Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue

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Specialized oxygen-sensing cells in the nervous system generate rapid behavioural responses to oxygen. We show here that the nematode *Caenorhabditis elegans* exhibits a strong behavioural preference for 5–12% oxygen, avoiding higher and lower oxygen levels. 3′,5′-cyclic guanosine monophosphate (cGMP) is a common second messenger in sensory transduction and is implicated in oxygen sensation. Avoidance of high oxygen levels by *C. elegans* requires the sensory cGMP-gated channel *tax-2/tax-4* and a specific soluble guanylate cyclase homologue, *gcy-35*. The GCY-35 haem domain binds molecular oxygen, unlike the haem domains of classical nitric-oxide-regulated guanylate cyclases. GCY-35 and TAX-4 mediate oxygen sensation in four sensory neurons that control a naturally polymorphic social feeding behaviour in *C. elegans*. Social feeding and related behaviours occur only when oxygen exceeds *C. elegans*' preferred level, and require *gcy-35* activity. Our results suggest that GCY-35 is regulated by molecular oxygen, and that social feeding can be a behavioural strategy for responding to hyperoxic environments.

All animals require oxygen as the essential electron acceptor in respiration, and respond to oxygen levels with behavioural and physiological changes. Soil, freshwater and marine animals encounter and avoid steep oxygen gradients in their natural environments[1,2,3]. In mammals, oxygen acts through the hypoxia-inducible transcription factor HIF-1 to regulate erythropoietin production, red blood cell development, angiogenesis and cardiovascular physiology[4]. However, behavioural responses to oxygen occur much more rapidly than can be explained by changes in transcription. For example, the mammalian carotid body regulates ventilatory and circulatory responses to hypoxia within seconds[5]. The molecular nature of rapid oxygen sensation in the nervous system is not well understood.

GCY-35 mediates oxygen sensation

To examine oxygen-related behaviours in the nematode *C. elegans*, an aerotaxis assay was developed. Washed wild-type animals were placed in a gas-phase oxygen gradient from 0–21% that was produced by diffusion in a microdevice made of poly(dimethylsiloxane) (PDMS)[6] and were allowed to move freely on an agar surface (Fig. 1a,b). Animals distributed themselves across the surface, avoiding low oxygen concentrations (<2%) as well as high oxygen concentrations (>12%) (Fig. 1c). The avoidance of hypoxia is consistent with previous studies[7], but avoidance of hyperoxia has not previously been described, to our knowledge.

cGMP has been implicated in oxygen responses in *Drosophila*, where a nitric oxide (NO)-sensitive, cGMP-dependent kinase pathway mediates behavioural avoidance of hypoxia[8]. In cGMP second-messenger cascades, cGMP is produced from GTP by either membrane-bound guanylate cyclases or soluble guanylate cyclases (sGCs). All active sGCs characterized until now contain a haem cofactor and are activated by NO, which is produced by NO synthase. Notably, however, other haemoproteins can bind oxygen, and prokaryotic haemoproteins mediate aerotaxis to preferred oxygen concentrations[9,10]. The genome of the nematode *C. elegans* contains seven predicted sGC homologues (*gcy-31* through *gcy-37*) but no predicted NO synthase, suggesting that these cyclases might detect ligands other than endogenously-produced NO[11,12]. The guanylate cyclases have the conserved histidine that ligates haem in mammalian β subunits (Supplementary Fig. 1)[12], and conservation of key catalytic residues from both α and β mammalian subunits suggests that *C. elegans* guanylate cyclases could be catalytically active (Supplementary Fig. 1)[13]. These genes were examined in more detail to determine whether they play a part in *C. elegans* oxygen sensing.

Previous studies demonstrated that *gcy-32* is expressed in URX, AQR and PQR sensory neurons and that *gcy-33* is expressed in BAG sensory neurons[11]. The expression patterns of *gcy-34, gcy-35, gcy-36* and *gcy-37* were examined in transgenic animals bearing reporter genes in which upstream sequences for each gene were fused to sequences encoding green fluorescent protein (GFP). Each transgene was expressed in a small number of neurons (Fig. 2 and data not shown). Expression of *gcy-34, gcy-35, gcy-36* and *gcy-37* was consistently observed in URX, AQR, and PQR sensory neurons; *gcy-35* expression was also observed in ALN, SDQ and BDU neurons and variably in AVM, PLM and PLN neurons, pharyngeal and body wall muscles, and the excretory cell. The cells that reliably express *gcy-31-gcy-37* have the morphology of sensory neurons, suggesting a sensory role for the guanylate cyclase proteins, but the sensory cues that activate these cells are unknown.

