Barstend and R. Waterston. All plasmid subcloning was done using pBlue-Script SK(--) (Stratagene, La Jolla, CA). DNA was sequenced by the dyeoxygen chain termination method (Sanger et al. 1977) using Sequenase Version 2.0 (United States Biochemical, Cleveland, OH). Deletion series of clones for sequencing were obtained by timed exonuclease III digestion (Henikoff 1987). Sequence analysis made use of the Genetics Computer Group (Madison, WI) sequence analysis package and the National Center for Biotechnology Information BLAST (Altschul et al. 1990) service. For Northern blot analysis, poly(A)-enriched RNA was isolated from staged populations of N2 animals as described by Starich et al. (1993).

**Cloning Tc1-tagged osm-6:** Genomic DNA was prepared as previously described (Li et al. 1992) from N2 and outcrossed strains carrying either of the spontaneous mutations m511 or m533 or a reversion of one of these mutations. Southern blots were probed with radioactively labeled Tc1 probe, as described by Starich et al. (1993), and a 2.6 kb Tc1-containing EcoRI restriction fragment derived from an osm-6(m533) strain was subcloned. Tc1 sequence in the clone was removed by EcoRV digestion (Rosenzweig et al. 1983) to create a plasmid carrying unique sequence flanking m533: Tc1, which was used to screen an N2 λEMBL4 genomic library provided by C. Link.

**Germline transformation and osm-6 rescue:** Hermaphrodites homozygous for osm-6(p811) were transformed by the method of Mello et al. (1991). DNA to be tested was injected at 10–15 μg/ml, and plasmid pRF4, which contains the semidominant mutation rol-6(su1006), was co injected at 80 μg/ml. Transgenic roller (Rol) hermaphrodites were picked, from which...
stably transformed lines were recovered and scored for dye filling. Lines that showed rescue of osm-6 exhibited essentially wild-type dye filling and amphid and phasmid neurons. The following four different genomic DNA fragments were tested for rescue of osm-6 (Figure 1C): pJC4 is a 5.9-kb BamHI restriction fragment; pJC5 was made by deleting 1.2 kb from one end of pJC4 by exonuclease III digestion (Henikoff 1987); pJC17 is a 3.7-kb EcoRI BamHI subfragment from pJC4; and pJC35 was made to carry a frameshift mutation within the proposed osm-6 coding region (which generates downstream stop codons) by digesting pJC4 with BglII, end-filling with Klenow enzyme and religating. We confirmed the DNA sequence of pJC35 in the vicinity of the frameshift mutation.

**DNA sequence determination of osm-6 mutations:** Purified DNA from osm-6 mutants was amplified by polymerase chain reaction (PCR) using Taq polymerase (Promega, Madison, WI). We amplified the genomic region extending from 85 bp upstream of the 5’ end of the osm-6 cDNA to 37 bp downstream of the deduced osm-6 translational stop. Cloned PCR products were sequenced using gene-specific oligonucleotide primers. Mutations were confirmed by sequencing products from at least two different PCRs.

**Testing amber suppressibility of osm-6(sa119):** The osm-6(sa119) mutation was originally identified (Stich et al. 1995) as a suppressor of the dauer constitutive phenotype Daf-c, conferred by dam-11(m87ts) at high temperature. Because osm-6 and dam-11 are only 0.2 map unit apart, the following steps were taken to recover osm-6(sa119) by itself. Unc non-Sma recombining segregating from unc-42 sma-1/daf-11 osm-6 hermaphrodites were picked, and self-progeny homozygous for osm-6 were identified by dye-filling assay. The presence or absence of dam-11(m87ts) was ascertained by mating animals to be tested with hin-8; dam-11(m47) males at 25°C and inspecting the cross-progeny for dauer larvae; four of 24 Unc non-Sma 0 sm recombinants were dam-11(+) a strain carrying only osm-6(sa119) was then obtained following mating of unc-42 osm-6 hermaphrodites with N2 males.

To test the ability of sup-5 to suppress osm-6(sa119), we crossed males of genotype sup-5 dpy-18/+ + to dpy-18; unc-42 osm-6(sa119) hermaphrodites. Dpy18 (one copy of sup-5 partially suppresses the homozygous dpy-18 mutation; see Waterston 1981) non-Unc progeny were picked, from which Unc non-Dpy (fully suppressed) self-progeny, genotype sup-5 dpy-18; unc-42 osm-6, were identified; these animals exhibited partial dye filling, much better than osm-6(sa119) but not completely wild type. Completely Dpy (osm-6) Unc animals, genotype unc-42 osm-6, were recovered from this stock following mating with N2 males.

**Expression experiments:** A translational fusion of osm-6 and a green fluorescent protein (GFP) gene (Chalfie et al. 1994), GFP, was constructed in the gfp expression vector pPD95.77 (A. Fire, S. Xu, J. Ahno and G. Seydoux, personal communication). Included upstream of gfp in the fusion gene was 5.1 kb of osm-6 genomic sequence, extending from 2.4 kb upstream of the 5’ end of the osm-6 cDNA through the first 10 exons and all introns into the last exon, such that only the last 13 codons, including the translational stop, of OS-6 were lacking. The osm-6 sequence was composed of three conjoined segments (from left to right in Figure 1C): a 2.68-kb BamHI genomic restriction fragment that extends upstream of the 5’ end of the osm-6 cDNA into the second osm-6 intron, a 2.17-kb BamHI/BglII genomic fragment that extends from the same BamHI site in the second intron to a BglII site in the last exon and a 0.22-kb cDNA fragment that extends from the same BglII site to a 3’ truncation produced by exonuclease III digestion that deleted the 12 C-terminal amino acid codons of OSM-6.

