Dietary vitamin B12 regulates chemosensory receptor gene expression via the MEF2 transcription factor in Caenorhabditis elegans

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Abstract

Dynamic changes in chemoreceptor gene expression levels in sensory neurons are one strategy that an animal can use to modify their responses to dietary changes. However, the mechanisms underlying diet-dependent modulation of chemosensory gene expression are unclear. Here, we show that the expression of the srh-234 chemoreceptor gene localized in a single ADL sensory neuron type of Caenorhabditis elegans is downregulated when animals are fed a Comamonas aquatica bacterial diet, but not on an Escherichia coli diet. Remarkably, this diet-modulated effect on srh-234 expression is dependent on the micronutrient vitamin B12 endogenously produced by Comamonas aq. bacteria. Excess propionate and genetic perturbations in the canonical and shunt propionate breakdown pathways are able to override the repressive effects of vitamin B12 on srh-234 expression. The vitamin B12-mediated regulation of srh-234 expression levels in ADL requires the MEF-2 MADS domain transcription factor, providing a potential mechanism by which dietary vitamin B12 may transcriptionally tune individual chemoreceptor genes in a single sensory neuron type, which in turn may change animal responses to biologically relevant chemicals in their diet.

Keywords: diet; vitamin B12; Caenorhabditis elegans; srh-234; chemosensory receptor gene; ADL; sensory neurons; plasticity

Introduction

Animals receive dietary inputs from their environment and their internal metabolic state, which allows them to modify their chemosensory response properties and behavioral outcomes (Sengupta 2013). One strategy that animals can use to trigger long-term changes in behavioral outcomes is by dynamically changing the expression of individual chemoreceptor genes localized in different chemosensory neuron types. These dynamic transcriptional changes in chemoreceptor gene expression in response to food and internal feeding state is observed in mosquitoes and play pivotal roles in their ability to seek food and reproduce (Fox et al. 2001; Hallem et al. 2004; Rinker et al. 2013; Ryan et al. 2014; Taparia et al. 2017; Khan et al. 2021), but the mechanisms controlling this plasticity in chemoreceptor gene expression are unclear.

The nematode Caenorhabditis elegans is an excellent model organism to study interactions between an animal and its dietary sources (Yilmaz and Walhout 2014; Zhang et al. 2017). C. elegans is a bacterivore, making it easy to expose these animals to different bacterial strains to study their effects on organismal health and physiology. Bacterially derived factors affect C. elegans in various ways; for instance, pathogenic factors are sensed by chemosensory neurons and trigger avoidance behaviors (Pradel et al. 2007; Meisel et al. 2014), while other bacterially derived factors are innocuous and contribute to physiology and development (Coolon et al. 2009; Gracida and Eckmann 2013). Recent work demonstrated that vitamin B12 obtained by C. elegans through its bacterial diet is an important nutritional factor in developmental growth and physiology of C. elegans (MacNeil et al. 2013). The vitamin B12 status of C. elegans can be easily assessed with help of the acdh-1p-GFP reporter, which is expressed in response to propionate accumulation resulting from B12 deficiency (Watson et al. 2013, 2014, 2016). When fed a vitamin B12-deficient Escherichia coli OP50 diet, acdh-1 is highly expressed in animals, whereas acdh-1 is lowly expressed when grown on the vitamin B12-producing Comamonas aq. DA1877 diet. The effects of these bacterial diets on acdh-1 promoter activity have led to important insights into the vitamin B12-dependent and independent propionate breakdown pathways.

Caenorhabditis elegans is also an ideal organism to study the plasticity in expression levels of individual chemosensory receptor genes in response to external and internal signals (Gruner and van der Linden 2015; Vidal et al. 2018). Our prior study showed that the expression levels of the srh-234 chemoreceptor gene in the ADL sensory neuron type is regulated by starvation. This starvation-mediated modulation of srh-234 expression levels is dependent on sensory inputs into ADL neurons perceiving food presence, and circuit inputs from RMG interneurons that are electrically connected to ADL perceiving internal state of starvation signals (Gruner et al. 2014). Circuit inputs from RMG into ADL
regulating srh-234 required the NPR-1 neuropeptide receptor acting in RMG, as well as insulin signals from other tissues acting on the DAF-2 insulin receptor in ADL (Gruner et al. 2014). In addition, starvation-mediated regulation of srh-234 expression levels in ADL is regulated by both cell- and cell-autonomous transcriptional mechanisms involving basic helix-loop-helix (bHLH) factors, including HLH-30 and Mxi-3 acting in the intestine, and HLH-2/3 acting together with the MEF-2 factor in ADL neurons (Gruner et al. 2016). Together, these findings demonstrated that expression of the srh-234 chemoreceptor gene in a single ADL sensory neuron type of C. elegans is regulated by multiple transcriptional modules, and revealed a neuron-to-intestine connection involving insulin signals in the modulation of chemoreceptor genes as a function of the C. elegans feeding state (Gruner and van der Linden 2015).