To ask whether *gcy-35* might participate in oxygen sensing, a *gcy-35(ok769)* mutant from the *C. elegans* knockout consortium was characterized in the aerotaxis assay. *gcy-35(ok769)* deletes sequences corresponding to amino acids 456–545 of GCY-35, including key residues in the GC catalytic domain (Supplementary Fig. 1), and should abolish any ability of the *gcy-35*(*ok769*) gene product to

produce cGMP. In contrast to wild-type animals, *gcy-35(ok769)* animals avoided hypoxia but not hyperoxia in gas-phase oxygen gradients (Fig. 1d, Supplementary Fig. 2). This defect did not appear to be due to a general locomotory deficit, because *gcy-35* animals were mobile and chemotaxis-proficient (data not shown). The aerotaxis defect was rescued by expression of a *gcy-35* complementary DNA in URX, AQR and PQR (Fig. 1d, Supplementary Figs 2, 3). Thus *gcy-35* can act in URX, AQR and PQR sensory neurons to mediate avoidance of hyperoxic conditions.

cGMP can depolarize neurons by activating cyclic nucleotide-gated channels[14]. A cGMP-gated sensory transduction channel in *C. elegans* is composed of two subunits encoded by the *tax-2* and *tax-4* genes, which are co-expressed with *gcy-35* in URX, AQR and PQR neurons[15,16]. To ask whether GCY-35 might act upstream of the cyclic nucleotide-gated channel, we tested *tax-4* and *tax-2* mutant and *tax-2; tax-4* double-mutant strains for aerotaxis behaviours. Like the *gcy-35* strain, *tax-4* and *tax-2* mutants failed to avoid hyperoxic conditions (Fig. 1e; Supplementary Figs 2, 3). The *tax-4* mutant defect was rescued by expression of *tax-4* in AQR, PQR and URX (Supplementary Figs 2, 3). These results indicate that the cGMP-gated channel is required for avoidance of hyperoxia, perhaps as the target for cGMP produced by GCY-35. In support of this model, a *tax-4; gcy-35* double mutant exhibited an aerotaxis defect resembling that of single mutants (Supplementary Figs 2, 3).

GCY-35 haem domain binds molecular oxygen

Gaseous ligands bind to sGCs through associated haem groups. To investigate the potential ligand-binding characteristics of GCY-35, we cloned, expressed and purified the amino-terminal-predicted haem-binding fragment GCY-35(1–252). This protein was soluble (unlike full-length GCY-35) and tractable for biochemical analysis. Previous studies have shown that N-terminal haem-binding regions of the rat sGC β1 subunit, β1(1–385) and β1(1–194), are spectroscopically similar to the full-length enzymes (data not shown and ref. 17). Therefore, the GCY-35(1–252) haem domain spectrum should be related to the ligand-binding characteristics of the full-length protein.

GCY-35(1–252) was characterized by ultraviolet/visible (UV/vis) spectroscopic analysis in the absence and presence of bound ligands. In an anaerobic environment, the purified protein was chemically treated with ferricyanide and dithionite to remove any ligands and to reduce the haem iron to its ferrous oxidation state. The Fe^{2+}-unligated, anaerobic spectrum of this protein exhibited a Soret maximum of 430 nm and a single, broad α/β region that was similar to ferrous-unligated sGC (Fig. 3a, c).

To test for oxygen binding, the unligated protein was exposed to air and immediately reanalysed by UV/vis spectroscopy. The resulting spectrum was characteristic of oxygen-bound haem, exhibiting a Soret maximum of 415 nm and a split α/β region similar to ferrous oxyhaemoglobin, indicative of a ferrous, low-spin complex (Fig. 3a, c). Like haemoglobin, GCY-35(1–252) was also able to bind NO and CO (Fig. 3c). In the presence of NO, GCY-35(1–252) exhibited a Soret maximum of 415 nm, similar to that of haemoglobin and a shoulder at 400 nm, similar to the Soret maximum of NO-bound

