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RESEARCH ARTICLE

Active uptake of artificial particles in the nematode *Caenorhabditis elegans*

Yuya Kiyama, Kohji Miyahara and Yasumi Ohshima*

Department of Applied Life Science, Sojo University, 4-22-1 Ikeda, Kumamoto City 860-0082, Japan

*Author for correspondence at present address: 1-17-16 Midorigaoka, Higashi-ku, Fukuoka City 813-0021, Japan (ohshima813@nifty.com)

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SUMMARY

Feeding and food choice are crucial to the survival of an animal. The nematode *Caenorhabditis elegans* feeds on various microorganisms in nature, and is usually fed *Escherichia coli* in the laboratory. To elucidate the mechanisms of food/non-food discrimination in *C. elegans,* we examined the accumulation of various fluorescent polystyrene microspheres in the absence and presence of bacterial food. In the absence of food and on agar plates, *C. elegans* worms actively accumulated 0.5 and 1 μm diameter microspheres, whereas those microspheres <0.5 μm or >3 μm were rarely accumulated. Carboxylate microspheres were accumulated more than sulfate or amine microspheres. These results of accumulation in the absence of food probably well simulate uptake of or feeding on the microspheres. Presence of food bacteria even at bacteria:nematode ratios of 1:100 or 1:10 significantly reduced accumulation of 0.5 μm microspheres, and accumulation was reduced to approximately one-fourth of that observed in the absence of bacteria at a ratio of 1:1. When accumulation of microspheres was examined with the chemical sense mutants *che-2*, *tax-2*, *odr-1* and *odr-2*, or the feeding mutant *eat-1*, all the mutants showed less accumulation than the wild type in the absence of food. In the presence of food, the *che-2* mutant showed more accumulation than the wild type. It is possible that *C. elegans* discriminates food both physically, based on size, and chemically, based on taste and olfaction.

Key words: nematode, *C. elegans*, food discrimination, microspheres, bacteria, chemical sense.

INTRODUCTION

In animals, various feeding methods are seen, such as absorption and endocytosis of nutrient molecules directly through exterior body surfaces, filter feeding, fluid feeding, and capturing large prey *via* the mouth (Randall et al., 2002). The nematode *Caenorhabditis elegans* is a filter feeder, although it does not have an obvious filter (Avery and Shtonda 2003; Fang-Yen et al., 2009). In nature, *C. elegans* likely feeds on microorganisms (Brenner, 1974), and it is usually fed *Escherichia coli* bacteria in the laboratory (Sulston and Hodgkin, 1988). It takes liquid containing suspended food particles into the pharynx, traps the bacteria, ejects the liquid and transports the bacteria into the intestine through the so-called grinder at the end of the pharynx (Avery and Shtonda, 2003; Fang-Yen et al., 2009). The mechanisms involved in the selective uptake and transport processes in the wild type have been studied extensively (*ibid.*), and many genes have been shown to be involved in these processes, such as *eat-1*, *eat-2*, *eat-4*, *eat-5*, *eat-6*, *eat-12*, *eat-18* and *exp-2* (Avery, 1993; Raizen and Avery, 1994; Avery and Thomas, 1997; Davis et al., 1999; McKay et al., 2004).

Food-seeking or food-choice behavior is important for, and might be closely related to, feeding in nematodes. Food-seeking behavior in *C. elegans* is known to be based on chemotaxis to water-soluble or volatile chemicals that represent taste or olfaction (Ward, 1973; Dusenbery et al., 1975; Bargmann et al., 1993). Food choice has been studied using various bacteria to examine chemotaxis, food transport and pathogenicity (Andrew and Nicholas, 1976; Avery and Shtonda, 2003; Zhang et al., 2005; Shtonda and Avery, 2006). Artificial particles have also been used to study the effects of starvation and drugs on feeding (Avery and Horvitz, 1990), mechanisms of selective uptake (Avery and Shtonda, 2003; Fang-yen et al., 2009) or incorporation into cells (Pluskota et al., 2009). However, it is not clear to what degree and how a natural food and a non-food are discriminated. In this report, we used fluorescent microspheres of various sizes and chemical natures in the absence and presence of food bacteria to elucidate the mechanisms of food discrimination in the nematode *C. elegans*.

MATERIALS AND METHODS

Cultivation of *C. elegans* worms

Caenorhabditis elegans wild-type strain N2, feeding-defective mutant DA531 *eat-1 (e2343)*, and chemotaxis mutants CB1033 *che-2 (e1033)*, FK100 *tax-2 (p671)*, CX2065 *odr-1 (n1936)* and CX2205 *odr-3 (n2150)* were obtained from the *Caenorhabditis* Genetics Center, University of Minnesota.