In this study, we discovered that feeding C. elegans vitamin B12-producing Comamonas aq. bacteria regulates the expression levels of the srh-234 chemoreceptor gene in ADL neurons. We show that srh-234 gene expression is repressed in ADL when animals are fed a high vitamin B12 diet of Comamonas aq. DA1877 bacteria relative to a low vitamin B12 diet of E. coli OP50 bacteria. This dietary effect of vitamin B12 on srh-234 in ADL appears to be distinct from the starvation response we reported previously (Gruner et al. 2014). Mutant bacteria of Comamonas aq. deficient in vitamin B12 production, indicated that Comamonas-supplied vitamin B12 regulates srh-234 expression levels in ADL. Similar to feeding C. elegans a Comamonas aq. diet, supplementing the E. coli diet with exogenous vitamin B12 also represses srh-234 expression in ADL neurons, in which E. coli may function as a vehicle for vitamin B12 uptake by C. elegans. The repressing effects of vitamin B12 on srh-234 expression in ADL neurons can be suppressed by excess propionate supplementation and genetic perturbations in the canonical and shunt propionate degradation pathways. Lastly, we show that the MEF-2 transcription factor and its binding site in the srh-234 cis-regulatory sequence are necessary for the vitamin B12-mediated repression of srh-234 expression in ADL. Together, these findings reveal that bacterially derived vitamin B12 turn individual chemoreceptor genes on and off at the level of transcription in sensory neurons that may inform our understanding of how animals fine-tune their chemosensory responses to biologically relevant chemicals in their diet.

Materials and methods

Caenorhabditis elegans strains and growth conditions

Strains used in this study were: wild-type N2 C. elegans variety Bristol, RB1774 pocc-1(ok2282), VC1307 pccb-1(ok1686), RB1434 mmcm-1(ok1637), VC1011 acdh-1(ok1489), RB2572 hphd-1(ok3580), RB755 met-1(ok521), VC1527 nhr-68(k708), JIN1375 hlh-30(3m1978), and KM134 met-2(g1u).

To analyze srh-234 expression in mixed bacterial diets, animals carrying the srh-234p::GFP reporter were exposed to mixed set ratios, i.e. 1:1, 9:1, and 99:1 ratio of E. coli OP50 to Comamonas aq. DA1877. To prepare plates, liquid bacterial cultures of OP50 and DA1877 were grown overnight at 37°C in LB and diluted with LB or concentrated to the same OD 600. Bacteria were seeded onto peptone-free NGM plates. Animals expressing the srh-234p::GFP reporter were transferred to plates and removed after laying eggs. Eggs were then allowed to develop to adults. The increased rate of development when fed Comamonas aq. DA1877 was accounted for, and levels of promoter::GFP expression of adult animals were then imaged and measured under a microscope equipped with epifluorescence as described previously (Gruner et al. 2014, 2016). Briefly, we mounted animals on 2% agarose pads containing 10 mM levamisole, and visualized them on a Leica DM5500 compound microscope equipped with epifluorescence and a Hamamatsu CCD-camera. Microscope and camera settings were kept constant between images of different genotypes and conditions used, unless indicated otherwise. The mean pixel intensity of GFP fluorescence in the entire cell body of ADL was quantified using Volocity software (version 6.3). Prior to measurement, images of ADL cell bodies were cropped for promoter::GFP expression level analysis.

Bacterial strains and growth conditions

Bacterial strains used in this study were: E. coli OP50, E. coli HT115 (DE3), E. coli HB101, E. coli BW25113, E. coli ΔtonB JW5195, Comamonas aq. DA1877, Comamonas aq. ΔchiA, and ΔchiB mutants.

Animals carrying chemoreceptor::GFP reporter genes (i.e. srh-234, sre-1) were cultivated at 20°C on NGM plates seeded with E. coli OP50 as the bacterial food source unless indicated otherwise. Gravid adults were transferred to assay plates and removed after laying eggs. The eggs were then allowed to develop to adults. The increased rate of development when fed Comamonas aq. DA1877 was accounted for, and levels of promoter::GFP expression of adult animals were then imaged and measured under a microscope equipped with epifluorescence as described previously (Gruner et al. 2014, 2016). Briefly, we mounted animals on 2% agarose pads containing 10 mM levamisole, and visualized them on a Leica DM5500 compound microscope equipped with epifluorescence and a Hamamatsu CCD-camera. Microscope and camera settings were kept constant between images of different genotypes and conditions used, unless indicated otherwise. The mean pixel intensity of GFP fluorescence in the entire cell body of ADL was quantified using Volocity software (version 6.3). Prior to measurement, images of ADL cell bodies were cropped for promoter::GFP expression level analysis.

Analysis of srh-234p::GFP expression

To analyze srh-234 expression in mixed bacterial diets, animals carrying the srh-234p::GFP reporter were exposed to mixed set ratios, i.e. 1:1, 9:1, and 99:1 ratio of E. coli OP50 to Comamonas aq. DA1877. To prepare plates, liquid bacterial cultures of OP50 and DA1877 were grown overnight at 37°C in LB and diluted with LB or concentrated to the same OD 600. Bacteria were seeded onto peptone-free NGM agar plates to minimize bacterial growth. Adults expressing the srh-234p::GFP reporter were transferred to plates and removed after eggs were laid. Eggs were allowed to develop to adulthood in the presence of the mixed bacterial diets, and srh-234p::GFP expression levels were measured and quantified as described above.