**Extrachromosomal arrays carrying the osm-6::gfp construct and either rol-6(su1006) (see section on germline transformation) or unc-36(+) (see section on mosaic analysis below) as visible markers were generated by germline transformation microinjection of DNA. Lines homozygous for an integrated array bearing osm-6::gfp and unc-36(+) were identified among F2 descendants of unc-36 nd-1 III; osm-6(p811) V; mnxEx64(osm-6::gfp unc-36(+)) hermaphrodites treated with γ-rays, by the procedure described by Melillo and Fire (1995).**

**Mosaic analysis:** Animals of genotype nd-1 unc-36 III; osm-6(p811) V were made transgenic for an extrachromosomal array, mnEx54, containing wild-type copies of osm-6 on pJC4, nd-1 on the cosmid C33C3 (Miller et al. 1996) and unc-36 on the plasmid R1p16 (obtained from L. Lobel). The mnEx54 array rescued the Dfy phenotype conferred by osm-6(p811). Non-Unc animals were exposed to DiO and examined by Nomarski differential interference contrast (DIC) microscopy to screen for mosaics in which some neurons of an aphid were Ncl (enlarged nucleolus) and others were non-Ncl. When mosaics were found, various cells were scored with respect to both dye filling and the Ncl phenotype. Nuclei were identified using published diagrams. (Albertson and Thomson 1976; Sulston and Horvitz 1977; Sulston et al. 1983; White et al. 1986). Cell names and lineages are given by Sulston and White (1988).

**RESULTS**

All five osm-6 mutant alleles confer a strong Dyf phenotype: The osm-6 gene is represented by five mutant alleles (Culotti and Russell 1978; Stich et al. 1995), all recessive to osm-6(+). The reference allele, p811, has been shown to cause defects in osmotic avoidance (Culotti and Russell 1978), chemotaxis (Perkins et al. 1986; Stich et al. 1995), dauer larva formation (Perkins et al. 1986) and mechanosensory perception (Kaplan and Horvitz 1993), and it was the allele that was used in the characterization of axonemal shortening caused by osm-6 mutation (Perkins et al. 1986). We have monitored another phenotype conferred by osm-6 mutation. When living wild-type animals are exposed to a fluorescent dye, either FITC or DiO, eight pairs of sensory neurons—six in head sensilla called amphids and two in phasmids in the tail—fill with the dye (Hedgecock et al. 1985); osm-6 mutation abolishes dye filling (Perkins et al. 1986), a phenotype referred to as Dyf. For each of the five osm-6 mutations, we have assayed hundreds of homozygous hermaphrodites for their ability to take up DiO. For either adults or larvae bearing any of the osm-6 mutations, more than 99% of the animals failed to exhibit dye filling of any orphid or phasmid neuron. By contrast, among more than 1000 wild-type hermaphrodites, the great majority exhibited characteristic dye filling, and no animal failed completely to dye fill. It was previously reported that osm-6(p811) over a deficiency for the osm-6 locus is fully Dyf (Stich et al. 1995). By dye-filling assay, therefore, all five osm-6 mutations are indistinguishable and appear to behave as null mutations; it is possible that they are not null, however, and that an osm-6 null mutant would exhibit additional defects.
**Transposon tagging of osm-6:** Two osm-6 alleles, m511 and m533, were identified (Starich et al. 1995) among spontaneous mutations arising in RW7097, a strain that exhibits enhanced germline transposition of the transposable element Tc1 (Mori et al. 1988). Several spontaneous phenotypic revertants of both m511 and m533 were identified, with the expectation that they could be generated by excision of Tc1. To reduce overall Tc1 copy numbers, strains harboring m511 or m533 were repeatedly outcrossed to N2 or N2-derived strains, and revertants of m511 or m533 were repeatedly outcrossed to multiply outcrossed m511 and m533 stocks, respectively (see materials and methods). Southern blots of DNA isolated from m511, m533, revertant and N2 stocks probed with Tc1 sequence were inspected for mutant-specific bands. Candidate Tc1-containing bands were cloned, and unique DNA fragments flanking the Tc1 elements were subcloned and used to probe Southern blots. It was found that strains bearing either m511 or m533 had a Tc1 element in the same 4.8-kb BamHI fragment (Figure 1). The corresponding BamHI fragment in N2 and all revertants had no Tc1 element and was 3.2 kb in size—reduced by 1.6 kb, the size of Tc1.

**Transformation rescue of osm-6:** Unique DNA flanking m533::Tc1 in a 2.8-kb EcoRI fragment was used to screen a genomic library, and hybridizing phage clones were identified. Two of these were placed on the C. elegans physical map by A. Coulson and J. Sulston (Coulson et al. 1995), in a region that corresponds well to the expected location of osm-6, on the basis of its genetic map location (Figure 1).