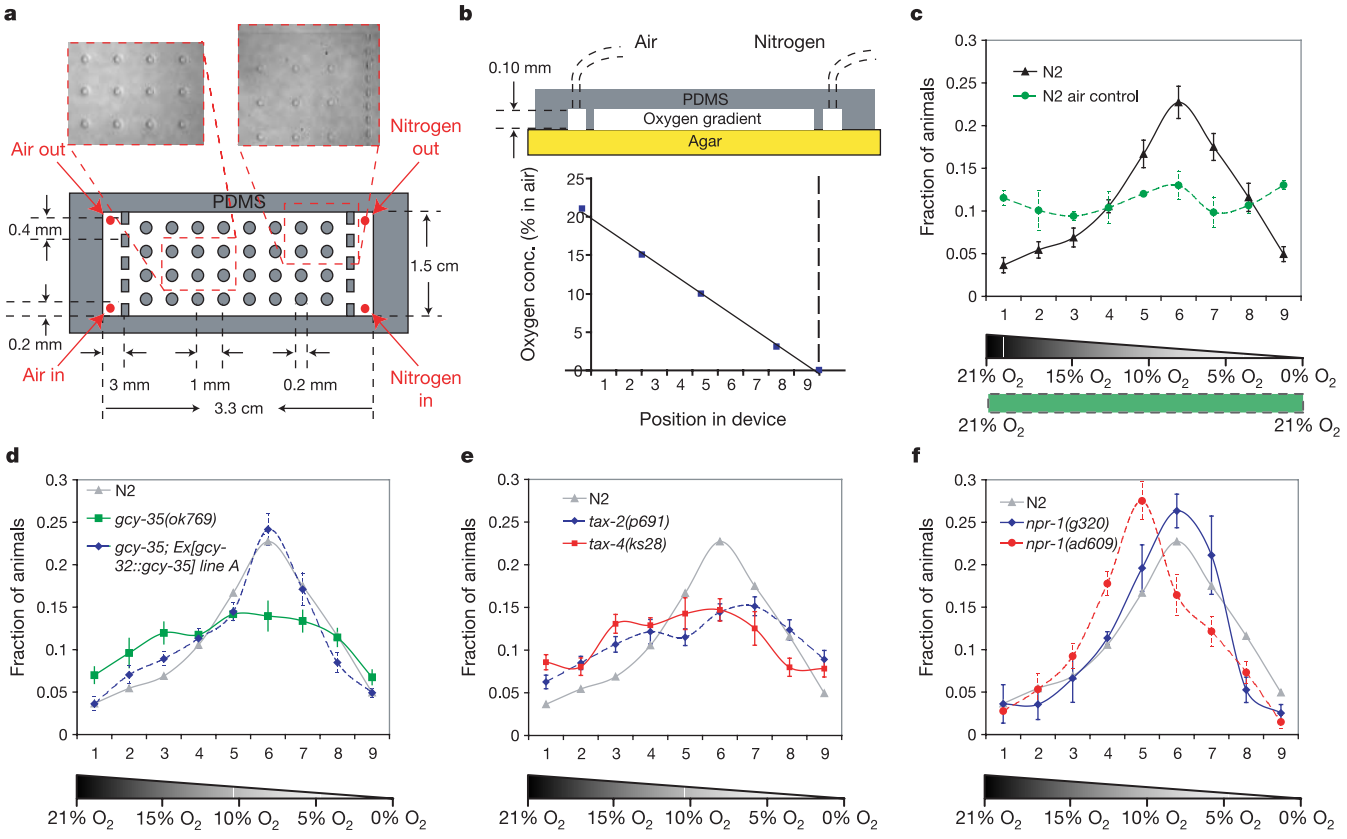


Figure 1 *gcy-35* mutants are defective in hyperoxia avoidance. **a**, Gas-phase PDMS aerotaxis device, top view. A gas-phase gradient was established by diffusion along the long axis of the device. **b**, Measured oxygen concentrations in aerotaxis device, and side view of device. **c**, Wild-type N2 animals accumulate at intermediate oxygen concentrations. **d**, Defective hyperoxia avoidance in *gcy-35* mutants, and rescue by *gcy-32::gcy-35* expression in URX, AQR, and PQR. **e**, Defective hyperoxia avoidance in *tax-4* and *tax-2* mutants. **f**, Similar oxygen preference of N2, *npr-1(g320)* and *npr-1(ad609)*. Error bars denote standard error of the mean (s.e.m.). For statistical analysis, see Supplementary Fig. 2.

sGC, suggesting that GCY-35(1–252) forms two stable nitrosyl complexes: a 5-coordinate high-spin complex that is similar to sGC and a 6-coordinate, low-spin complex that is similar to haemoglobin. The ligand-binding characteristics of the GCY-35 haem domain were most similar to oxygen-binding proteins like haemoglobin, and suggest that this protein could act as an oxygen sensor. No other native sGCs or sGC haem domain fragments characterized to date have been found to bind O_2. GCY-35(1–252) is unique in this respect.

Oxygen regulates social feeding behaviour

The URX, AQR and PQR neurons that co-express *gcy-35* and *tax-4* have previously been implicated in cGMP-mediated behaviours. The cyclic nucleotide-gated channel TAX-4 is required in these neurons to promote social feeding (or aggregation on a bacterial lawn) and bordering (the accumulation of animals on the thickest part of a bacterial lawn)[18]. Aggregation, bordering, burrowing into agar, and hyperactive locomotion represent a cluster of related behaviours that are not pronounced in the standard *C. elegans* laboratory strain, N2. However, social feeding behaviours are prominent in naturally isolated *C. elegans* strains that differ from N2 at the *npr-1* locus and in N2 strains that are deficient for the function of *npr-1*[19]. *npr-1* encodes a G-protein-coupled receptor for FMRF amide-like neuropeptides, and high levels of *npr-1* activity suppress aggregation and bordering behaviours[20]. *tax-4* cGMP signalling stimulates aggregation and bordering by activating the URX, AQR and PQR neurons (as well as other neurons), whereas *npr-1* functions in URX, AQR and PQR to inhibit aggregation and bordering[18]. These reciprocal results suggest that the activity of URX, AQR and PQR regulates aggregation and bordering behaviours.