The nematodes were handled essentially as described by Sulston and Hodgkin (Sulston and Hodgkin, 1988). Three young adult worms were placed, using a platinum wire picker, on a nematode growth medium (NGM) agar plate in a 6 cm plastic Petri dish seeded with *E. coli* strain OP50, cultured at 20°C for 3–4 days, and maintained by this cycle of cultivation. OP50 was cultured in Luria–Bertani (LB) liquid medium (1% polypeptone, 0.5% Difco Yeast Extract, 1% NaCl) at 37°C.

Assay for accumulation of microspheres

As microspheres, we mainly used Fluoresbrite® Polystyrene Carboxylate Size Range Kits I and II (yellow-green fluorescent; Polysciences Inc., Warrington, PA, USA; Kit I: 0.116±0.005, 0.210±0.013, 0.516±0.011, 0.748±0.021 and 0.968±0.028 μm diameters; Kit II: 1.67±0.032, 1.83±0.061, 2.89±0.15, 4.87±0.25 and 6.60±0.60 μm diameters). In some experiments, Fluoresbrite®

Carboxy BB microspheres (blue fluorescent, 0.483±0.010 μm; Polysciences Inc.) were also used; in the experiments to examine discrimination based on the surface chemical nature of the microspheres, carboxylate, amine or sulfate-modified latex (polystyrene) beads (yellow-green fluorescent, φ1.0 μm; Sigma-Aldrich, St Louis, MO, USA) were used. Apart from the original stocks, the microspheres were stored in the refrigerator as 1/10 diluted stocks in 90% ethanol and 1/100 diluted stocks in 9% ethanol.

For the assay of microsphere accumulation, a 100 μl suspension of 1.0×10^9 ml^{-1} of 0.5 μm or 1.0×10^8 ml^{-1} of 1.0 μm microspheres was mixed with 100 μl S-basal buffer (Sulston and Hodgkin, 1988), and the mixture was put on a 3 cm NGM agar plate (Sulston and Hodgkin, 1988) and spread by tilting the plate on a clean bench. The plate was left for liquid absorption for 30–60 min and stored in a 20°C incubator. For an uptake assay in the presence of food, *E. coli* OP50 cells were recovered from an NGM culture plate into S-basal, the density was adjusted and the cell suspension was mixed with a microsphere suspension. The density of microspheres or cells was measured with a bacterial counter and a microscope.

For the assay, the worms that had been cultured for 4 days were recovered from a 6 cm NGM plate in 1 ml S-basal, and after the worms sedimented, washing was repeated three times in total by removal of the supernatant, addition of 1 ml of new S-basal and self-sedimentation. Fifteen microliters of the washed worm suspension was put on a 6 cm NGM plate seeded with OP50, and the worms were cultured for 2 h at 20°C. The worms were then recovered as before with two washings, and 5 μl of the worm suspension was put on a 3 cm NGM plate seeded with microspheres prepared as above, and the plate was incubated for uptake of the microspheres. After incubation, three worms in the center of the plate were separated from the microspheres by washing in 50 μl of S-basal on Parafilm for 2 min. The worms were then anesthetized on an agar pad (Sulston and Hodgkin, 1988) containing 200 mmol l^{-1} Na azide (NaN_3) and placed on a slide glass. The worms on the agar pad were examined under a Nikon ECLIPSE 80i microscope (Tokyo, Japan), and black-and-white fluorescent microphotographs of the microspheres were taken with a digital camera (Digital Sight DS-2MBWc-U2, Nikon) with the neutral density filter 4 (excitation light intensity at 25% of the maximum) as the standard condition. The fluorescence of microspheres accumulated in a worm was estimated using the image-analysis software WinROOF (Mitani Corp. Ltd, Tokyo, Japan), and was corrected by adding the fluorescence of the microspheres expelled from the worm during anesthetization. Means ± s.e.m. were derived from the fluorescence of 10 worms. In some experiments without this correction, 50 μl of 50 mmol l^{-1} Na azide was added to the plate to anesthetize the worms after uptake, and then 15 adult worms were placed into 200 μl of 50 mmol l^{-1} Na azide. Eight hundred microliters of S-basal was added, the mixture was centrifuged at 1700 ***g*** for 1 min, and 15 μl of the sedimented worms was placed inside a 1 cm^2 space cut out of a piece of double-sided adhesive tape affixed to a slide glass. The fluorescence was examined as described above. The number of microspheres accumulated by a worm was estimated by dividing the total fluorescence of the worm by the mean fluorescence of a microsphere, which was directly measured for microspheres down to 0.5 μm and estimated for 0.2 and 0.1 μm microspheres by the linear relationship between fluorescence and volume for spheres 0.5 to 2 μm in size.