Fragments of one of the physically mapped phage clones, SP#LST12, were subcloned and used to generate transgenic lines by germline transformation of osm-6(p811) (Figure 1). Transgenic lines were tested for rescue of the Dyf phenotype conferred by osm-6(811). A 3.7-kb EcoRI/BamHI fragment, which included the sites of insertion of both m511::Tc1 and m533::Tc1, was capable of transformation rescue (Figure 1). We determined the DNA sequence of the rescuing fragment. The DNA sequence of the entire region was recently determined by the C. elegans DNA Sequence Consortium (Wilson et al. 1994); large portions of the two cosmids shown in Figure 1B, R31 (GenBank accession number Z75956) and F58H1 (accession number Z75954), were used in the genomic sequencing.

**A 1.5-kb osm-6 cDNA:** The 3.7-kb EcoRI/BamHI rescue fragment was used to screen a mixed-stage, amplified cDNA library, and four identical hybridizing clones were isolated. One of these was used to screen a second mixed-stage cDNA library. One hybridizing clone was isolated; it contained less osm-6 sequence than that found in the other cDNA clones. We determined the nucleotide sequence of both strands of one
Comparison of the cDNA and genomic nucleotide sequences indicated that the cDNA is composed of 11 exons, five of which (exons 4–8), as well as the coding region of exon 11, were predicted by the Sequencing Consortium (as part of predicted gene \textit{R31.3} under GenBank accession number Z75956). All 10 introns have consensus \textit{C. elegans} donor and acceptor splice sites (Bulumenthal and Steward 1997).

We have not clearly identified the 5\textsuperscript{\textprime} end of the \textit{osm-6} message. The 1476-bp length of the cDNA corresponds to the approximately 1.5-kb length of the \textit{osm-6} transcript detected on Northern blots probed with the cDNA (Figure 3). The 5\textsuperscript{\textprime} end of the cDNA begins with an initiation codon AUG and an open reading frame that extends for 1416 nucleotides. Four codons upstream of the 5\textsuperscript{\textprime} end of the cDNA in the genomic sequence is a stop codon, with no intervening AUG codons but with a possible intervening 3\textsuperscript{\textprime} splice acceptor site. The rescuing DNA fragment pJC17 (Figure 1C) extends 190 nucleotides upstream of the start of the cDNA. There is only one AUG codon within this 190 nucleotides; the third in-frame codon following it is a stop, however, and no pattern of splicing with consensus splice sites puts the AUG in-frame with the rest of the \textit{osm-6} message. We suggest that the \textit{osm-6} translational start is included in the cDNA. The second AUG codon of the cDNA open reading frame is the ninth codon. Introduction of frameshift mutation between this AUG and the third AUG in the open reading frame (Figure 1C, codon number 64) abolished transformation rescue. We suggest that translation is initiated at a position corresponding to one of the first two AUG codons of the cDNA. The second AUG codon gives a...
better match to previously identified C. elegans translational initiation sites (Krause 1995), but there is no strong consensus sequence for C. elegans translational initiation. The 3’ end of the cDNA contains a poly(A) stretch, 18 bp upstream of which is a consensus polyadenylation signal, AATAAA.

The osm-6 transcript is most abundant during the first larval stage (Figure 3).

**DNA alterations in osm-6 mutants:** We found sequence alterations associated with each of the five known osm-6 mutant alleles (Figure 4). Each of the two Tc1 insertion mutations was within the osm-6 coding sequence, both at TA target sites, which have been found for all Tc1 insertions (Van Luenen and Plasterk 1994). The other three mutations, all induced with ethyl methanesulfonate (EMS), were G/C-to-A/T transitions, the most common class of mutation caused by EMS in C. elegans (Anderson 1995). Two of the transition mutations affected splice sites: p811 altered the splice site acceptor of the first intron, and m201 altered the splice site donor of the seventh intron. Finally, sa119 was associated with the change of codon 377 from glutamine encoding to a UAG or amber stop codon. Consistent with this result is our finding that the dye-filling defect conferred by sa119 is suppressed by the amber suppressor sup-5 (see materials and methods). As expected, neither of two other osm-6 mutations tested, osm-6(m511) and osm-6(m533), was amber suppressible. The genetic characterization of sa119 as an amber-suppressible mutation confirms our molecular identification of osm-6.

**OSM-6 is 40% identical to a predicted mammalian protein:** Conceptual translation of the osm-6 cDNA predicts a polypeptide (OSM-6) of 472 amino acids. A hydrophathy profile (Kyte and Doolittle 1982) indicates that the protein has neither a signal peptide nor a transmembrane domain. Overall the protein is acidic, with a calculated isoelectric point of 4.64. The region extending from amino acid 325 through 358 is strikingly rich in proline: 13 of 34 residues. Within this segment is an exact six-residue repeat: ELPMPP appears at both 336-341 and 353-358 (Figure 2).

A search of protein databases revealed that OSM-6 is 40% identical to the conceptual translation product of a cDNA clone, called NGD5, isolated from a neuroblastoma-glioma rat-mouse hybrid cell line called NG108-15 (Wick et al. 1995). Comparison of the translation products of the osm-6 cDNA and the published NGD5 sequence suggested that there were sequencing errors in the published NGD5 sequence. We therefore obtained the NGD5 cDNA clone and determined its nucleotide sequence; conceptual translation of the corrected sequence (updated GenBank accession number L38481) gives a better match to OSM-6 (Figure 5). Transcripts corresponding to NGD5 are reported to be decreased after prolonged treatment of NG108-15 cells with opioid (Wick et al. 1995). The transcript is also reported to be expressed in rat brain. The similarity between OSM-6 and the NGD5 protein extends throughout the entire length of the NGD5 protein, which is somewhat shorter than OSM-6 (Figure 5). The two N-termini would be more closely matched if translation of the osm-6 mRNA began at the position corresponding to the second AUG of the osm-6 cDNA. The similarity between OSM-6 and the NGD5 protein throughout the full extent of the latter is also apparent in a dot plot comparison of the two proteins (data not shown). Four regions of particularly high similarity, all with at least 75% amino acid identity, are marked in Figure 5. The longest of these, with 34 of 44 identical residues in the segment that includes OSM-6 residues 327-370, includes nearly all of the proline-rich segment.

