Bacterial diet affects vulval organogenesis in Caenorhabditis elegans Mediator kinase module mutants

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Abstract

Diverse intrinsic and extrinsic signals converge on gene expression regulation during animal development. The Mediator complex is a transcriptional co-regulator complex that integrates such signals to coordinate gene expression changes. Here, we show that ectopic vulval organogenesis in *Canorhabditis elegans* Mediator kinase module mutants, a hallmark of derepressed epidermal growth factor receptor-Ras-extracellular signal regulated kinase (EGFR-Ras-ERK) signaling, is influenced by dietary signals. Feeding *C. elegans* two different strains of *Escherichia coli*, the standard laboratory strain OP50 and the feeding RNA interference strain HT115, causes substantial changes in the penetrance of ectopic vulva formation in kinase module mutants. Conversely, a preferred bacterial food source of *C. elegans*, *Comamonas aquatica*, causes no change in ectopic vulva formation penetrance compared to the standard OP50 food source. Thus, the bacterial diet of *C. elegans* can influence a developmental phenotype driven by EGFR-Ras-ERK signaling in Mediator kinase module mutants. We discuss the possible mechanisms by which *E. coli* OP50 and HT115 may influence vulval organogenesis in this system.

Introduction

The Mediator complex is a multi-subunit transcriptional coregulator that integrates signals from multiple pathways to coordinate gene expression output [1] [2] [3]. Individual Mediator subunits are thus capable of modulating specific developmental and physiological processes. In the nematode worm *Caenorhabditis elegans*, the Mediator kinase module, consisting of the subunits cyclin-dependent kinase 8 (CDK-8), CIC-1/cyclin C, MDT-12/MED12, and MDT-13/MED13, inhibits vulval organogenesis [4] [5]. *C. elegans* vulval organogenesis is induced by epidermal growth factor receptor-Ras-extracellular signal regulated kinase (EGFR-Ras-ERK) signaling [6]. Kinase module loss-of-function mutants display ectopic induction of EGFR-driven vulval cell fates (multivulva phenotype, Muv) and show hallmarks of decreased transcriptional repression by the EGFR signaling effector transcription factor LIN-1/Ets as well as increased transcriptional activation by other Mediator subunits; thus, the kinase module normally restrains transcription downstream of the EGFR-Ras-ERK pathway [5].

The standard laboratory diet of *C. elegans* is the *Escherichia coli* strain OP50, which is derived from the *E. coli* B strain. Alternatively, to perform feeding RNA interference (RNAi), *C. elegans* is commonly fed the specifically engineered *E. coli* strain HT115, which is derived from the *E. coli* K-12 strain [7]. Feeding on these distinct *E. coli* strains impacts the *C. elegans* host. For example, OP50 is higher in triacylglycerols (TAGs) than HT115, and worms show a corresponding change in TAG storage [8]. Moreover, OP50 and HT115 show distinct interactions with specific genes of the *C. elegans* host; for example, worms carrying a mutation in the gene encoding nuclear hormone receptor *nhr-114* are sterile on an OP50 diet, but fertile when feeding on HT115 or on various other bacterial species such as the soil bacterium *Comamonas aquatica* [9], a preferred *C. elegans* food source compared to *E. coli* [10]. Compared to worms fed OP50, worms fed *C. aquatica* show substantially altered gene expression, including downregulation of many metabolic enzymes; moreover, worms fed *C. aquatica* develop more rapidly and live shorter than worms fed *E. coli* OP50 or HT115 [11]. In sum, the bacterial food source of *C. elegans* can alter its metabolism, development, and longevity, including mutant-specific phenotypes.

Objective

We aimed to investigate whether the bacterial food source, that is, different *E. coli* strains and *C. aquatica*, would affect vulval phenotypes of *C. elegans*, especially in mutants of the Mediator kinase module.
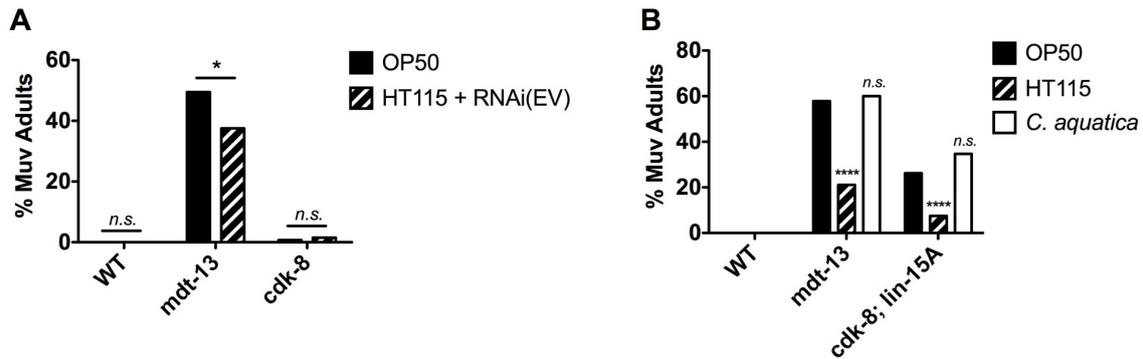
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Figure Legend

Figure 1. Bacterial food source influences vulva development in kinase module mutants.