For the assay of accumulation by first instar (L1) larvae, worms cultured for 4–5 days were treated with an alkaline bleach to prepare nematode eggs (Sulston and Hodgkin, 1988). The eggs were incubated on a 3 cm NGM plate without bacteria, and the resultant L1 larvae were recovered, washed and incubated for uptake for 30 min at 20°C.

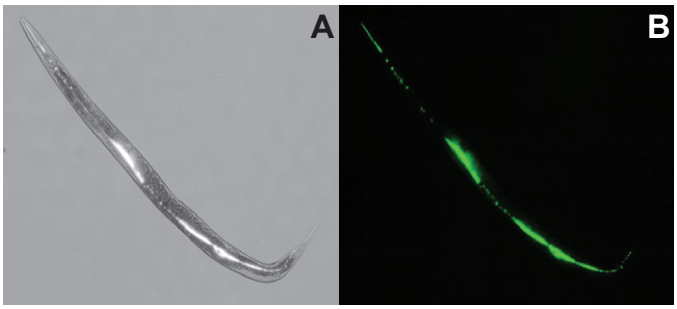

Fig. 1. Bright field (A) and fluorescence (B) images of a *Caenorhabditis elegans* adult worm that accumulated 0.5 μm microspheres of yellow-green fluorescence for 15 min at 20°. The photographs were taken at ×100 magnification.

RESULTS

Conditions for accumulation of microspheres

To study the uptake of artificial particles, we initially examined accumulation of fluorescent polystyrene microspheres of 1.0 or 0.5 μm diameter by *C. elegans* worms in a buffer solution, as in the first study on the uptake of artificial particles (5 μm iron particles) by *C. elegans* (Avery and Horvitz, 1990). However, when we tested accumulation on an agar plate, far more microspheres were accumulated. In addition, the worms are usually fed on an agar plate. Therefore, subsequently we examined accumulation on agar plates instead of in a buffer solution. Fig. 1 shows typical images of a worm that took up 0.5 μm microspheres on an agar plate. Most of the microspheres were found in the intestine, and some in the pharynx. To determine the standard conditions for accumulation, we varied time and temperature for accumulation of the 0.5 μm microspheres at a density of 10^8 microspheres plate^{-1} (3 cm diameter) and in the absence of food (Fig. 2A,B). Similar results were obtained with 1.0 μm microspheres (data not shown). Based on the results, we chose 15 min and 20°C as the standard conditions. The results of accumulation at various microsphere densities under the standard conditions are shown in Fig. 2C. Although more microspheres were accumulated at a density of 10^9 microspheres plate^{-1}, we chose 10^8 microspheres plate^{-1} as the standard because this was sufficient for the assay and the lower density allowed us to save on materials.

Effects of the size and chemical nature of the microspheres

We examined accumulation of microspheres of various sizes under the standard time and temperature conditions and using an equal volume of microspheres per plate (Fig. 3A). The highest fluorescence was observed with 1 μm spheres; spheres of 5 μm or <0.5 μm were accumulated much less by *C. elegans*. The fluorescence intensities of these microspheres are roughly proportional to their volumes for the 0.5 to 2 μm range, and so Fig. 3A simulates accumulation on a volume or mass basis. On a number basis, more 0.5 μm spheres were accumulated than 1 μm spheres (estimated to be 2.5×10^4 *vs* 3.7×10^3 microspheres worm^{-1}). Fig. 3B shows the results of accumulation by L1 larvae, which are much smaller than adults used in the experiment shown in Fig. 3A, and most other experiments. The larvae actively accumulated 0.5 μm spheres, but few spheres larger than 0.5 μm were accumulated. It is interesting that even the small larvae took up spheres smaller than 0.5 μm only rarely, as in the case of adults. Fig. 3C,D shows the results of experiments with