To analyze srh-234 expression in the presence of exogenous vitamin B12 and propionic acid (aka propionate), animals carrying the srh-234p::GFP reporter were transferred to NGM plates seeded with E. coli OP50 supplemented with or without vitamin B12 (methylcobalamin or Me-Cbl, Sigma, Cat. M9756; adenosylcobalamin or Ado-Cbl, Sigma, Cat. C0884) and propionic acid (Sigma, Cat. P1386). Stocks were made in either ethanol (for Me-Cbl) and water (for Ado-Cbl) and water (for Ado-Cbl and propionic acid) to the maximum soluble concentration. Vitamin B12 and propionic acid was diluted to a final 64 mM and 40 mM concentration, respectively, in NGM agar prior to plate pouring. For E. coli OP50 supplementation assays with increasing Me-Cbl concentrations, we created a dilution series from a 1-mM Me-Cbl stock. To confirm vitamin B12 action,
acdh-1p::GFP reporter animals were used as a control in parallel to the srh-234p::GFP expression analysis. For bacterial olfactory assays, we exposed srh-234p::GFP reporter animals to a 1-cm² NGM agar square soaked with 1 mM Me-Ctl placed on petri dish lids. For the quadrant petri dish assay, NGM plates were seeded in each quadrant with a 100-μl spot of either E. coli OP50 or Comamonas aq. DA1877 diets. srh-234p::GFP reporter animals were then transferred to a single quadrant of the plate allowing only a single diet for food ingestion, while allowing olfactory cues of the surrounding diets.

Dye filling of ADL sensory neurons

A stock dye solution prepared in DMSO containing 5 mg/ml red fluorescent lipophilic dye Dil (Sigma, Cat. 42364) was diluted in M9 buffer to a final concentration of 5 μg/ml for optimal signal intensity. Animals carrying the srh-234p::GFP reporter (VDL3) were soaked in Dil for 1 h and then rinsed with M9 buffer twice. Stained animals were recovered for 1 h on NGM plates seeded with either E. coli OP50 or Comamonas aq. DA1877 before examination of dye-filled ADL neurons with a Leica DMS500 microscope equipped with epifluorescence.

Analysis of ADL neuron morphology

To examine the integrity of ADL neurons, animals carrying the integrated sire-1p::GFP reporter (VDL497) were cultivated at 20°C on NGM plates seeded with Comamonas aq. DA1877. Briefly, we mounted animals on 2% agarose pads containing 10 mM levamisole and visualized them on a Leica Thunder 3D Tissue microscope equipped with a DFC 9000 GT/C sCMOS camera. Z-stack images were obtained at 500 ms exposure time. Small volume computational clearing was applied to the image data set, and a maximum intensity projection was generated.

Statistical analysis

All results are expressed as means with 95% confidence intervals. Data sets were first analyzed for Gaussian distribution using a normality test (alpha = 0.05, P > 0.05) using either the Shapiro–Wilk test or D’Agostino and Pearson normality test to determine whether a parametric or nonparametric statistical test should be performed. Statistical comparisons made for 2 groups include an unpaired t-test (parametric) or the Mann–Whitney t-test (non-parametric). For more than 2 groups, the ordinary 1-way ANOVA (parametric) or the Kruskal–Wallis test (non-parametric) was used followed by a posthoc multiple comparisons test. Specific statistical tests and P-values are reported in the figure legends. All data were graphed and analyzed using Prism 9 software.

Results

The Comamonas aq. DA1877 diet represses srh-234 expression in ADL neurons

To study how bacterial diet regulates chemoreceptor gene expression levels in C. elegans, we used the candidate srh-234 chemoreceptor gene specifically expressed in a single sensory neuron type, ADL. We previously found that GFP expression driven by only 165 bp cis-regulatory sequence of srh-234 (referred here as srh-234p::GFP) is rapidly (<1h) downregulated in starved animals (Gruner et al. 2014). While testing the srh-234p::GFP reporter in different bacterial diets, we observed that animals fed a Comamonas aq. DA1877 diet repress srh-234 expression in ADL neurons similar to that observed in starved animals (Gruner et al. 2014); that is srh-234p::GFP expression levels in adult animals is strongly decreased when fed a Comamonas aq. DA1877 diet compared to a E. coli OP50 diet (Fig. 1a). This Comamonas-mediated repression of srh-234 expression levels is rapid as adult animals raised on E. coli OP50 and then transferred to a DA1877 diet decrease srh-234p::GFP expression in ADL by 50% about 2 h after the transfer (Supplementary Fig. 1a). Animals fed other E. coli diets such as the K12/B-type hybrid HB101 strain, and the K12-type HT115 strain commonly used in C. elegans research showed a srh-234 expression phenotype intermediate to that of E. coli OP50 and Comamonas aq. DA1877 diets (Supplementary Fig. 1b).

The dietary effect of Comamonas aq. DA1877 on srh-234 expression appears to be distinct from the starvation response, because mixing the E. coli OP50 diet with Comamonas aq. DA1877 diet 1:1 resulted in animals in which srh-234 expression levels remained strongly decreased similar to starvation (Fig. 1b). Moreover, smaller concentrations of Comamonas aq. DA1877 by diluting it in E. coli OP50 (i.e. 9:1 and 99:1 OP50/DA1877) was sufficient to strongly repress srh-234 expression. Others have reported that Comamonas aq. DA1877 bacteria are not a nutrient-poor diet for C. elegans (Shtonda and Avery 2006; MacNeil et al. 2013), suggesting that Comamonas aq. DA1877 may generate a bacterial signal that regulates srh-234 expression levels. This dietary effect of...
Comamonas on srh-234 may be specific since expression of another ADL-specific sre-1 chemoreceptor is not affected (Supplementary Fig. 1c). Since we previously showed that lack of functional ADL cilia can dramatically decrease srh-234p::GFP expression levels (Gruner et al. 2014), it is possible that the Comamonas diet affects the integrity of ADL neurons resulting in the reduced srh-234 expression. However, we found that wild-type animals fed a Comamonas aq. DA1877 diet show normal ADL morphology determined by sre-1p::GFP expression (Supplementary Fig. 1, d and e) and wild-type dye filling (Supplementary Fig. 1f) ruling out cilia defects of ADL neurons fed on the Comamonas aq. DA1877 diet. Together, these results suggest that in addition to starvation, a dilutable bacterial metabolite produced by Comamonas aq. DA1877 bacteria represses srh-234 expression levels in ADL neurons.

