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# A Mediator subunit imparts robustness to a polyphenism decision

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**Polyphenism is a type of developmental plasticity that translates continuous environmental variability into discontinuous phenotypes. Such discontinuity likely requires a switch between alternative gene-regulatory networks, a principle that has been borne out by mechanisms found to promote morph-specific gene expression. However, whether robustness is required to execute a polyphenism decision has awaited testing at the molecular level. Here, we used a nematode model for polyphenism, *Pristionchus pacificus*, to identify the molecular regulatory factors that ensure the development of alternative forms. This species has a dimorphism in its adult feeding structures, specifically teeth, which are a morphological novelty that allows predation on other nematodes. Through a forward genetic screen, we determined that a duplicate homolog of the Mediator subunit MDT-15/MED15, *P. pacificus* MDT-15.1, is necessary for the polyphenism and the robustness of the resulting phenotypes. This transcriptional coregulator, which has a conserved role in metabolic responses to nutritional stress, coordinates these processes with its effects on this diet-induced polyphenism. Moreover, this MED15 homolog genetically interacts with two nuclear receptors, NHR-1 and NHR-40, to achieve dimorphism: Single and double mutants for these three factors result in morphologies that together produce a continuum of forms between the extremes of the polyphenism. In summary, we have identified a molecular regulator that confers discontinuity to a morphological polyphenism, while also identifying a role for MED15 as a plasticity effector.**

developmental plasticity | MED15 | switch genes | threshold traits

## Significance

The ability to produce multiple phenotypes from a single genotype, or developmental plasticity, allows organisms to match phenotypes to their surroundings within their lifetime. For this response to be adaptive, perception of the environment must be predictably processed into those phenotypes. This response's reliability is exemplified in the case of dimorphism, where phenotypes are stereotypical alternatives that result from a decision made during development. Reliability likely requires mechanisms for robustness, especially where decision-making factors ("switch genes") influence those that build the phenotype itself ("effector genes"). Our finding that a well-known mediator of gene transcription, together with two other factors, ensures the outcomes of a dimorphism gives a molecular context to predictions about how plastic developmental decisions are realized.

Developmental plasticity is a fundamental feature of the complex route from genotype to phenotype in animals and plants (1, 2). The degree to which environmental influence is represented in the distribution of plastic trait values, however, varies. This is well demonstrated by discontinuous plasticity, or polyphenism, an exemplar for the nonlinearity between upstream signals and traits whose development is influenced by the environment (3, 4). Because of this nonlinear relationship, polyphenism has been predicted to have distinctive and especially strong evolutionary impacts (5). For example, it can hide genetic variation that influences plasticity, since different genotypes converge on the same, canalized phenotypes of a threshold response (6–9). Moreover, polyphenism may, through conditional (i.e., not constitutive) expression of alternative morphs, control how gene products influenced by the threshold response are exposed to selection (10–12). To test such predictions, what distinguishes polyphenism from continuous plasticity, or reaction norms, must be understood in terms of defined molecular mechanisms.

Polyphenisms have been proposed to arise from continuous plasticity, either through a steepening of the threshold between phenotypes or by environmental exclusion of intermediate forms (4). The continuum between smooth reaction norms and threshold phenotypes is supported by known evolutionary transitions, such as in wing spots in *Bicyclus* butterflies (13, 14) and head horns in *Onthophagus* dung beetles (15). What defines a threshold, and thus how a plastic response reaches a binary decision, has been increasingly tested through molecular manipulations (16–21). Unlike continuous plasticity, even when it is marked by a steep threshold, fully discontinuous alternatives likely require a transcriptional switch between developmental networks, demanding an additional step in their regulatory logic (22). The switch-like activation of polyphenism gene networks has long been supported by molecular studies, such as of the dauer decision of *Caenorhabditis elegans* and the wing polyphenism of ants (23, 24). Yet even reaction norms may hold modularity in their gene regulation, and thus it is possible that they are not incompatible with, and might give rise to, discontinuous plasticity (25, 26). By this scenario, the molecular factors that keep the execution of these networks distinct should inform which general principles ensure the robustness of polyphenic traits.

An example of a "hard" polyphenism controlled by a switch is in the nematode *Pristionchus pacificus*. This species has two adult morphs that differ in their feeding structures: One morph ("stenostomatous," St) is microbivorous, while the alternative

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("eurystomatous," Eu) is predatory on other nematodes. These morphs are alternatively induced by, and favored under, microbe-rich and starved conditions, respectively (27–30). The two morphs differ in the shape of their feeding structures, specifically teeth, which are an evolutionary novelty in the family that includes *P. pacificus* (Diplogastridae). In *P. pacificus*, the polyphenism switch involves a series of enzymes and a nuclear receptor (NHR-40), each of which can fully convert between alternative morphs (16, 31–35) (Fig. 1*A*). Downstream of this molecular switch, the suites of "effectors" that execute the ultimate phenotypes have also been described (36–38). In addition, some factors—specifically, the heat-shock chaperone Hsp90 and the nuclear receptor NHR-1—increase morphological variation within morphs when defective, showing the possibility to uncover buffering mechanisms in this system (38, 39). Given defined switch genes and downstream effector networks, perturbations of this system could, in principle, reveal which and how molecular factors maintain a threshold trait in this species.

