Alanine tRNA translate environment into behavior in *Caenorhabditis elegans*

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Abstract

Caenorhabditis elegans **nematodes produce and keep imprints of attractive chemosensory cues to which they are exposed early in life. These imprints enhance adult chemo-attraction to the same cues. Depending on the number of odor-exposed generations, imprinting is transiently or stably inherited.**

Strinkingly, we have found that early odor-exposed *C. elegans* produce odor-specific forms of the transfer RNA^{Ala} (UGC). Naive animals fed on these tRNAs acquire transient or stable odor-specific imprinting.

The tRNA^{Ala} (UGC) controls *C. elegans* chemo-attractive responses through the multifunctional Elongator complex. Mutations that affect the functions of Elongator sub-units 1 or 3, either impaired chemo-attraction, or definitely abolish responses to the odors nematodes were exposed.

We hypothesize that early olfactory experiences translate into tRNA^{Ala} (UGC) bearing odor-specific signatures. These diffusible odor memory, together with Elongator, can stably reprogram the *C. elegans* chemo-attractive behavior.

Keywords:

C. elegans chemosensory behavior, Olfactory imprinting, Odor-specific memory, Transfer RNA (tRNA), Elongator complex, Odor-specific anosmia, Chemoreceptors, Stable behavioral changes, Non-genetic inheritance.

INTRODUCTION

Nutritional, sensory or emotional parental experiences can be inherited by non-genetic mechanisms. If adaptive responses to different environmental challenges can be transmitted to future generations, the mechanisms by which biological organisms actually translate external signals into heritable information are largely unknown.

Non-coding RNAs had been involved in the memory and transgenerational transmission of environmental informations in the *C. elegans* nematode (***Rechavi et al., 2014; Juang et al., 2013; Hall et al., 2013***) and in the mouse (***Gapp et al., 2014; Grandjean et al., 2015; Benito et al., 2018***).

Understanding how non-coding transcriptomes are modified in response to environmental changes and how these modifications impact germ-line cells and translate into inherited phenotypes non-genetic inheritance represents an important challenge.

In this paper, we identified an Alanine transfer RNA (tRNA) as a molecular link between chemosensory environment and chemosensory responses in *C. elegans*. The behavioral adaptation of *C. elegans* worms to his early olfactory environment can be inherited. We show that this single tRNA molecule could be modified according to the different odors present during the first larval stage L1. Once modified in response to odor stimuli, these tRNAs act as diffusible odor-specific memories. They can transfer odor-specific adaptations to naive worms via feeding, and transfer stable alterations of odor-specific responses to the progeny.

RESULTS AND DISCUSSION

Olfactory imprinting and its inheritance is RNA-mediated.

Olfactory imprinting is a long-term inherited behavioral change induced by the early olfactory environment. We therefore wanted to test if RNAs could convey olfactory imprints. Olfactory imprinting was induced, as previously described (***Remy and Hobert, 2005***), after exposure to two different olfactory cues, citronellol (CI) or benzaldehyde (BA) (*Figure 1a*, odor-exposure). The efficiency of this induction was tested using the chemotaxis assay described in Methods and in ***Supplement Figure Method 1***. Total RNA was then extracted from CI-exposed, BA-exposed or water-exposed control worms. These RNAs were fed to naive larvae (***Figure 1a***, RNA-feeding). Once RNA-fed larvae reached the adulthood, they were subjected to the chemotaxis assays. We observed the naive worms fed on RNA from CI or BA-

exposed worms migrate significantly faster toward CI or BA, as if they had been themselves odor-exposed (***Figure 1b***).

We observed that odor-specific imprinting can be elicited by the exogenous addition of RNA extracted from odor-exposed worms to the food of naive unexposed worms. This observation shows that worms exposed to odors during the L1 larval stage produce RNA populations able to alter chemosensory responses of naive animals via ingestion.

Olfactory imprints co-purify with the transfer RNAs fraction.

To identify which RNA molecules are able to transfer odor imprints, we started by separating the large from the small RNAs. We studied the transfer of imprinting by feeding naive worms on either the "large RNAs fraction" or the "small RNAs fraction". We observed that only RNAs smaller than 200 nucleotides (nt) are able to trigger imprinting (***Figure 2a***). Migration on 3.5% agarose gels separates small RNAs into five fractions (A to E bands, insert on ***Figure 2b***). After RNA-feeding, we observed that olfactory imprints were exclusively transmitted by the "D" small RNA fraction (***Figure 2b***). Based on co-migration with a double-stranded RNA ladder (L), the "D" RNA population migrates with an apparent mean size of 45 nt on this non-denaturing electrophoresis condition.

Further fractionation on denaturing 7 M urea-15 % polyacrylamide gel (***Figure 3a)*** revealed the "D" fraction is composed of several RNA species we named fraction 1 to fraction 8. Strikingly, we noticed fractions 2 to 7 represent a typical profile of transfer RNAs (tRNAs) (***Figure 3a, left panel***). Performing Northern-blot analyses

using two *C. elegans* specific probes for $tRNA^{Gly}$ (UCC) and $tRNA^{Leu}$ (AGG), we confirmed that fractions 2 to 7 indeed contain the *C. elegans* tRNA population (***Figure 3a, right panel***).

We next wanted to know if the imprinting activity is spread over the whole tRNA containing population or linked to specific fractions. We cut and eluted each of the indicated 1 to 8 fractions from the polyacrylamide gels. We reconstituted the whole « D » RNA population by mixing fractions 1 to 8, and the whole tRNA co-migrating populations by mixing fractons 2 to 7. As expected, both 1 to 8 and 2 to 7 mixes extracted from CI-exposed worms (CI Mix 1-8 and CI Mix 2-7) transferred a CI-specific imprint to naive worms, while the corresponding NA mixes (NA Mix 1-8 and NA Mix 2-7) did not (***Figure 3b***).

Strikingly, after a detailed fraction by fraction analyses, we found that only the RNA molecules that co-purify with fractions 5 and 6 contained and transferred the imprinting activity (***Figure 3c***).

Alanine tRNAs transfer olfactory imprinting.

596 functional tRNA genes have been predicted in the *C. elegans* genome (***Duret, 2000; Chan and Lowe, 2009***). To be fully active, tRNAs need to be heavily modified post-transcriptionally (***Agris, 2015; Duechler et al., 2016***). Indeed, the high abundance of tRNA post-transcriptional modifications blocks the progression of reverse-transcriptases, thus cDNA synthesis, making quantitative tRNA sequencing very challenging. In order to obviate these limitations, alternative methods have been proposed in the recent literature (***Pang et al., 2014; Zheng et al., 2015; Cozen et al., 2015; Shigematsu et al., 2017***), however none of them is readily available. For this

reason, instead of a sequencing strategy, we used a biochemical approach to identify which tRNA molecules are able to transfer olfactory imprinting.

To identify the imprinting tRNAs, we combined streptavidin microbeads purification with Northern blot analysis. According to the genomic tRNA database (GtRNAdb) predictions, *C. elegans* uses 46 different anticodons to decode the 20 aminoacids. Five anticodons are used for Ser, Arg and Leu, three for Ala, Gly, Pro, Thr and Val, two for Lys, Glu, Gln and Ile, and one for Phe, Asp, His, Met, Tyr, Cys and Trp.

From ***Figure 3c***, we know that imprinting is transferred only by fractions 5 and 6. tRNAs bearing different anticodons decoding the same amino-acid, or isoacceptors, display a high degree of sequence homology. Since the denaturing gel shown in ***Figure 3a*** separates tRNA molecules on the basis of nucleotide sequences, we hypothesized that each fraction, including the imprinting fractions 5 and 6, may contain a limited number of isoacceptor tRNAs.

For microbeads purifications, we used anticodon-specific (isoacceptor tRNAs) tDNA probes of 37 nucleotides each, complementary to the respective tRNAs 3' halves (described in Methods). Except two out of the five decoding Ser and Arg and one out of the five decoding Leu, all anticodon-specific tRNAs used by *C. elegans* are represented in our set of tDNA probes. For Northern blots analysis, we used shorther aminoacid-specific (isotype tRNAs) probes (described in Methods).

We reasoned that if a mixture of tRNAs eluted from a pool of tDNA probes bound to microbeads transfers imprinting, then this population should necessary contain the imprinting tRNAs.

tRNAs were purified from odor-exposed (CI or BA) worms using 14 different pools

made of 7 to 11 different tDNA probes ***(A to N, Table 1)***. The pools were designed such as each isotype (amino-acid decoding) tRNA is present in three different pools. Out of the 14 pools, only the A, J and N pools purified tRNAs able to transfer imprinting.

The A pool is made of Ala, Arg, Asn, Asp and Cys tDNA probes: Arg, Asn, Asp and Cys probes did not purify the imprinting tRNAs, as they are part of the imprinting negative pools F and K. The J pool is made of Tyr, Val, Ala and Arg probes: Tyr and Val probes are part of the imprinting negative E pool. The N pool contains the Thr, Trp, Tyr, Val and Ala probes: Thr, Trp, Tyr and Val probes are part of the negative E and I pools. Altogether, we concluded that none but the Ala probes present in the imprinting positive pools A, J and N purified the imprinting tRNAs.

We further proceeded with tRNA purifications by isotype-specific probes, and confirmed that only the tRNAs eluted from Alanine tDNA probes imprint naive worms, as shown in ***Figure 4a***.

To identify which RNAs are present in the imprinting fractions, we performed Northern blot analysis (***Figure 4b***). We show here the analysis of naive (N3 to N6), CI-exposed (C3 to C6) and BA-exposed (B4 to B7) fractions using tDNA probes for most tRNA isotypes probes used in ***Figure 4a.*** While $tRNA^{Leu}$ co-migrate with fractions 3, $tRNA^{Lys}$ migrates mostly in fractions 6, but are also present in fractions 5, and $tRNA^{Gln}$ co-migrate with $tRNA^{Glu}$ mostly in fraction 7, but are also present in fractions 6. Northern blots support the data obtained after microbeads purification: Alanine tRNAs are mostly present in the imprinting fractions 5 and 6.

The $tDNA^{Ala}$ probes 1 to 4, as described in Methods, purify the three different

tRNA^{Ala} isoacceptors used by *C. elegans*, tRNA^{Ala} (AGC), tRNA^{Ala} (CGC) and tRNA^{Ala} (UGC). To further discriminate between these Alanine tRNA isoacceptors, we used isoacceptor-specific probes. tRNAs eluted from the microbeads bearing the tRNA^{Ala} (AGC) or the tRNA^{Ala} (CGC) probes, do not transfer imprinting. By contrast, the tRNAs bound to the two probes corresponding to, respectively, the 5' and the 3' halves of the tRNA^{Ala} (UGC) transfer imprinting to naive worms (***Figure 4c).***

Alanine tRNA (UGC) transfers olfactory imprints for three different odorants

Our findings suggest the codon-specific tRNA^{Ala} (UGC) is able to transfer imprinting for two different attractive odorants, benzaldehyde and citronellol. To further prove tRNA^{Ala} (UGC) can transfer multiple odor-specific imprints, we exposed worms to isoamyl alcohol (IA), another attractive odorant (***Bargmann et al., 1993***), for which imprinting after early exposure has been demonstrated (***Remy, 2005***).

Using microbeads bearing the same tDNA probe, we purified tRNA^{Ala} (UGC) from, respectively, naive, CI-exposed, BA-exposed and IA-exposed worms. Naive worms were fed on these tRNAs and assayed for BA, IA or CI chemotaxis. Feeding naive worms on tRNA^{Ala} (UGC) from BA-exposed (BA) increases BA chemo-attraction, compared to feeding tRNA^{Ala} (UGC) from naive unexposed (None), while it has no effect on IA or CI responses. Feeding on tRNA^{Ala} (UGC) from IA or CI-exposed (IA, CI) respectively increases IA or CI responses specifically, compared to feeding tRNA^{Ala} (UGC) from naive (None) (***Figure 5*).** This suggests odor-stimulated worms produce odor-specific forms of the tRNA^{Ala} (UGC) molecule, each of it bearing an

odor-specific code, according to their early olfactory experience.

Multigenerational feeding on tRNA^{Ala} (UGC) from odor-exposed leads to stable imprinting inheritance.

Enhanced response after odor-exposure is inherited by the F1 unexposed worm generation, while it is lost in the F2 generation. However, olfactory imprinting is stably fixed and stably inherited in worm populations after five worm generations were odor-exposed (***Remy, 2010***). A tRNA^{Ala} (UGC) molecule able to transfer the imprinting behavior might be able to recapitulate the inheritance pattern of odor-triggered imprinting. To assess this question, we fed naive N2 wt worms through seven successive generations on the CI-tRNA^{Ala} (UGC) that transfer a CI imprint, CI-tRNA, to naive. As schematically outlined (***Figure 6a***), we compared CI responses of the seven CI-tRNA fed generations and of their naive progeny grown without tRNA addition up till the fourth generation. We found that a CI imprint is passed to the first but not to the second naive generation issued from one to five generations of CI-tRNA fed animals. However, CI imprinting is stably maintained in naive generations issued from worms fed on CI-tRNA at least for six successive generations (***Figure 6b***). In order to assess the stability of inheritance after the 6th CI-tRNA fed generation, we grew more generations without adding tRNAs to worm food. We observed that multigenerationally tRNA-triggered imprinting, as odor-triggered imprinting is stably maintained in worm progeny. Although it takes six instead of five generations, odor-tRNAs feeding elicit the same long-term stably inherited behavioral change as early odor-exposure. tRNA^{Ala} (UGC) from odor-exposed are

able to stably reprogram odor-specific responses, producing worm populations displaying odor-specific hyperosmia, compared to parental worms.

Odor-specific tRNAs added to worm food might enter worm tissues through the intestinal cells, diffuse to olfactory neurons to alter odor responses and migrate to germ-line cells to imprint the next generation. Uptake and diffusion of double-stranded RNA (dsRNA) support systemic silencing by RNA interference in *C. elegans*. We hypothesized tRNAs could take the paths used by dsRNA.

We studied imprinting and its inheritance in mutant worms bearing amino-acid substitutions in one of two *C. elegans* double-stranded RNA selective transporters SID-1 or SID-2 (***Jose et al., 2009; McEwan et al., 2012)***. The dsRNA selective transporter SID-2 is exclusively localized to the apical membrane of intestinal cells, where it is responsible for the initial binding and internalization of dsRNA from the intestinal lumen (***Marré et al., 2016)***. Due to its high expression in germ-line cells, the dsRNA selective importer SID-1 has been involved in the transgenerational diffusion of neuronally expressed mobile dsRNAs (***Devanapally et al., 2014)***.

Worms with the *sid-1 (qt2)*, the *sid-1 (pk3321)* and the *sid-2 (qt13)* mutations show a wild-type imprinting behavior after an early odor exposure (**Table 2**, Odor-exposure). While odor-triggered imprinting was F1 inherited in N2 wt and *sid-2 (qt13)* worms, imprinting inheritance was impaired by the two *sid-1 (qt2)* and *sid-1 (pk3321)* substitutions mutations (**Table 2**, Inheritance). Furthermore, while CI-tRNA feeding do imprint naive wild-type, *sid-1 (qt2)* and *sid-1 (pk3321)* worms, the naive *sid-2 (qt13)* worms do not acquire a CI imprint via feeding (**Table 2**, tRNA feeding).

Table 2 data suggest intertissular and intergenerational imprinting tRNAs use the

SID-1 and SID-2 dependent paths described for linear dsRNA motility in *C. elegans.*

tRNA^{Ala} (UGC) controls *C. elegans* chemo-attraction through the Elongator complex sub-unit 3.

