constraint. After all, change endures, but the slate is not wiped clean.

1. Mizrahi, A. & Katz, L.C. *Nat. Neurosci.* **6**, 1201–1207 (2003).
2. Shepherd, G.M. & Greer, C.A. in *The Synaptic Organization of the Brain* (ed. Shepherd, G.M.) 159–203 (Oxford Univ. Press, New York, 1998).
3. Wong, R.O. & Ghosh, A. *Nat. Rev. Neurosci.* **3**, 803–812 (2002).
4. Sin, W.C., Haas, K., Ruthazer, E.S. & Cline, H.T. *Nature* **419**, 475–480 (2002).
5. Brennan, P.A. & Keverne, E.B. *Prog. Neurobiol.* **51**, 457–481 (1997).
6. Kosaka, K., Aika, Y., Toida, K. & Kosaka, T. *J. Comp. Neurol.* **440**, 219–235 (2001).
7. Kasowski, H.J., Kim, H. & Greer, C.A. *J. Comp. Neurol.* **407**, 261–274 (1999).
8. Luskin, M.B. *Neuron* **11**, 173–189 (1993).
9. Murray, R.C. & Calof, A.L. *Semin. Cell Dev. Biol.* **10**, 421–431 (1999).
10. Sanes, J.R. & Lichtman, J.W. *Annu. Rev. Neurosci.* **22**, 389–442 (1999).
11. van Praag, H. *et al. Nature* **415**, 1030–1034 (2002).
12. Grutzendler, J., Kasthuri, N. & Gan, W.B. *Nature* **420**, 812–816 (2002).
13. Trachtenberg, J.T. *et al. Nature* **420**, 788–794 (2002).
14. Gan, W.B., Kwon, E., Feng, G., Sanes, J.R. & Lichtman, J.W. *Nat. Neurosci.* **6**, 956–960 (2003).

# Food for thought: a receptor finds its ligand

Christopher J Potter & Liqun Luo

In *C. elegans*, social and solitary feeding behavior can be determined by a single amino acid change in a G protein–coupled receptor. A new study identifies ligands for this receptor and suggests how changes in behavior evolve at the molecular level.

Organisms alter their behavior to accommodate changes in the environment. But how do genetic changes alter neuronal activity and thereby influence behavioral output? With a circuitry of 302 invariant neurons[1], a quick generation time and a plethora of genetic tools, *Caenorhabditis elegans* is an ideal model system for studying the interplay among genes, neurons, circuits and behavior. Studies on foraging behavior[2–4] are a prime example. The N2 laboratory strain of *C. elegans* exhibits 'solitary' feeding behavior—individual worms tend to disperse across their bacterial food source and forage alone. In contrast, other wild-type strains of *C. elegans* tend to accumulate where food is thickest and are termed 'social' feeders. These two variations are due to a single amino acid change in a putative G protein–coupled receptor, NPR-1, a protein that shows high homology to the neuropeptide Y (NPY) receptor family[2]. Yet how could this single amino acid change in NPR-1 lead to altered behavior *in vivo*? In this issue, Rogers *et al.*[5] shed light on this question by identifying the ligands for the NPR-1 receptor and showing that these ligands differentially activate the solitary versus social variants of NPR-1.

Using a screen for mutants that can convert the solitary N2 strain into social feeders, previous work identified multiple loss-of-function mutations in the *npr-1* gene[2]. Closer inspection of the *npr-1* gene from many naturally occurring social and solitary *C. elegans*

strains revealed that all solitary strains, isolated from different regions of the world, contain a valine residue at position 215 (*npr-1 215V*), whereas all social strains contain a phenylalanine at this position (*npr-1 215F*). In addition, *npr-1 215V* is dominant over *npr-1 215F*, as transgenic expression of *npr-1 215V* in *npr-1 215F* strains converts them from social into solitary feeders, but expression of *npr-1 215F* in the solitary N2 strain does not affect feeding behavior.