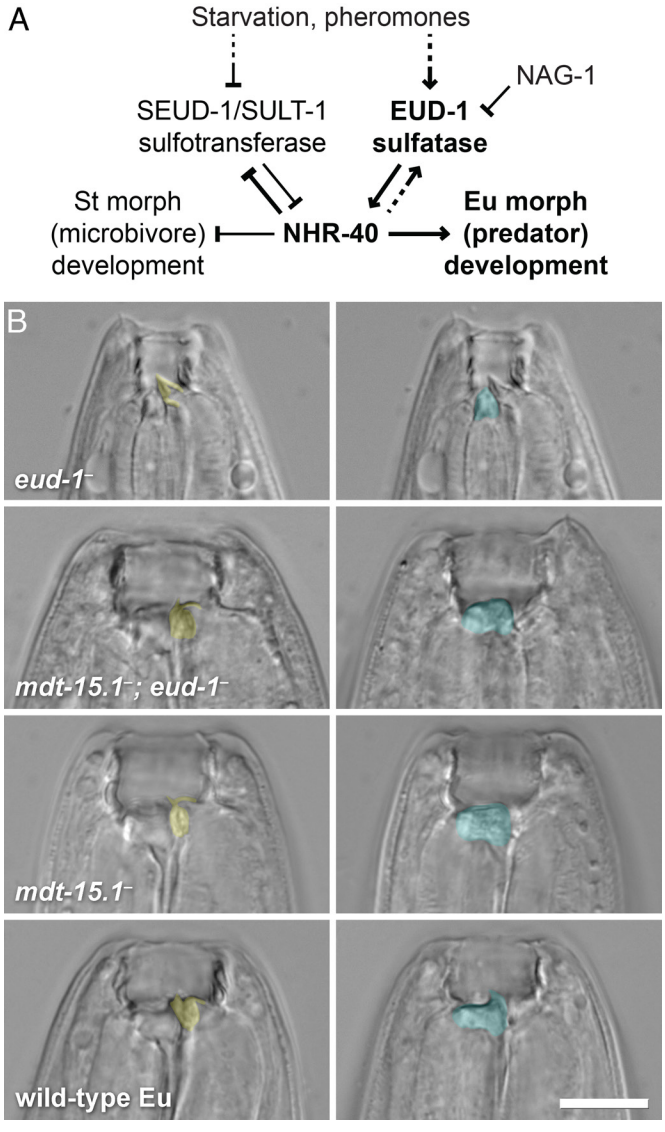


**Fig. 1.** Disruption of a hard polyphenism in the nematode *Pristionchus pacificus*. (*A*) A step-response model for polyphenism regulation. (*B*) Morphologies of polyphenism mutants compared to the wild-type Eu morph, from top to bottom: St-constitutive line *eud-1(tu445)*, which was suppressed in forward screen; aberrant Eu-like morphology of suppressor mutant *mdt-15.1(iub10)* in *eud-1(tu445)* background; *mdt-15.1(iub19)* mutant phenotype, similar to double-mutant phenotype above; wild-type Eu morph of reference strain PS312. Each individual is shown in a row of two focal planes: left column is in plane of dorsal tooth (false-colored yellow), right column in plane of right subventral tooth or ridge (aqua). (Scale bar, 10 μm, for all images.)

Here, we took an unbiased search for genes that are necessary to realize a polyphenism decision. Our search revealed a recently duplicated homolog of a transcriptional coregulator that is conserved across eukaryotes. Queries of this gene's function show that this coregulator has, in diplogastrid nematodes, expanded its functional repertoire to control plasticity in a novel morphology. Consequently, our findings give molecular insight into how developing organisms ensure discrete alternatives in threshold traits.

## Results and Discussion

**Mutants in a Mediator Subunit Suppress a Polyphenism Switch.** To identify molecular factors needed to execute a polyphenism, we conducted a forward screen for suppressors of the polyphenism switch-gene *eud-1*. Because *eud-1* loss-of-function mutants are, regardless of environmental conditions, unable to produce the predatory Eu morph (i.e., are only St), this screen was designed to reveal mutants that fully reverse the constitutive phenotype (i.e., are only Eu) as well as other mutants that depart from the St phenotype. Following random mutagenesis of the mutant line *eud-1(tu445)*, nine mutants were isolated from a screen of ~10,300 haploid genomes. While six of these mutants simply converted the phenotypes to being constitutively Eu, as previously described (31, 33), three of them were constitutive for an alternative, Eu-like morphology. Specifically, mutants showed characteristics intermediate between morphs as previously defined (28), particularly a weakly sclerotized dorsal tooth and loss of the subventral tooth's cusp (Fig. 1*B*). These three mutants, *sup(iub9)*, *sup(iub10)*, and *sup(iub12)*, fell into a single complementation group. After backcrossing the mutants and resequencing their genomes, we found a single gene with potentially harmful lesions in all three lines, specifically one nonsense and two splice-site mutations (*SI Appendix*, Fig. S1 and Table S1). This gene, located on Chromosome III, encodes a homolog of the Mediator subunit MED15, a transcriptional coregulator conserved from yeast to humans (40). In *C. elegans*, MDT-15/MED15 regulates, together with numerous nuclear hormone receptors, responses to metabolic and other stresses (41, 42). Even in yeast, MED15/Gal11p interacts with a nuclear-receptor analog (Oaf1p) to regulate fatty acid-dependent regulation of fat-metabolism genes, indicating a conserved function for MED15 in lipid homeostasis (43). Thus, this finding suggests that an ancient mechanism for responding to nutrient availability has acquired a role in the novel feeding polyphenism of diplogastrid nematodes.