Our data suggest that attractive odorants, when present during the first *C. elegans* larval stage, trigger the production of different forms of tRNA^{Ala} (UGC), each bearing an odor-specific signature.

It is now largely admitted that the nucleosides of all forms of coding and non-coding RNAs can be chemically modified. Over 130 RNA nucleoside modifications have been already described (***Machnicka et al., 2013; Sarin and Leidel, 2014; Schaefer et al., 2017; Jonkhout et al., 2017*),** which, theoretically, would allow a huge combinatorial number of chemical variants for a single RNA sequence.

Such combinatorial complexity defines the « epitranscriptome », and would greatly extent the functional diversity of RNA molecules. Due to the growing number of human diseases linked to RNA modification defects, understanding the biological significance of the epitranscriptome is becoming a major challenge.

The technology available to analyse known identified tRNA chemical modifications is making progress and rapidly developing (***Schaefer et al., 2017; Jonkhout et al., 2017*)**. However, to date, there is no straight forward available method able to accurately describe the whole quantitative and qualitative pattern of modifications that would encode odor-specific signatures on tRNA^{Ala} (UGC).

The *C. elegans* Elongator complex is involved in chemo-attraction

Amongst the most studied tRNA chemical modifications are those affecting Uridine at the wobble position, the first base of the anticodon. Wobble U modifications are considered of critical functional importance in codon-anticodon recognition as they might improve tRNA aminoacylation kinetics and prevent translational frame-shifts (***Larsen et al., 2015; Nedialkova and Leidel, 2015; Deng et al., 2015***). Some of these tRNA modifications are thought to be modulated by the activity of the Elongator (ELP) complex (***Karlsborn et al., 2014***). Elongator is an evolutionarily conserved dodecamer complex made of two copies of six ELP-1 to ELP-6 subunits (***Glatt et al., 2012, Dauden et al., 2017***). Besides the chemical modifications of tRNA bases (***Chen et al., 2009)***, Elongator has been involved in a great variety of nuclear and cytoplasmic cellular functions, including transcription elongation, chromatin remodeling, exocytosis, zygotic paternal DNA demethylation (***Okada et al., 2010***), and neuronal development (***Solinger et al., 2010; Creppe et al., 2009; Tielens et al., 2016; Dalwadi and Yip, 2018)***.

How Elongator performs its multiple functions and the respective role of its different subunits remains poorly understood.

The multifunctionality of Elongator could be partly explained by structural analysis of the yeast complex (***Dauden et al., 2017).*** In particular, the yeast ELP3 contains a C-terminal Lysine Acetyl Transferase (KAT) domain and a domain with sequence homology with the S-adenosylmethionine (SAM) domain. tRNA binding sites are present on ELP1 and ELP3, while the ELP 4/5/6 subcomplex possesses an ATP-

modulated tRNA binding activity. The precise molecular mechanism that underlies tRNA modifications by this complex is yet to be elucidated. Based on sequence homology, worm and mammalian Elongator sub-units may carry the same functional domains described in yeast. Strikingly, the radical SAM domain but not the KAT domain has been involved in paternal genome demethylation in the mouse, suggesting the ELP3 functional domains play different roles (***Okada et al., 2010***).

Two studies reported the effects of mutations inactivating the *C. elegans* Elongator complex sub-units 1 (ELPC-1) and 3 (ELPC-3). Worms harboring different mutations in the *elpc-1* or *elpc-3* genes display neuronal and behavioral phenotypes, despite the ubiquitous expression of ELPC-1 and ELPC-3. Importantly, all analysed *elpc-1* and *elpc-3* mutations produced identical phenotypes (***Chen et al., 2009; Solinger et al., 2010***). We used worms carrying the same chromosomal deletions or punctual mutations to ask if and how they also affect the *C. elegans* chemo-attractive behavior. We first compared the behavior of worms carrying the elpc-1 deletion *elpc-1 (ng10)*, the elpc-3 deletion *elpc-3 (tm3120)*, and the point mutation *elpc-3 (ng15)* to the behavior of wild-type worms (***Figure 7***).

The *elpc-3 (tm3120)* mutants display significantly reduced chemo-attractive responses to all tested dilutions of CI, BA and IA, compared to wild-type N2 (***Figure 7a***). By contrast, the *elpc-3* R327C substitution in *elpc-3 (ng15)* mutants or the 2050 bp deletion *elpc-1 (ng10)* mutants display a wild-type chemotaxis (***Figure 7b***).

elpc-3 (*tm3120*) is a 355 bp deletion within *elpc-3*, which supresses both the ELPC-3 KAT and SAM domains. Chemotaxis is unaffected by the *elpc-3* point mutation in *elpc-3 (ng15)* that may inactivate the KAT domain but preserve the SAM domain.

SAM proteins have been indeed involved in the activity of several tRNA-modifying enzymes as the methyl-thiotransferases YqeV and MiaB or the 4-demethylwyosine synthase TyW1 (***Hori, 2014***). However, nothing is known about a putative tRNA-modifying activities of the ELPC-3 SAM domain.

Feeding *elpc-3 (tm3120)* deletion mutants on wild-type tRNA^{Ala} (UGC) restores a wild-type chemo-attractive behavior.

We reasonned that if the chemotaxis defects *elpc-3 (tm3120)* worms are due to impaired tRNA bases modifications, then providing wild-type tRNAs through feeding would rescue the phenotype.

We cut and eluted the NA (naive) tRNA co-migrating fractions 2 to 7 (as seen in ***Figure 3a*** gel). We fed *elpc-3 (tm3120)* worms on the pooled 2 to 7 fractions and on each fraction, separately. We found that the whole population of naive tRNA and the Alanine tRNAs containing fractions 5 and 6 (N_5 and N_6 on ***Figure 4b***) indeed restored CI chemotaxis ***(Figure 8a)***. Moreover, feeding on microbead-purified wild-type tRNA^{Ala} (UGC), but not wild-type tRNA^{Ala} (AGC) nor wild-type tRNA^{Ala} (CGC), fully rescue chemo-attractive responses to CI, BA and IA of *elpc-3 (tm3120)* worms to wild-type levels ***(Figure 8b)***.

We showed that tRNA^{Ala} (UGC) is both responsible for odor-specific imprinting and required for chemoattraction. In the absence of sequencing, it is important to demonstrate the specificity of the microbead tRNA isolation method. Comparing the

C. elegans tRNA gene sequences reveals that among an isoacceptor-specific population of tRNAs, those bearing the same anticodon tends to display the highest level of homology. The tDNA probe for Alanine tRNAs with the AGC anticodon differ from the probe for Alanine tRNAs with the TGC anticodon by only 4 nucleotides (***Supplemental Figure Method 2A***). All other tDNA probes differ by more than 4 nucleotides. The tRNA isolation protocol uses a 0.5 M concentration of NaCl in the Binding and Washing buffers. We reasonned that the Ala AGC probe would co-purify both the $tRNA^{Ala}$ (AGC) and the $tRNA^{Ala}$ (UGC) at lower ionic strength. Indeed, the tRNAs purified from wild-type naive tRNAs fraction 5 (N5) using Ala AGC probe and NaCl concentrations lower than 0.2 M, do rescue CI chemotaxis in *elpc-3 (tm3120)* mutants (***Supplemental Figure Method 2B***).

Altogether, these observations suggest the chemotaxis defects of *elpc-3 (tm3120)* worms is due to their inability to synthesize the wild-type form of $tRNA^{Ala}$ (UGC), required for the establishment of chemo-attractive responses.

According to the study of Chen et al., 2009, the tRNAs isolated from *elpc-3* and *elpc-1* deletion mutants (*elpc-3 (tm3120)* and *elpc-1 (2149))* lack the same Uridine side-chain modifications. The *elpc-1 (2149)* deletion remove 275 bp from *elpc-1*, whereas the *elpc-1 (ng10)* deletion we use in this study, remove a much larger 2050 bp *elpc-1* fragment. Therefore both *elpc-1* deletions should lack the same Uridine side-chain tRNA modifications. We observed that the *elpc-1 (ng10)* worms display a wild-type chemotaxis behavior, suggesting these Uridine modifications are not required for the development of chemo-attraction.

Elongator prevents the stable suppression of odor-specific responses

The *elpc-1 (ng10)* and *elpc-3 (ng15)* mutations do not affect chemo-attractive responses. We assessed olfactory imprinting in these two mutants. By contrast to wild-type, early odor-exposure or odor-specific $tRNA^{Ala}$ (UGC) feeding do not enhance odor responses, but stably suppress responses to odors (or odor-tRNAs) worms were exposed.

CI-exposure of *elpc-1 (ng10)* worms abolished CI responses ***(Figure 9a)***, while IA exposure of *elpc-3 (ng15)* abolished IA responses ***(Figure 9b)***.

However, BA or IA exposed *elpc-1 (ng10)* remain attracted by CI ***(Figure 9a)***, while CI or BA exposed *elpc-3 (ng15)* remain attracted by IA ***(Figure 9b)***.

Strikingly, we found that extinctions of odor responses after exposure to a single odorant is stably inherited over generations in the two mutants. We therefore submitted worms to sequencial exposures. One generation was exposed to a single odorant, the next generation was (or not) exposed to a second odorant, and the third generation exposed (or not) to a third odorant. By doing this, we were able to obtain worm populations unable to chemotax to a single (CI or BA or IA) odor, to two (CI+BA, CI+IA or BA+IA) different odors, or to the three (CI+BA+IA) odors ***(Figure 9a, 9b)***.

Odor-specific suppression of chemo-attraction was also obtained after feeding $tRNA^{Ala}$ (UGC) from odor-exposed. Microbeads purified $tRNA^{Ala}$ (UGC) - but not $tRNA^{Ala}$ (AGC) or $tRNA^{Ala}$ (CGC) - from CI-exposed or from BA-exposed wild-type worms decrease chemo-attraction in the two elongator mutants (***Figure 9c***). As after odor-exposure, inhibition of chemo-attraction after odor-tRNA feeding is odor-

specific, as shown here for CI-tRNA^{Ala} (UGC) or BA-tRNA^{Ala} (UGC) fed *elpc-3 (ng15)* worms. Moreover, as after odor-exposure, extinctions of odor responses via odor-tRNA feeding was found stably inherited in the progeny (data not shown).

The synthesis of odor-specific tRNA^{Ala} (UGC) does not require Elongator.

We next asked if ELPC plays a role in the generation of odor-specific tRNA^{Ala} (UGC). To address this question, we purified tRNA^{Ala} (UGC) from naive and CI-exposed N2, *elpc-1 (ng10),* and *elpc-3 (ng15)* worms. The behavioral effects of these tRNA^{Ala} (UGC) were assessed by feeding to naive N2, naive *elpc-1 (ng10)* and naive *elpc-3 (ng15)* (***Figure 10)***. Results are expressed as Mean Imprinting Index (MII): a positive imprinting index indicates that CI-tRNA fed migrate faster than NA-tRNA fed worms toward CI, while a negative imprinting index indicates by contrast that CI-tRNA fed migrate slower than NA-tRNA fed animals toward CI.

Figure 10 data show the genotype of the fed worms, but not the genotype of worms from which tRNA^{Ala} (UGC) were originated, determined the imprinting behavior. Indeed, tRNA^{Ala} (UGC) purified from CI-exposed wt, from CI-exposed *elpc-1 (ng10)* or from CI-exposed *elpc-3 (ng15),* all increase CI chemo-attraction of fed wt N2 (Fed worms N2).

The same tRNAs by contrast decreased CI chemo-attraction of fed *elpc-1 (ng10)* and the *elpc-3 (ng15)* mutants (Fed worms *elpc-1 (ng10)* or *elpc-3 (ng15)*).

The same pattern of responses was obtained after BA exposure, meaning tRNA^{Ala} (UGC) from BA-exposed wt, *elpc-1 (ng10)* and *elpc-3 (ng15)* increased BA chemo-attraction in wt while they decreased BA chemo-attraction in *elpc-1 (ng10)* and *elpc-*

3 (ng15) (data not shown).

Figure 10 data indicate $tRNA^{Ala}$ (UGC) from odor-exposed wt and from odor-exposed *elpc* mutants transfer the same behavioral changes to naive animals. It seems thus that the ELPC functions altered by the *elpc-1 (ng10)* and *elpc-3 (ng15)* mutations are not involved in the odor-triggered modifications of $tRNA^{Ala}$ (UGC).

A wild-type form of $tRNA^{Ala}$ (UGC) seems to be required for the establishment of chemo-attraction ***(Figure 8)***. This form would be made by wt as well as by *elpc-1 (ng10)* and *elpc-3 (ng15)*, but not by the *elpc-3 (tm3120)* worms. Upon odor-exposure, wt, *elpc-1 (ng10)* and *elpc-3 (ng15)* would produce identical odor-modified forms of $tRNA^{Ala}$ (UGC) ***(Figure 10)***.

These two latter mutants are however unable to perform imprinting. Instead, they respond to odor-exposure and to feeding odor-modified $tRNA^{Ala}$ (UGC) by a definitive suppression of odor-specific chemo-attraction ***(Figure 9)***.

We observed that olfactory stimuli lead to transient or stable hyperosmia (enhanced chemo-attraction) in wt worms, or to stable anosmia (suppressed chemo-attraction) in *elpc-1 (ng10)* and *elpc-3 (ng15)* worms.

Anosmic worms do not produce odor-modified $tRNA^{Ala}$ (UGC).

In *C. elegans*, only two pairs of chemosensory neurons, AWA and AWC, are required for chemotaxis to volatile attractants (***Bargmann, 1993***). The *C. elegans* genome encodes around 1300 functional seven transmembrane receptors, presumably coupled to G-proteins, called Serpentine Receptors (SR). When expressed in

chemosensory neurons, these receptors are thought to interact with odorant molecules and support odor specificity of the chemoattractive responses ***(Hart et al., 2010***). The AWC olfactory neurons are responsive to a high number of chemically different molecules that includes the three chemoattractants BA, CI and IA used in this study (***Bargmann, 1993***). AWC would therefore express the BA, CI and IA specific chemo-receptors.

One could speculate that the stable hyperosmia or anosmia described here would rely on the differential expression of odor-specific receptors. The identity of these receptors is however unknown, so that expression levels cannot be directly assessed.

The AWC neurons of the anosmic worms for one, two or three odorants, would not express the respective olfactory receptors. We reasonned that in the absence of receptors, odor-stimuli would not lead to the production of odor-modified $tRNA^{Ala}$ (UGC). Anosmic worms for CI, BA or IA odorants were respectively called CI^-, BA^- or IA^-.

We fed N2 wt on $tRNA^{Ala}$ (UGC) purified from naive, CI or BA-exposed, and CI or BA-exposed CI^-, BA^-, BA^-IA^- or BA^-CI^- *elpc-1 (ng10)* and *elpc-3 (ng15)* worms, as indicated. As shown on ***Figure 11***, $tRNA^{Ala}$ (UGC) from odor-exposed *elpc* mutants enhance chemo-attraction of wt worms, compared to $tRNA^{Ala}$ (UGC) from naive *elpc* mutants. By contrast, $tRNA^{Ala}$ (UGC) from CI-exposed worms bearing a CI^- negative imprint or from BA-exposed worms bearing a BA^- negative imprint, do not respectively enhance CI or BA chemo-attractive responses of wt.

Figure 11 results indicate odor-stimuli do not trigger the production of odor-specific

$tRNA^{Ala}$ (UGC) in negatively imprinted anosmic worms.