**Vitamin B12 produced by Comamonas aq. represses srh-234 expression**

The strain Comamonas aq. DA1877 produces the dilutable metabolite vitamin B12, while the E. coli OP50 strain is not able to synthesize vitamin B12 (Watson et al. 2014). To test the hypothesis that vitamin B12 represses srh-234 expression levels in ADL neurons, we examined C. elegans animals fed an E. coli OP50 diet supplemented with 2 biologically active and interconvertible forms of vitamin B12, adenosylcobalamin (Ado-Cbl), and methylcobalamin (Me-Cbl). We found that animals fed an E. coli OP50 diet supplemented with either 64 nM vitamin B12 (Ado-Cbl or Me-Cbl) repress srh-234p::GFP expression in ADL neurons (Fig. 2a). Moreover, supplementing E. coli OP50 with increasing concentrations (nM doses) of vitamin B12 (Me-Cbl) resulted in a dose-dependent decrease in srh-234p::GFP expression (Supplementary Fig. 2a), which fits with our observation that diluting Comamonas aq. DA1877 into the E. coli OP50 diet is sufficient to repress srh-234 expression (Fig. 1b). As a control, we found similar dose-dependent repressive effects of vitamin B12 (Me-Cbl) using the acdh-1p::GFP dietary reporter expressed primarily in the intestine (MacNeil et al. 2013; Watson et al. 2014) when fed either the vitamin B12-producing Comamonas aq. DA1877 or the E. coli OP50 diet supplemented with vitamin B12 (Me-Cbl) (Supplementary Fig. 2b). These results are also consistent with the observed decrease in srh-234 expression when animals were fed on E. coli HT7115 and E. coli HB101 diets (Supplementary Fig. 1b), which are better sources of vitamin B12 levels than E. coli OP50 (Revtovich et al. 2019). Thus, vitamin B12 supplementation to the E. coli OP50 diet can repress srh-234 expression in ADL neurons.

To further test whether Comamonas-supplied vitamin B12 regulates srh-234 expression in ADL, we took advantage of 2 mutant strains of Comamonas aq. bacteria (ΔcbiA and ΔcbiB) that are deficient in vitamin B12 production (Watson et al. 2014). We found that feeding animals ΔcbiA and ΔcbiB bacterial mutants fail to repress srh-234p::GFP expression compared to DA1877-fed animals (Fig. 2c). As expected, ΔcbiA and ΔcbiB bacterial mutants also fail to repress acdh-1 expression as determined by the acdh-1p::GFP reporter (Supplementary Fig. 2d). Together, these findings suggest that vitamin B12 synthesized by Comamonas aq. DA1877 bacteria repress srh-234 expression in ADL.

**Escherichia coli may act as a conduit for vitamin B12 uptake by C. elegans, to in turn regulate srh-234 expression**

Since E. coli OP50 bacteria supplemented with exogenous vitamin B12 can repress srh-234 expression, we reasoned that E. coli may function as a vehicle for the provision and uptake of vitamin B12 by C. elegans, to in turn regulate srh-234 expression in ADL neurons. Alternatively, it remains possible that the availability of vitamin B12 in these supplementation experiments can also be directly perceived by ADL and regulate srh-234. To distinguish between these possibilities, we first tested whether vitamin B12 can be perceived as a volatile olfactory cue by ADL neurons to regulate srh-234. We exposed animals to live Comamonas aq. DA1877 which they cannot eat or touch, while feeding live E. coli OP50, and vice versa (Fig. 3a). In addition, we exposed animals expressing srh-234p::GFP to plates seeded with live E. coli OP50 that were covered with petri-dish lids containing NGM agar squares soaked with 1 mM of vitamin B12 (Me-Cbl) placed above the animals (Supplementary Fig. 3a). In both olfactory assays, we found that srh-234 expression in ADL was not significantly altered when exposed to volatile cues of Comamonas aq. bacteria or the addition of exogenous vitamin B12. Thus, vitamin B12 may not act as a volatile chemical cue to repress srh-234 expression, but we cannot exclude the possibility that vitamin B12 may still be directly sensed by ADL neurons.

We next tested whether E. coli bacteria could act as a vehicle for vitamin B12 uptake by C. elegans in vitamin B12 supplementation experiments. The tolR transporter in E. coli has been shown to be vital for vitamin B12 uptake from its extracellular environment (Bassford et al. 1976; Kadner 1990). Recent work showed that C. elegans animals fed an E. coli BW25113 diet in which the
tonB transporter gene is deleted (ΔtonB) can block, at least in part, the repression of the acdh-1p::GFP reporter when the growth media is supplemented with vitamin B12 (Revtovich et al. 2019). Based on these observations, we hypothesized that the effects of vitamin B12 on repressing srh-234 expression would also be dependent on the E. coli TonB pathway. We found that supplementing the E. coli BW25113 diet with vitamin B12 (Ado-Cbl or Me-Cbl) repressed srh-234p::GFP expression similar to that observed for E. coli OP50-fed animals supplemented with vitamin B12 (Fig. 3b). When tonB was deleted (ΔtonB), we found a small, but statistically significant (P < 0.05) block in the repression of srh-234 by Ado-Cbl but less so with Me-Cbl (Fig. 3b). Thus, E. coli bacteria via the TonB transporter may be an important determinant for the vitamin B12 uptake by C. elegans to in turn regulate srh-234 expression. However, because ΔtonB did not completely block the effects of vitamin B12 on repressing srh-234, it is likely that there are alternative TonB independent routes to obtain vitamin B12 from the media.