**A Polyphenism Effector Evolved Through Turnover of a Conserved Physiological Regulator.** To identify the origins of this polyphenism effector, we identified homologs of this gene across several diplogastrid nematodes and outgroups. We found that, despite the conservation of *mdt-15/MED15* across eukaryotes, *P. pacificus* carries a second homolog of the gene, on the opposite arm of Chromosome III. Phylogenetic inference revealed that an ancestral *mdt-15* gene duplicated into two copies that have both persisted in several *Pristionchus* species (Fig. 2*A*), showing unexpected evolutionary turnover in this highly connected regulator. To test whether the gene we identified from our forward screen, which we named *mdt-15.1*, has specialized to assume its polyphenism function in *P. pacificus*, we created knockout mutants for this gene and its sister copy, *mdt-15.2* (*SI Appendix*, Fig. S1 and Table S2). Specifically, we created mutants that have the same genetic background ("wild-type" reference strain, PS312), such

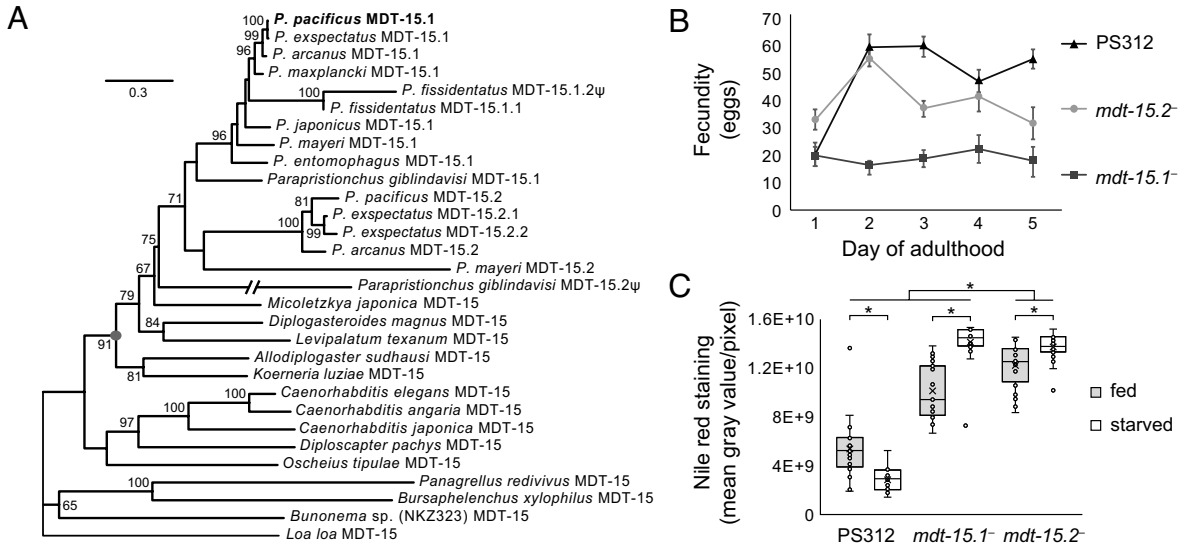


**Fig. 2.** Evolution and specialization of *mdt-15* in *Pristionchus* nematodes. (*A*) Gene history of *mdt-15* in diplogastrid nematodes and nonpolyphenic outgroups. *mdt-15* was duplicated at least once in Diplogastridae, with both duplicates persisting in several *Pristionchus* species. (*B*) Fecundity as a measure of the physiological effects of *mdt-15.1* and *mdt-15.2* mutants. Whiskers represent the SE. (*C*) Fat storage of *mdt-15.1* and *mdt-15.2* mutants as measured by staining with the vital dye Nile red. Values represent the product of area and fluorescence intensity measured across the bodies of individual, young adult nematodes. Asterisks indicate significant differences ($P_{adj}$ < 0.001, Wilcoxon rank-sum).

that they would not have any other polyphenism switch-gene defects; this test would allow us to determine whether mutant phenotypes simply modify the mouth morphology, while still producing two morphs, or suppress the switch itself (i.e., show an aberrant and constitutive phenotype). Further, we created the double mutants *mdt-15.2⁻; eud-1⁻* and *mdt-15.1⁻; mdt-15.2⁻* to test for epistasis of *mdt-15.2* over the switch and execution of the mouth polyphenism. Finally, we knocked out an exon missing from an alternative isoform ("b") of *mdt-15.1* to test the possibility that this and the other isoform ("a") differ in their influence on the polyphenism.

From these tests, we found that mutants in regions common to both *mdt-15.1* isoforms had the same constitutive phenotypes as the suppressor mutant (*mdt-15.1⁻; eud-1⁻*) lines from our screen (Table 1). Additionally, the knockout specific to *mdt-15.1a* had the same mutant phenotype, indicating this isoform to be indispensable for the polyphenism. In contrast to the fixation of a single morph in *mdt-15.1* mutants, *mdt-15.2⁻* animals were indistinguishable from the wild type in both their morphology and the ability to produce the St morph (*SI Appendix*, Figs. S2 and S3). Consistent with *mdt-15.2* having no observed effects on the polyphenism switch, *mdt-15.2⁻; eud-1⁻* double mutants were, like *eud-1* single mutants, constitutively St. The relatively few *mdt-15.1⁻; mdt-15.2⁻* double mutants that reached adulthood were constitutive for the same aberrant, Eu-like morphology of *mdt-15.1* single mutants (Table 1 and *SI Appendix*, Figs. S2 and S3).