In *C. elegans*, only the developing first larval stage L1 can be imprinted by attractive (this study) or by aversive (***Jin et al., 2016)*** signals present in worm's environment. Environment-responsive mechanisms must be present at this stage to adapt future worm's behaviors through up or down regulation of genes expressed in sensory neurons. A single chemo-attractive odor signal specifically enhances, in wt, or suppresses, in *elpc* mutants, adult attraction to a single odorant molecule, while all other AWC-mediated responses remain unchanged. Odor-specificity strongly supports the existence of a physical insulation of olfactory signaling within the AWC neurons, as already hypothesized for other forms of odor-induced adaptation in *C. elegans* (***O'Halloran et al., 2009; Juang et al., 2013***).

The production of odor-modified tRNAs requires a functional olfactory transduction pathway.

In *C. elegans*, odor to receptor interaction activates an AWC-specific olfactory transduction pathway made of several G proteins, the DAF-11/ODR-1 guanylyl cyclase, and the TAX-2/TAX-4 cGMP-gated channel. Worms with mutations inactivating members of this pathway are defective for all AWC-mediated chemosensory responses, that include responses to the three attractive odorants used in this study (***Bargmann, 2006***).

We asked whether a functional AWC olfactory transduction pathway is required for the synthesis of odor-tRNAs. We purified $tRNA^{Ala}$ (UGC) from CI-exposed worms harboring the *odr-1 (n1936)*, the *tax-2 (p671)* or the *tax-4 (p678)* mutations,

respectively inactivating the ODR-1 guanylyl cyclase, the TAX-2 or the TAX-4 subunits of the cGMP-gated channel. Through feeding wt or *elpc-1 (ng10)* mutants, we compared the imprinting activity of these tRNAs to the imprinting activity of $tRNA^{Ala}$ (UGC) from naive wt and from CI-exposed wt worms ***(Figure 12)***.

As already shown, feeding on $tRNA^{Ala}$ (UGC) from CI-exposed wt increase CI MMI in wt, and decrease CI MMI in *elpc-1 (ng10)*, compared to feeding on $tRNA^{Ala}$ (UGC) from naive wt (***Figure 12***, CI-exp wt, Naive wt). By contrast, none of the three CI-exposed transduction mutants produce a $tRNA^{Ala}$ (UGC) able to transfer a CI-imprint to naive ***(Figure 12***, CI-exposed *odr-1*, *tax-2, and tax-4)*.

Figure 13 schematize our main findings. Only olfactory cues present during the first larval stage of *C. elegans* development are memorized as to influence and adapt future chemo-sensory behavior. Three different attractive odorant molecules would bind to yet unknown odor-specific chemoreceptors expressed by a single olfactory receptor neurons AWC. Each odor stimuli would trigger odor-specific modifications of the $tRNA^{Ala}$ (UGC). Early odor-exposure and feeding odor-modified $tRNA^{Ala}$ (UGC), imprint worms as to enhance odor-specific chemo-attraction (hyperosmia). Odor-modified $tRNA^{Ala}$ (UGC) act as mobile olfactory mnemonics able to spread olfactory information to naive and to next generations. Odors and odor-tRNAs triggered alterations of chemosensory responses are odor-specific, supporting the idea of a spatial insulation of odor signaling in AWC cilia.

The wild-type form of $tRNA^{Ala}$ (UGC) is required for the development of AWC sensed chemo-attractive responses. Mutant worms carrying a chromosomal deletion of the Elongator sub-unit 3 gene, are chemo-attraction defective. Adding the wild-

type form of $tRNA^{Ala}$ (UGC) to the food of this mutant fully restore the chemoattractive responses.

Imprinting require some Elongator functions: deletion of ELPC-1 in *elpc-1 (ng10)* or of the ELPC-3 KAT domain in *elpc-3 (ng15)* worms impair imprinting. Instead of a transient or stable hyperosmia, odors or odor-tRNAs specifically suppress odor-responses, resulting in stably inherited odor-specific anosmia.

Conclusion:

Nematodes respond to many volatil odorants but has only three pairs of olfactory neurons, AWA, AWB and AWC, suggesting each neuron would express a large number of olfactory receptors. It has been shown that the type of behavioral response elicited by an odorant is not specified by the olfactory receptors, but by the olfactory neuron in which they are expressed and activated **(*Troemel et al., 1997*)**. Our results suggest specific olfactory receptors for attractive cues are expressed and already functional, i.e. coupled to the olfactory transduction pathway, at the first stage of larval development. The way each worm's odor-responsive neuron expresses specific sets of OR, and represses the expression of all others, is still unknown. In mammalian species, each olfactory neuron expresses only one allele of a member of the OR gene family; the precise mechanisms by which such monogenic monoallelic receptor choice is achieved is however not yet fully undestood. These mechanisms involve nuclear compartimentalization, repression/derepression through epigenetic marks made by the chromatin-modifying enzyme Lysine demethylase 1 (LSD-1) and stabilization of functionally expressed OR by the odor-signaling pathway (reviewed

in ***Degl'Innocenti A and D'Errico A, 2017***).

In all species, $tRNA^{Ala}$ display streches of consecutive guanine residues at their 5' ends. This conserved motif may favor the formation of intermolecular G-quadruplex structures (***Lyons et al., 2017***). The existence of G-quadruplex structures in worm's $tRNA^{Ala}$ might be of importance regarding the epigenetic control of chemo-attractive behavior. Indeed the chromatin-remodelling Lysine Demethylase-1 (LSD-1), the main epigenetic regulator of olfactory receptor choice in mouse olfactory neurons, interacts with RNA G-quadruplexes (***Lyons et al., 2013***).

In human, 5'-$tiRNA^{Ala}$ fragments containing the guanine rich region, can be produced after cleavage of mature $tRNA^{Ala}$ within the anticodon loop by the human-specific enzyme angiogenin. This fragment has been shown to inhibit translation via the displacement of the m7GTP cap structures from cap-binding translation initiation complexes (***Lyons et al., 2017***). As other tRNAs, Alanine tRNAs can be cleaved by the endonuclease Dicer (***Schimmel, 2018***), present in *C. elegans*. G-rich Dicer generated 5'-$tRNA^{Ala}$ fragments might inhibit translation through the same mechanism.

Our data suggest the *C. elegans* $tRNA^{Ala}$ (UGC) form produced by naive wild-type worms is required for the implementation of chemo-attractive responses. Expression of a specific set of OR in a sensory neuron needs all others to be repressed. Whether such repression occurs at the translation level, and if inhibitory fragments of $tRNA^{Ala}$ (UGC) are involved in this process remains to be proven.

tRNAs and tRNAs genes not only control translational (***Kirchner and Ignatova, 2015; Avihu et al., 2013***) but also transcriptional fine-tuning (***Woolnough et al.,***

2015; Pratt-Hyatt et al., 2009; Kirkland and Kamakaka, 2015). Importantly for epigenetic inheritance, tRNAs also control the plasticity of genome architecture via the dynamic positioning of nucleosomes and the modulation of chromatin domains boundaries (***Raab et al., 2012; McFarlane and Whitehall, 2009; Talbert and Henikoff, 2009***).

In this work, we produced worm strains with stable non-genetic alterations of odor-specific responses, compared to wild-type. Although not yet proven, odor-specific hyperosmia or anosmia might rely on the differential expression of odor-specific OR. Through unknown epigenetic mechanisms, the nuclear localization of OR gene alleles may have change, moving from either active or repressed chromatin domains (***Armelin-Correa et al., 2014***). Knowledge of the OR genes involved is a prerequisite to delineate these mechanisms. The hyper or anosmic worm strains described in this study might facilitate the identification of these genes.

Evidences for inheritance of acquired adaptations to local environments accumulated recently (***Bohacek and Mansuy, 2015; Jablonka and Lamb, 2015***). However, still little is known about how environmental conditions are translated into epigenetic information, which could lead to stably inherited phenotypic changes.

This work highlights the role of a specific tRNA and the multifunctional Elongator complex at the interface between environmental inputs and stably inherited behavioral changes. It set the stage of elucidating how environmental informations, here olfactory cues, could regulate the secondary and tertiary tRNA structures, which, in turn, will influence worm behavior. More investigations are needed to understand

how the high combinatorial potential of (epi)transcriptomes could organize the interplay between environments and (epi)genomes.

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Material and Methods

Strains and culture conditions

We used the wild-type *C. elegans* reference strain N2, unless otherwise stated. Worms with the *sid-1 (qt2)*, *rrf-1 (ok589)*, *rrf-1 (pk1417)* and *rrf-3 (pk1426)* mutations were obtained from the *Caenorhabditis* Genetics Center (CGC). The *sid-2 (qt13)* mutant was obtained from Craig Hunter. The elongator mutants ***elpc-1 (ng10)***

or *ikap-1 (ng10), elpc-3 (ng15)* and *elpc-3 (tm3120)* were from Jachen A. Solinger. Worms were cultured on E. coli OP50 at 20°C using standard protocols.

Odor-exposure for olfactory imprinting

Benzaldehyde and ®-citronellol (Sigma-Aldrich) were diluted to the 1/300 in water. Odor-exposures were done by suspending a 4 µl drop of these dilutions on the lids of worm culture dishes during the critical plasticity period corresponding to the first 12 hours of post-hatch development (***Remy and Hobert, 2005***).

Chemotaxis assay

The chemotaxis assay used in this study is schematically outlined in the Supplement Figure Method. It is based on the population chemotaxis assay originally described by C. I. Bargmann (***Bargmann, 2006***). Several modifications were made in the procedure and in chemotaxis index calculation. Changes aimed at more accurately compare the chemo-attraction of worm populations to moderately attractive odorants (the 1/300 dilution of benzaldehyde or citronellol used here), using 20 adult worms per condition.

40 ml of low-salt (1mM Ca^{++}, 1mM Mg^{++}, 5mM KPO_4) agar (20 g/l) were poured in 12x12 cm square plates. Assay plates were allowed to dry at room temperature for at least three days before use. Worms were individually transferred from culture dishes to the middle of the square assay plates. To establish a homogeneous odor gradient, so that all worms were submitted to the same olfactory stimulus, 3 drops of odor-dilution (4 µl each) were suspended on the lids at one side, each placed at a distance

of 3 cm from the others. At the opposite side of the lids were placed 3x 4 µl of water. 6 drops of 4 µl of 1M NaN3 were added at both sides of the agar plate to immobilize animals that reached edges.

At time 0, 20 worms were placed on the middle line of the squared plate, every worm being at a distance of 6 cm from odor sources. Assays performed on squared plates allowed the indexation of all worm positions between the starting line (time 0, position 0 cm) and the odor source (position + 6 cm), usually four times at 10, 20, 30 and 40 minutes from time 0. The mean value of all indexed positions (in cm from the starting line) of each of the 20 worms represented the Mean Migration Index (MMI). Each experiment shown in the paper was performed at least 4 times. MMI (Means +/- S.E.) values were compared using unpaired data with unequal variance Student t-tests performed with the KaleidaGraph program.

Assays were always performed so as to compare synchronized worm populations. In ***Figure 10***, results are expressed as Mean Imprinting Indices, which is a subtraction of two MMI. For instance, MMI of CI-tRNAs fed population *minus* MMI of naive-tRNAs fed population.

RNAs and tRNAs fractionation and purification

The large and small RNAs (< 200 nt) were separated using the NORGEN RNA Purification Kit (Norgen Biotek Corp). Small RNAs were further size-fractionated on 3.5% low-melting agarose (Nusieve GTG) gels. RNA was quantified with the Nanodrop 2000 (ThermoScientific). To purify *C. elegans* transfer RNAs, small RNA

fractions (under 150 nt in size) were prepared as described (***Maréchal-Drouard et al., 1995***). In brief, RNA was incubated in a 2 M lithium chloride solution over-night at 4°C. After a centrifugation at 16000 g for 30 min at 4°C, the supernatants containing the tRNAs were recovered. Transfer RNAs were thereafter precipitated with 0.1 volume of 1 M sodium acetate pH 4.5 solution and 2.5 volumes of ethanol over-night at -20°C. Pelleted tRNAs were dissolved in water and loaded on 15 % polyacrylamide with 7 M Urea and 1 X TBE gels. After Ethidium bromide staining, gel slices were cut from the gels. The tRNAs were eluted over-night at room temperature in a solution composed of 0.5 M ammonium acetate, 10 mM magnesium acetate, 0.1 mM EDTA and 0.1% SDS. After phenol extraction, tRNAs were ethanol precipitated and finally recovered in 10 µl water.

Specific tRNA molecules isolation

To purify tRNA molecules, we used the microMACS Streptavidin MicroBeads Kit (Miltenyi Biotec). We synthesized 37 nt long 3'-biotinylated DNA probes complementary to the 3' half of the *C. elegans* tRNAs.

Oligonucleotide sequences of the tDNA probes were deduced from the *C. elegans* tRNA genes predictions (GtRNAdb, Lowe lab, Biomolecular Engineering, University of California Santa Cruz).

The sources of RNA from which specific tRNAs were isolated were either the whole small RNA populations or the gel-eluted tRNA fractions showed in ***Figure 3***.

Procedure was as recommended by the microMACS Kit, except the annealing buffer was made of 10 mM Tris HCl pH 7.5, 5 mM $MgCl_2$. Binding/Wash buffer 5 X was 50 mM Tris HCl pH 7.5, 5 mM EDTA pH 8, and 2.5 M NaCl, as indicated.

Isolation of tRNAs through this procedure is highly discriminative as it can separate Alanine tRNA (AGC) from Alanine tRNA (TGC), the two tRNA molecules displaying the highest level of sequence homology (***Supplemental Figure Method 2***).

Elutions from microbeads were in 200 ⌈l TE. For each feeding and behavioral assay, we used 10⌈l eluate per worm culture dish, as described in the RNA-feeding section below.