To understand how the NPR-1 215F and NPR-1 215V receptors differentially alter feeding behavior, Rogers *et al.*[5] sought to identify the NPR-1 ligand. The *C. elegans* genome does not contain any NPY ligands, but it does contain a structurally related class of neuropeptides called 'FMRFamide and related peptides' (FaRPs) that can stimulate NPY-like receptors in other species[6]. Through alternate splicing, the 22 FaRP genes (*flp1-22*) can potentially encode 59
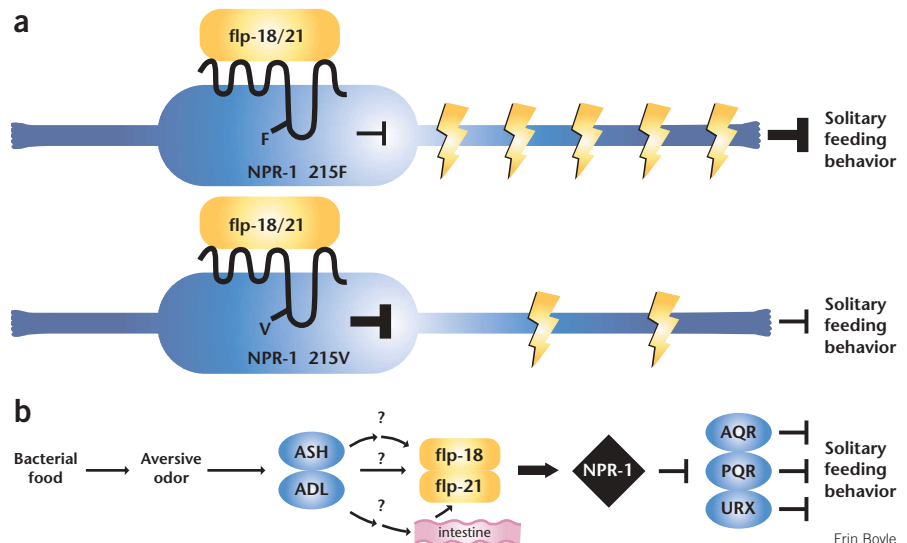
Figure 1 Differential responses of NPR-1 215F and NPR-1 215V receptors to ligand result in changes in feeding behavior. (**a**) The G protein–coupled receptor, NPR-1, likely functions to inhibit neuronal activity in neurons that normally repress solitary feeding behavior[4,5,8]. The social NPR-1 215F variant (top) responds poorly to FLP-18 and FLP-21 ligands, resulting in less inhibition of activity and greater inhibition of solitary feeding behavior. In contrast, the solitary NPR-1 215V variant (bottom) responds strongly to ligand, resulting in greater inhibition of neuronal activity and promotion of solitary behavior. (**b**) Schematic of a possible NPR-1 neural circuit. Bacterial food emits an aversive odor that stimulates the ASH/ADL neurons. The neurons may induce, directly or indirectly, expression and release of FLP-18 and FLP-21 ligands into the body fluid, where they activate the NPR-1 receptor expressed on the AQR/PQR/URX neurons, inhibiting their normal repression of solitary feeding behavior.

Christopher Potter and Liqun Luo are in the Department of Biological Sciences, Stanford University, Stanford, California 94305, USA.
e-mail: cpotter@stanford.edu

NEWS AND VIEWS

FaRP peptides[6,7]. Using this genomic information as their guide, Rogers et al.[5] tested the ability of synthesized FaRP peptides to activate NPR-1 215V and NPR-1 215F in a heterologous *Xenopus laevis* oocyte system. The authors found that both NPR-1 215F and NPR-1 215V were activated by the peptide encoded by *flp-21*, whereas only NPR-1 215V was activated by the six peptides encoded by *flp-18*. An independent set of experiments[8] in mammalian CHO cells also identified FLP-21 peptide as a ligand for NPR-1. In both studies, the NPR-1 215V receptor responded more readily (by about five times) than the NPR-1 215F receptor to the FLP-21 peptide, suggesting that NPR-1 215V functions as a more potent receptor in activating downstream signaling. Increasing the NPR-1 signal by overexpressing the *npr-1 215F* receptor *in vivo* promotes solitary behavior to a similar degree as does low expression of the *npr-1 215V* allele[2], further supporting the conclusion that NPR-1 215V responds more acutely to ligand binding (**Fig. 1a**).

In a heroic effort, Rogers et al.[5] also assayed the ability of all 59 FaRPs to activate either NPR-1 215F or NPR-1 215V *in vivo*, by expressing the receptors ectopically in the *C. elegans* pharynx and measuring the frequency of pharyngeal action potentials. As in the *in vitro* assays[5,8], only the FLP-18 and FLP-21 peptides stimulated the NPR-1 receptors, confirming that these peptides can function as NPR-1 ligands *in vivo*. However, in contrast to the *in vitro* work, FLP-18 peptide activated both NPR-1 215F and NPR-1 215V in the pharynx. Thus, ligand/receptor specificity *in vivo* could vary based on the intracellular context of the G protein–coupled receptor. Because residue 215 of NPR-1 is in the third intracellular loop in a region that is implicated in G-protein coupling[2], ligand-induced NPR-1 activation might depend on the specific G proteins that are coupled to the NPR-1 receptor. Alternatively, the change in ligand specificity could reflect extracellular conditions. For example, NPR-1 215V, when compared to NPR-1 215F, has an increased binding affinity for the FLP-21 ligand[8], which should lead to greater activation. Therefore, in the pharyngeal assay, FLP-18 ligand might activate NPR-1 215F because it might bind more effectively to NPR-1 215F *in vivo*.

Rogers et al.[5] also examined the effects of increasing (via overexpression) or decreasing (via mutation) *flp-21* activity *in vivo*. Increasing FLP-21 expression in NPR-1 215F strains led to solitary behavior, but did not affect social behavior of *npr-1* null animals, suggesting that the FLP-21 ligand functions via NPR-1 to downregulate social feeding.