**Table 1. Polyphenism phenotypes of mutants for *mdt-15.2* and two isoforms of *mdt-15.1* in *P. pacificus* (*n* = 120 unless otherwise indicated)**

| Mutant line | Phenotype |
|---|---|
| PS312 (wild type) | Eu-biased (93%) |
| *eud-1(tu445)* | Fixed St |
| *mdt-15.1(iub10); eud-1(tu445)* | Fixed aberrant "Eu" |
| *mdt-15.1(iub18)* | Fixed aberrant "Eu" |
| *mdt-15.1(iub19)* | Fixed aberrant "Eu" |
| *mdt-15.1a(iub23)* | Fixed aberrant "Eu" |
| *mdt-15.2(iub32)* | Eu-biased (89%) |
| *mdt-15.2(iub35)* | Eu-biased (96%) |
| *mdt-15.2(iub35); eud-1(tu445)* | Fixed St |
| *mdt-15.1(iub42); mdt-15.2(iub35)* | Aberrant "Eu" (*n* = 19) |

Although we did not detect a role for *mdt-15.2* in the polyphenism, we examined whether *mdt-15.1* and *mdt-15.2* have specialized in their roles in regulating other, physiological processes. First, we measured a basic life-history trait, fecundity, known to be affected by *mdt-15* knockdowns in *C. elegans* (44). As in the latter species, *mdt-15.1* mutants had lower fecundity than PS312 individuals ($t$ = 5.17, $P$ = 1.06 × $10^{-6}$, LMM; Fig. 2*B*). Although *mdt-15.2* single mutants did not have significantly lower fecundity than the wild type ($t$ = 0.12, $P$ = 0.91), *mdt-15.1⁻; mdt-15.2⁻* double mutants were mostly sterile, and the few eggs they produced showed lethality of the embryos, indicating overlap in the two genes' physiological function. Second, we tested whether fat storage is regulated by *mdt-15.1* or *mdt-15.2* in response to fasting, a mouth-polyphenism induction cue in *P. pacificus* (27). In *C. elegans*, knockdown of *mdt-15* interferes with fatty-acid ratios, leading to altered fat storage, as detected by increased staining by the lipophilic dye Nile red (44). When we likewise assayed fat storage in *P. pacificus*, we found that fasting induced the depletion of intestinal fatty acids in the wild type ($P_{adj}$ = 5.6 × $10^{-5}$, Wilcoxon rank-sum). In contrast, this response was defective in both *mdt-15.1* and *mdt-15.2* mutants, which instead showed more staining in starved over fed individuals ($P_{adj}$ < 0.001 for both; Fig. 2*C*). Further, both mutants showed increased staining, whether starved or fed, over the wild type ($P_{adj}$ < 0.001 for all comparisons). Thus, both *mdt-15* duplicates have retained a role in fatty-acid metabolism, despite only *mdt-15.1* influencing the mouth polyphenism. Together, our results show that *mdt-15.1* expanded its functional repertoire to controlling polyphenism while, together with its semiortholog *mdt-15.2*, maintaining its physiological importance in *P. pacificus*.

**Polyphenism-Biased Genes Are Enriched in Both the Gut and Polyphenic Tissues.** To investigate the molecular function of *mdt-15.1* as a polyphenism effector, we studied its activity on the networks executing dimorphic morphologies. Using RNA-seq to identify transcriptomic differences of *mdt-15.1(iub19)* mutants from PS312, we determined the set of genes whose expression *mdt-15.1* regulates in *P. pacificus* (Dataset S1). Because we expected *mdt-15.1* to be pleiotropic in its transcriptional signatures, we also identified which *mdt-15.1*-regulated genes were involved in the polyphenism per se. Specifically, we determined the genes' overlap with networks of polyphenism-biased genes, as previously defined by transcriptomic comparisons of three Eu-constitutive lines (*seud-1/sult-1*$^-$, *nhr-40* gain-of-function, *eud-1* over-expression) to a St-constitutive one, *eud-1*$^-$ (36, 37). As predicted, *mdt-15.1*-regulated expression included many genes previously confirmed or inferred to be part of the *P. pacificus* plasticity pathway (Dataset S2). Moreover, the proportion of shared genes was higher than expected by chance ($\chi^2$ = 47.9, df =1, $P = 4.5 \times 10^{-12}$). Among shared genes were those encoding the environmental sensor TAX-2, a cGMP-activated cation channel (45), a suite of astacins regulated by both NHR-40 and NHR-1 in pharyngeal gland cells (38), and several nuclear receptors (36), as well as several collagen genes possibly involved in the morphology itself.

Having identified that overlap, we then inferred the organism-wide function of *mdt-15.1*, mapping its targets to the spatial transcriptome of *P. pacificus*. This transcriptional map, which was previously built using RNA-tomography, identified genes from across the genome that were enriched in specific body regions (46). We found *mdt-15.1*-regulated genes to be overrepresented among these enriched "regional genes" across most of the body, although overrepresentation of *mdt-15.1*-regulated genes that were also polyphenism biased was restricted to five of eleven defined body regions. One of these was the mouth, which was not enriched among *mdt-15.1* targets alone and is the site of the dimorphic mouthparts; the others were the brain, gut (two, anterior and posterior), and tail (Fisher's exact test; Fig. 3*A*; *SI Appendix*, Table S3; and Dataset S3). Upregulation of polyphenism-biased targets of *mdt-15.1* specifically in the gut suggests that this factor influences not only plastic morphologies but also the anticipated diets associated with them. Thus, genetic manipulation of *mdt-15.1* alone, in the absence of starvation cues, is sufficient to trigger transcription likely associated with both developmental and metabolic responses. To additionally characterize *mdt-15.1* activity, we expressed reporters of *mdt-15.1* both by transgenic arrays and by insertion of an epitope tag directly into the endogenous copy of the gene. Although expression reports varied within and among lines, some individuals showed expression in facial cells that produce the mouth itself (*SI Appendix*, Fig. S4 and Table S4). Together, these results indicate that *mdt-15.1* has retained its predicted role as a physiological mediator while also assuming the regulation of feeding morphologies specific to diplogastrid nematodes.