Northern blots analyses

Northern blots were performed as described in ***Cognat et al., 2017***. Roughly, RNAs fractionated on a denaturing 7M Urea/15 % polyacrylamide gel were transferred onto Hybond-N+ membrane (Amersham Pharmacia Biotech). Membranes were then hybridized with ^{32}P labeled probes specific to *C. elegans* tRNAs:

$tRNA^{Ala}$(AGC) : 5'- CTACCACTGAGTTATACCCCC - 3'

$tRNA^{Ala}$(CGC) : 5' - TACCCCTGAGCTATACCCCC - 3'

$tRNA^{Ala}$(UGC) : 5' - TATGGGGAATCGAACCCCA - 3'

$tRNA^{Leu}$(AAG) : 5'- TGGTGAAGAGAGTGGGATTCGAAC - 3'

tRNA^{Gly}(UCC) : 5'- TGGTGCGTTCGGGGGGAATCGAAC - 3'

tRNA^{Lys}(UUU) : 5'- ACCAACTGAGCTAAGGAGGC -3'

tRNA^{Glu}(UUC) : 5'- AACCACTAGACCACATGGGA -3'

tRNA^{Gln}(UUG) : 5'- AACCGCTACACCATGGAACC - 3'

RNA-feeding

NGM agar plates were loaded with 40 µl of OP50 culture, which, after drying, formed approximately a 100 mm^2 spot. Volumes of 10 µl RNA to be assayed were deposited per *E. coli* spot and shortly dried. Synchronised naive embryos were spawned on the RNA-loaded plates, and worms grown at 20°C until adulthood. For imprinting inheritance after multi-generational tRNA feeding, N2 worms were grown from the spawn embryo to the adult laying stage on 1 ng CI-tRNA-loaded cultures plates (F1). Part of the next generation was grown on a new CI-tRNA-loaded culture plates - the second tRNA-fed generation F2 -, another on a regular plate without CI-tRNA - the first naive generation F1+1. F3 is the progeny of F2 grown on tRNA while F2+1 is the progeny of F2 grown without tRNA. The CI chemo-response of the naive generations from the seven successive tRNA fed generations (F1 to F7) was determined as described.

tDNA oligonucleotides probes used for microbeads tRNAs purification:

1) Ala AGC : 5’ TGGAGGTTTGGGGAATTGAACCCCAGCCCTCTCCCAT 3’

2) Ala CGC : 5’ TGGAGGCACGGGGGATTGAACCCCGGACTTCCCGCAT 3’

3) Ala TGC : 5’ TGGAGGTATGGGGAATCGAACCCCAGACTTCTCGCAT 3’

4) Ala TGC : 5’ ATGCAAAGCCAGCGCTCTACCCCTGAGCTATACCCCC 3’

5) Arg ACG : 5’ CGACCACGGCAGGATTCGAACCTACAATCTTCTGC 3’

6) Arg CCG : 5’ AGCTCGCGGAGGGACTTGAACCCCCATTCCCGGTTCC 3’

7) Arg CCT : 5’ CGACCGAGGCAGGACTCGAACCTGCTGTCTTCGGTTT 3’

8) Asn GTT : 5’ CGCTCCCGGTGGGCTCGAATCACCTTTCGGTTAA 3’

9) Asp GTC : 5’ CTCCCCGGCCGGGAATTGAACCCGGGTCTCGCATGTG 3’

10) Cys GCA : 5’ CTAGCTCTCCAGGGACCAAGTTGAGGCCCACGGGGGA 3’

11) Gln CTG : 5’ CTTAGGACGCTGGGCTCAAGTTTAGAGCCACCCTGGA 3’

12) Gln TTG : 5’ CTTAGGACGCTGGGCTCAAGTTTAGAGCCACCTTGGA 3’

13) Glu CTC : 5’ GTGGGTATTCCGGCCCCAAGCTAAGGGGCGTTGCTTT 3’

14) Glu TTC : 5’ GTGGGTGCGCCGGGCCCAAGCTAAGGGCCGTACCCTT 3’

15) Gly CCC : 5’ ACCATTGTCTCGCGCCCAAGCTTAGGGCAGGTGGCGT 3’

16) Gly TCC : 5’ GTTCGTAAGCTGCCCCCAAGCTAAGGGGAGCTTGCGT 3’

17) His GTG : 5’ CCGGCACCGCTGCGACCAAGCTAAGGTCGTCGTCCGT 3’

18) Ile AAT : 5’ TTCGGGTTCCAGCGTCCAAGCTGGGGACGACCGCCGT 3’

19) Ile TAT : 5’ CAATTTGGTCAGCGCCCAAGCTTAGGGCGGGCCCCGT 3’

20) Leu AAG : 5’ GGGAAGCCCCCGCACCCAAGCTTAGGGAGAGAGAAGT 3’

21) Leu CAA : 5’AGCATACCCACGCACCCAAGCTTAGGGTGAAGCACGT 3’

22) Leu CAG : 5’AGAGGCCTCCCGCGTCCAAGCTTAGGACGCCTGCCGT 3’

23) Leu TAA : 5’ GGGAGGCCCCCGCACCCAAGCTTAGGGTGAGAGTAGT 3’

24) Lys CTT : 5’ ATTAGACCAACAGCGCCAAAGCTCGGGGCGTAACCCA 3’

25) Lys TTT : 5’ TTAGAATTCCAGTCCCCAAGCTCAGGGGATCCACCGA 3’

26) Met CAT : 5’ TTGGGTCTCCAGCCACCTAGCTTTGGTGAGCGACGAT 3’

27) Met CAT : 5’ TTAGACTTCCAGCACTCAAGCTCGGAGTGGCCCTCGT 3’

28) Phe GAA : 5’ TTTAGCAATCCAGTGGTCAAGCTAGGACCAAGCCCGT 3’

29) Pro AGG : 5’ CACGTTCTCTAGGGCCCAAGCTAGGGGCCAAGCTGGG 3’

30) Pro CGG : 5’ CACGCTCTCCAGGGCCCAAGCTAAGGGCCAAGCCGGG 3’

31) Pro TGG : 5’ CACGCTCTCCAGGGACCAAGTTAGGGGCCAAGCCGGG 3’

32) Ser AGA : 5’ CCGAGACGGGCGCATCCAAGCTTAGGACGACTGACGC 3’

33) Ser CGA : 5’ CCGAGACGGGCGCATCCAAGCTTAGGACGACTGACGC 3’

34) Ser GCT : 5’ CCCAAAGGGGCGCACTCAAGCTTAGAGTAGAACTAGC 3’

35) Thr AGT : 5’ TTTGTCTTCCAGCGACCAAGCTAAGGTCGTACTCCGT 3’

36) Thr CGT : 5’ TTTGTCTTCCAGCTGCCAAGTTAGGGCAGACCCCCGT 3’

37) Thr TGT : 5’ TAGTTATCCAGGCCCCAAGCTGGGGAGCATTCCCAGT 3’

38) Trp CCA: 5’ CTAGCTTTCCATCCCGCAAGCTAGGCGAGTCACCAGT 3’

39) Tyr GTA : 5’ TAGGAATCCAGTGACCAAGCTTAGGCCAAGCTGCCT 3’

40) Val AAC : 5’ TGTGTCTTCCAGCCACCAAGCTCGGGCGGGCTCTAGT 3’

41) Val CAC : 5’ TGCGTCTTCCAGCGGCCAAGCTTGGGCCGGTCCTGGA 3’

42) Val TAC : 5’ TGCGTCTTCTAGCGGCCAAGCTTGGGCCGATCCTGGA 3’

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Figure Legends

Supplement Figure Method 1

Schematic outline of the chemotaxis assay.

Chemotaxis assays were performed on square plates in order to index the worms' positions between the starting line (time 0, position 0 cm) and the odor source (position + 6 cm) or water source (position - 6 cm). 3 ml of odor solution or water are added to the lids of the plates. 20 synchronised worms are placed at the starting line at time 0. Worms start their migration and their position is carefully noted, usually at four time-points, at 10, 20, 30 and 40 minutes past time 0. Worms that migrate up the odor gradients are positively indexed (from 0 to 6). Worms that migrate down the odor gradients towards water are negatively indexed (from 0 to – 6). The mean value of all indexed positions at all noted times (in cm from the starting line) of each of the 20 worms involved in the assay is the Mean Migration Index (MMI).

Figure 1. RNA extracted from odor-exposed worms transfer olfactory imprinting to naive unexposed worms via feeding.

(1a) Schematic depiction of worms odor-exposure and RNA-feeding. Upper part: a drop of 4 ⌈l of citronellol (CI) or benzaldehyde (BA) or water (for control unexposed) is placed on the lid of each culture plate. Worms produce long-lasting odor-specific imprints when exposed to odors for 12 hours post-hatch at 20°C, the critical period for olfactory imprinting (*Remy and Hobert, 2005*). Worms exposed to odors during this period are collected at the adult stage. RNA is extracted from each collected population. Bottom part: a 10 ⌈l drop of purified RNA is placed on worm food (*E. coli* 0P50) to be ingested by naive larvae.

(1b) Chemotaxis assays performed on naive adults fed on different RNA populations. Worms fed on RNA from CI-exposed (CI-RNA, blue column) and worms fed on

RNA from BA-exposed (BA-RNA, red column) migrate faster toward a CI source and a BA source, respectively, compared to worms fed on RNA from naive animals (Naive-RNA, white columns). Mean Migration Index (MMI) was determined as described in Supplemental Material (experimental repeats > 4, ***p-value<0.001).

Figure 2. A specific population of small RNA transfers olfactory imprinting.

(2a) RNA from BA-exposed and unexposed naive was fractionated into two populations: the larger than 200 nucleotides fraction (Large-RNA) and the smaller than 200 nucleotides fraction (Small-RNA). Worms fed on 10 ng of Large BA-RNA migrate in a BA gradient as worms fed on 10 ng of Large Naive-RNA. By contrast, worms fed on 10 ng of Small BA-RNA display enhanced BA responses, compared to worms fed on 10 ng of Small Naive-RNA or to worms fed on 10 ng of Large BA-RNA (experimental repeats > 4, ***p-value < 0.001).

(2b) 3.5% low-melting agarose gels fractionate BA-RNA and Naive-RNA into five A to E discrete bands (left side of insert). Naive worms were fed on each of the five gel-purified NA-RNA and BA-RNA populations. Compared BA 1/300 MMI shows that only the BA-RNA band D is able to transfer a BA-imprint to naive (experimental repeats > 4, ***p-value < 0.001). (L), NEB double stranded RNA ladder.

Figure 3. Olfactory imprinting is triggered by the transfer RNAs (tRNAs) containing fraction.

(3a) Left part: Total small RNA from naive (NA) and from CI-exposed (CI) were fractionated on a 7M Urea 15% polyacrylamide gel and stained with ethidium

bromide (EtBr). Band 1 is the 5S rRNA, bands 2 to 7 are tRNAs, while band 8 is made of unknown RNAs. The L well contains the NEB single stranded RNA ladder. Right part: Northern blot analyses were performed on NA and CI RNA blots with two radiolabeled probes specific to the *C. elegans* $tRNA^{Leu}$(AAG) and $tRNA^{Gly}$(UCC). **(3b)** After elution from the polyacrylamide gels, fractions 1 to 8 or fractions 2 to 7 from naive (NA Mix 1-8 and NA Mix 2-7) and from CI-exposed (CI Mix 1-8 and CI Mix 2-7) were pooled. Naive worms were fed on each pool and assayed for CI response. Both of the pooled CI mixes trigger a faster migration (MMI) in CI gradients than the corresponding NA mixes (experimental repeats > 4, **p=0.006; ***p<0.001).

(3c) Naive N2 were fed on each of the 1 to 8 individual fractions from naive and from CI-exposed. Only feeding on the tRNA fractions 5 and 6 from CI-exposed enhances migration in CI gradients (MMI), compared to all other tRNA fractions (experimental repeats > 4, ***p<0.001).

Figure 4 and Table 1 : Alanine tRNA (UGC) transfers olfactory imprinting

(4a) 37 nt long isotype and isoacceptor-specific DNA probes corresponding to the 3' halves of *C. elegans* tRNAs were designed (Methods). Probes were 3'-biotinylated and used for purification on streptavidin microbeads, as described in the Methods. Small RNAs or gel-eluted fractions 5 or 6 from odor-exposed worms were hybridized to pools of isotype-specific probes bound to microbeads. The tRNA eluted from each pool was assayed to test the imprinting transfer to naive. The CI imprinting Fraction 5 CI-tRNAs were hybridized to the isotype-specific 3'-biotinylated DNA probes for the

3' halves of $tRNA^{Glu}$, $tRNA^{Lys}$, $tRNA^{Cys}$, $tRNA^{Gln}$, $tRNA^{Ala}$ or $tRNA^{Leu}$ bound on streptavidine-microbeads. Naive worms were fed on tRNAs eluted from the microbeads and tested for migration in CI 1/300 gradients (***p-value < 0.001).

(4b) Northern blot analysis on gel-eluted tRNA fractions from Naive (N3 to N6), on tRNA fractions from CI-exposed (C3 to C6) and on tRNA fractions from BA-exposed (B4 to B7) N2 worms. Hybridizations were performed with radiolabeled oligonucleotides specific for $tRNA^{Lys}$ (UUU), $tRNA^{Gln}$ (UUG), $tRNA^{Glu}$ (UUC), $tRNA^{Leu}$ (AAG), and $tRNA^{Ala}$ (AGC, CGC, UGC).

(4c) Fraction 5 CI-tRNAs were eluted from microbeads bound to the four Alanine codon-specific (3'-AGC, 3'-CGC, 3'-UGC and 5'-UGC) probes. Naive worms were fed on tRNAs eluted from the microbeads and tested for migration in CI 1/300 gradients (***p-value < 0.001).

Figure 5 : A unique $tRNA^{Ala}$ (UGC) probe purifies BA, CI or IA specific imprints.

Small RNAs were purified from naive, BA-exposed, CI-exposed or IA-exposed N2 wt worms using LiCl precipitation as described in Methods. The tRNAs eluted from microbeads bound to the tDNA Alanine TGC probe, were added to the food of naive worms during egg to adult development. At the adult stage, worms fed on these tRNAs were assayed for BA, CI or IA chemotaxis (**p-value < 0.01).

Figure 6 : $tRNA^{Ala}$ (UGC) triggered imprinting can be fixed and stably inherited after feeding al least six generations.

(6a) Imprinting segregation in the progeny of multigenerational imprinting tRNA fed worm populations (schematic representation). CI imprint (CI-tRNA) produced by CI-exposed worms was eluted from microbeads bound to the Alanine tRNA 3'-UGC specific probe. Naive worm populations were fed 1 ng CI-tRNA for one (F1) to seven (F7) successive generations. Naive generations grown without addition of RNAs were obtained from each of the seven (F1+n to F7+n, here n=4) CI-tRNAs fed populations. CI-tRNAs fed worms passed a CI-imprint to the first naive generation. Imprinting is definitely lost at the second naive generation issued from one to five CI-tRNAs fed generations; it is maintained in worm populations issued from six and from seven CI-tRNAs fed generations.

(6b) Behavior of the fourth naive generations issued from worms fed on CI-tRNAs for one to seven generations (F1+4 to F7+4) was compared to behavior of Naive-tRNA fed worms. The F6+4 and F7+4 worms migrate significantly faster than Naive-RNA fed animals in CI gradients ($n > 4$, **p-value < 0.01).

Figure 7. The development of chemo-attraction requires the elongator complex sub-unit 3 (ELPC-3).

(7a) Mean Migration Indices (MMI) of N2 wt and *elpc-3 (tm3120)* mutant worms in gradients of the chemo-attractive odors Citronellol (CI), Benzaldehyde (BA) and Isoamyl alcohol (IA) at the indicated dilutions. The *elpc-3 (tm3120)* mutation is a 355 base pair deletion in the Elongator complex sub-unit 3 gene. MMI were determined

as described for 4 days old worms (experimental repeats ≥ 4, **p-value < 0.01, **p-value < 0.01).

(7b) Mean Migration Indices (MMI) of N2 wt, *elpc-3 (ng15)* and *elpc-1 (ng10)* mutant worms in gradients of the chemo-attractive odors Citronellol (CI), Benzaldehyde (BA) and Isoamyl alcohol (IA) at the indicated dilutions.

elpc-3 (ng15) worms carry a mutation leading to the substitution of arginine (R) 327 into a cysteine (C) in the ELPC-3 protein. *elpc-1 (ng10)* is a 2050 base pair deletion in the Elongator sub-unit 1 (ELPC-1) gene. MMI was determined as described on 4 days old worms (experimental repeats ≥ 4, **p-value < 0.01).

Figure 8. tRNA^{Ala} (UGC) purified from wild-type naive worms fully rescues chemo-attraction of the chemotaxis defective *elpc-3 (tm3120)* mutants.

tRNA co-migrating 2 to 7 RNA fractions from the naive (NA) lane shown in ***Figure 3a*** were gel-eluted. *elpc-3 (tm3120)* worms were grown during their whole development in the presence of pooled NA 2-7 fractions, of individual NA 2 to 7 fractions, or without RNA (No tRNA). MMI to CI 1/300 was determined as described on 4 days old worms (experimental repeats ≥ 4, **p-value < 0.01).