To further validate that E. coli bacteria acts as a vehicle for vitamin B12 uptake by C. elegans, we asked whether heat-killed E. coli bacteria supplemented with vitamin B12 could repress srh-234 expression. Growing worms on heat-killed E. coli bacteria is challenging, because heat destroys some of the nutrients needed for worms to develop and renders the bacteria less edible, resulting in larvae that arrest their development (Qi et al. 2017). We therefore exposed animals to live E. coli OP50 until adulthood, and then transferred young adults to plates seeded with E. coli OP50 killed by high heat (>75 °C) with and without vitamin B12 (Me-Cbl). Adults fed heat-killed E. coli OP50 without vitamin B12 (HK-OP50) grew slowly as expected and resulted in animals with significantly reduced srh-234p::GFP expression 48h after their transfer (Supplementary Fig. 3b), mimicking the starvation-induced

Fig. 3. Escherichia coli bacteria act as a vehicle for vitamin B12 uptake by C. elegans, to in turn regulate srh-234 expression. a) Top panel: Schematic of a bacterial olfactory assay setup using quadrant assay plates. Adult animals are placed into 1 quadrant allowing only 1 diet to be ingested, while surrounding quadrants are seeded with either the E. coli OP50 diet (control) or the Comamonas aq. DA1877 diet. Lower panel: Relative expression of srh-234p::GFP in the ADL cell body of adults fed on either the E. coli OP50 or Comamonas aq. DA1877 diet on quadrant plates with surrounding inaccessible diets. Data are represented as the mean ± SEM (n > 23 animals for each diet). The Kruskal–Wallis with Dunn multiple-comparisons test was used to determine the statistical significance of differences vs wild-type animals fed E. coli (OP50-OP50), with brackets indicating statistical differences between 2 specific conditions. ns, not significant, ***P < 0.001. b) Relative expression of srh-234 in the ADL cell body of adults fed the E. coli ΔtonB mutant (strain JW5195) compared to its parental wild-type strain (BW25113) supplemented with or without Ado-Cbl and Me-Cbl (64 nm final concentrations). Data are represented as the mean ± SEM (n = 14–28 animals for each condition). The Kruskal–Wallis with Dunn multiple-comparisons test was used to determine the statistical significance of differences vs wild-type animals fed E. coli BW25113, with brackets indicating statistical differences between 2 specific bacterial genotypes and conditions. ns, not significant, *P < 0.05, **P < 0.01. Right panel: srh-234p::GFP is weakly expressed in adults when fed E. coli BW25113 compared to E. coli OP50. a, b) Representative cropped images of srh-234p::GFP expression in the ADL cell body of adults. Images were acquired at the same exposure time for comparison.
repressing effects of vitamin B12 on breakdown pathways also lead to changes in propionate buildup due to genetic perturbations in these propionate activity. We found that expression of also block the repressive effects of vitamin B12. Similarly, mutation of effects of vitamin B12 on (Fig. 4c). Thus, consistent with excess propionate overriding the vitamin B12 as a cofactor is not significantly different in animals encoding the methylmalonyl-CoA mutase that requires DA1877. Interestingly, acdh-1 in propionate shunt pathway genes, (Watson 2013, 2014, 2016) (Fig. 4a). The balance between vitamin B12 and propionyl-CoA levels involved in this propionate breakdown controls promoter activity of the acdh-1 gene; that is excess propionate is able to override the repressing effects of vitamin B12 on acdh-1 expression (Watson et al. 2016). Similarly, we found that animals fed an E. coli OP50 diet supplemented with both vitamin B12 (Me-Cbl, 64 nM) and excess propionate (40 mM) can override the repressing effects of vitamin B12 on srh-234p::GFP expression (Fig. 4b).

Genetic perturbation of the canonical and propionate shunt breakdown pathway can also lead to propionate accumulation, such that loss of both pcca-1 and acdh-1 function results in synthetic lethality likely due to toxic effects of propionate buildup (Watson et al. 2013, 2014, 2016). We therefore tested whether propionate buildup due to genetic perturbations in these propionate breakdown pathways also lead to changes in srh-234 promoter activity. We found that expression of srh-234p::GFP in mutants of mccc-1 encoding the methylnaloyl-CoA mutase that requires vitamin B12 as a cofactor is not significantly different in animals fed E. coli OP50 with or without exogenous vitamin B12 (Me-Cbl) (Fig. 4c). Thus, consistent with excess propionate overriding the effects of vitamin B12 on srh-234 expression, mccc-1 mutations also block the repressive effects of vitamin B12. Similarly, mutations in pcco-1 and pccb-1 genes (Supplementary Fig. 4, a and b) or in propionate shunt pathway genes, acdh-1 and hphd-1 (Fig. 4d and Supplementary Fig. 4c) can also, at least in part, block the repressing effects of vitamin B12 on srh-234 when fed on Comamonasaq. DA1877. Interestingly, srh-234p::GFP expression is slightly increased in mutants of mccc-1, pcco-1 and pccb-1 compared to wild-type animals when fed on E. coli OP50, which could be explained by a further accumulation of propionate in these conditions. Mutations in the methionine/SAM cycle gene, metr-1, did not show significant differences in srh-234p::GFP expression compared to wild-type animals when fed on Comamonasaq. DA1877 (Fig. 4e). All mutants tested showed a normal dye filling of ADL neurons (Supplementary Fig. 4d). These findings suggest that mainly genetic perturbations in the canonical and propionate shunt breakdown pathways can alter srh-234 expression levels in ADL.