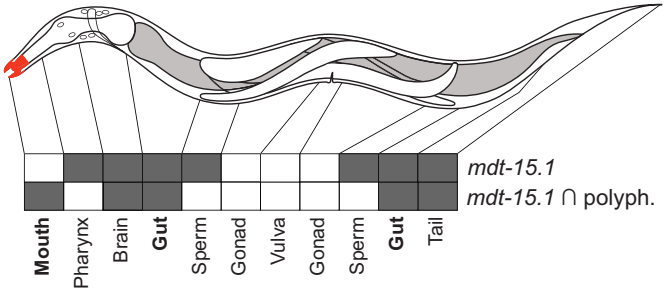


**Fig. 3.** *Pristionchus pacificus mdt-15.1*-biased gene expression across body regions. As informed by a previously built transcriptomic map for *P. pacificus* (45), dark boxes show expression significantly enriched for all *mdt-15.1*-regulated genes (above) and that for *mdt-15.1*-regulated genes that overlap with targets common to three polyphenism switch genes (*eud-1, nhr-40, seud-1/sult-1*).

**Conserved Activity of *mdt-15.1* Was Integrated into a Novel Developmental Response.** Given the importance of a Mediator subunit in regulating a novel morphology and its associated plasticity, we compared this response to a species that retains the ancestral state in lacking these features, the model nematode *C. elegans*. To do this, we found where the transcriptome regulated by both *P. pacificus mdt-15.1* and other polyphenism control genes overlaps with *mdt-15* regulated transcription in *C. elegans* (Dataset S4). Specifically, we compared our results with a previous transcriptomic contrast with *mdt-15* mutants and the wild type in that species (47). We found that some polyphenism-biased, *mdt-15.1*-regulated genes in *P. pacificus* are likewise regulated by MDT-15 in *C. elegans*, including the fatty acid-Δ9-desaturase genes *fat-6* and *fat-7*. These genes, expressed in the gut of *C. elegans*, are regulated by MDT-15, together with the nuclear receptor NHR-49, and are essential for fat storage (44, 48). As in *C. elegans mdt-15* knockdowns, *P. pacificus mdt-15.1* mutants showed repression of *fat-6* and *fat-7* homologs (Dataset S1), indicating a fasting-like response even when well fed. This result is consistent with our finding that *mdt-15.1* influences fat storage (Fig. 2*C*) and suggests that *mdt-15.1* may coordinate metabolic and developmental responses to the environment in *P. pacificus*. In summary, *P. pacificus*-specific expression reflects the role of MDT-15.1 in a developmental decision as well as the integration of this role with other, metabolic responses to nutritional cues.

***mdt-15.1*, *nhr-1*, and *nhr-40* Defects Together Produce a Continuum of Forms.** Having inferred molecular functions of MDT-15.1 as a polyphenism effector in *P. pacificus*, we then determined how these functions interfere with the polyphenism switch itself. The aberrant mouth morphology of *mdt-15.1* mutants suggested that, in contrast to genes that switch between wild-type developmental programs, is necessary for the robustness of the switch as a binary decision. Because *nhr-1* mutants in *P. pacificus* also have a constitutive mouth phenotype with apparent defects, we studied how these two genes interact to ensure the integrity of the switch. Specifically, we quantified and compared the morphologies of both mutant lines, as well as an *mdt-15.1; nhr-1* double mutant to test for epistasis between the genes. Additionally, we quantified the wild-type (PS312) Eu morph and a *nhr-40* (St-constitutive) mutant to capture the extremes of the polyphenism. Finally, we tested for the possible interaction of *mdt-15.1* with *nhr-40*, which acts upstream of *nhr-1*, by including a double mutant we created. We measured the lines' mouth phenotypes by geometric morphometrics, using a set of landmarks based on individual cell homologies, on which we performed principal component analysis (49, 50) (*SI Appendix*, Fig. S5).

Our analysis showed that the combined activity of *mdt-15.1*, *nhr-1*, and *nhr-40* is responsible for channeling a plastic response into discontinuous phenotypes (Fig. 4*A*). This conclusion is supported by three key findings. First, the morphologies of *mdt-15.1* and *nhr-1* mutants were significantly different from each other (FDR-adjusted $P_{adj} = 2.6 \times 10^{-5}$, Procrustes ANOVA) and from the wild-type Eu (PS312) and an St-constitutive (*nhr-40*$^-$) line, respectively ($P_{adj} = 2.6 \times 10^{-5}$, $P = 2.3 \times 10^{-3}$). Second, we found that *mdt-15.1* and *nhr-40* do not show complete epistasis but