(8b) RNA from the naive worms fraction 5 (N5) was submitted to purification on microbeads bound to tDNA probes corresponding to the 3' half of tRNA^{Ala}(AGC), the 3' half of tRNA^{Ala} (CGC), the 3' half of tRNA^{Ala} (UGC) or the 5' half of tRNA^{Ala} (UGC). *elpc-3 (tm3120)* worms were fed on tRNAs eluted from each probe, or unfed

as control (No tRNA). MMI to CI 1/300, to BA 1/600 or to IA 1/600 were determined at the adult stage as described (experimental repeats ≥ 4,**p-value < 0.01).

Supplement Figure Method 2

Specificity of the tRNA isolation method

(A) The tDNA probe for tRNA (AGC) differs from the tDNA probe for tRNA (TGC) by only 4 nucleotides. **(B)** Microbeads were coupled to the Ala 1 (tRNA (AGC)) tDNA probe. Procedures of tRNA purification were as indicated in the microMACS kit procedure, except the ionic strength of the Binding/Washing buffer varied from 0.5 M, the regular concentration used for all tRNA isolations, to 0.05 M. Wild-type naive tRNAs eluted from the Alanine tDNA probe 1 (AGC) do not rescue chemotaxis of *elpc-3 (tm3120)* mutant worms using the regular 0.5 M or a 0.2 M NaCl Binding/Wash buffer. By contrast, the same probe purified tRNAs able to rescue CI chemotaxis, when lower concentrations (0.1 M or 0.05 M) NaCl were used. Using low ionic strength leads to the co-purification of both inactive $tRNA^{Ala}$(AGC) and active rescuing $tRNA^{Ala}$ (UGC).

Figure 9. Odors or odor-specific $tRNA^{Ala}$ (UGC) specifically and stably suppress odor-specific chemo-attraction in the *elpc-1 (ng10)* and *elpc-3 (ng15)* elongator mutants.

(9a) *elpc-1 (ng10)* larvae were exposed or not (Naive) to a single odorant CI or BA or IA. The progeny of worms exposed to a single odorant was or was not exposed to a second odorant. The progeny of doubly-exposed CI+BA, CI+IA, BA+IA worms was or was not exposed to the third odorant to obtain CI+BA+IA worms. MMI to CI 1/300 was determined as described on 4 days old worms (experimental repeats ≥ 4, **p-value < 0.01).

(9b) *elpc-3 (ng15)* larvae were exposed or not (Naive) to a single odorant CI or BA or IA. The progeny of worms exposed to a single odorant was or was not exposed to a second odorant. The progeny of doubly-exposed CI+BA, CI+IA, BA+IA worms was or was not exposed to the third odorant to obtain CI+BA+IA worms. MMI to IA 1/600 was determined as described on 4 days old worms (experimental repeats ≥ 4, **p-value < 0.01).

(9c) *elpc-3 (ng15)* worms were fed on microbeads-purified $tRNA^{Ala}$ (UGC) from, respectively, naive, CI-exposed or BA-exposed N2 wt animals, as described. MMI to BA 1/600 and to CI 1/300 was determined on 4 days old worms (experimental repeats ≥ 4, ***p-value < 0.001, **p-value < 0.01).

Figure 10. $tRNA^{Ala}$ (UGC) from odor-exposed or odor-tRNA fed *elpc-1 (ng10)* and *elpc-3 (ng15)* worms transfer imprinting to naive.

Wild-type (wt) N2, *elpc-1 (ng10)* and *elpc-3 (ng15)* larvae were or not early exposed to CI 1/300. $tRNA^{Ala}$ (UGC) was purified from adult worms using microbeads bound

to its complementary tDNA probe, as described. Naive wt, naive *elpc-1 (ng10)* and naive *elpc-3 (ng15)* worms were fed on $tRNA^{Ala}$ (UGC) from CI-exposed wt, CI-exposed *elpc-1 (ng10)* or CI-exposed *elpc-3 (ng15)*. Their Mean Imprinting Index (MII) to CI 1/300 was determined at the adult stage, as described. Results are expressed as Mean Imprinting Index (MII). MII represents the difference between mean migration index (MMI) after feeding on $tRNA^{Ala}$ (UGC) from CI-exposed MMI after feeding on $tRNA^{Ala}$ (UGC) from naive. A positive MII indicates $tRNA^{Ala}$ (UGC) from CI-exposed increased worm migration to CI, compared to $tRNA^{Ala}$ (UGC) from naive. A negative MII results by contrast indicates $tRNA^{Ala}$ (UGC) from CI-exposed decreased worm migration to CI compared to naive $tRNA^{Ala}$ (UGC).

Figure 11. Chemotaxis suppression in *elpc-1 (ng10)* and *elpc-3 (ng15)* also abolished the odor-triggered production of odor-tRNAs.

Stable suppression of CI response in *elpc-1 (ng10)*, and stable suppression of CI, BA, BA+IA or BA+CI responses in *elpc-3 (ng15)* were obtained after odor-exposed or odor-tRNA feeding, as in ***Figure 9.*** Worm populations for which chemotaxis to CI, to BA or to IA were stably abolished are respectively annoted CI^-, BA^- or IA^-.

BA^-IA^- are worms with abolished BA and IA responses, while BA^-CI^- worms do not chemotax to BA and to CI.

N2 wt were fed on $tRNA^{Ala}$ (UGC) respectively extracted from (left to right):

Naive, CI-exposed, stable CI^- CI-exposed *elpc-1 (ng10)*, Naive, CI-exposed, stable CI^- CI-exposed *elpc-3 (ng15)*, stable BA^-IA^- CI-exposed *elpc-3 (ng15)*, stable BA^- CI^- CI-exposed *elpc-3 (ng15)*, Naive, BA-exposed, stable BA^- BA-exposed *elpc-3 (ng15)*. MMI to BA 1/300 and to CI 1/300 were determined as described (experimental repeats ≥ 4,**p-value < 0.01).

Figure 12. Odor-exposed olfactory transduction mutants do not produce odor-imprinting $tRNA^{Ala}$ (UGC).

$tRNA^{Ala}$ (UGC) was microbeads purified from Naive unexposed, CI 1/300-exposed wt N2 worms, CI 1/300-exposed *odr-1 (n1936)*, CI 1/300-exposed *tax-2 (p671)*, and from CI 1/300-exposed *tax-4 (p678)* mutant worms. Naive wt and naive *elpc-1 (ng10)* worms were fed on these $tRNA^{Ala}$ (UGC). Mean Migration Index to CI 1/300 was determined at the adult stage as described (**p-value < 0.01).

Figure 13. Summarized hypothesis

During the first laval stage of *C. elegans* development, attractive odorants sensed by olfactory neurons AWC, bind putative odor-specific membrane chemoreceptors coupled to a functional odor-signal transduction pathway.

Odor-specific activation triggers the production of odor-specific forms of $tRNA^{Ala}$ (UGC), each carrying an odor signature, able to transfer olfactory information to

naive animals via feeding. Odor-specificty of imprinting support the existence of a physical insulation of odor signalling within the AWC neurons.

Different functions associated to the Elongator complex sub-units 1 and 3 (ELPC-1, ELPC-3) control the chemo-sensory behavior of *C. elegans*. Worms with a deleted *elpc-3* gene lose all AWC-sensed responses; addition of the wild-type naive form of $tRNA^{Ala}$ (UGC) fully rescue the *elpc-3* mutant phenotype. Worms with a deleted *elpc-1* gene or a punctual substitution in *elpc-3* perform chemotaxis as wt. These mutations do not affect the production of odor-specific forms of $tRNA^{Ala}$ (UGC). They however impair imprinting: as opposed to wt, odor-exposure or odor-tRNA feeding result in stably inherited suppression of odor response.

Table 1 : Alanine tRNAs from odor-exposed transfer imprinting to naive.

Pooled tDNA Probes		RNA from odor-exposed worms	Behavior after feeding eluates	tRNA isotypes
Ala 1+2+3 Arg 1+2+3 Asn, Asp, Cys	**A**	Small RNAs or Gel-eluted fractions 5 and 6	**Imprinting**	**Ala, Arg, Asn, Asp, Cys**
Gln 1+2, Glu 1+2 Gly 1+2, His Ile 1+2	**B**	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Gln, Glu, Gly, His, Ile
Leu 1+2+3+4 Lys 1+2, Met 1+2	**C**	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Leu, Lys, Met
Phe, Pro 1+2+3 Ser 1+2+3	**D**	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Phe, Pro, Ser
Thr 1+2+3, Trp Tyr, Val 1+2+3	**E**	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Thr, Trp, Tyr Val

Arg 1+2+3 Asn, Asp, Cys Gln 1+2, Glu 1+2 **F**	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Arg, Asn, Asp Cys, Gln, Glu
Gly 1+2, His **G** Ile 1+2 Leu 1+2+3+4	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Gly, His, Ile Leu
Lys 1+2 **H** Met 1+2 Phe, Pro 1+2+3	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Lys, Met, Phe Pro
Ser 1+2+3 **I** Thr 1+2+3, Trp	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Ser, Thr, Trp
Tyr, Val 1+2+3 **J** Ala 1+2+3 Arg 1+2+3	Small RNAs or Gel-eluted fractions 5 and 6	**Imprinting**	**Tyr, Val, Ala** **Arg**
Asn, Asp, Cys **K** Glln 1+2, Glu 1+2 Gly 1+2, His	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Asn, Asp, Cys Gln, Glu, Gly, His
Ile 1+2 **L** Leu 1+2+3+4 Lys 1+2	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Ile, Leu, Lys
Met 1+2 **M** Phe, Pro 1+2+3 Ser 1+2+3	Small RNAs or Gel-eluted fractions 5 and 6	Naive	Met, Phe, Pro Ser
Thr 1+2+3, Trp **N** Tyr, Val 1+2+3 Ala 1+2+3	Small RNAs or Gel-eluted fractions 5 and 6	**Imprinting**	**Thr, Trp, Tyr** **Val, Ala**

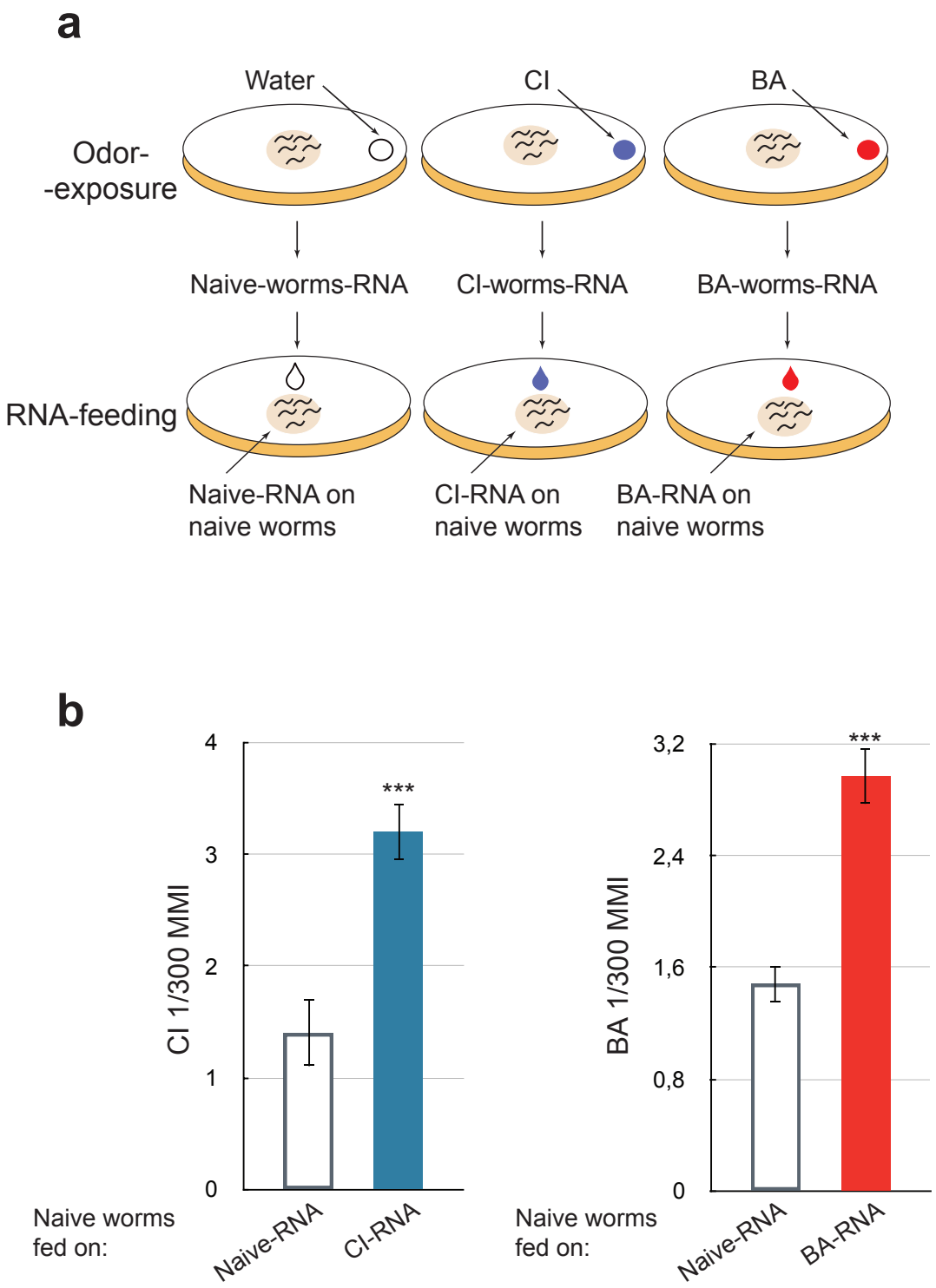
Table 2 : tRNAs use the SID-1 and SID-2 dsRNA-specific transporters to, respectively, support imprinting inheritance and imprinting transmission via feeding.

(a) Wild-type N2 larvae and larvae with mutations inactivating the *sid-1* or *sid-2* genes were odor-exposed to CI 1/300 or not exposed, as control naive. The Mean

Migration Indices of naive and of CI-exposed wt N2, *sid-1 (qt2)* and *sid-2 (qt13)* worms in CI 1/300 gradients are shown. (experimental repeats > 4, ***p-value<0.001).

(b) *sid-1 (qt2)* and *sid-2 (qt13)* mutants were fed on the indicated amounts (from 0 to 100 ng) of total RNA extracted from CI-exposed wt N2 animals. Mean Migration Indices to CI 300 were determined at the adult stage (experimental repeats > 4, ***p-value<0.001).