If the guanylate cyclases expressed in URX, AQR and PQR are molecular oxygen sensors, then oxygen should regulate the activity of these cells. To test this hypothesis, aggregation and bordering behaviours were examined in animals exposed to a constant flow of gas with different concentrations of oxygen. Initial experiments were conducted by shifting animals from 21% to 7% oxygen, the concentration that was preferred by *C. elegans* in aerotaxis experiments. *npr-1(ad609)* is a loss-of-function mutation, and *npr-1(g320)* is the reduced-function allele of *npr-1* present in natural social strains[19]. *npr-1(ad609)* mutants shifted to 7% oxygen rapidly suppressed both aggregation and bordering behaviours (Fig. 4b, d, f). Suppression was evident within three minutes of shifting to 7% oxygen and was stable for at least thirty minutes after the shift (Fig. 4d, f). A return to 21% oxygen led to the reappearance of aggregation and bordering behaviours within three minutes (Fig. 4d, f). Similar effects were seen with *npr-1(g320)* (data not shown).

A smaller shift from 21% oxygen to 15% oxygen or 10% oxygen led to a similar, but less marked suppression of aggregation and bordering behaviours (Fig. 5a, b). In all cases, the change in behaviour was reversed by returning to 21% oxygen. Thus decreases in oxygen lead to a dose-dependent suppression of social feeding behaviour, suggesting that oxygen serves as a quantitative regulator of social feeding by URX, AQR and PQR.

Bacteria alter oxygen levels and responses

All behaviours in the social feeding cluster are most pronounced in the presence of bacterial food[19]. The aerotaxis assay is conducted in the absence of food, and under these circumstances *npr-1* strains exhibited aerotaxis with preferred concentrations similar to those preferred by N2 animals (Fig. 1f). Because social behaviours are food-induced, we also tested aerotaxis in N2 and *npr-1* strains in the presence of food (Fig. 5c, Supplementary Fig. 2). On a thin bacterial lawn, aerotaxis behaviours in N2 were blunted, with a greatly

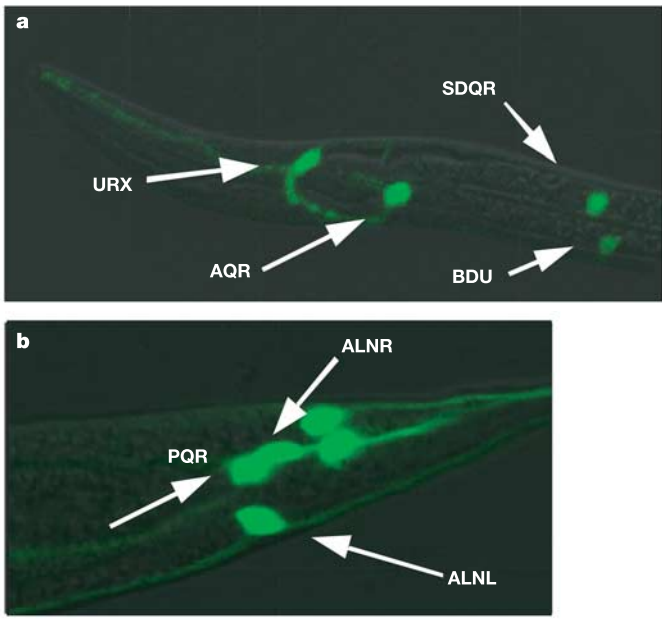
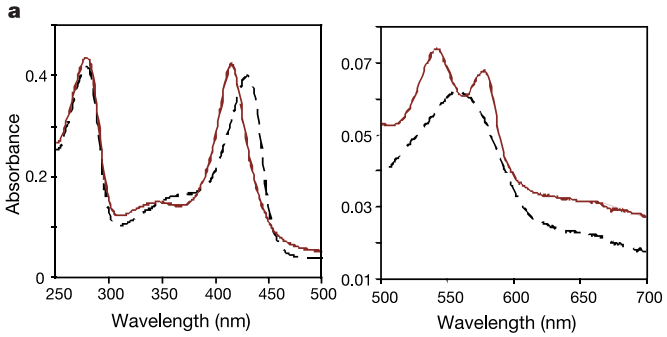


Figure 2 *gcy-35::gfp* is expressed in URX, AQR, PQR and other sensory neurons. **a**, Lateral view of the anterior body showing URX, AQR, SDQR and BDU neurons. Anterior is at left and ventral is down. **b**, Ventral view of the tail showing PQR and ALNL/R neurons. The more posterior cells may be PLM neurons, the sisters of ALNL or ALNR, or PLN neurons.



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Ligand	Protein	Soret	α	β
None	sGC (α1β1)	431	555	
	*Tt*Tar4H	431	565	
	Hb	430	555	
	GCY-35(1-252)	430	559	
O_2	sGC (α1β1)	Does not bind		
	*Tt*Tar4H	416	556	591
	Hb	415	541	576
	GCY-35(1-252)	415	542	578
NO	sGC (α1β1)	398	537	572
	*Tt*Tar4H	420	547	575
	Hb	418	545	575
	GCY-35(1-252)	415/400	533	568
CO	sGC (α1β1)	423	541	567
	*Tt*Tar4H	424	544	565
	Hb	419	540	569
	GCY-35(1-252)	421	538	564