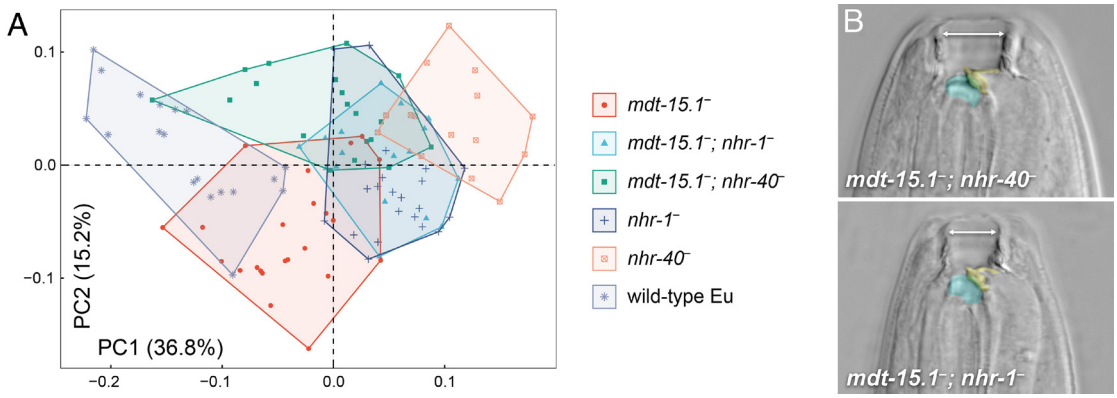


**Fig. 4.** A continuum of plastic phenotypes regulated by *mdt-15.1*, *nhr-1*, and *nhr-40*. (*A*) Principal component analysis of geometric morphometric data of wild-type (PS312) Eu morph, the St-constitutive line *nhr-40(lof)*, and single- and double-mutants that influence the mouth polyphenism of *Pristionchus pacificus*. Functional copies of *mdt-15.1*, *nhr-1*, and *nhr-40* are collectively needed to ensure the robust, binary outputs of the polyphenism. (*B*) Qualitative morphology of *mdt-15.1; nhr-40* and *mdt-15.1; nhr-1* double mutants. Dorsal tooth and right subventral tooth are false-colored yellow and aqua, respectively.

instead produce an alternative phenotype that was significantly different from either *mdt-15.1* or *nhr-40* mutant phenotypes alone ($P_{adj} = 4.5 \times 10^{-5}$ and $P_{adj} = 1.1 \times 10^{-4}$, respectively; Fig. 4*B*). This result indicates that *mdt-15.1* mutants interfere with but do not wholly mask the activity of *nhr-40*. Third, *mdt-15.1; nhr-1* double mutants were indistinguishable from *nhr-1* single mutants in their morphology ($P_{adj} = 0.09$; Fig. 4*B*), indicating complete epistasis of *nhr-1* over *mdt-15.1*. One interpretation of the latter result is that the intermediate, St-like morphology of *nhr-1* mutants may represent a default phenotype in the absence of a functional polyphenism switch or its transcriptional effectors, including NHR-1 and the still unknown polyphenism factor that physically interacts with MDT-15.1. In summary, these results show that *mdt-15.1*, *nhr-1*, and *nhr-40* are jointly responsible for canalizing alternative morphs into a hard polyphenism in *P. pacificus*.

**A Conserved Transcriptional Mediator Canalizes a Polyphenic Response to the Environment.** The essential role of *mdt-15.1* in maintaining polyphenism puts the integrity of discontinuous developmental plasticity into molecular terms. The concept that a distinct switch can achieve complete "developmental conversion" (*sensu* 51) between alternative morphologies (52, 53) has been supported by studies of many animal polyphenisms. The largest body of evidence comes from studies correlating gene expression with differentially induced morphs (e.g., 54–58). Further, the concept has been supported by the discovery of genes that show switch-like activity (16, 17). While studies of polyphenism generally assume the operation of a switch, the question has remained how switch-like outcomes are realized. Here, we have addressed this question by directly perturbing the boundary separating the outcomes of discontinuous plasticity.

The simplest model for polyphenism assumes that the degree of continuity of a plastic response is due to the shape of the threshold itself. According to this model, the discontinuity of environments or differences among hormone titers would be enough to ensure binary outcomes, given a steep enough threshold (59). Consequently, feedback between hormonal controls upstream of a transcriptional switch should result in a rarity of intermediates between the two sides of a threshold. Molecular studies have shown how plasticity thresholds can be steepened by endogenous signaling: In the brown planthopper, the microRNA miR-34 maintains a positive feedback loop between insulin signaling and juvenile hormone signaling pathways to reinforce the binary distinction of alternative wing-length morphs (60); in *Onthophagus* dung beetles, the opposing influences of insulin and Hedgehog signaling has implied their antagonism in promoting horned or hornless males (61, 62). In terms of polyphenism switch mechanisms, thresholds shaped by upstream signaling might be achieved through a compound or "ganged" switch, such that incomplete activation of individual switches result in mosaics, or intermediates, between morphs (5). Once a switch decision has been made, this model assumes that the decision stays true through the alternative gene networks that execute the ultimate phenotypes, down through a precise series of genetic controls (22). However, the possibility remains that downstream buffering of those alternative modules may also ensure a discontinuous response. For example, polyphenism controlled by a master switch might act through subordinate switches, which, if not coordinated, could potentially confound otherwise hierarchical gene expression (5). If so, intermediate phenotypes may result.

To test the possibility of downstream effects on polyphenism outcomes, we screened for suppressors of a gene (*eud-1*) that fully converts between morphs in *P. pacificus*. As a result, we found that a switch-like outcome can indeed be effected through buffering between discrete morphs downstream of a switch network. This result complements an earlier finding in this system, that the robustness of ultimate forms was relaxed through perturbations of Hsp90 (39), a general buffer for developmental variation (63). Although the loss of Hsp90 and subsequent heat-shock left the polyphenism intact (i.e., without intermediates), that study raised the question of what part of a polyphenism, whether in the switch-gene network or phenotype execution networks, is needed to buffer the ultimate phenotypic outcomes (64). Our mutant screen revealed a gene with epistasis over the switch, supporting the idea that integrity of a threshold can occur in the effector-gene network controlled by that switch. The identity of this gene as a transcriptional mediator, which influences the phenotype differently from another transcriptional regulator of the polyphenism, NHR-1, is consistent with the activity multiple, subordinate switches with incomplete control over the ultimate phenotypes. Defects in either regulator results in a failure of the polyphenism, exposing continuity between normally discrete morphs.