Behavior / **Genotypes**		Imprinting after odor-exposure	Imprinting Inheritance	Imprinting after tRNA feeding
N2 wt	**1.6 +/- 0.2**	**2.8 +/- 0.3** **p<0.001**	**2.8 +/- 0.3** **p<0.001**	**2.9 +/- 0.3** **p<0.001**
sid-1 (qt2)	**1.8 +/- 0.3**	**3.2 +/- 0.2** **p<0.001**	***1.7 +/- 0.3*** ***(= naive)***	**3.0 +/- 0.2** **p<0.001**
sid-1 (pk3321)	**1.8 +/- 0.3**	**2.9 +/- 0.2** **p<0.001**	***1.8 +/- 0.3*** ***(= naive)***	**2.8 +/- 0.3** **p<0.001**
sid-2 (qt13)	**0.4 +/- 0.2**	**1.5 +/- 0.2** **p<0.001**	**1.6 +/- 0.2** **p<0.001**	***0.5 +/- 0.2*** ***(= naive)***
	Naive **CI MMI**	**CI-exposed generation** **CI MMI**	**Unexposed F1 generation** **CI MMI**	**CI-tRNA fed generation** **CI MMI**



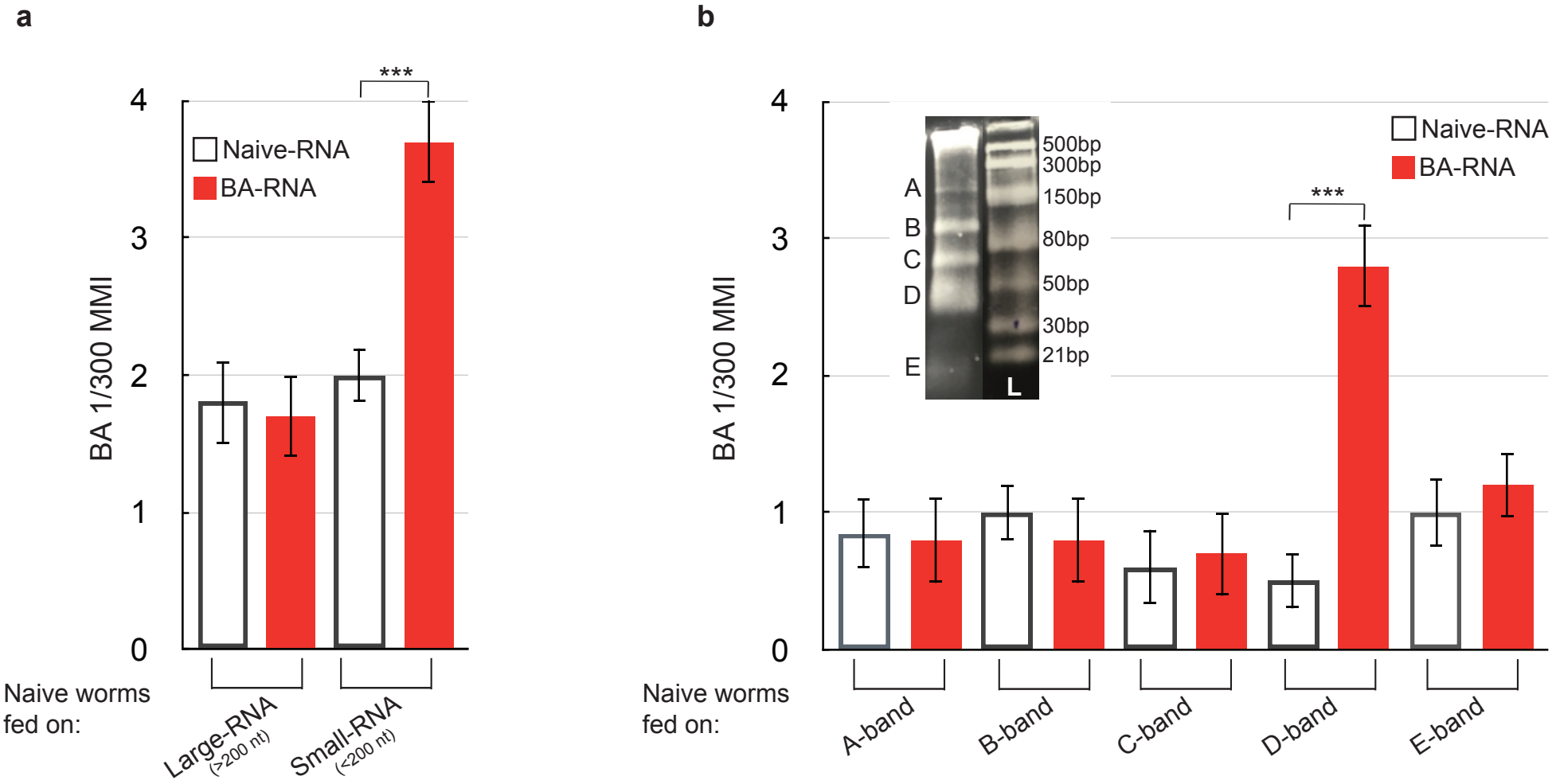

a
b
BA 1/300 MMI
Naive-RNA
BA-RNA
***
Naive worms fed on:
Large-RNA (>200 nt)
Small-RNA (<200 nt)
A
B
C
D
E
L
500bp
300bp
150bp
80bp
50bp
30bp
21bp
A-band
B-band
C-band
D-band
E-band