The nuclear hormone receptor, nhr-68, is involved in activating shunt gene expression in the C. elegans intestine in response to excessive propionate levels, and mutations in nhr-68 are proposed to mitigate propionate toxicity (Balcha et al. 2019). We found that animals with mutations in nhr-68 show reduced srh-234p::GFP expression when fed E. coli OP50 supplemented with or without exogenous vitamin B12 (Me-Cbl) compared to wild-type animals (Supplementary Fig. 4e). Feeding nhr-68 mutants E. coli OP50 supplemented with both vitamin B12 (Me-Cbl) and excess propionate (40 mM) resulted in significantly higher srh-234p::GFP expression levels (P < 0.0001) than compared to wild-type (Supplementary Fig. 4e). That is, adding excess propionate to nhr-68 mutants could modestly override the repressing effects of vitamin B12 on srh-234 expression, but not to the same extent as in wild-type animals. Thus, nhr-68 is an important determinant in mitigating the response to excessive propionate levels on srh-234 expression.

Together, these findings suggest that excess propionate can override the repressing effects of vitamin B12 on srh-234 expression in ADL neurons.

MEF-2 is required for the vitamin B12-mediated repression of srh-234 expression

To further interrogate the mechanisms underlying the vitamin B12-dependent regulation of srh-234 gene expression in ADL neurons, we examined candidate components and pathways. We previously reported that the MEF-2 transcription factor acts together with bHLH factors to regulate the starvation-dependent regulation of srh-234 expression (Gruner et al. 2016). In this mechanism, MEF-2 acts cell-autonomously with bHLH factors HLH-2/HLH-3 in ADL neurons, while HLH-30 and MLX-3 bHLH factors function in the intestine to non-cell-autonomously regulate srh-234 expression in ADL in response to starvation signals. We found that a mutation in mef-2 but not in hlh-30 can fully suppress the vitamin B12-dependent reduction in srh-234 expression when animals were fed a Comamonasaq. DA1877 diet, suggesting that the MEF-2 factor is required for the vitamin B12-dependent regulation of srh-234 (Fig. 5a and Supplementary Fig. 5a). We next took advantage of srh-234p(-MEF-2)::GFP reporter animals in which the core of the MEF2 binding in the srh-234 promoter is mutated (Gruner et al. 2016), and found that vitamin B12-supplemented E. coli OP50 bacteria fail to repress srh-234p(-MEF-2)::GFP expression compared to srh-234p(WT)::GFP expressing animals (Supplementary Fig. 5b). These results suggest that the MEF-2 factor and its binding site in the srh-234 promoter are necessary for the vitamin B12-dependent repression of srh-234 expression.

We next examined whether the MEF-2 binding site in the srh-234 promoter was sufficient for the vitamin B12-dependent regulation of srh-234. To test this, we used a transgenic reporter strain of the srh-1 promoter fused to gfp with or without the MEF-2 binding site identified in the srh-234 promoter (AGTTATATTATAA) (Supplementary Fig. 5c) (Gruner et al. 2016). The srh-1 promoter is specifically and highly expressed in ADL neurons, but levels of srh-1 expression are not significantly changed in animals fed the vitamin B12-producing Comamonasaq. DA1877 bacteria (Supplementary Fig. 1c). Surprisingly, we found that animals carrying a transgene of the srh-1 promoter with the inserted srh-234 MEF-2 site (srh-1p(+MEF2)::GFP) showed similar srh-1 expression levels in ADL neurons when fed Comamonasaq. DA1877 compared to wild-type srh-1p::GFP animals (srh-1p(WT)::GFP) on the same diet (Fig. 5b). These results suggest that in contrast to the starvation-dependent regulation of srh-234 (Gruner et al. 2014), insertion of the MEF2 binding site alone is not sufficient for the vitamin B12-dependent regulation of srh-234 expression levels in ADL neurons, suggesting the requirement of another yet
unknown factor that may act together with the MEF-2 factor (Fig. 5c).

Taken together, these findings show that the repressing effects of vitamin B12 on the expression of srh-234 in ADL neurons are dependent on the MEF-2 transcription factor.

Discussion

In this study, we show that the expression levels of the srh-234 chemoreceptor gene in the ADL sensory neuron type is regulated by dietary vitamin B12. In a low vitamin B12 E. coli diet, srh-234 is highly expressed in ADL but not when C. elegans is fed a high vitamin B12-producing Comamonas ag. DA1877 diet (Fig. 6). Excess propionate and genetic perturbations in the propionate breakdown pathways are able to override the repressing effects of vitamin B12 on srh-234 expression. In addition, the vitamin B12-mediated regulation of srh-234 is dependent on the MEF-2 transcription factor. The mechanisms by which dietary vitamin B12 transcriptionally tunes srh-234 could provide C. elegans the means to modify long-term changes in ADL-mediated responses.