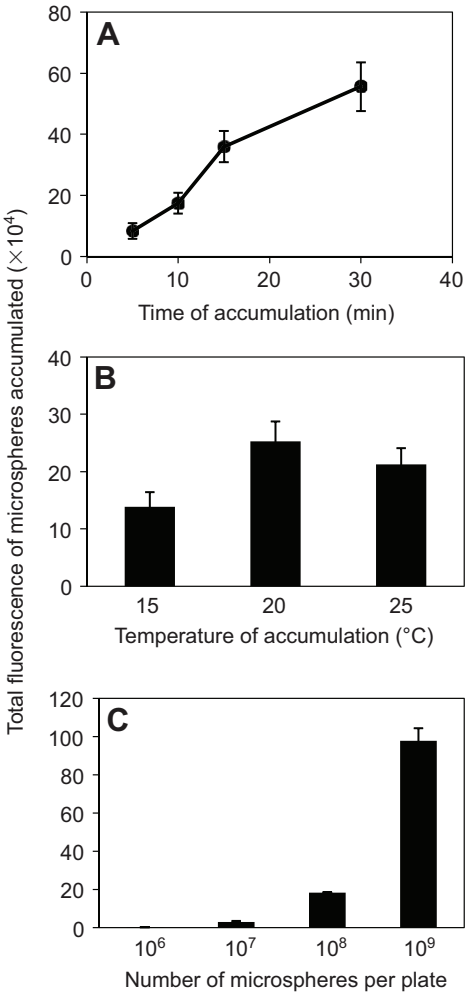

Fig. 2. Accumulation of 0.5 μm microspheres under various conditions. Time (A), temperature (B) or microsphere density (C) was varied, and the other conditions were 15 min, 20°C and 10^8 microspheres plate^{-1}. Data are means ± s.e.m. of 10 worms.

adult worms that tested whether the presence of 0.5 μm spheres, which are actively accumulated, stimulates accumulation of 0.1 μm or 0.2 μm spheres, which alone are accumulated to a much lesser degree. The 0.5 μm spheres significantly stimulated accumulation of 0.2 μm spheres, but not of 0.1 μm spheres. When 1 μm spheres were used in some of the following experiments, they were used at a density of 10^7 microspheres plate^{-1}, which nearly corresponds to the sphere density of 10^8 microspheres of 0.5 μm diameter plate^{-1}.

Accumulation of sulfate- or amine-modified polystyrene microspheres was compared with that of the standard carboxylate-modified spheres (Fig. 4). The three kinds of spheres showed significantly different accumulation, indicating that the surface chemical nature of the spheres has a role in the mechanisms of accumulation.

Effects of food bacteria

We tested whether the presence of food bacteria affected the accumulation of the 0.5 μm spheres (Fig. 5A), and found that accumulation exhibited gradual inhibition depending on the ratio of bacterium/sphere; at a 1:1 ratio the accumulation was reduced to approximately one-fourth of that observed in the absence of bacteria. The experiments with 1.0 μm spheres showed similar results (data not shown). Presence of bacteria did not affect the accumulation of 0.2 μm spheres, but drastically reduced the accumulation of 0.1 μm spheres (Fig. 5B,C).

Examination of genes possibly involved in the discrimination of food and non-food

Many mutants abnormal in the taste for water-soluble substances or in the smell or olfaction for volatile substances have been isolated and characterized (reviewed by Bargmann and Mori, 1997). Some of these, and a feeding-defective mutant (see the Introduction) were selected and examined for accumulation of the spheres in the absence and presence of food bacteria (Fig. 6). In the absence of food, all the mutants tested showed reduced accumulation of the spheres. In the presence of food, all the mutants except *eat-1* showed a significant reduction of sphere accumulation compared with those in the absence of food, but the degree of reduction was less in all of the mutants than that in the wild type. Among them, only *che-2* showed increased accumulation compared with that of the wild type (P=0.0080).

DISCUSSION

Accumulation, uptake and defecation

The main objective of our study was to elucidate the mechanisms of food/non-food discrimination in the feeding process. For this purpose, we should examine uptake of or feeding on microspheres, which may be different from their actual accumulation. Accumulation of food in a worm is generally considered to be the result of uptake minus digestion and defecation. For the polystyrene microspheres, digestion does not take place. In the absence of food bacteria, the time course of accumulation shown in Fig. 2A indicates that accumulation clearly slows down after 15 min. Also, defecation is drastically suppressed in the absence of food (Thomas, 1990; Avery and Thomas, 1997), and our preliminary observation shows that defecation of the microspheres rarely takes place (data not shown). Therefore, we think that our results of microsphere accumulation during 15 min in the absence of food (Figs 1–4, 6) closely represent uptake of the spheres. In the presence of food, defecation may take place and we should carefully consider the relationship between accumulation and uptake, as described later.

Mechanisms of size selection

The results shown in Fig. 3A,B suggest that the adult worms prefer food 0.5 to 1 μm in size, whereas L1 larvae cannot ingest food 1 μm in size. The difference in the results between adults and larvae is clear and must be related to the difference in their sizes (approximately 1.4 mm long and 4 nl in volume for an adult *vs* 0.4 mm and 0.1 nl for an L1 larva). For the food bacterium *E. coli* strain OP50, we obtained mean (±s.d.) sizes of 0.76±0.18 μm, 0.53±0.07 μm and 0.23±0.06 μm^2 for the length, diameter and sectional area, respectively, in our laboratory (So et al., 2011) (S. So, K.M. and Y.O., unpublished results). The shape of OP50 cell is nearly spherical and its volume estimated from these data is 0.11 μm^3, between that of 0.5 and 1 μm spheres (0.072 and 0.48 μm^3, respectively). Active uptake of 0.5 μm and 1 μm spheres by adults may be reasonable as the size and shape of the *E. coli* cells are close to those of these spheres. Also, the results are consistent with the fact that L1 larvae can take up *E. coli* cells, the size of which is close to that of a 0.5 μm sphere.