(A) Adult Muv penetrance in wild type (WT), *mdt-13*, and *cdk-8* mutants fed *E. coli* OP50 or HT115 transformed with RNAi empty vector (EV) (n >180 for each condition). *p <0.05 for indicated comparison, Fisher's exact test.

(B) Adult Muv penetrance in wild type (WT), *mdt-13*, and *cdk-8; lin-15A* mutants fed *E. coli* OP50, untransformed HT115, or *C. aquatica* (n > 140 for each condition). ****p <0.0001 vs. OP50 within same genotype, Fisher's exact test.

C. elegans strains, culture, and genetic methods

C. elegans strains were cultured at 23℃. Wild type was the N2 Bristol strain; strains XA7703 *cdk-8(tm1238) I*, STE74 *cdk-8(tm1238) I; lin-15A(n767) X*, and HS310 *let-19(mn19)/mIn1[dpy-10(e128) mIs14] II* were maintained and studied as described [5]. We used nematode growth medium (NGM)-lite (0.2% NaCl, 0.4% tryptone, 0.3% KH_2PO_4, 0.05% K_2HPO_4) agar plates supplemented with 5 µg/ml cholesterol to culture *E. coli* OP50, untransformed *E. coli* HT115, or *C. aquatica* DA1877 [11]. We used NGM-lite supplemented with 5 µg/ml cholesterol, 12.5 µg/ml tetracycline, 25 µg/ml carbenicillin, and 2 mM IPTG to culture HT115 + RNAi(EV). RNAi empty vector plasmid (L4440) has been described [7].

Multivulva phenotype penetrance

Analysis of Muv morphology was performed as described [18]. Muv phenotype penetrance was scored in synchronous day 1 adult animals under a dissection microscope at 200x magnification (*mdt-13* mutants) or at 56x magnification (all other strains). Worms with ectopic vulval protrusions were scored as Muv. Statistical analysis was performed using Fisher's exact test using GraphPad Prism. **Results & Discussion**

While studying the role of Mediator in EGFR signaling in *C. elegans*, we observed striking differences in Muv penetrance of *mdt-13* mutants fed different bacterial food sources: *mdt-13* mutants fed HT115 transformed with an empty RNAi vector (HT115 + RNAi(EV)) showed significantly lower Muv penetrance than those fed OP50 (Fig. 1A). All 4 Mediator kinase module subunits are required to repress vulval organogenesis in *C. elegans*, but *mdt-12* and *mdt-13* mutants display a much more penetrant Muv phenotype than *cdk-8* or *cic-1* mutants [5]. Unlike the effect seen in *mdt-13* mutants, the Muv penetrance of the *cdk-8* mutant did not change significantly on HT115 + RNAi(EV) vs. OP50 (Fig. 1A); however, this could be due to the very low penetrance of the Muv

phenotype in the *cdk-8* mutant (Fig. 1A). Therefore, for further investigation, we utilized a sensitized genetic background, *cdk8; lin-15A*, which shows enhanced Muv penetrance compared to the *cdk-8* single mutant [5].

Using *mdt-13* and *cdk-8; lin-15A* double mutants, we tested whether the HT115 bacterial food source itself, rather than the RNAi(EV) vector or the selective media used for RNAi experiments, was responsible for the observed changes in *mdt-13* Muv penetrance (Fig. 1A). Indeed, *mdt-13* mutants fed untransformed HT115 bacteria and grown on standard media displayed a substantially and significantly reduced Muv penetrance compared to *mdt-13* mutants fed OP50 (Fig. 1B). We observed a similar effect in *cdk-8; lin-15A* mutants (Fig. 1B). Thus, different *E. coli* strains can modulate ectopic vulval organogenesis in two Mediator kinase module mutants.

C. aquatica has profound effects on the developmental rate of *C. elegans* [11], and, like an HT115 diet, completely suppresses the sterility of *nhr-114* mutants [9]. Therefore, we reasoned that *C. aquatica* might also affect the formation of ectopic vulvae during development. Surprisingly, we observed no change in Muv penetrance of *mdt-13* or *cdk-8; lin-15A* mutants fed *C. aquatica* vs. OP50 (Fig. 1B). This experiment demonstrates that 2 strains of *E. coli* compared in this study, OP50 and HT115, can have more disparate effects on vulva formation in Mediator kinase module mutants than species from two different bacterial genera, *E. coli* OP50 vs. *C. aquatica.*

The results presented here demonstrate that the bacterial diet of *C. elegans* can influence ectopic vulva formation in Mediator kinase module subunit mutants. Ectopic vulva formation in these mutants arises due to derepression of EGFR-Ras-ERK signaling-driven cell fates [5]. Thus, our findings imply that the worm diet may modulate EGFR-Ras-ERK signaling pathway activity, at least in Mediator kinase module mutants. As human Mediator kinase module subunit mutations have been identified in several tumor types [12] [13] [14] [15] [16], it would be of interest to investigate whether such tumors are sensitive to changes in nutrient availability.