Besides probing the boundary that maintains discontinuous plasticity, another insight of our study is to expand the known function of the Mediator complex, specifically MED15. Mediator is well characterized as a master coordinator of RNA Polymerase II gene transcription, including with roles in development (65, 66). Mediator's tail module, which includes MED15, is the part

of the protein complex that transcription factors recruit to their target genes (67). Our finding that a *MED15* homolog is a polyphenism effector expands the known functional repertoire of this gene in animals. In *C. elegans*, MDT-15 has multiple roles in responding to environmental stress, including through lipid metabolism (43, 68), xenobiotic detoxification (69), and response to oxidative stress (70). Additional roles for MED15 in both early and postembryonic development have been found in other organisms, specifically to facilitate TGFβ/Nodal signaling in frog mesendoderm (71), Decapentaplegic signaling in *Drosophila* wing formation (72), and salicylic acid signaling in *Arabidopsis* floral transition (73). Here, we have identified a role for a *MED15* homolog that combines environmental sensitivity with a developmental process. Consequently, this finding makes a critical link between known mechanisms for signaling, through nuclear receptors, and transcription of environmentally sensitive genes. Because *P. pacificus mdt-15.1* acts on genes involved in metabolic homeostasis, such as those encoding FAT-6 and FAT-7, our finding also explains how a coordinated response of both development and metabolism is achieved. The numerous, validated physical interactions of MDT-15 in *C. elegans* offer a suite of obvious candidate factors, especially nuclear receptors (74), to pursue in detailing the mechanism of this response.

In conclusion, laboratory manipulations of a morphological dimorphism revealed an effector essential for imparting robustness to discontinuous plasticity. That this effector is a conserved, well-characterized transcriptional coregulator opens a route to knowing the proximal mechanisms of robustness in switch-like developmental responses.

## Materials and Methods

**Forward Screen for Polyphenism Mutants.** To generate mutants suppressing a polyphenism switch, the null mutant *eud-1(tu445)*, which confers an St-constitutive phenotype in *P. pacificus*, was mutagenized by ethyl methanesulfonate as previously described (31). Mutagenized hermaphrodites (i.e., self-fertilizing, morphological females) were allowed to each produce about 20 $F_1$ offspring, which themselves produced $F_2$ on the same culture plates. After most $F_2$ had reached adulthood, the stage at which the polyphenism is expressed in wild-type nematodes, individuals that deviated from the St phenotype as previously defined (28) were isolated. Specifically, the phenotype screened for was a wide mouth and two teeth (one dorsal, one subventral). The potentially mutant individuals found were then cloned, upon which >100 $F_3$ offspring were screened to confirm the phenotype as conferred by recessive mutant alleles. Three mutants were similar in showing an aberrant phenotype, and factorial, noncomplementation tests were performed between lines. These crosses failed to recover the St phenotype of the mutagenized line, placing all three mutant alleles (*iub9*, *iub10*, and *iub12*) into a single complementation group. Each mutant line was backcrossed to the *eud-1(tu445)* line six times as follows: Mutant hermaphrodites were crossed to *eud-1(tu445)* males, after which $F_1$ males were crossed to the double-mutant *pdl-2(tu463); eud-1(tu445)*, which shows a recessive morphological ("dumpy") phenotype that allowed identification of $BC_1F_1$; the latter were then self-crossed, the Eu $BC_1F_2$ offspring of which were cloned, confirmed to be homozygous by phenotyping, and then backcrossed further as above.

**Genomic Resequencing of Mutant Lines.** Whole-genomic library preparation and sequencing were performed as described in *SI Appendix, Materials and Methods*.

**Identification of Mutant Lesions.** Variants called for each of the resequenced mutant genomes were filtered to background mutations present either in the *eud-1(tu445)* line used for mutagenesis or the *pdl-2(tu463)* line (voucher EJR1018) used to make our marked, double-mutant line for backcrossing. Variants in the filtered lists were then assigned to gene predictions based on the El Paco v. 3 annotation for the El Paco genome assembly (75). The three lists of variants were intersected by their annotation names, producing a list from which we manually validated all homozygous SNPs by inspection of individual, mapped reads in the Integrative Genomics Viewer (76). According to gene predictions, only one gene (gene ID: ppa_stranded_DN24725_c0_g1_i8) was found to have deleterious variants in all three mutant lines, thereby revealing the causal alleles. The ortholog of this gene *C. elegans* was identified as *mdt-15* by reciprocal best BLAST similarity.

**Identification and Phylogenetic Analysis of *mdt-15* Homologs.** Potential homologs of *C. elegans mdt-15* in *P. pacificus* and other Diplogastridae were identified by either one-way or reciprocal best BLAST matches with gene predictions in available genome assemblies for *Pristionchus* species (77), other Diplogastridae (37, 77), and representatives of other clades of Rhabditida. Gene fragments predicted to be missing part of the Mediator functional domain were labeled as putative pseudogenes but still included in our analysis. Phylogenetic inference was performed on amino acid sequences and under the maximum likelihood criterion, as described in *SI Appendix, Materials and Methods*.