To determine whether spheres of different sizes were discriminated even when they were present together, we tested whether uptake of the small spheres of 0.1 or 0.2 μm diameter was

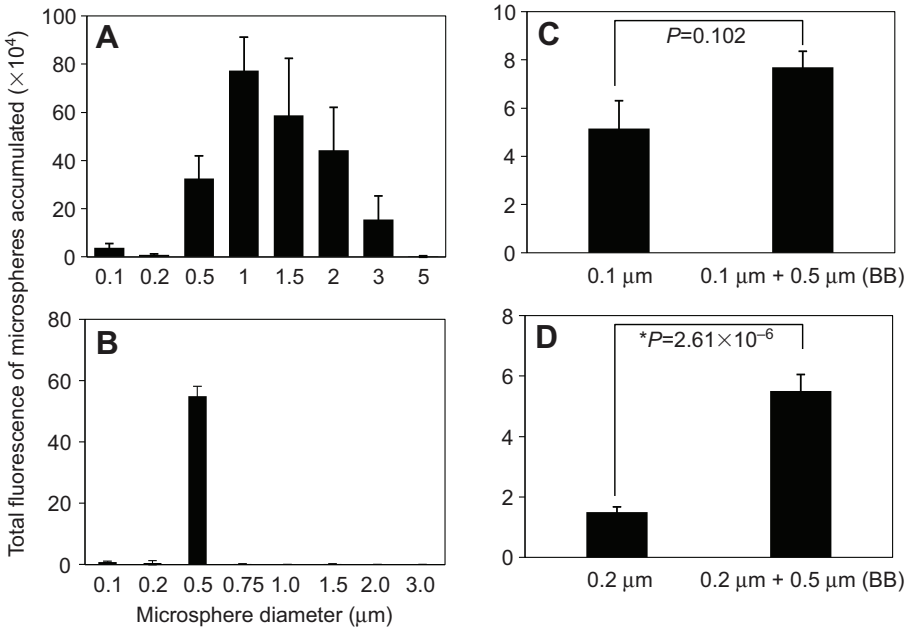

Fig. 3. Effect of the size of microspheres on accumulation. (A,B) Accumulation of microspheres of various sizes by an adult worm (A) or an L1 larva (B). (C,D) Effect of the presence of 0.5 μm microspheres on the accumulation of 0.1 μm (C) or 0.2 μm (D) microspheres. The 0.5 μm spheres used in C and D carry a different fluorescence (blue) from the 0.1 or 0.2 μm spheres (yellow-green), so that only fluorescence of the latter is measured. Data are means ± s.e.m.; *N*=10 (A,B) and 15 (C,D). *P*-values were obtained from *t*-tests.

stimulated by the presence of 0.5 μm spheres that were actively taken up (Fig. 3C,D). For 0.1 μm spheres, the presence of 0.5 μm spheres did not result in a significant difference in the uptake, whereas it clearly stimulated uptake of 0.2 μm spheres. The results indicate that the worms have some difficulty in discriminating 0.2 μm spheres from 0.5 μm spheres when they are mixed, whereas 0.2 μm spheres are taken up less than 0.1 μm spheres when they are present alone. Uptake of few 5 μm spheres (Fig. 3A) is consistent with the results of studies by Fang-Yen et al. (Fang-Yen et al., 2009), in which virtually no 4.5 μm polystyrene spheres were taken up, and Avery and Horvitz (1990), in which less than one 5 μm iron particle per worm was taken up under similar conditions (well-fed worms in the absence of food).

The results shown in Fig. 3 are mostly novel, although dependence of uptake on the particle size of Fig. 3A is partially similar to the data reported by Fang-Yen et al. (Fang-Yen et al., 2009), which were based on the fraction of the worms carrying each of various spheres and not on quantitation of the spheres. However, they distinguished the location of the spheres within a worm and did extensive kinetic studies on uptake and transport, enabling them to reveal the mechanisms, which we have not done.

