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Altered behaviour following RNA interference knockdown of a *C. elegans* G-protein coupled receptor by ingested double stranded RNA

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Abstract

Using a systemic and continuous delivery method based on feeding on a particular strain of transformed *Escherichia coli* to induce double stranded RNA-mediated interference, we targeted the product of the *npr-1* gene, a putative *Caenorhabditis elegans* homologue of a neuropeptide Y receptor, a G-protein coupled receptor. We were able to reproduce the social behaviour observed for the naturally occurring *npr-1* mutant when wild type N2 Bristol eggs developed in a lawn of bacteria producing double stranded RNA for *npr-1*. This facile approach may also be useful when studying the function of other worm G-protein coupled receptors.

Keywords: G-protein coupled receptor; RNA-mediated interference; *npr-1*

In *Caenorhabditis elegans*, ''undetectable'' phenotypes are very frequent when the expression of a particular gene is inhibited and this may be particularly the case for genes expressed in the nematode nervous system (Bargmann and Kaplan, 1998). Redundant genes, genes that are non-essential for survival in laboratory conditions and low or temporal gene expression patterns are factors that may account for the lack of a detectable phenotype. This situation may become evident when one is attempting to elucidate the function of a novel gene after reducing its expression by double stranded RNA-mediated interference.

Double stranded RNA-mediated interference is a new but already widely used and powerful technique to interfere with gene function that has very recently been further developed and refined (Timmons and Fire, 1998; Tabara et al., 1999) since it was first described (Fire et al., 1998). The molecular mechanism behind this phenomenon is gradually being elucidated (Fire, 1999; Tabara et al., 1999; Ketting et al., 1999). One of the latest developments of the technique involved the feeding of the nematodes with *Escherichia coli* that have been transformed with a plasmid containing the gene of interest, surrounded by two T7-RNA polymerase promoter regions (Timmons and Fire, 1998). Especially when using an RNase III-deficient strain of *E. coli*, and in the presence of isopropylthyo-β-D-galactoside (IPTG) to induce the expression of the T7-RNA polymerase and ampicillin for bacterial selection, high concentrations of double stranded RNA for the gene of interest are produced by the bacteria (Tabara et al., 1999). By feeding on these bacteria, the nematodes are exposed to double stranded RNA continuously and through several generations. The other methods of delivery of double stranded RNA, namely, injection or soaking, may miss a particular window during development when the gene is expressed or very few progeny might be obtained to allow for a thorough study. The feeding method, allowing continuous delivery to a large number of worms, may overcome this problem. One is nevertheless still limited to the study of genes that, when knocked down, do give rise to a phenotype that can be observed.

G-protein coupled receptors and many other proteins expressed in the worm nervous system fall frequently in this class of genes that are thought to be ''untractable'' (Bargmann, 1998). One naturally occurring mutant, however, shows a characteristic phenotype because of a defect in a worm homologue of the neuropeptide Y receptor, the *npr-1* gene product (DeBono and Bargmann, 1998). Muta-

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tions in this receptor have been shown to cause otherwise ''solitary'' nematode strains to become ''social'', i.e., to exhibit a bordering phenotype at the rim of the bacterial lawn and to form clumps of worms in the presence of abundant food. Clumping is frequently observed in wild type worms when the bacterial lawn on which they feed has been cleared. In addition, these mutants do not slow down their locomotion in the presence of food as wild type strains do.