Mutation of *flp-21,* however, only mildly suppressed the solitary phenotype of *npr-1* 215V. This is surprising because if FLP-21 were the major *in vivo* ligand for NPR-1, then the *flp-21*;*npr-1* 215V double mutant should act just like loss-of-function alleles of *npr-1*. This suggests that another ligand, most likely FLP-18, functions redundantly to provide the signal for social behavior in this mutant genetic background. Furthermore, the ability of FLP-18 to activate NPR-1 215V, but not NPR-1 215F, in the oocyte system[5] suggests that NPR-1 215V may induce solitary behavior due to its ability to be activated by FLP-18. This raises the possibility that FLP-18, and not FLP-21, is the major *in vivo* ligand for inducing solitary behavior via NPR-1 215V. Experimental confirmation of this possibility will require identification of an *flp-18* mutant.

The expression pattern of the NPR-1 ligands might also shed light on the neural circuitry underlying foraging behavior. Two classes of neurons regulate NPR-1-mediated feeding behavior[3,4]. The first class consists of the ASH and ADL neurons, which directly respond to noxious stimuli emitted by the bacterial food source[3]. The second class of neurons, AQR, PQR and URX, express NPR-1 and are exposed to the body fluid[4]. Decreasing the activity of AQR/PQR/URX neurons leads to an increase in solitary behavior that mimics the phenotype of the *npr-1 215V* allele[4]. Because NPR-1 215V is a more potent receptor than 215F[5,8] and functions to promote solitary behavior[2–4], NPR-1 likely functions to inhibit the activity of the AQR/PQR/URX neurons, which results in solitary behavior (**Fig. 1b**).

Rogers et al.[5] found expression of *flp-21* in the ASH/ADL neurons and in the intestine (which secretes to the body fluid). In addition, *flp-18* is expressed in neurons that are in close proximity to AQR/URX. Thus, the ASH/ADL neurons, when stimulated by an aversive odor, might induce secretion of FLP-18/FLP-21 ligands into the body fluid, where they would hyperactivate NPR-1 215V receptors on AQR/PQR/URX neurons. This, in turn, would lead to inactivation of these neurons and the promotion of solitary behavior (**Fig. 1b**). Upregulation of *flp-18* or *flp-21* expression when animals are exposed to a bacterial food source would support this model.

Another question still remains: which variant, *npr-1 215V* or *npr-1 215F*, represents the ancestral form? That is, has solitary behavior evolved from a social strain, or vice versa? Rogers et al.[5] sequenced the *npr-1* gene from three different species of *Caenorhabditis* and

found that *npr-1* encoded only the *215F* allele. Given that *C. elegans* and these three additional *Caenorhabditis* species diverged from a common ancestor, the authors concluded that the common ancestor contained the *npr-1 215F* allele and that the *npr-1 215V* variant arose as a relatively recent gain-of-function mutation in the *C. elegans* branch. However, although the other *Caenorhabditis* species contain the 'social' *npr-1 215F* allele, it was not clear from this study whether each of these species exhibits social feeding behavior. If some are actually solitary, then additional gene(s), or residues within NPR-1 besides 215, might dominantly regulate feeding behavior much like the 215V mutation does. Such additional *Caenorhabditis* species would be a rich source from which to identify additional components involved in feeding behavior. Nonetheless, it is amusing that the 'wild-type' laboratory N2 strain actually represents a mutant for NPR-1. This highlights the importance of examining the evolutionary relationships among species when defining 'wild-type' behavior.

Although it seems unlikely that other FaRPs beside FLP-18 and FLP-21 could activate NPR-1, it is possible that these ligands might activate other G protein–coupled receptors. Overexpression of *flp-18* results in an uncoordinated phenotype that is likely to be caused by activation of a G protein–coupled receptor other than NPR-1. The techniques described by Rogers et al.[5] and others[8] could help to link other G protein–coupled receptors encoded in the *C. elegans* genome to their cognate ligands. Such a technique has recently identified a G protein–coupled receptor encoded in the *C. elegans* genome that can function as an FLP-15 receptor[9]. A next step will be to determine the biological relevance of such newly discovered ligand–receptor pairs, which might modulate other interesting behaviors. Since the initial discovery of NPR-1, elegant studies including the current work have made it clear that *C. elegans* has much to offer in understanding how genes, neurons and circuits control animal behavior.

1. White, J.G., Southgate, E., Thomson, J.N. & Brenner, S. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **314**, 1–340 (1986).
2. de Bono, M. & Bargmann, C.I. *Cell* **94**, 679–689 (1998).
3. de Bono, M., Tobin, D.M., Davis, M.W., Avery, L. & Bargmann, C.I. *Nature* **419**, 899–903 (2002).
4. Coates, J.C. & de Bono, M. *Nature* **419**, 925–929 (2002).
5. Rogers, C. et al. *Nat. Neurosci.* **6**, 1178–1185 (2003).
6. Li, C., Nelson, L.S., Kim, K., Nathoo, A. & Hart, A.C. *Ann. NY Acad. Sci.* **897**, 239–252 (1999).
7. Li, C., Kim, K. & Nelson, L.S. *Brain Res.* **848**, 26–34 (1999).
8. Kubiak, T.M. et al. *J. Biol. Chem.* **278**, 33724–33729 (2003).
9. Kubiak, T.M. et al. *J. Biol. Chem.* doi:10.1074/jbc.M304056200 (2003).