OSM-6 was also found to be very similar to the (incomplete) conceptual translation products of 16 mammalian expressed sequence tags (ESTs). All 16 of the ESTs are highly similar, generally more than 85% iden-

![Figure 4.—DNA changes in osm-6 mutants. Open boxes indicate exons; the stippled box represents the 3’ untranslated region of the osm-6 mRNA. Two of the mutations were insertions of the transposable element Tc1, between nucleotides 540 and 541 of the cDNA sequence (Figure 2) for m533 and nucleotides 819 and 820 for m511. All three EMS-induced mutations were G/C-to-A/T transitions, p811 and m201 affecting splice sites and sa119 generating an amber nonsense mutation at codon 377 (Figure 2).](image-url)
tical at the nucleotide level to different segments of the NGD5 coding sequence. One of the ESTs was from a cDNA generated from rat PC12 cells (Lee et al. 1995). Two others were derived from mouse brain and mouse embryos. The remaining 13 were from cDNAs derived from several different human tissues.

**osm-6::gfp is expressed in ciliated neurons:** To elucidate the pattern of expression of osm-6, we fused a GFP gene (Chalfie et al. 1994), gfp, in-frame to the 3' end of an osm-6 genomic segment that included 2.5 kb upstream of the beginning of the cDNA sequence and extended through the osm-6 coding region into the last exon such that the osm-6 translation product lacked only the 12 C-terminal amino acids. This osm-6::gfp translational fusion gene was injected into the germline of osm-6 hermaphrodites, and extrachromosomal arrays were generated. Some arrays carried a dominant rol-6 allele as a marker and others carried unc-36(+) (see materials and methods). Two rol-6-bearing lines and one unc-36(+)-bearing line were studied with respect to patterns of GFP expression. From the unc-36(+)-bearing line, several independent lines were created in which the osm-6::gfp array was integrated and homozygous in an otherwise unc-36; osm-6(p811) genome. Three of these lines were studied with respect to patterns of GFP expression. The patterns of GFP expression were analyzed in more detail for the three integrated lines, but the patterns were essentially identical for all the lines we studied. We tested one of the rol-6-bearing lines and an integrated unc-36(+)-bearing line for rescue of the DiO dye-filling defect; both exhibited excellent rescue. The same integrated unc-36(+)-bearing line was also tested for its abilities to chemotax up a radial gradient of NH₄Cl and to make dauer larvae; it was well rescued, exhibiting wild-type behavior, with respect to both of these characteristics (data not shown).

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**Figure 5**—Comparison, based on the Gap program of the Genetics Computer Group, of conceptual translation products of osm-6 and a mammalian cDNA clone, NGD5. Gaps in either sequence introduced to optimize alignment are indicated by intervening dots in sequence. Identical residues are joined by lines; related residues are joined by single or double dots. In the alignment shown, 40% of the amino acid residues in the NGD5 sequence are identical to OSM-6 residues. Four ungapped segments in which more than 75% of the residues are identical are indicated by boxes.

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**Figure 6**—Localization of OSM-6::GFP in wild-type and mutant animals, as detected by fluorescence microscopy. The images are composites of fluorescence and DIC micrographs. (A) GFP fluorescence is apparent in the cell bodies of two wild-type right phasmid neurons, PHAR and PHBR, and also in the region of the dendritic endings of the neurons (indicated by the arrow), posterior to the cell bodies. The cell nuclei tend to exclude OSM-6::GFP; this is an L2 animal. (B) GFP fluorescence in the anterior of an L4 wild-type hermaphrodite. The large patch of fluorescence correspond to the positions of cilia of other sensory neurons. The cell bodies of these neurons are at more posterior positions (to the right and out of the field of the micrograph). (C) GFP fluorescence in an L4 che3 mutant. Enhanced fluorescence is concentrated in a patch associated with amphid neurons and is much brighter than the corresponding region in wild-type animals (exposure times for the different fluorescent images were not equivalent). Magnifications in all panels are the same; scale bar is 10 μm.
GFP in the osm-6::gfp-bearing animals was first apparent at about the twofold stage of embryonic elongation. L1 animals showed strong expression, which then gradually weakened during larval development. This pattern is consistent with the appearance of osm-6 transcript assessed by Northern blot analysis (Figure 3). By switching between Nomarski and fluorescence microscopy while maintaining a particular field of view, we were able to identify all GFP-expressing cells in hermaphrodites. The assignments were based primarily on the relative positions of nuclei, as shown in published diagrams (Sulston et al. 1983; White et al. 1986), but we were also aided by the appearance of GFP in neuronal processes, the characteristic shapes of which have been documented for every neuron in the hermaphrodite (Warner et al. 1975; Warner et al. 1975; White et al. 1986; Hall and Russell 1991).