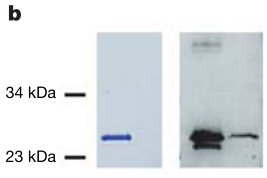

Figure 3 Characterization of GCY-35(1–252) binding to gases. **a**, UV/vis spectroscopy of GCY-35(1–252). The black broken trace shows anaerobic spectrum of ferrous-unligated complex (peaks at 430 and 559 nm). The solid red trace shows the same sample after exposure to air, and is indicative of a ferrous-oxy complex (peaks at 415, 542 and 578 nm). **b**, Purification of GCY-35(1–252):His. Left, Coomassie blue-stained gel. Right, western blot with affinity-purified anti-GCY-35 antisera, lanes at tenfold different dilutions. **c**, Comparison of spectroscopic data for GCY-35(1–252), soluble guanylate cyclase[32], haemoglobin[33], and *Thermoanaerobacter tengcongensis* Tar4H[25] unliganded and in the presence of CO, NO and O_2.

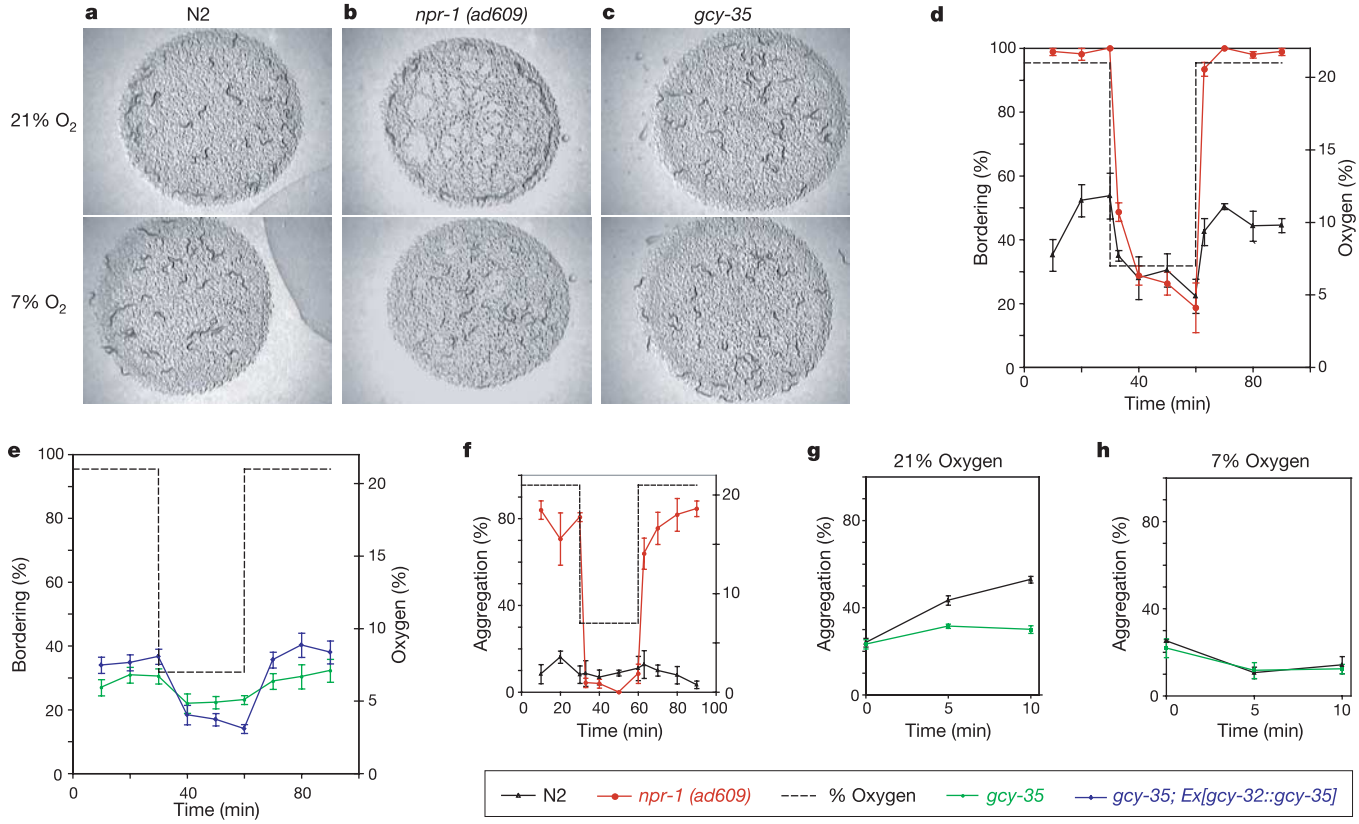

Figure 4 Oxygen stimulates GCY-35-dependent aggregation and bordering. **a**–**c**, Animals equilibrated at 21% oxygen or 7% oxygen for 30 min. **a**, N2; **b**, *npr-1(ad609)*; **c**, *gcy-35(ok769)*. **d**–**f**, Oxygen shifts from 21%–7%–21% (dotted lines). **d**, **f**, 7% oxygen suppresses bordering in N2 and *npr-1* strains and aggregation in *npr-1* strains. **e**, Reduced oxygen sensitivity of bordering in *gcy-35(ok769)*, and rescue by *gcy-32::gcy-35* transgene. **g**, **h**, Aggregation of N2 and *gcy-35(ok769)* immediately after transfer to a fresh bacterial lawn in 21% or 7% oxygen. Error bars denote s.e.m. For statistical analysis see Supplementary Information.

reduced avoidance of hyperoxia. By contrast, *npr-1* strains exhibited robust hyperoxia avoidance in the presence or absence of food.