Figure 3a

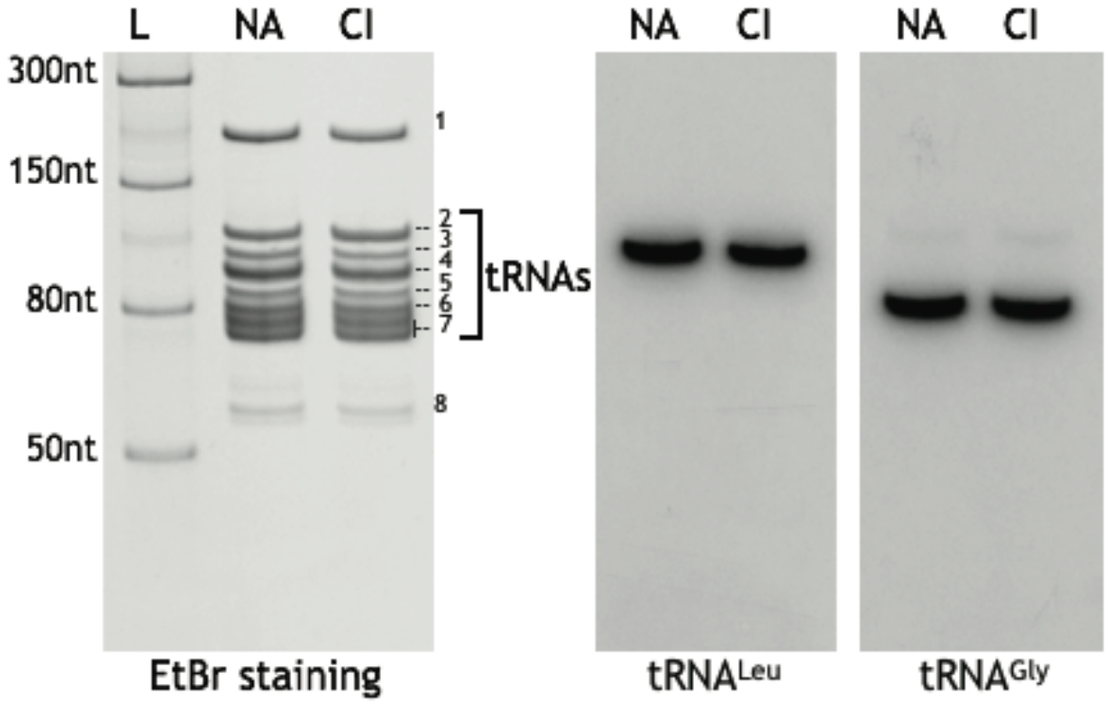


Figure 3b

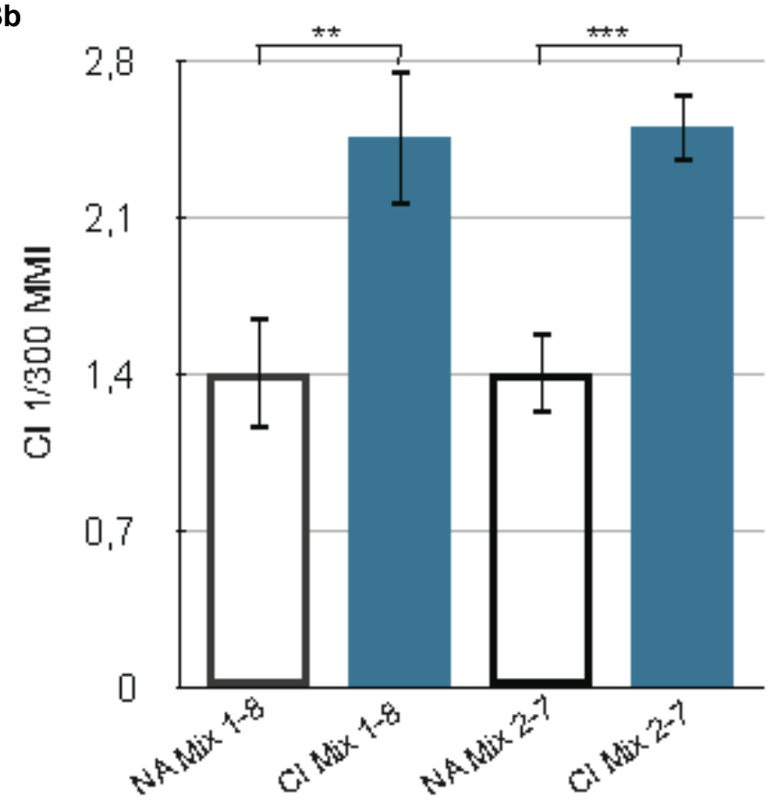


Figure 3c

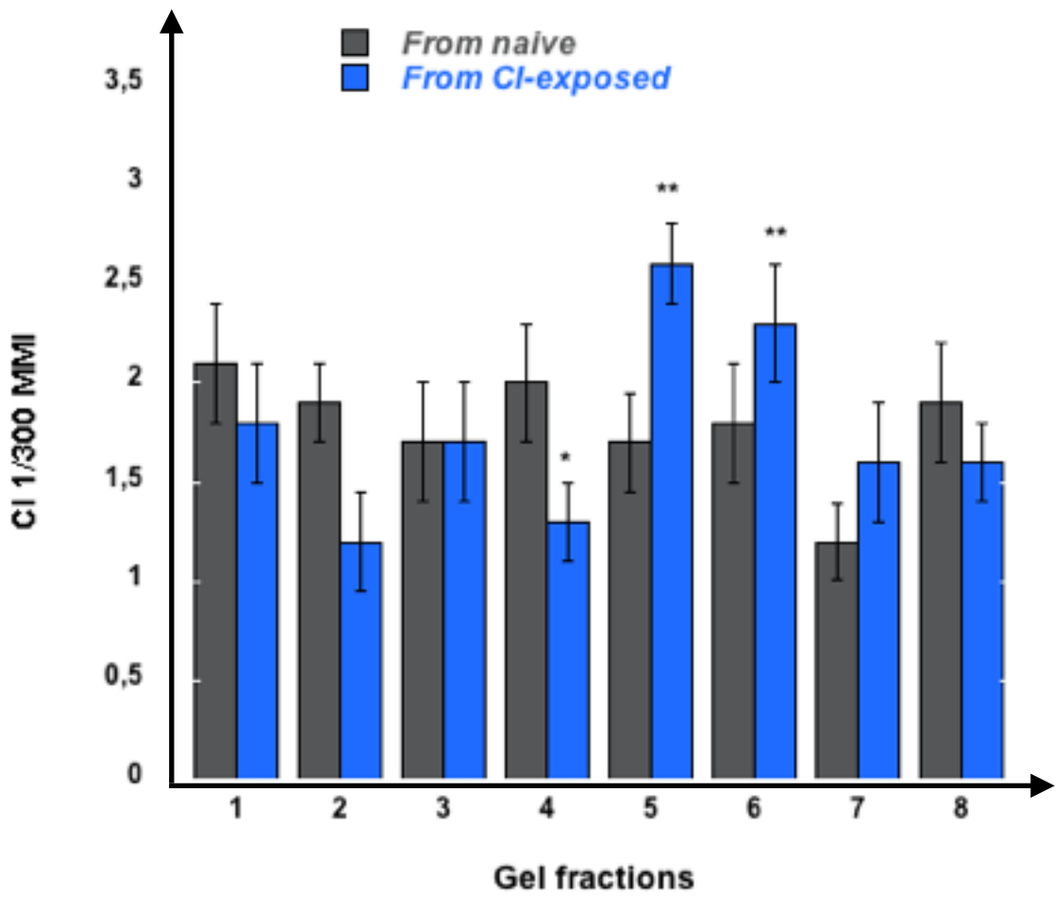


Figure 4a

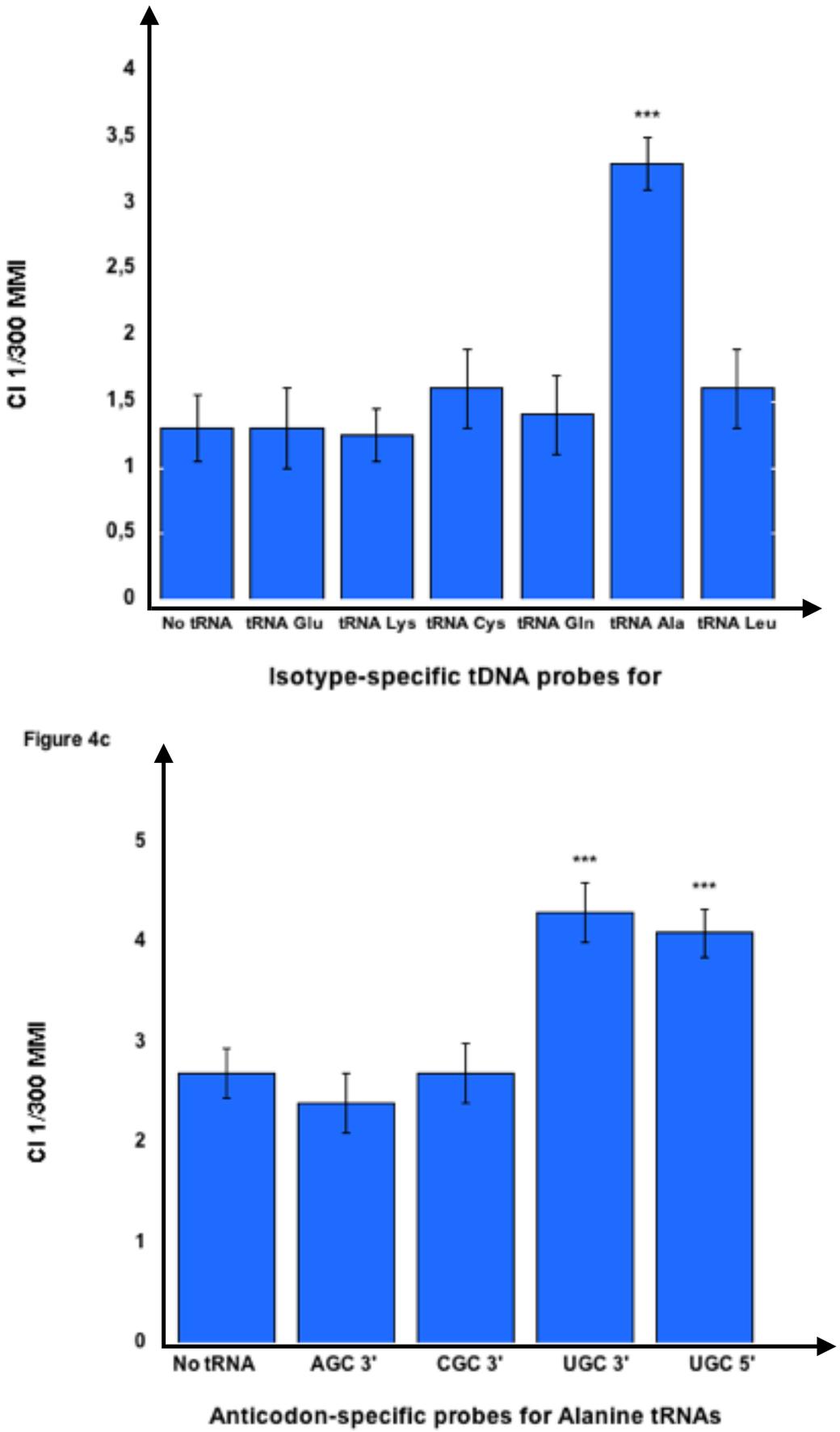


Figure 4b

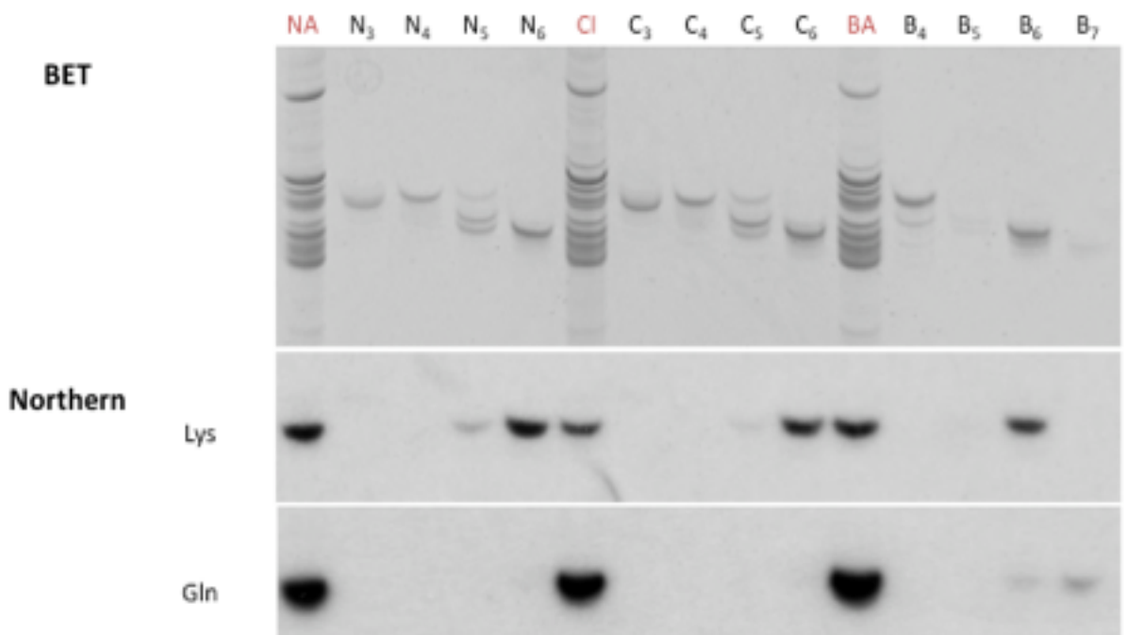


Figure 4c

Figure 5

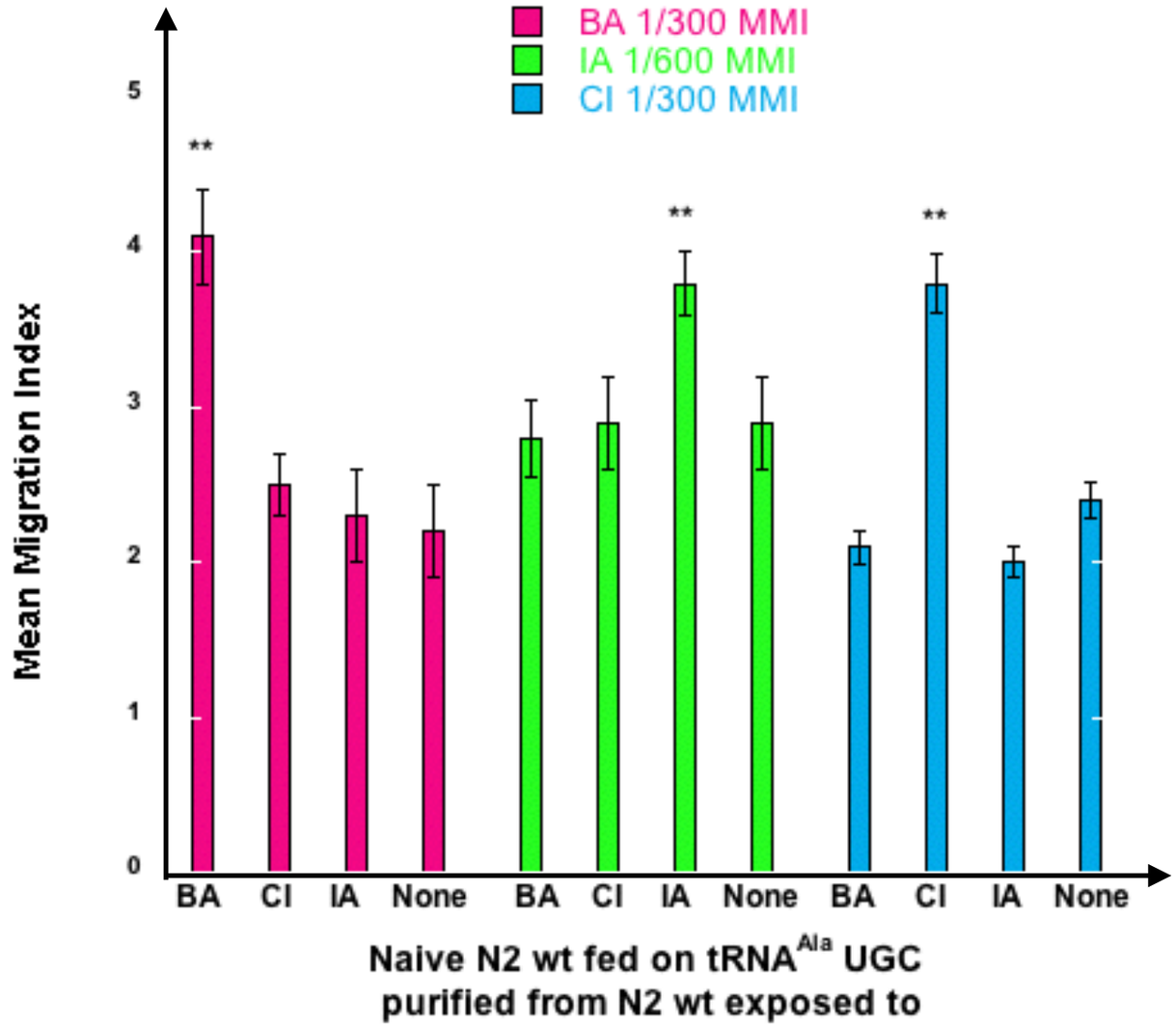
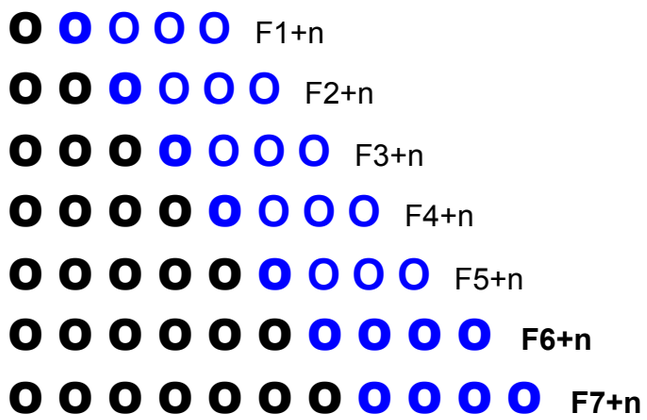

BA 1/300 MMI
IA 1/600 MMI
CI 1/300 MMI
Mean Migration Index
0
1
2
3
4
5
**
**
**
BA
CI
IA
None
BA
CI
IA
None
BA
CI
IA
None
Naive N2 wt fed on tRNA^Ala UGC
purified from N2 wt exposed to

Figure 6a



Generations of N2 wt worms

O Feeding tRNA ^{Ala} UGC from CI-exposed, Imprinting behavior

O No tRNA feeding, Imprinting behavior

O No tRNA feeding, Naive behavior

Figure 6b

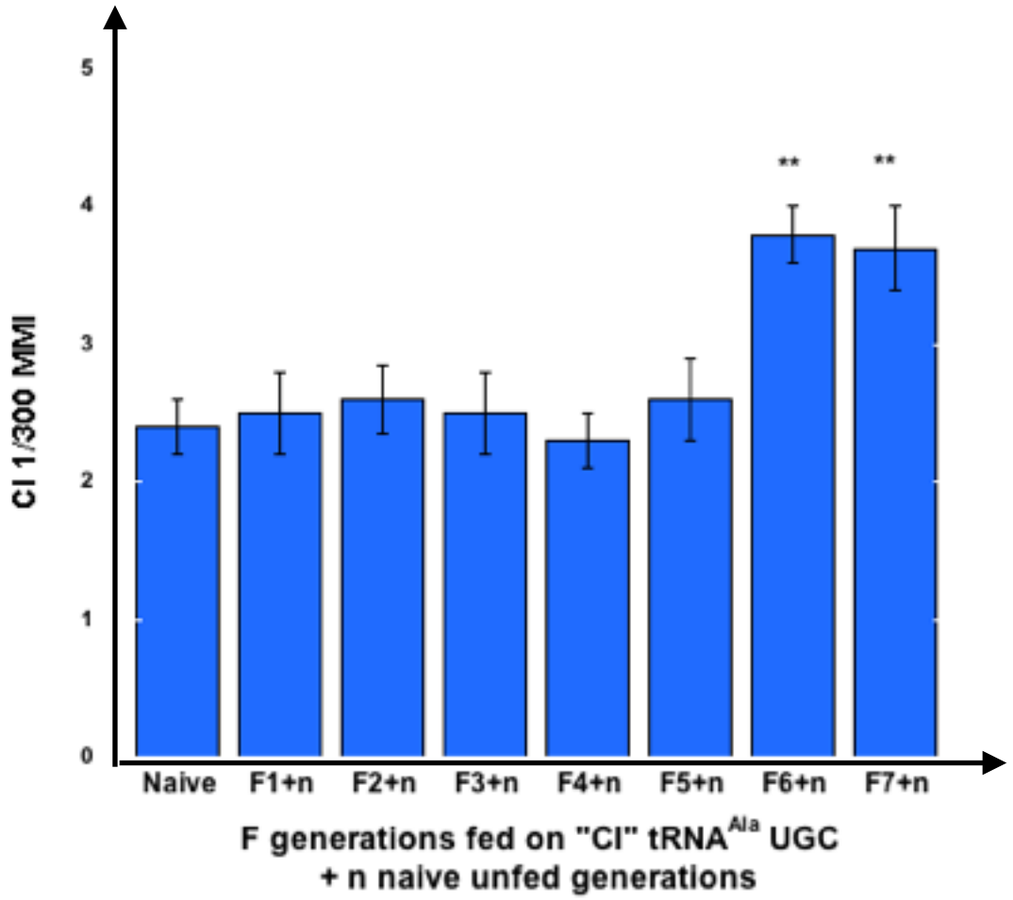
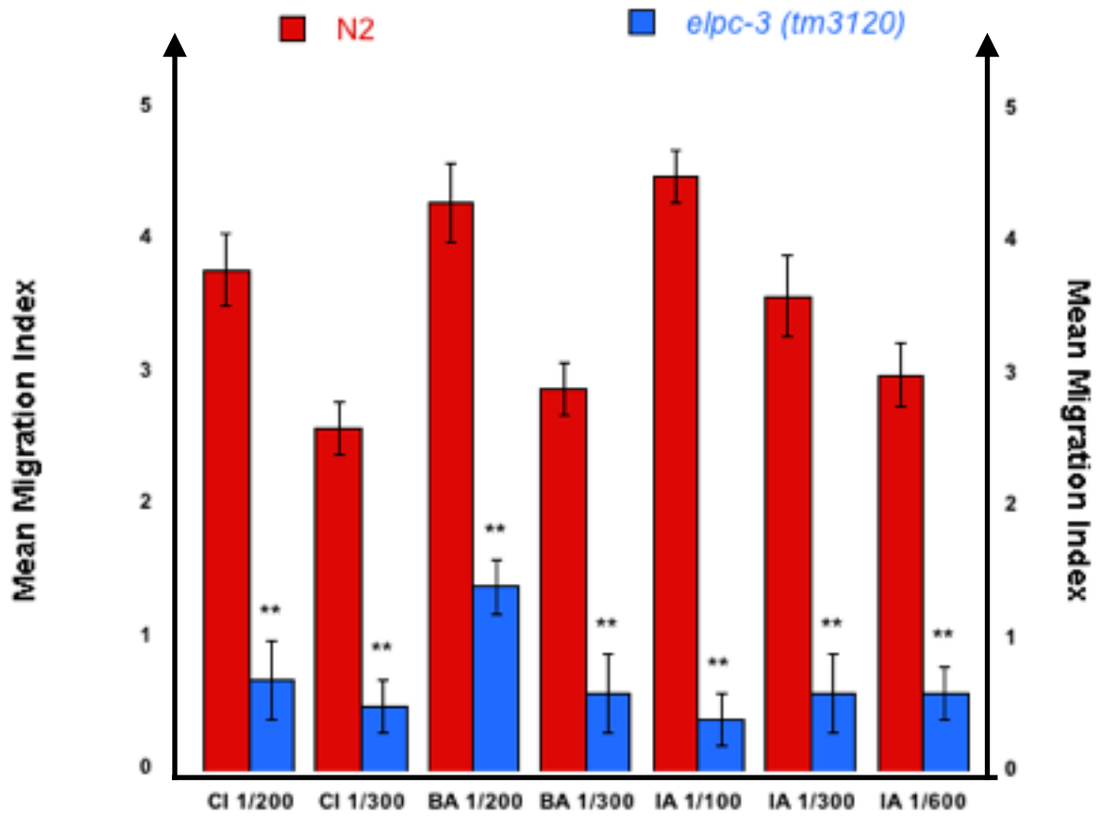