We have detected GFP expression in a total of 56 cells of the hermaphrodite, all of the ciliated neurons except the BAG and FLP pairs (indicated in the drawing by open circles). The localization of OSM-6::GFP near sensory cilia is affected by mutations in other genes that affect cilia structure. The appearance of GFP within cells that expressed the osm-6::gfp fusion gene was clearly cytoplasmic, as could be seen in the cell bodies of the GFP-expressing neurons (Figure 6A). In addition to the appearance of GFP within cell bodies, there was GFP along processes and near the ciliated endings (Figure 6). This is particularly apparent in the nose, which is abundantly endowed with ciliated endings.

We have analyzed, in less detail, osm-6::gfp expression in males. It appears that all of the cells that expressed GFP in hermaphrodites also did so in males, but malespecific GFP-expressing cells were also apparent. Four of these were ciliated neurons in the head, the CEM or male-specific cephalic neurons, which differentiate from cells that are eliminated in the hermaphrodite by embryonic cell death (Sulston et al. 1983). Additional cells in the male tail expressed GFP. At least some of these cells appear to be sensory neurons of the copulatory spicules and sensory rays (Sulston et al. 1980), since we observed GFP fluorescence associated with the endings of the spicules and rays (Figure 8). The sensory ray and spicule dendrites terminate in short cilia (Sulston et al. 1980; Chou et al. 1995; Hall, personal communication).

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The localization of OSM-6::GFP near sensory cilia is affected by mutations in other genes that affect cilia structure: The appearance of GFP in wild-type and osm-6 mutant backgrounds was indistinguishable; however, the osm-6::gfp array rescues the osm-6 mutant phenotype. For other mutant backgrounds, we chose reference mutations in other genes that affect the structure of ciliated neurons. For che-2, che-13, osm-1 and osm-5 mutants, whose sensory cilia, like those of osm-6, are all severely foreshortened (Lewis and Hodgkin 1977; Perkins et al. 1986), the intensity of GFP fluorescence in the tips of the neurons was greatly reduced, although GFP accumulation in the neuron cell bodies...
appeared to be the same as in the wild-type genetic background. We did not discern an altered pattern of OSM-6::GFP expression in osm-3 animals, but only amphid and phasmid cilia are foreshortened in the osm-3 mutant and not to the same degree as in che-2, che-13, osm-1 and osm-5 mutants (Perkins et al. 1986). The osm-3 gene, which encodes a kinesin-like protein, appears to be expressed in a limited subset of ciliated neurons, including eight pairs of amphid neurons (Tabish et al. 1995). Animals mutant for che-3, which encodes a dynein heavy chain (W. Grant, personal communication), showed enhanced GFP accumulation in two large bilateral patches near the region of the dendritic endings of the amphid neurons (Figure 6C) and also near the phasmid endings. Ultrastructural analysis of che-3 mutants has shown that the amphid (and phasmid) cilia are reduced in length and have bulb-shaped endings filled with electron-dense material (Lewis and Hodgkin 1977; Albert et al. 1981); we thus suggest that OSM-6::GFP is associated with the bulb-shaped endings. OSM-6::GFP expression in daf-10 and che-11 mutants also showed patches of GFP expression in the neuronal endings that were thicker than wild-type, although they were not as large as the patches seen in che-3 mutants. This pattern is also consistent with electron microscopic studies, which have shown that at least some cilia are enlarged in diameter and contain ground material in their centers (Albert et al. 1981; Perkins et al. 1986).

All sensory dendrites in daf-19 mutants are devoid of cilia, including transition zones (Perkins et al. 1986). The number of sensory neurons exhibiting GFP accu-

Figure 8.—GFP accumulation in tips of sensory rays of male tail promoted by osm-6::gfp. (A) Nomarski micrograph. (B) Fluorescence, showing fluorescent ray tips (and a patch of autofluorescence in the region of the cloaca). Scale bar is 10 μm.

Figure 9.—Example of an osm-6 genetic mosaic. The partial cell lineages of 21 cells are shown. Cell and lineage nomenclature follows Sulston and White (1988). The zygote P0 contained an extrachromosomal array carrying nd-1(+), unc-36(+) and osm-6(+) and was otherwise nd-1 unc-36; osm-6. The Ncl phenotypes of various scored cells are given as minus or plus, for Ncl and non-Ncl, respectively. It is concluded that the cell ABpraap, indicated in the lineage diagram by a circled p, failed to transmit the array to its descendants, which were as a consequence all Ncl. Four bilateral pairs of amphid neurons were scored with respect to their DiO-filling phenotype. Two neurons in the right amphid, ADLR and ASJR, both of which are descended from ABpraap, failed to fill with dye, as indicated by the double-lined boxes. The other six neurons, ASKR and ASHR in the right amphid and all four left amphid neurons, did fill with DiO, as indicated by the heavily lined boxes. Note that both the right amphid sheath, AMshR, and socket, AMsoR (although not scored explicitly with respect to its Ncl phenotype), must have carried osm-6(+), but did not prevent the loss of dye filling by the two osm-6 right amphid neurons. This and other examples led to the conclusion that osm-6 affects dye filling cell autonomously. The two right phasmid neurons, PHAR and PHBR, were both non-Ncl and, as expected, filled with DiO (for simplicity, their dye-filling phenotypes are not indicated in the diagram).
mulation in daf-19 animals was less than half that found in wild-type. We scored only a few cells, but amphid neurons ASK, ADL and ASI and phasmid neurons PHA and PHB rarely, if ever, accumulated detectable GFP in daf-19 animals.