This study complements our previous work (Gruner et al. 2014, 2016), which explored the dynamics in srh-234 expression upon...
starvation, which was dependent on MEF-2 function and its respective MEF2 binding site present in the cis-regulatory sequence of srh-234. Similarly, we show that loss of MEF-2 function can inhibit the repression of srh-234 expression in ADL in response to vitamin B12 produced by Comamonas aq. DA1877 diets, suggesting that MEF-2 has dual roles in regulating srh-234 expression in response to both starvation and dietary vitamin B12. However, unlike starvation (Gruner et al. 2016), artificial introduction of the srh-234 MEF2 binding site into the cis-regulatory sequence of the sre-1 gene close to its E-box site that drives expression in ADL.

**Fig. 5.** mef-2 is required for vitamin B12-dependent regulation of srh-234. 

a) Relative expression of srh-234 in the ADL cell body of mef-2 and hli-30 mutants when adults are fed a Comamonas aq. DA1877 diet compared to an E. coli OP50 diet. Data are represented as the mean ± SEM (n > 22 animals). The Kruskal–Wallis with Dunn multiple-comparisons test was used to determine the statistical significance of differences vs wild-type animals fed E. coli OP50, with brackets indicating statistical differences between 2 specific genotypes and conditions. ns, not significant, **P < 0.01, ***P < 0.001. Right panel: Representative cropped images of srh-234p::GFP expression in the ADL cell body. Images were acquired at the same but at a lower exposure time.

b) Relative expression of wild-type sre-1p::GFP (sre-1p[WT]::GFP) or the sre-1 promoter with the inserted MEF2 binding site sequence from the srh-234 promoter (sre-1p(+MEF2)::GFP) in the ADL cell body of adults fed a Comamonas aq. DA1877 diet compared to an E. coli OP50 diet. Data are represented as the mean ± SEM (n = 21–40 animals). For sre-1 expression, data were normalized to the sre-1p::GFP reporter for animals fed E. coli OP50. For srh-234 expression, data were normalized to srh-234p::GFP for animals fed E. coli OP50. The Kruskal–Wallis with Dunn multiple-comparisons test was used to determine the statistical significance of differences vs wild-type animals fed E. coli OP50, with brackets indicating statistical differences between 2 specific genotypes and conditions. ns, not significant, *P < 0.05, ***P < 0.001. Right panel: Representative cropped images of GFP expression under control of the srh-234 or sre-1 promoter with or without the srh-234 MEF2-binding site in the ADL cell body. Images were acquired at the same exposure time.

c) Model based on findings shown in panel (b) explaining the observed expression changes for srh-234 in mef-2 mutants, and sre-1 with a srh-234 MEF2-binding site artificially inserted in its promoter upstream and close to the identified E-box that drives sre-1 expression in the ADL neuron. +, ++, and +++(+ +++) indicates low, high, and highly increased expression levels, respectively.
levels of srh-234 in C. elegans animals is high (++) when fed a low vitamin B12 diet of E. coli OP50 bacteria (a) but low (+) when fed a high vitamin B12 diet of Comamonas aq. DA1877 bacteria (b). An unknown factor (X) may act together with the MEF-2 transcription factor to repress srh-234 expression levels under conditions of high vitamin B12 via a bHLH/E-box module important to promote expression of srh-234 in ADL.

neurons (McCarroll et al. 2005) did not confer vitamin B12-induced repression via MEF-2. Instead, insertion of the srh-234 MEF-2 site in the sre-1 promoter showed higher sre-1 expression levels when fed the high vitamin B12 Comamonas aq. DA1877 diet compared to the low vitamin B12 E. coli diet. One possible explanation for the increased sre-1 expression levels in ADL is that MEF-2 acts to promote (or enhance) sre-1 transcription via its respective bHLH/E-box module after introducing the MEF2 site near the sre-1 E-box site. In mammals, it is known that MEF2 factors can act either to activate or repress transcription depending on its interacting cofactors (Molkentin et al. 1995; Black et al. 1996). Thus, MEF-2 may act to repress or promote expression based on the different bHLH factors that drive srh-234 and sre-1 transcription in ADL via their respective E-box sites contained in their promoters (McCarroll et al. 2005; Gruner et al. 2016).

Based on these findings, we propose a model (Fig. 6) in which animals fed a high vitamin B12 Comamonas aq. DA1877 diet regulates srh-234 expression via a transcriptional module consisting of a MEF-2 factor and its respective MEF2 binding site, together with a yet unknown factor (X) stimulated by dietary vitamin B12. In this turn may repress bHLH factors through an E-box site that promotes srh-234 in ADL neurons via a complex mechanism involving a combination of different bHLH heterodimer pairs (Gruner et al. 2016). When animals are fed a low vitamin B12 diet of E. coli OP50, MEF-2 activity no longer represses srh-234 expression in ADL. Thus, MEF-2 activity is necessary to repress srh-234 in response to vitamin B12, but the exact nature of factor X that may act with MEF-2 remains to be determined.