How does oxygen sensation relate to the cluster of social feeding behaviours? We observed that the thick lawns of *E. coli* that are usually fed to *C. elegans* consume oxygen more quickly than oxygen diffuses through the lawn. The border of a thick lawn has an effective oxygen concentration of 12.8%, compared to 17.1% in the centre of the lawn (Fig. 5d). Thus bordering behaviours may be caused, in part, by the strong preference of *npr-1* strains for lower oxygen concentrations.

Moderate bordering behaviour is evident in N2 animals grown on thick bacterial lawns, consistent with the moderate hyperoxia avoidance that N2 exhibits on food. N2 bordering behaviour was suppressed by a shift from 21% to 7% oxygen (Fig. 4a, d) or from 21% to other low oxygen concentrations (Fig. 5a). Conversely, bordering in the N2 background was stimulated by a shift from 7% to 21% oxygen (Fig. 4d). N2 animals aggregate for the first 20 min after transfer to a fresh bacterial lawn (that is, a lawn with no worms on it)[21]. This aggregation was suppressed at 7% oxygen (Fig. 4 g, h). Thus oxygen acts in parallel to *npr-1*, regulating social feeding behaviours regardless of the *npr-1* genotype of the animal.

In the N2 genetic background, *gcy-35* mutants exhibited lower levels of aggregation and bordering than wild-type animals (Fig. 4c, e, g). Changes in oxygen concentration between 21% and 7% had little effect on bordering behaviour in *gcy-35* mutants (Fig. 4e). Moreover, *gcy-35* mutants aggregated less than N2 when placed on a fresh bacterial lawn under 21% oxygen (Fig. 4g). Expression of a *gcy-35* cDNA in URX, AQR and PQR restored the oxygen sensitivity of these mutants (Fig. 4e). These results suggest that oxygen acts through *gcy-35* to regulate aggregation and bordering, a process that is antagonized by *npr-1* activity. Indeed, an independent study recently showed that *gcy-35; npr-1* double mutants do not border or aggregate[22]. Like *gcy-35* mutants, *tax-4* mutants responded poorly to changes in oxygen levels, suggesting that oxygen regulation of social feeding behaviour depends on cGMP-gated channels (Supplementary Fig. 3).

Discussion

These behavioural results demonstrate that GCY-35 is required for avoidance of hyperoxia and for oxygen-induced aggregation and bordering. Biochemical evidence suggests that GCY-35 forms a stable ferrous-oxy complex. Canonical NO-sensitive sGCs do not bind oxygen; indeed, their inability to bind oxygen is essential to their ability to sense NO, given that the NO:O_2 ratio in tissues is about 1:1,000 (refs 23, 24). By analogy with the NO-sensitive sGCs, we suggest that oxygen modulates GCY-35 guanylate cyclase activity, either directly or by competing with other activators like NO. Although catalytic activity remains to be demonstrated, several bacterial and archaeal haem domains mediate aerotaxis[9], and one other sGC-like haem domain, Tar4H from *Thermoanaerobacter tengcongensis*, has been found to bind oxygen[25]. Our results suggest that the soluble guanylate cyclase GCY-35 may represent one member of a new class of oxygen-sensitive sGCs.

GCY-35 mediates oxygen sensing in URX, AQR and PQR. The URX sensory neurons have dendrites that extend to the tip of the nose, suggesting that URX detects external stimuli. The AQR and PQR neurons extend dendrites with ciliated endings into the pseudocoelom, an internal, fluid-filled cavity immediately under