Considering that the particular phenotype observed for the null mutant of the receptor is rather complex (and not so easy to observe with the number of worms obtained using injections or soaking for delivery of the double stranded RNA), we set out to test the feeding technique to deliver double stranded RNA to knock down the *npr-1* gene. We exposed Bristol N2 eggs (approximately 30 μl of compacted eggs per plate, in double at all times) to a lawn of HT115 (DE3) *E. coli* transformed with the double T7 vector encoding the *npr-1* cDNA, as described (Timmons and Fire, 1998). After 2 days at 15°C, the hatched worms formed a strong bordering phenotype and ''beads of worms'' surrounding the bacterial lawn (Fig. 1C,D). This is comparable to the actual null mutant of *npr-1* (ky13) (Fig. 1A). The same phenotype was observed when the experiment was performed at 20°C or when clumps of agar from a cleared plate, containing a large number of worms that had previously been exposed to the transformed bacteria, were moved to a new plate with the same bacterial lawn. As a negative control, we transformed the bacteria with the same vector lacking the insert and no such behaviour was observed (Fig. 1B). Due to the large number of worms used per plate (in three independent transformations of bacteria, plates, and sets of eggs), we can only give an estimate of the number of worms involved in clumping on the *npr-1* RNA-mediated interference and the negative control plates, starting from wild type eggs. It is not possible to quantitate so easily when a clump of agar containing worms is transferred to a fresh plate of the same type. While in the *npr-1* RNA-mediated interference plates, one could not count more than 150–170 worms in the center of the lawn (typically around 3 cm in diameter) while all the other worms were clumped, in the negative control plates, the worms spread in large numbers (more than 500) throughout the lawn. We used *unc-22* cloned in the double T7 vector as a positive control (Fire et al., 1998; Timmons and Fire, 1998) and the twitching phenotype was observed when the N2 eggs developed (not shown). However, we observed that the twitching phenotype was stronger at 20°C than at 15°C, approximately 90% and 40%, respectively. We used plates containing around 100 worms each to test *unc-22*, as individual worms were to be observed and such a large population as that required to observe the *npr-1* social behaviour was not necessary. Note that the 100 worms for *unc-22* were picked (after hatching) onto a plate with the same type of bacterial lawn and scored for twitching. One should thus be aware of the possibility of varying penetrance of a particular phenotype, as a result of different experimental conditions, including temperature. As a cautionary note when setting up an experiment of RNA-mediated interference by feeding, we nevertheless recommend that the more traditional methods of delivery of double stranded RNA (injections or soaking) should be performed in parallel and the results of the various forms of double stranded RNA delivery compared.

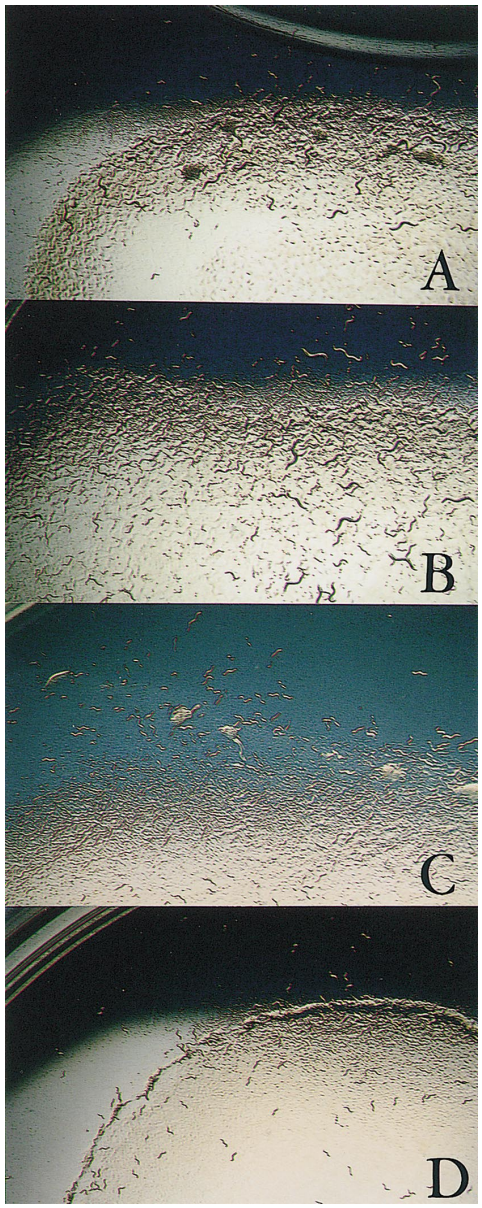

Fig. 1. Nematode behaviour under the dissection microscope. (A) *npr-1* (ky13) null mutant. Note the clumps of nematodes formed at the border of the bacterial lawn. (B) N2 Bristol after ''ingestion'' of bacteria transformed with double T7 vector without insert. No clumping formed. (C,D). Wild type N2 Bristol after ''ingestion'' of bacteria producing double stranded RNA for *npr-1* cDNA. Note the clumps (C) and bordering behaviour (D).

The results obtained with the knockdown of *npr-1* by feeding delivery of double stranded RNA are encouraging for future applications when studying genes that give rise to complex phenotypes. For example, in the case of novel candidate worm G-protein coupled receptors, this technique may generate informative data in the initial phase of characterization.

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