**osm-6 acts cell autonomously with respect to dye filling of amphid neurons:** Our expression studies, which indicate that OSM-6 is a cytoplasmic protein present in precisely those neurons that exhibit ultrastructural defects in osm-6 mutants, predict that osm-6 functions cell autonomously. We tested this prediction by generating osm-6 genetic mosaics and asking about the osm-6 focus of action with respect to dye filling of amphid neurons.

To generate osm-6 mosaics, we made unc-36 ncl-1 III; osm-6 V animals transgenic for an extrachromosomal DNA array, mnEx54, that contained wild-type copies of unc-36, ncl-1 and osm-6. The extrachromosomal array was subject to mitotic loss. Each loss generated a clone of mutant cells, and the ncl-1 mutation, which results in enlarged nucleoli, a phenotype referred to as Ncl, was used as a cell-autonomous marker for specifying the nature of mosaic animals (Hedgecock and Herman 1995; Miller et al. 1996). The focus of action of unc-36 is among the descendants of both daughters of ABp (Kenyon 1986), the posterior daughter of AB, which is one of the two descendants of the first embryonic cleavage (Sulston et al. 1983; Figure 9). Non-Unc-36 animals therefore necessarily carry unc-36(+) and mnEx54 in at least some descendants of both ABpl and ABpr.

We exposed the progeny of unc-36 ncl-1; osm-6; mnEx54 hermaphrodites to the fluorescent dye DiO, picked non-Unc-36 amphids, mounted them on agar pads on microscope slides and screened for animals in which at least one amphid neuron, generally ASK, ADL or ASI, was Ncl. When a genetic mosaic was found, amphid neurons were scored with respect to both DiO filling and the Ncl phenotype. For several animals, we scored the Ncl phenotype of many additional cells to pinpoint positions in the lineage at which the extrachromosomal array was lost; one such mosaic animal is described by Figure 9.

All of the neurons and support cells—sheaths and sockets—of the amphid and phasmid sensilla are descendants of AB. In wild-type animals, six neurons of each amphid fill with DiO, but only four fill strongly. ASI fills very weakly and AWB fills somewhat weakly (Starrich et al. 1995). We therefore scored the dye-filling properties of the four that fill strongly—ADL, ASH, ASJ and ASK—in mosaic animals. The results confirmed the prediction of cell autonomy for each of these four neurons. We scored a total of 80 neurons in 22 mosaic amphids with respect to both Ncl and Dyf phenotypes (in some cases the only Ncl neuron in a mosaic amphid was ASI, which was not scored with respect to dye filling, and in other cases, not all neurons were identified and scored): 21 neurons were Ncl and dye-filling negative, 58 were non-Ncl and filled with dye normally, and one exceptional neuron was Ncl and filled with dye weakly. The exceptional Ncl neuron exhibiting weak dye filling may have picked up its dye from dye-filled neighbors, although we saw other examples in which the same neuron, ADL, was surrounded by dye-filled neighbors and did not fill even weakly. The four neurons assayed for dye filling are not closely related by lineage (Figure 9); as a consequence, various classes of mosaic amphids were found, supporting our conclusion of cell autonomy. The different classes of mosaic also led us to conclude that the genotypes of the amphid sheath and socket cells had no effect on dye filling; thus, we found examples (and no counter-examples) in which a mutant sheath cell did not block dye filling of a wild-type neuron in the same sensillum, and we found examples (one of which is given in Figure 9) in which a wild-type sheath did not promote the dye filling of a mutant neuron.

**DISCUSSION**

The evidence that we have correctly identified osm-6 molecularly is based, first, on the transformation rescue, by defined genomic fragments, of the osm-6 dye-filling defective phenotype and, second, on the molecular characterization of the five known osm-6 mutations. The region of genomic DNA necessary for rescue was delimited to a 2.9-kb segment that encodes the cDNA we characterized as representing the osm-6 message. One end of the 2.9-kb segment was delimited by a rescuing genomic fragment that extended only 190 nucleotides upstream of the 5' end of the cDNA. The other end was delimited by a rescuing osm-6::gfp construct that included all but the last 13 codons, including the natural termination codon, of the osm-6 cDNA. As expected, the insertion of a frameshift mutation near the beginning of the open reading frame defined by the cDNA abolished the ability of a genomic fragment to rescue. Finally, each of the five osm-6 mutant alleles was associated with an alteration that affected the coding capacity of the osm-6 message. Two were transposon insertions within coding sequence, two affected consensus splice sites between coding exons, and one was a nonsense mutation of the amber class, which was suppressed by an amber suppressor. We conclude that we have identified osm-6.

We suggest that all five osm-6 mutations are null or nearly null. All five mutations are recessive to osm-6(+), and abolish dye filling of amphid and phasmid neurons: osm-6 opposite a deficiency has the same Dyf phenotype. The amber suppressor sup-5, when homozygous at 20°C, restores approximately 10% of the wild-type level of expression to a suppressed locus (Waterston and Brenner 1978; Waterston 1981). When osm-6(sa119) was suppressed by sup-5 at 20°C, animals exhibited considerable dye filling, albeit not completely wild-type. (We also saw some dye filling in animals that
The best candidate for a null mutation on the basis of molecular evidence is sa119, a nonsense mutation at codon 377, which would omit 96 amino acid residues from the C-terminal end of the protein. Although omission of the last 12 amino acids did not block osm-6 function in the osm-6::gfp construct, it seems likely that omission of the larger C-terminal segment would, particularly since most of it is similar to the mammalian NGD5 protein.