On the mechanisms of selective uptake and size selection of food, Avery and Shtonda (Avery and Shtonda, 2003) found that the contraction–relaxation cycle of the middle section of the pharynx (isthmus) is delayed relative to that of the anterior section (corpus), and proposed that this delay causes net particle transport. They also proposed that the particles (0.8 μm latex beads) in the center of the pharyngeal lumen move faster than the fluid on average. Fang-Yen et al. (Fang-Yen et al., 2009) reported that the stoma or buccal cavity at the beginning of the pharynx and the relaxation of the anterior tip of the corpus (metastomal flaps) exclude excessively large particles from entering, and that the latter also works as a valve to prevent food-sized particles from getting out. They also showed radial filtering of food bacteria and polystyrene particles, by which bacteria and particles 0.5 μm or larger are restricted to the center of the pharyngeal lumen while 0.03 and 0.1 μm particles diffuse into peripheral channels of the lumen. A constriction of 0.1–0.2 μm separating the channels from the central lumen, which was observed in electron micrographs, was proposed to function as the radial filter (Fang-Yen et al., 2009). Exclusion of most microspheres larger than 3 μm in adults and those larger than 0.5 μm in L1 larvae in the present study can be explained by the exclusion by the stoma and metastomal flaps described by Fang-Yen et al. (Fang-Yen et al., 2009). Based on these results, the diameter of the stoma of an adult seems to be between 3 and 5 μm and that of an L1 larva between 0.5 and 0.75 μm. Fang-Yen et al. (Fang-Yen et al., 2009) did not examine the behavior of 0.2 μm spheres, and we showed that 0.2 μm spheres, as well as 0.1 μm spheres, were not taken up significantly (Fig. 3A). Based on their radial filtering mechanism, this result suggests that the constriction separating the central lumen and the channels is larger than 0.2 μm *in vivo*. It is interesting that even very small L1 larvae do not take up 0.1 and 0.2 μm spheres. For nematodes, which mostly live in soil, excluding small soil particles may be important. If the explanation for the difference in the behavior of 0.1 and 0.2 μm spheres in the presence of 0.5 μm spheres (Fig. 3C,D) is related to this radial filtering mechanism, then 0.5 μm particles in the central lumen may form a kind of barrier, preventing the 0.2 μm particles

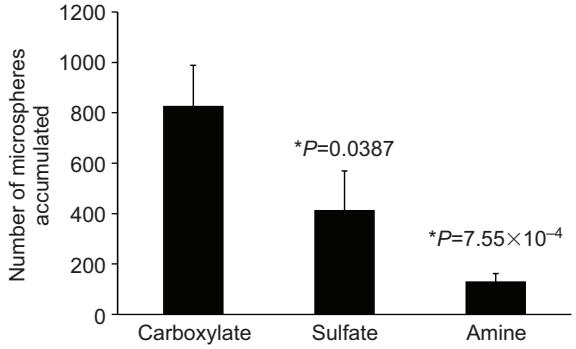

Fig. 4. Accumulation of 1.0 μm microspheres carrying each of three different chemical modifications on the surface. The number of each kind of sphere accumulated by a worm was estimated from the fluorescence intensity of a single sphere, which is different among the three kinds of spheres. Direct counting with a bacteria counter and a microscope of the spheres taken by 10 worms after lysis of the worms showed similar results. Data are means ± s.e.m.; *N*=15. *P*-values were obtained from *t*-tests.

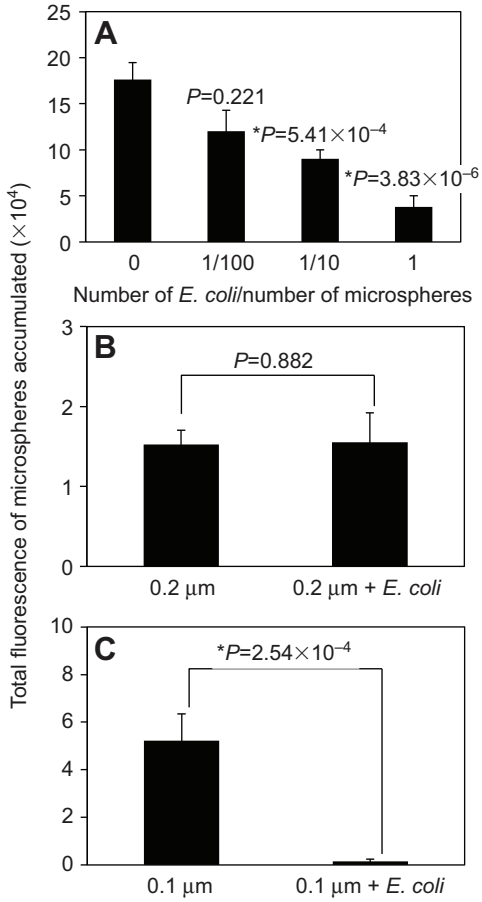

Fig. 5. Effect of the presence of food bacteria (*Escherichia coli*) on the accumulation of 0.5 μm (A), 0.2 μm (B) or 0.1 μm (C) microspheres. In A, *E. coli* cells were added at the indicated ratio to 10^8 microspheres. In B and C, 0.1 ml of the 1/200 diluted microspheres (>>10^8 spheres) and 10^8 *E. coli* cells were used. Data are means ± s.e.m.; *N*=10 (A) and 15 (B,C). *P*-values were obtained from *t*-tests.