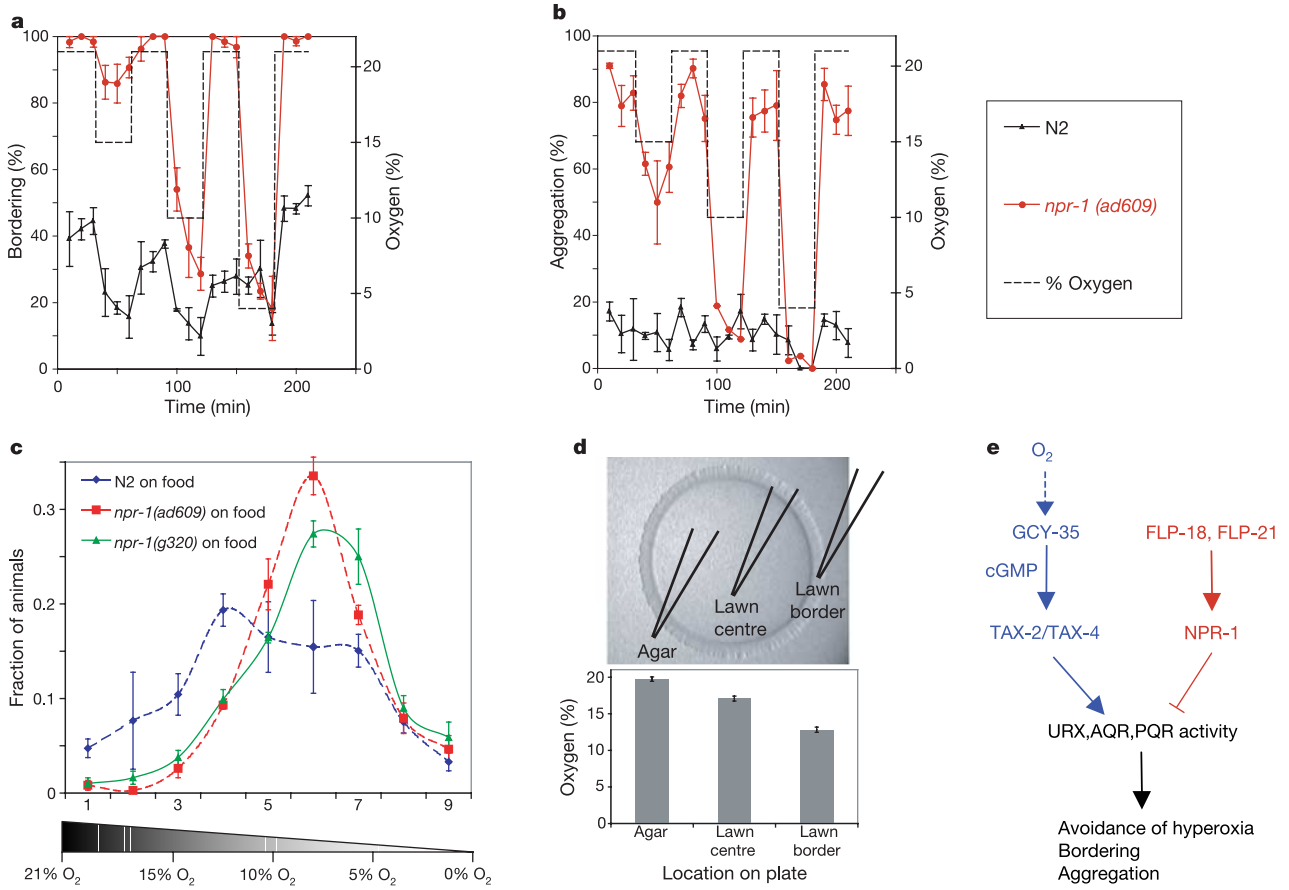

Figure 5 Regulation of oxygen responses by food. **a**, **b**, Bordering and aggregation during oxygen shifts (dotted line). **c**, Aerotaxis on a thin bacterial lawn. N2 aerotaxis is suppressed. **d**, Oxygen concentrations in and near a thick OP50 bacterial lawn. Error bars denote s.e.m. **e**, Model for oxygen regulation of behaviour. Oxygen directly or indirectly regulates GCY-35; cGMP activates the TAX-2/TAX-4 cGMP-gated channel in URX, AQR and PQR to promote hyperoxia avoidance, bordering and aggregation. The neuropeptides FLP-18/21 (possibly released by pharyngeal neurons during feeding[20]) activate the neuropeptide receptor NPR-1, which inhibits URX, AQR and PQR.

the epithelium[26]. AQR and PQR may detect internally generated stimuli or internal levels of externally produced stimuli. On the basis of its rate of diffusion through water, molecular oxygen should diffuse from the environment to the AQR and PQR sensory endings in less than a second.

Because oxygen diffuses rapidly through small animals like *C. elegans*, it does not become a limiting factor for respiration until it reaches external concentrations below 4% (refs 27, 28). Above 4% oxygen, additional increases in oxygen do not affect respiration, but are likely to increase cellular oxidative damage, because the propensity to produce reactive oxygen species will increase with oxygen concentration[29]. One mechanism for protecting against such damage may be a behavioural preference for lower oxygen environments. This strategy has been well characterized in bacteria, which distribute themselves along oxygen gradients in characteristic bands at their preferred concentration[30]. In *C. elegans*, behavioural preference for lower oxygen concentrations could also result from factors unrelated to oxidative stress; for example, low oxygen may signal the presence of food in the form of actively growing bacteria that consume oxygen more quickly than the oxygen can diffuse through soil.

Our results suggest that social feeding behaviour represents an integrated behavioural response to aversive hyperoxic conditions (Fig. 5e). Accumulation at the border of the lawn may represent direct aerotactic avoidance of hyperoxia. By contrast, small groups of animals are unlikely to create oxygen gradients sufficient to attract more animals. We propose that high oxygen levels and other aversive chemical stimuli[21] serve as sensory triggers that can initiate social behaviour by activating chemotaxis or mechanotaxis to other animals.