The nutritional signals that alter vulval organogenesis in Mediator kinase module mutants remain unidentified, but comparison of lipid composition of OP50 vs. HT115 may provide some clues. OP50 is higher in TAGs than HT115, which causes accumulation of TAGs in *C. elegans* fed OP50 compared to HT115 [8]. Interestingly, the TAG content of the *C. elegans* diet correlates inversely with the accumulation of branched-chain fatty acids (BCFAs) in worms [8]. Feeding mice a high-fat diet has been linked to a decrease in short chain fatty acids (SCFAs), which are precursors of BCFAs. This decrease in SCFAs alters the microbiota composition of the intestinal tract of mice expressing a Ras gain-of-function allele in the intestine (RasG12Dint), and intestinal dysbiosis increases tumor incidence in RasG12Dint mice via activation of immune signaling [17]. It is thus tempting to speculate that the differences we observed in Muv penetrance of kinase module mutant worms, akin to tumor incidence in mice, are due to alterations in BCFAs caused by a high-fat diet, which may favor activation of an intestinal immune response. Clearly, many aspects of this model must be tested, such as whether EGFR-Ras-ERK signaling-driven cell fates occur at higher incidence in worms fed OP50 vs. HT115, whether TAGs are the main driver of the observed phenomenon, and whether blocking an immune response can reverse the observed effects of OP50. If these predictions hold true, then it will provide compelling evidence to test whether human tumor cell lines with mutations in Mediator kinase module subunits are responsive to nutritional cues.

Conclusions

We have shown that ectopic vulval organogenesis in *C. elegans* Mediator kinase module mutants is altered by bacterial diet.

Limitations

We have not directly assessed whether bacterial diet modulates EGFR-Ras-ERK signaling-driven vulval cell fates, or whether it modulates vulva development via a parallel pathway such as Notch or Wnt signaling, which are also known to affect vulva development [6].

It is possible that, compared to an OP50 diet, an HT115 diet reduces Muv penetrance

because it generally slows down development; however, larval development as judged by the fraction of individual worms reaching specific L3, L4, or adult stages, was not significantly different between worms fed OP50 or HT115 [11].

Our analysis was limited to the phenotype of a mutant, as wild type animals essentially never show the Muv phenotype. Nevertheless, it is likely that EGFR signaling is modulated by different food sources in wild-type worms as well. Assessing other EGFR-dependent organogenesis events such as the excretory duct cell [18] could shed light on food signal modulation of EGFR signaling in wild type worms.

We do not know whether food source components directly affect EGFR signaling. It is alternatively possible that changes in Tor signaling, which is responsive to nutrient content, influences EGFR-Ras-ERK output. Indeed, wild type animals fed with the *E. coli* HB101 strain develop faster than those fed OP50, and developmental acceleration depends on *rict-1*, a component of TORC2 [19]. Although as noted HT115 does not accelerate development [11], it may still affect Tor signaling. A genetic and biochemical analysis of this pathway in the Mediator kinds module mutants should be informative. As two strains of the same bacterial species had more disparate effects on vulval organogenesis than strains from two different bacterial genera, our findings hint at a role for dietary lipid content, which is known to differ between the two studied *E. coli* strains. As ectopic vulva formation in kinase module mutants stems from derepression of EGFR-Ras-ERK signaling-driven cell fates, our findings suggest a role for dietary lipids in modulating this oncogenic pathway in *C. elegans*.

Additional Information

Methods
C. elegans strains, culture, and genetic methods
C. elegans strains were cultured at 23℃. Wild type was the N2 Bristol strain; strains XA7703 *cdk-8(tm1238) I*, STE74 *cdk-8(tm1238) I; lin-15A(n767) X*, and HS310 *let-19(mn19)/mIn1[dpy-10(e128) mIs14] II* were maintained and studied as described [5]. We used nematode growth medium (NGM)-lite (0.2% NaCl, 0.4% tryptone, 0.3% KH_2PO_4, 0.05% K_2HPO_4) agar plates supplemented with 5 µg/ml cholesterol to culture *E. coli* OP50, untransformed *E. coli* HT115, or *C. aquatica* DA1877 [11]. We used NGM-lite supplemented with 5 µg/ml cholesterol, 12.5 µg/ml tetracycline, 25 µg/ml carbenicillin, and 2 mM IPTG to culture HT115 + RNAi(EV). RNAi empty vector plasmid (L4440) has been described [7].
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Supplementary Material
Please see https://sciencematters.io/articles/201605000012.

Ethics Statement
Not applicable.

Citations

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