Tc1 insertion mutations within coding sequence are not invariably null. Splicing of the transcript from the Tc1-bearing gene can remove most or all of the Tc1 sequence to give messages that contain small insertions, deletions or substitutions, and some of these mature transcripts may contain in-frame mRNA that encodes protein with small deletions, insertions or substitutions of amino acid residues (Rushforth and Anderson 1996). If the function of the protein is tolerant of such alterations, then some residual function may be retained. We suggest that the two Tc1 insertions in osm-6 are in regions (m511 between codons 273 and 274 and m533 between codons 180 and 181) that are intolerant of such changes.

Splice site mutations can also retain some residual function. Although the dinucleotide AG is found at the 3' end of essentially all eukaryotic introns, Aroian et al. (1993) showed that when such AGs were mutated to AA in two C. elegans genes, each gene retained residual function by virtue of some correct splicing of the mutant intron, although a variety of incorrect splices were also made as a consequence of the mutations. Zhang and Blumenthal (1996) have observed some splicing to AA at the 3' end of a synthetic intron. These workers also found that the efficiency of splicing to the AA was reduced when UUUC immediately 5' of the AA dinucleotide was changed to UUCC; the latter sequence immediately precedes the AG to AA change at the 3' end of the first intron in p811. Because it is not yet possible to predict with any confidence an expected pattern of splicing for the p811 mutant, we cannot say from the molecular evidence that osm-6(p811) is null. Similarly, we cannot be sure from the molecular evidence that the 5' splice site mutation, m201, is null. Although the 5' end of the seventh intron of the osm-6 transcript is changed from the virtually invariant GU to AU, Rushforth and Anderson (1996) have detected the use of an AU 5' splice donor in the production of aberrant splice products by unc-54 Tc1 insertion mutants.

Our mosaic analysis indicates that the effect of osm-6 on the ability of amphid neurons to fill with dye is cell autonomous. We therefore suggest that the ultrastructural defects in osm-6 sensory axonemes detected by electron microscopy (Perkins et al. 1986) are also cell autonomous. The osm-6::gfp expression results, which indicate that OSM-6 is a cytoplasmic protein present near sensory axonemes, clearly support this view. Perkins et al. (1986) observed an increased number of unfused matrix-filled vesicles in the cytoplasm of the amphid sheath cells in the osm-6 mutant (as well as in other mutants with shortened cilia) compared to wild-type animals. The amphid sheath cell is a nonneuronal support cell that makes a cylindrical channel that surrounds a bundle of neuronal processes. Ciliated dendrites project into one end of the channel; the other end is extended by connection of the sheath to a nonneuronal socket cell and terminates at a pore to the outside. The wild-type sheath cell contains vesicle-bound material that is thought to be exported to the extracellular channel matrix (Perkins et al. 1986). We suggest that the presence of excess vesicles in osm-6 sheath cells is a secondary consequence of the axonemal defect in the neighboring neurons and is not the cause of the ciliary defects; thus, a mutant sheath cell did not block normal dye filling by an osm-6(+) neuron in the same sensillum.

The presence of OSM-6::GFP near sensory cilia and its perturbed localization in these regions in various mutants with aberrant sensory cilia support the proposal of Perkins et al. (1986) that OSM-6 plays a role in promoting the growth of axonemes. In the absence of functional OSM-6 protein, the base and intermediate zones of the cilia appear to be normal, but the distal parts of the axonemes are foreshortened, and ectopic membrane-attached microtubules assemble at sites proximal to the cilia. It has been suggested that the ectopic microtubules are misassembled components of the foreshortened axoneme (Perkins et al. 1986). All cilia in the head of an osm-6 mutant were found by electron microscopy to be defective (Perkins et al. 1986). In hermaphrodites carrying our osm-6::gfp construct, we detected expression of GFP by 56 ciliated neurons. The only ciliated neurons that did not show expression were two pairs in the head called BAG and FLP. It is possible that these neurons also require osm-6 function and that either the level of expression was below our limit of detection or the osm-6::gfp construct we used lacked a regulatory feature required for expression in
these cells. Additional cells exhibited GFP expression in males, including the ciliated CEM neurons in the head and several unidentified cells in the tail, some of which, at least, have endings in the male spicules and sensory rays. The pattern of OSM-6::GFP localization suggests that OSM-6 is itself a component of the sensory cilia. Consistent with this suggestion was our finding of reduced OSM-6::GFP localization in the dendritic endings of animals carrying mutations in che2, che13, osm-1 and osm-5. All four of these mutants have shortened cilia, lacking middle and distal segments (Perkins et al. 1986). Also consistent with the idea that OSM-6 is a ciliary component was our observation that OSM-6::GFP accumulation was enhanced near the bundled endings of che3 amphid and phasmid neurons; the amphid and phasmid neurons in che3 mutants have been shown to have shortened but enlarged-diameter endings filled with electron-dense material (Lewis and Hodgkin 1977; Albert et al. 1981), which we suggest includes OSM-6 and other ciliary components.