In C. elegans, vitamin B12 is exclusively obtained from its diet, and our findings support that vitamin B12 produced by Comamonas aq. can repress srh-234 expression in ADL neurons. We further showed that E. coli bacteria via the TonB transporter may function as a vehicle for vitamin B12 uptake by C. elegans, and its uptake in turn may regulate srh-234. However, tonB mutations did not completely block the repression of srh-234 expression when vitamin B12 was added to the growth media. These findings suggest that E. coli may have alternative tonB independent routes to obtain vitamin B12 from the media consistent with previous reports using the acdh-1p::GFP reporter (Revtovich et al. 2019). Alternatively, the increased availability of exogenous vitamin B12 added to the media may be directly perceived by ADL sensory neurons, which could play additional roles in the repression of srh-234 expression in ADL. Using olfactory assays, we showed that vitamin B12 does not appear to act as a volatile chemical cue to regulate srh-234 in ADL neurons, but we cannot exclude the possibility that vitamin B12 is also directly sensed by ADL in the vitamin B12 supplementation experiments. Our previous work showed that elimination of all sensory inputs into ADL neurons strongly decrease srh-234 expression in E. coli OP50-fed conditions similar to the effects of vitamin B12 (Gruner et al. 2014; Gruner and van der Linden 2015), and thus it would be challenging to investigate whether physical or genetic manipulations of ADL sensory dendrites and/or cilia important for sensing the environment can alter the effects of vitamin B12 on srh-234 expression. Future studies investigating intracellular calcium dynamics in ADL in the presence of vitamin B12 using a genetically encoded Ca^{2+} sensor have the potential to reveal whether ADL neurons can also directly sense vitamin B12.

The balance between vitamin B12 and propionate levels in C. elegans is important for tuning srh-234 promoter activity in ADL neurons. Mutations in the canonical (pcca-1, pccb-1, and mmm-1) and the shunt propionate (acdh-1, hpd-1) breakdown pathways, the propionate persistence detector (nhr-68), as well as propionate supplementation, were all able to override the repressing effects of vitamin B12 on srh-234 expression. In mammalian models of propionic acidemia, animals lacking the propionyl CoA-carboxylase (PCCA) were found to have elevated propionate levels shortly after birth (Miyazaki et al. 2001). Similarly, pcca-1-mutant animals in C. elegans have naturally elevated propionate levels that cannot be restored to normal levels by vitamin B12 sufficient diets alone (Watson et al. 2016). Consistent with a persistent accumulation of propionate in C. elegans in regulating srh-234 promoter activity, we show that mutants of mmm-1, pcca-1, pccb-1 significantly enhance the levels of srh-234 expression on an E. coli OP50 diet that is unable to efficiently breakdown propionate by the canonical pathway. Conversely, srh-234 expression levels are strongly reduced in ADL when exposed to low propionate levels; for instance, in animals that are food deprived (starved) or exposed to high vitamin B12 conditions. Studies in rats demonstrated that after 2 days of starvation, propionate levels are rapidly decreased but again restored after refeeding (Illman et al. 1986).

What are the functional consequences of the regulation of chemoreceptor gene expression by dietary-supplied vitamin B12?
The nociceptive ADL neuron where srh-234 is specifically expressed mediates avoidance responses to a wide variety of environmental signals such as odors (Troemel et al. 1995, 1997; Chao et al. 2004), pheromones (Jang et al. 2012), and heavy metals (Sambongi et al. 1999; Wen et al. 2020). Since chemoreceptor genes expressed in a specific sensory neuron type are generally linked to a common chemical response determined by the identity of the neuron in *C. elegans*, with a few exceptions in which neurons switch their preference toward odors (Tsunozaki et al. 2008), it is probable that the srh-234 chemoreceptor may detect aversive stimuli perceived by ADL. Interestingly, vitamin B12 in mammals has anti-nociceptive properties (Erfanparast et al. 2014), and the activity of certain olfactory receptors in tissues other than neurons can respond to propionate (Pluznick et al. 2013), which is a metabolic byproduct produced by gut bacteria in mammals (Morrison and Preston 2016). It is therefore tempting to speculate that dietary-supplied vitamin B12 can alter ADL-mediated nociceptive responses to specific cues by changing the expression levels of chemoreceptor genes. However, nothing is known about whether vitamin B12 or propionate levels affects ADL-mediated responses in *C. elegans*. Other than growth, development, and lifespan (Bito et al. 2013; MacNeil et al. 2013), only recently vitamin B12 in the diet has been reported to be an important micronutrient in the regulation of predatory behaviors between nematodes (Akduman et al. 2020).

In summary, the srh-234 chemoreceptor gene is one of a large repertoire of over 1,300 chemoreceptor genes (Robertson 2000), many of which are localized in a relatively small subset of chemosensory neurons (Vidal et al. 2018), such that each neuron expresses multiple chemoreceptor genes. We show here dynamic changes in the expression levels of the srh-234 chemoreceptor gene localized in the ADL sensory neuron type in response to changing dietary vitamin B12. Other studies have illustrated that dynamic expression changes in individual chemoreceptor genes can have profound effects on behavioral outcomes. For instance, changes in the expression levels of the odr-10 olfactory receptor required to sense diacetyl (Sengupta et al. 1996) in the male *C. elegans* contributes to its plasticity in food detection and feeding/exploration decisions in order to locate mates (Ryan et al. 2014). Further research will determine what the functional consequences are of the plasticity in srh-234 chemoreceptor gene expression in ADL neurons in response to dietary vitamin B12.

## Data availability

All data are available as part of this manuscript. Strains and plasmids are available upon request. Supplementary data and information are available online.

Supplemental material is available at G3 online.

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## Conflicts of interest

None declared.

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