An independent study found that mutations in either *gcy-35* or the related gene *gcy-36* suppress bordering and aggregation in *npr-1* mutants[22]. Together, these results suggest that oxygen regulates social behaviour by modulating the guanylate cyclase activity of GCY-35 and GCY-36. It will be interesting to see whether soluble guanylate cyclases act as neuronal oxygen sensors in other animals. □

Methods

Standard techniques used for genetics, molecular biology, biochemistry and statistics are described in the Supplementary Methods.

Oxygen binding of bacterially-produced GCY-35(1–252)

Purified bacterially produced protein was made anaerobic in an O_2-scavenged gas train with ten cycles of alternate evacuation and purging with purified argon and brought into an anaerobic glove bag. Ferricyanide (~100 equivalents) was added and then removed using a PD10 desalting column that had been equilibrated with 50 mM TEA pH 7.5 and 50 mM NaCl (Buffer C). The protein was then reduced using dithionite (~100 equivalents). The dithionite was then removed in the same manner. A ferrous-unligated UV/vis absorption spectrum was recorded in an anaerobic cuvette on a Cary 3E spectrophotometer equipped with a Neslab RTE-100 temperature controller set at 10 °C. Spectra were recorded from protein in Buffer C. Fe^{2+}–O_2 protein and other gas-bound proteins were generated by exposing Fe^{2+}-unligated GCY-35(1–252) to air or other gases before recording a spectrum. Bacterial haem can assemble with recombinant eukaryotic haem-binding proteins, and because *C. elegans* cannot itself synthesize haem[31], bacterial

haem is probably the natural form for *C. elegans* guanylate cyclases.

Behavioural assays

For gas-phase aerotaxis assays, microdevices were fabricated using the PDMS rapid prototyping technique[6]. The photolithography masks were laser-printed on silver halide films with 0.625-μm resolution, and used to produce the prototype masters in a photo-patternable epoxy (SU-8-50, Microchem) on silicon wafers using standard UV photolithography. The masters were silanized using vapour-phase tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane (United Chemical Technologies). The PDMS devices were micromoulded using two-part Sylgard 184 silicone elastomer (Dow Corning) against the masters. Before the assay, the devices were cleaned in ethanol followed by deionized water and dried overnight at 65 °C.

In each assay, 30–200 washed adult animals were placed on an agar plate before the device was placed over them. Air and nitrogen gas (at ~21–22 °C, 1 atm) were delivered to the source and drain chambers under laminar flow at 1 ml min^{-1} for 15–30 min from gas-tight syringes using a syringe pump (PHD2000, Harvard Apparatus). Although PDMS and agar are both oxygen-permeable, the diffusion rate of gases through these media is substantially smaller than the gas flux between the source and drain, and did not disrupt the oxygen gradient in gas phase. Animals at nine equally sized regions in the device were counted at the end of the assay, determined by at least two consecutive scorings (five minutes apart), yielding similar spatial distributions. Each aerotaxis data point represents 3–8 assays with 80 or more animals per assay. In assays with fewer than 80 total animals, counts from experiments done on the same day were combined for data analysis. The oxygen concentration gradient in the agar was measured using a Clark-style oxygen microelectrode with guard cathode (Chemical microsensor #1201, Diamond Microsensors), which was calibrated with standards immediately before each use.

For aggregation and bordering assays, 40 animals were picked onto a bacterial lawn, allowed to equilibrate for an hour, placed into a flow chamber with a constant gas flow of 150 ml min^{-1}, and exposed to 21% oxygen for at least 30 min. Oxygen was then adjusted to different concentrations for various periods of time at a constant flow rate of 150 ml min^{-1}. The flow chamber was a 100 mm × 15 mm Petri dish with a modified cover: two female luers (Biorad) were melted and glued into the ends of the cover. One luer served as the gas inlet and was connected to tubing, while the other luer was always exposed to air and served as the outlet. Gas mixes with varying concentrations of oxygen were obtained by mixing oxygen and nitrogen (99.997%) in a flow meter (Cole-Parmer). All gases were obtained from Airgas. Images were captured every 10 min with an Ultrachip CCTV camera (JE-7442, Javelin) mounted on a stereomicroscope (Wild M3Z, Leica). The analogue camera output was connected to the RCA port on a Macintosh Power PC 7600/132 equipped with an on-board analogue/digital converter. Movies and photographs were captured with Adobe Premiere software (version 4.0.1). Bacterial lawns (OP50 strain) were grown for four days before the assay and were 10–13 mm in diameter. Animals within 1 mm of the edge of the bacterial lawn were scored to be in the border. Any animal that was touching at least one other animal across at least 30% of its body was scored to be in an aggregate.

For the transient aggregation experiments in Fig. 4g, h, animals were placed in the flow chamber and counted immediately after transfer to a fresh bacterial lawn. Fresh bacterial lawns were 4-day-old strain OP50 lawns that had never encountered worms.

In Fig. 5c, a thin bacterial lawn (bacterial strain OP50) was produced by seeding 10-cm NGM plates that were used 8–10 h later.

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