**Creation of Knockout Mutants by CRISPR/Cas9.** Putative loss-of-function alleles for *mdt-15.1* and *mdt-15.2* were generated by microinjection of the germline of young adult hermaphrodites, as described in *SI, Appendix, Materials and Methods*. Guide RNAs for CRISPR/Cas9 were designed to ablate Exon 4 (*SI Appendix*, Fig. S1), which incurred a nonsense mutation in an *mdt-15.1* allele (*iub10*) isolated from our forward screen. In the case of *mdt-15.1*, lesions on this exon targeted both isoforms detected for that gene. To create a mutant specific to the isoform *mdt-15.1a*, the exon that was targeted is missing from the alternative isoform, *mdt-15.1b* (*SI Appendix*, Fig. S1). *mdt-15.1* mutants were both generated in the wild-type (PS312) genetic background. To generate a double mutant with *eud-1*, the CRISPR/Cas9-generated *mdt-15.1* allele was crossed into the *eud-1(tu445)* mutant line used in the suppressor screen. To generate double mutants with *mdt-15.2*, *nhr-1*, and *nhr-40* loss-of-function alleles, the Exon 4 was targeted (as above) lines for the latter three mutants. Because *mdt-15.1; mdt-15.2* double mutants were sterile, requiring the allele to be maintained through heterozygotes, phenotypes were scored for individuals who were subsequently genotyped to confirm homozygosity of the *mdt-15.1(lof)* allele. Descriptions of mutants are given in *SI Appendix*, Table S2. Mouth phenotypes, which were scored as above, are reported for adult hermaphrodites and were observed in live individuals by DIC microscopy on a Zeiss Axio Imager.

**Geometric Morphometrics of Mutants.** Shape was quantified for nematode mouthparts in two analyses: first, to confirm qualitative observations that *mdt-15.2* mutants are indistinguishable from the wild type in their mouth morphology (*SI Appendix*, Fig. S3) and, second, to compare the morphologies of known regulators of the mouth polyphenism (Fig. 4). Measurements were of live specimens mounted on 5% agar pads and immobilized with heat. Individuals were observed using a 100× oil objective on a Zeiss Axio Imager, and vertical stacks of images, to allow placement of landmarks in different focal planes, were captured using a Zeiss Axiocam supported by Zeiss ZEN microscopy software. Using Fiji (78), a two-dimensional set of 20 landmarks were then recorded from these images, all observed from their right side and confirmed to be lying laterally by inspection of their cheilostomatal plates. Of the landmarks used, 18 landmarks followed a previous study (39), with two (anterior tips of dorsal and ventral pro/mesostegostom) being omitted to increase reliability of landmark placement and two new landmarks being added to capture i) the dorsal base of the dorsal tooth and ii) the medial apex of the pm1 muscle's insertion on the dorsal tooth (*SI Appendix*, Fig. S5). Procrustes alignment of landmarks, PCA to visualize morphospace, and Procrustes ANOVA to test for differences among nematode lines were performed using the R package geomorph (79), following a previously described protocol for *Pristionchus* nematodes (48). Adjusted P-values were FDR-corrected for multiple tests.

**Measurements of Mutant Fitness.** Fecundity was measured for strains PS312, *mdt-15.1(iub19)*, and *mdt-15.2(iub35)*. To take these measurements, 10 individuals from each strain were transferred as preadults (J4 larvae) to their own agar plates and fed on a bacterial lawn grown from 50 μL *Escherichia coli* OP50. These individuals were then transferred to a new plate every day for four more days, over the course of which all eggs on each plate were counted. Because maturation was only complete in all individuals by day 2, differences were tested among lines across days 2 to 5. Differences were tested using a linear mixed model, as implemented in R v. 4.1.1 (80).

**Measurement of Fat Storage as Regulated by *mdt-15.1* and *mdt-15.2*.** Because knockdown of *mdt-15* and the associated deregulation of fatty acid-Δ9-desaturases was previously shown to lead to increased lipophilic staining by Nile red in *C. elegans* (44), this technique was used to test for similar effects *mdt-15.1(iub19)* and *mdt-15.2(iub32)* mutants, as compared with the wild type (PS312), under both well-fed and starvation conditions. Experimental details are given in *SI Appendix, Materials and Methods*.

**Transcriptomic Analyses to Characterize *mdt-15.1* Function.** RNA was extracted from the wild-type strain PS312 and *mdt-15.1(iub19)* strain as previously described (36). Procedural details for RNA extraction and sequencing are given in *SI Appendix, Materials and Methods*. Differential expression analyses were carried out in R v. 4.1.1 using DESeq2 (81). The FDR-adjusted P-value cutoff for differential expression was set to 0.01. To test for enrichment of *mdt-15.1* targets and polyphenism-biased *mdt-15.1* targets by body region, those lists of genes were intersected with a dataset for which gene expression was determined to be enriched by body region in *P. pacificus* (45). For statistical tests, numbers of target genes across the genome (i.e., not locally enriched) were set as the null. Statistical significance was determined using Fisher's exact test with a Bonferroni correction for multiple comparisons ($\alpha = 0.00313$).

**Comparative Transcriptomics to Study Conserved Function of *mdt-15*.** Raw reads from a previously published *C. elegans* dataset (82) were processed as described above for *P. pacificus*. Three biological replicates of the wild-type N2 and the *mdt-15(tm2182)* mutant strains were used. Reads were aligned to the WS281 *C. elegans* genome. Because we expected a weaker signal in the *C. elegans* dataset, since *mdt-15(tm2182)* reduction-of-function rather than a null mutation, we maximized the chances of identifying shared MDT-15 targets by relaxing the FDR-adjusted P-value cutoff for differential expression to 0.05. To identify homologs of *P. pacificus*, we performed a reciprocal best-hit BLASTp against the *C. elegans* genome.

**Localization of *mdt-15.1* Expression.** Spatial expression of *mdt-15.1* was studied as described in *SI Appendix, Materials and Methods*.

**Data, Materials, and Software Availability.** Raw sequencing reads have been deposited in the NCBI Sequence Read Archive under accession PRJNA922571 (83). All study data are included in the article and/or supporting information.

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