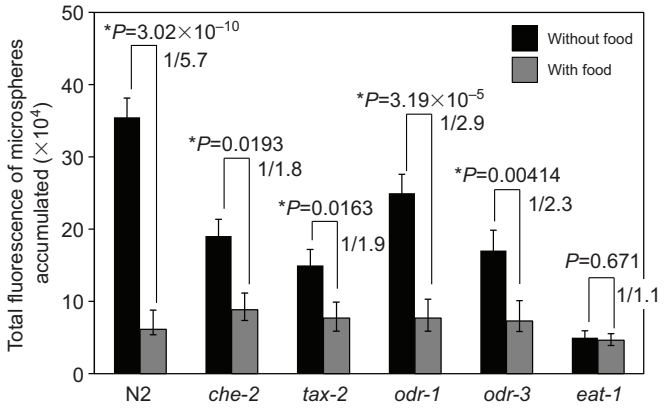

Fig. 6. Effect of each of several mutations on the accumulation of 0.5 μm microspheres. Accumulation was assayed with or without the same number of *E. coli* cells. Data are means ± s.e.m.; *N*=10. The fraction given for each pair of bars represents the ratio of the value with food to that without food. *P*-values were obtained from *t*-tests.

from diffusing freely into the peripheral channels, but preventing diffusion only slightly for the smaller 0.1 μm spheres.

Chemical discrimination

The results shown in Fig. 4 suggest that chemical modification on the surface of the spheres affects their uptake. Reduction in the accumulation of 0.5 μm spheres by the presence of *E. coli* cells (Fig. 5A) indicates that *E. coli* cells are moderately selected for accumulation against the carboxylate polystyrene spheres of a similar size. This result suggests that chemical discrimination occurs based on the nature of the surfaces in the uptake. Alternatively, chemical stimulation in the uptake may not have actually occurred, instead, stimulation of defecation by the presence of food may have led to the present results. We think that the effects of *E. coli* cells on the accumulation of 0.2 and 0.1 μm spheres (Fig. 5B,C) favor the former possibility because, if *E. coli* cells significantly stimulate defecation of spheres, the accumulation of both 0.1 and 0.2 μm spheres is likely to be affected similarly. Also, the results shown in Fig. 5B,C differ from the effects of 0.5 μm spheres shown in Fig. 3C,D, and must be related to a difference in the chemical nature of the bacteria and the spheres. An explanation for this may be that 0.1 μm spheres are easier to be excluded from uptake in the presence of *E. coli* cells based both on physical and chemical selection, whereas uptake of 0.2 μm spheres is stimulated physically by the addition of 0.5 μm spheres (Fig. 3D) and is compensated by a possible reduction based on chemical discrimination.

Genes possibly involved in the discrimination of food

All the mutations tested reduced accumulation of the spheres in the absence of food (Fig. 6), suggesting that the corresponding genes function in the uptake of the spheres. In the presence of food, accumulation of the spheres was further reduced in all the mutants except *eat-1*, but the rate of reduction was significantly lower than that in the wild-type N2. Among the mutants tested, only the *che-2* mutant showed a significant difference (increase) in the uptake of the spheres in the presence of food from that of the wild type, suggesting a unique and important role of the *che-2* gene in the negative discrimination of the spheres from food. Because the common function of the *che-2*, *tax-2*, *odr-1* and *odr-3* genes is the chemical sense (taste or olfaction) and because no abnormal phenotypes in pumping rate or defecation are reported (WormBase, www.wormbase.org), we suggest that they function to distinguish chemically between the spheres and the food bacteria in the uptake. The *che-2* mutant is defective in chemotaxis to water-soluble substances (taste) and in the structure of sensory cilia, and the gene is known to encode a WD40 repeat protein (Fujiwara et al., 1999). The *tax-2* mutant is defective in chemotaxis and thermotaxis, and the gene encodes a β-subunit of the cyclic nucleotide-gated ion channel expressed in sensory neurons (Coburn et al., 1996; Komatsu et al., 1996). The *odr-1* and *odr-3* mutants are defective in chemotaxis to volatile substances (olfaction), and the genes encode a guanylyl cyclase and an α-subunit of a tripartite G-protein complex, respectively (Bargmann et al., 1993; L'Etoile and Bargmann, 2000; Roayaie et al., 1998). The *eat-1* mutant showed the lowest uptake of the spheres among the mutants tested in the absence of food (Fig. 6), and the uptake changed little in the presence of food, suggesting that the *eat-1* gene has an important role both in the uptake and discrimination of food. The *eat-1* mutant is known to be severely defective in food uptake and to show irregular pharyngeal pumping and sluggish movement (Avery, 1993). The *eat-1* gene encodes an Alp-Enigma family protein (McKeown and Beckerle, 2001).