Bargmann and Horvitz (1991b) showed that when ciliated amphid neurons ADF, ASG and ASI were killed early in the L1 stage, wild-type animals formed dauer larvae constitutively, i.e., despite the presence of food. This finding indicates that these sensory neurons normally signal to prevent dauer formation in the presence of food. All cilium-defective mutants except daf-19 retain this inhibitory function, since they do not form dauers under normal growth conditions. Indeed, when the sensory neurons were killed in the cilium-defective mutants che2 and daf-10, which are normally defective in dauer formation, they formed dauers constitutively (Bargmann and Horvitz 1991b). The exceptional cilium-defective daf-19 mutant lacks cilia (Perkins et al. 1986), whereas the other mutants have malformed cilia, and rather than being defective in dauer formation, daf-19 animals form dauers constitutively. Although it is possible that defects in cells other than the sensory neurons are responsible for the dauer constitutive phenotype of daf-19 animals, it seems simpler to suppose that the sensory neurons are responsible. Possibly it is the complete absence of cilia in daf-19 animals that leads to constitutive dauer formation. According to this view, the shortened cilia in osm-6 mutants impair the ability to form dauer larvae under conditions of starvation and limited food, but retain enough cilia to repress inappropriate dauer formation. Another possibility is that daf-19(+) is required to specify at least two distinct sensory cell functions: the formation of cilia and the repression of inappropriate dauer formation. In any case, it is interesting that the accumulation of OSM-6::GFP is drastically curtailed in daf-19 animals.

A corollary of our suggestion that osm-6 serves the single role of promoting distal outgrowth of all sensory axonemes is the suggestion that all of the behavioral problems exhibited by osm-6 animals—defective chemotaxis to various water soluble compounds and volatile odorants, defective dauer larva formation, defective responses to touch in the head and poor male mating (Hodgkin 1983)—are caused, directly or indirectly, by defective sensory cilia. Different sets of sensory neurons are implicated in different behaviors, as noted in the introduction, but we suggest that all of these behaviors require intact cilia. Not all mechanosensation in C. elegans requires intact cilia; mechanosensory receptors in the body and tail of the animal are not ciliated (Chalfie and Sulston 1981), and osm-6 animals do respond to touch along the body and in the tail.

We searched the OSM-6 amino acid sequence using the Motifs and ProfileScan programs of the Genetics Computer Group and did not identify any protein motifs or domains. We also applied the PHD program (Ros et al. 1996) for predicting secondary structure; the most striking prediction was that the OSM-6 segment from residues 332 to 370 is devoid of both α-helix and β-strand. This segment includes most of the proline-rich region and overlaps almost exactly the longest region of high similarity (>75% identical) between OSM-6 and the NGD5 protein (Figure 5). We suggest that this segment may have domains that take on a threefold left-handed helical structure referred to as the left-handed poly-L-proline II (PPII) helix. Adzhubei and Sternberg (1993) have shown that PPII helices are common in globular proteins. Although not obligatory components of PPII helices, proline residues strongly favor their formation (Adzhubei and Sternberg 1993). Among the least favored amino acid residues in PPII helices are tryptophan, isoleucine, glycine, tyrosine, histidine and cysteine, all of which are absent from the 39-residue segment from residues 332 to 370. PPII helices tend to be exposed to solvent and are therefore thought to be ideal for interactions with other proteins (Adzhubei and Sternberg 1993; Cohen et al. 1995). Peptide regions that bind to SH3 domains have PxxP motif (Cohen et al. 1995), which is present twice in this region of OSM-6 and once in the corresponding region of the NGD5 protein. Residues surrounding the PxxP motif are thought to confer specificity of binding of the ligand to particular SH3 domains. The PxxP motif present in the NGD5 protein is embedded in a sequence that is very similar to the proposed consensus of one class of SH3 binding sequence, RxLPP(L/R)P (Feng et al. 1994). The SH3 domain, which contains approximately 60 amino acids, is found in a wide variety of proteins, including signal transduction proteins and cytoskeleton proteins such as spectrin (Cohen et al. 1995). Possibly OSM-6 binds to an SH3-containing cytoskeletal protein.

The original NGD5 cDNA (Wick et al. 1995) was identified in a library constructed from a hybrid cell line, NG108-15, made from mouse neuroblastoma and...
rat glioma cell lines. Northern blot analysis indicated that NGD5 transcripts are present in both parental cell lines as well as rat brain. Neither NG108-15 cells (Daniels and Hamprecht 1974) nor rat PC12 cells (Greene and Tischler 1976), from which an NGD5-like CDNA was derived (Lee et al. 1995), appear to be ciliated. After the NG108-15 cells were exposed to an opioid agonist for 48 hr, the level of NGD5 transcript was reduced to about 60% the pretreatment level. NG108-15 cells express high levels of opioid receptors, which mediate, in at least some cases through the action of GTP-binding regulatory proteins, a variety of physiological effects (Reisine and Bel I 1993). Many ESTs—one derived from rat, two from mouse and more than a dozen from a variety of human tissues—have been identified that are very similar to NGD5. NGD5 protein has not been identified, and its function is unknown. We suggest from our work on OSM-6 that it may interact with the cytoskeleton, perhaps through the proline-rich region that is highly similar to OSM-6.

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LITERATURE CITED


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