In conclusion, our results form a basis to elucidate the mechanisms of discrimination between food and non-food in the nematode. Further studies will be needed to understand more detailed mechanisms in the future.

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REFERENCES

Andrew, P. A. and Nicholas, W. L. (1976). Effect of bacteria on dispersal of *Caenorhabditis elegans* (Rhabditae). *Nematologia* **22**, 451-461.

Avery, L. (1993). The genetics of feeding in *Caenorhabditis elegans*. *Genetics* **133**, 897-917.

Avery, L. and Horvitz, H. R. (1990). Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. *J. Exp. Zool.* **253**, 263-270.

Avery, L. and Shtonda, B. B. (2003). Food transport in the *C. elegans* pharynx. *J. Exp. Biol.* **206**, 2441-2457.

Avery, L. and Thomas, J. H. (1997). Feeding and defecation. In *C. elegans II*, 2nd edn (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 679-716. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Bargmann, C. I. and Mori, I. (1997). Chemotaxis and thermotaxis. In *C. elegans II*, 2nd edn (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 717-737. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

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

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

Coburn, C. 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.

Davis, M. W., Fleischhauer, R., Dent, J. A., Joho, R. H. and Avery, L. (1999). A mutation in the EXP-2 potassium channel that alters feeding behavior. *Science* **286**, 2501-2504.

Dusenbery, D. B., Sheridan, R. E. and Russell, R. L. (1975). Chemotaxis-defective mutants of the nematode *Caenorhabditis elegans*. *Genetics* **80**, 297-309.

Fang-Yen, C., Avery, L. and Samuel, A. D. T. (2009). Two size-selective mechanisms specifically trap bacteria-sized food particles in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **106**, 20093-20096.

Fujiwara, M., Ishihara, T. and Katsura, I. (1999). A novel WD40 protein, CHE-2, acts cell-autonomously in the formation of *C. elegans* sensory cilia. *Development* **126**, 4839-4848.

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.

L'Etoile, N. D. and Bargmann, C. I. (2000). Olfaction and odor discrimination are mediated by the *C. elegans* guanylyl cyclase ODR-1. *Neuron* **25**, 575-586.

McKay, J. P., Raizen, D. M., Gottschalk, A., Schafer, W. R. and Avery, L. (2004). *eat-2* and *eat-18* are required for nicotinic neurotransmission in the *Caenorhabditis elegans* pharynx. *Genetics* **166**, 161-169.

McKeown, C. R. and Beckerle M. C. (2001). eat-1 encodes two members of the ALP-Enigma family of proteins and is required for normal muscle function in *C. elegans*. In Abstracts of 13th International *C. elegans* Meeting, pp.267, Los Angels.

Pluskota. A., Horzowski, E., Bossinger, O. and von Mikecz, A. (2009). In *Caenorhabditis elegans* nanoparticle-bio-interactions become transparent: Silica-nanoparticles induce reproductive senescence. *PLoS ONE* **4**, e6622.

Raizen, D. M. and Avery, L. (1994). Electrical activity and behavior in the pharynx of *Caenorhabditis elegans*. *Neuron* **12**, 483-495.

Randall, D., Burggren, W. and French, K. (2002). *Eckert Animal Physiology. Mechanisms and Adaptation*, 4th edn. New York: W. H. Freeman and Co.

Roayaie, K., Crump, J. G., Sagasti, A. and Bargmann, C. I. (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.

Shtonda, B. B. and Avery, L. (2006). Dietary choice behavior in *Caenorhabditis elegans*. *J. Exp. Biol.* **209**, 89-102.

So, S., Miyahara, K. and Ohshima, Y. (2011). Control of body size in *C. elegans* dependent on food and insulin/IGF-1 signal. *Genes to Cells* **16**, 639-651.

Sulston, J. and Hodgkin, J. (1988). Methods. In *The Nematode Caenorhabditis elegans* (ed. W. B. Wood and the Community of *C. elegans* Researchers), pp. 587-606. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Thomas, J. H. (1990). Genetic analysis of defecation in *Caenorhabditis elegans*. *Genetics* **124**, 855-872.

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

Zhang, Y, Lu, H. and Bargmann, C. I. (2005). Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature* **438**, 179-184.