Figure 7a



Elongator sub-unit 3 mutant ***elpc-3 (tm3120)***

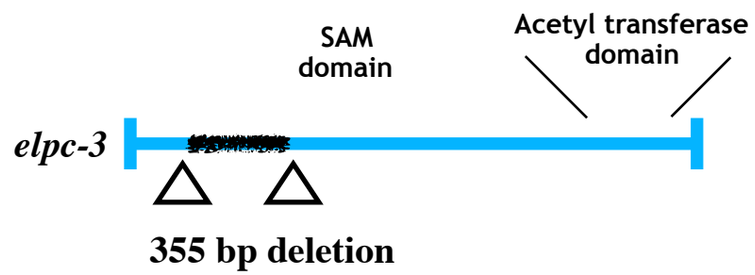
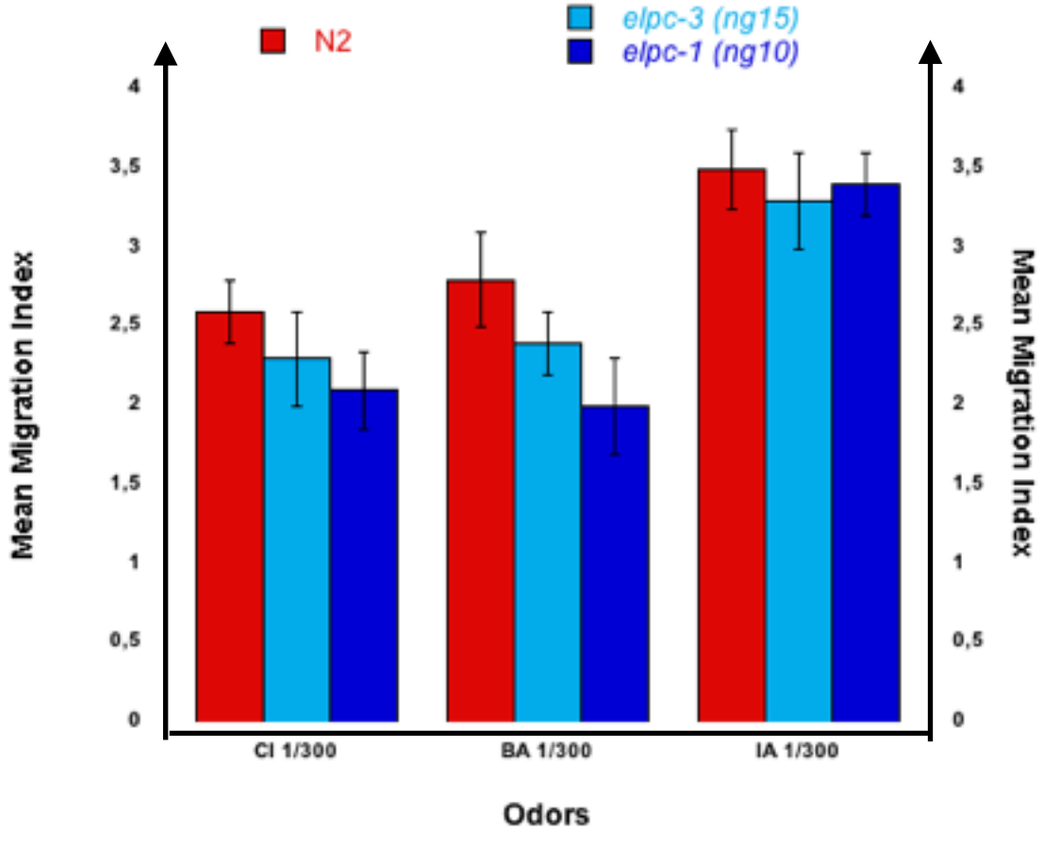
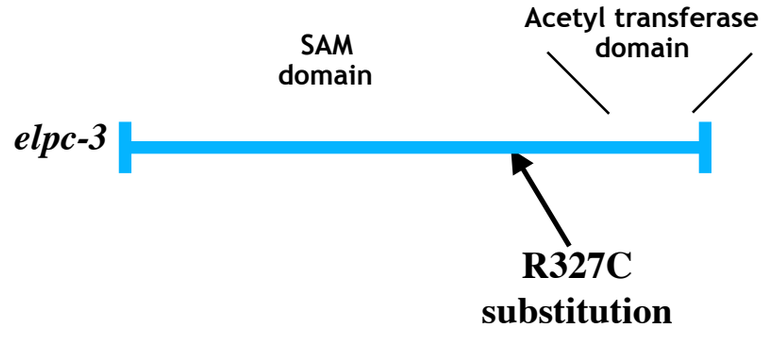


Figure 7b



Elongator sub-unit 3 mutant ***elpc-3 (ng15)***



Elongator sub-unit 1 mutant ***elpc-1 (ng10)***

Figure 8a

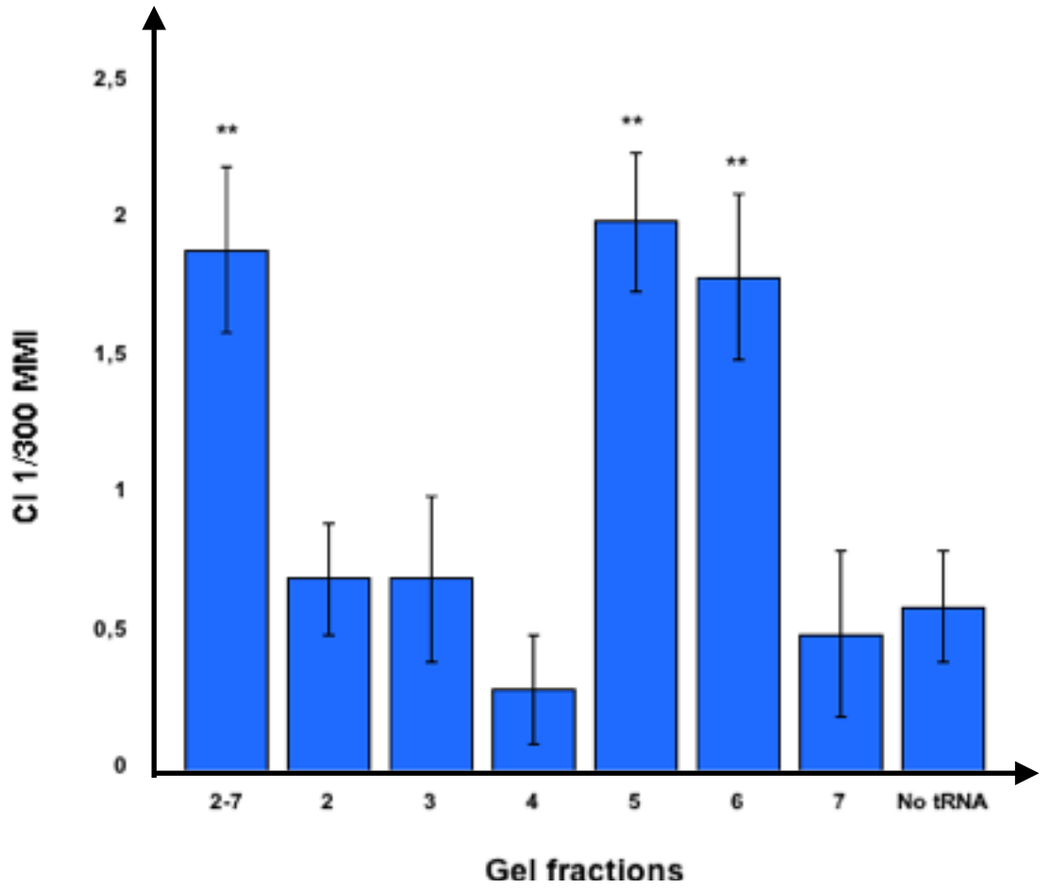


Figure 8b

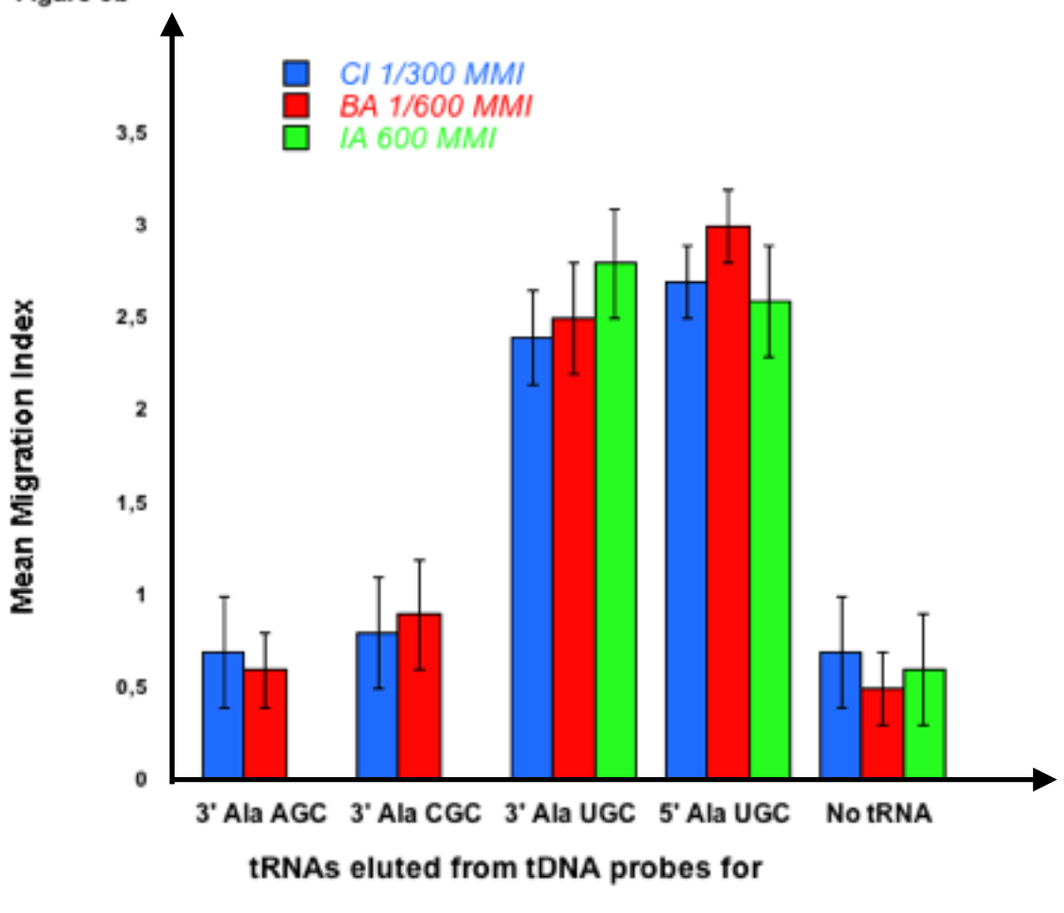


Figure 9a

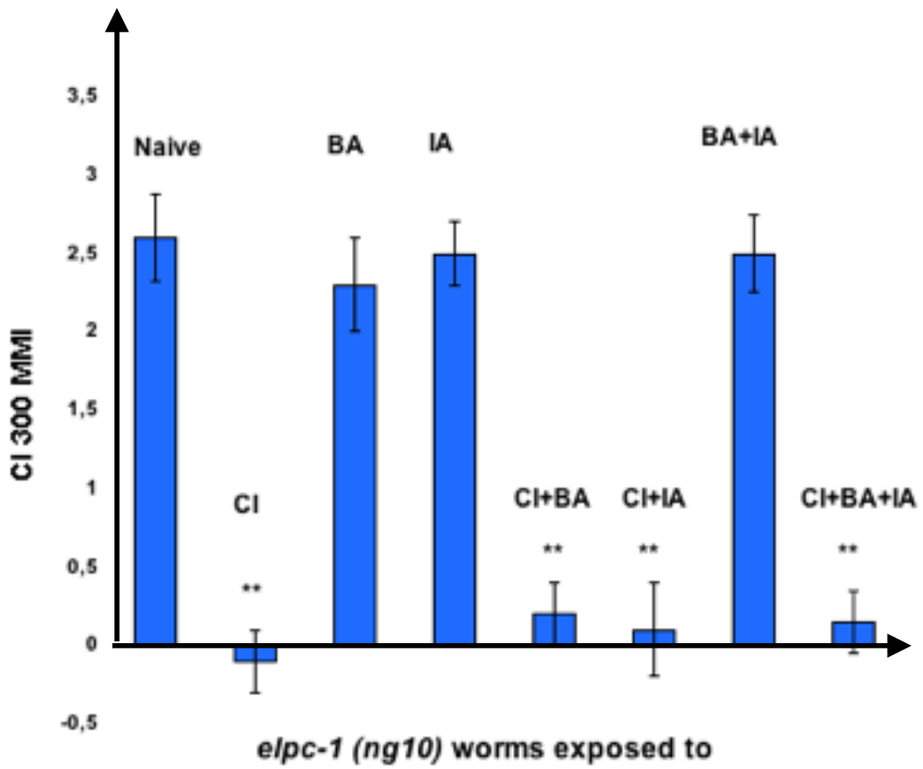


Figure 9b

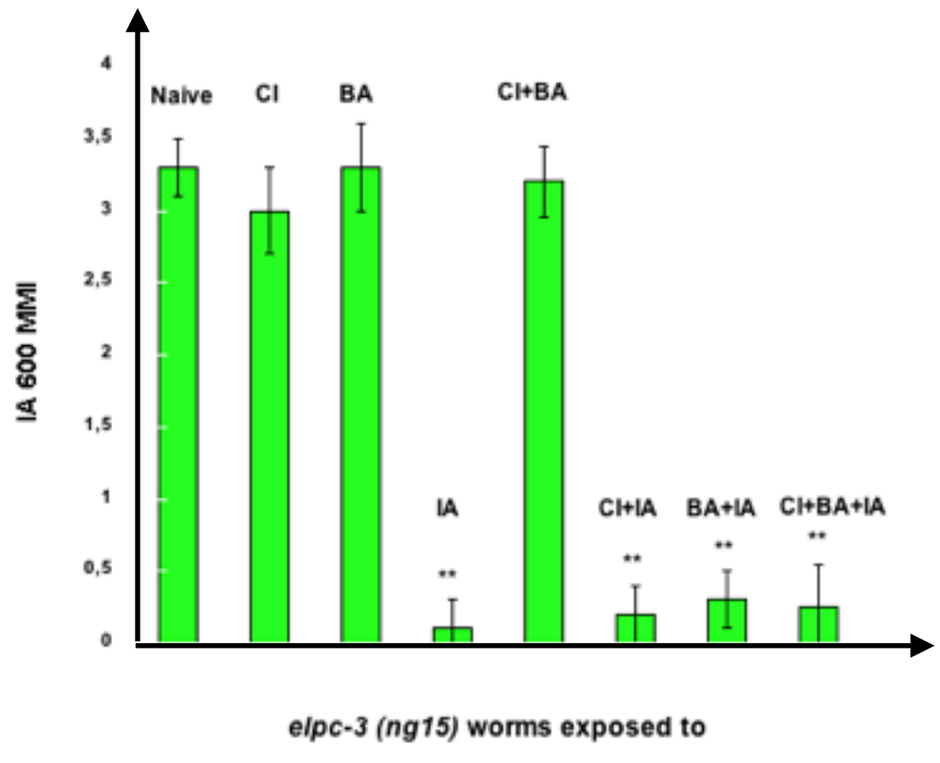


Figure 9c

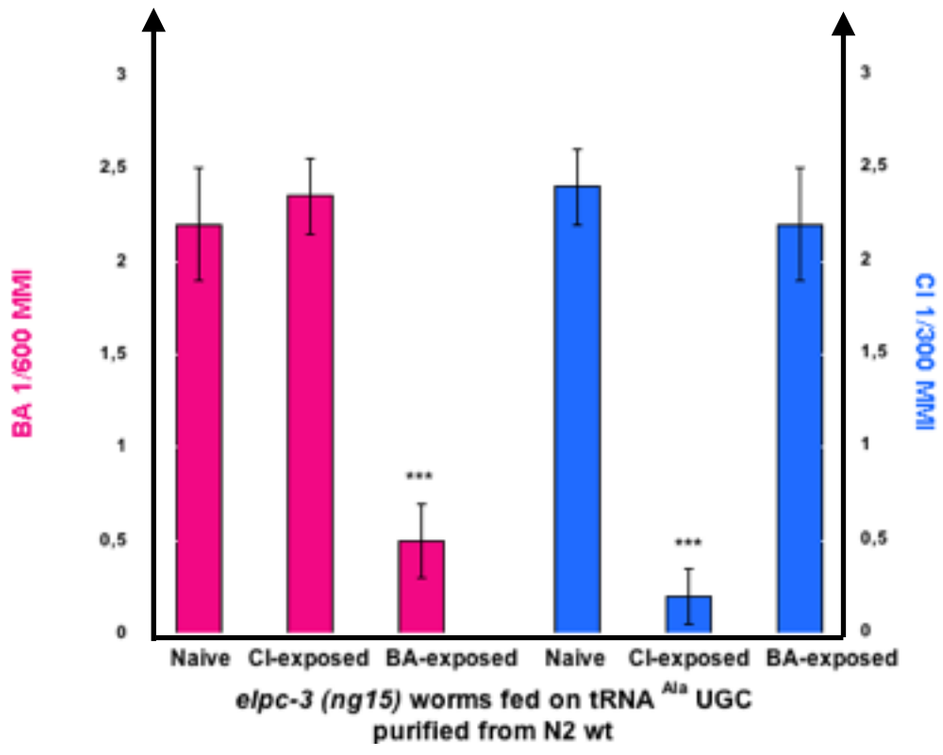


Figure 10

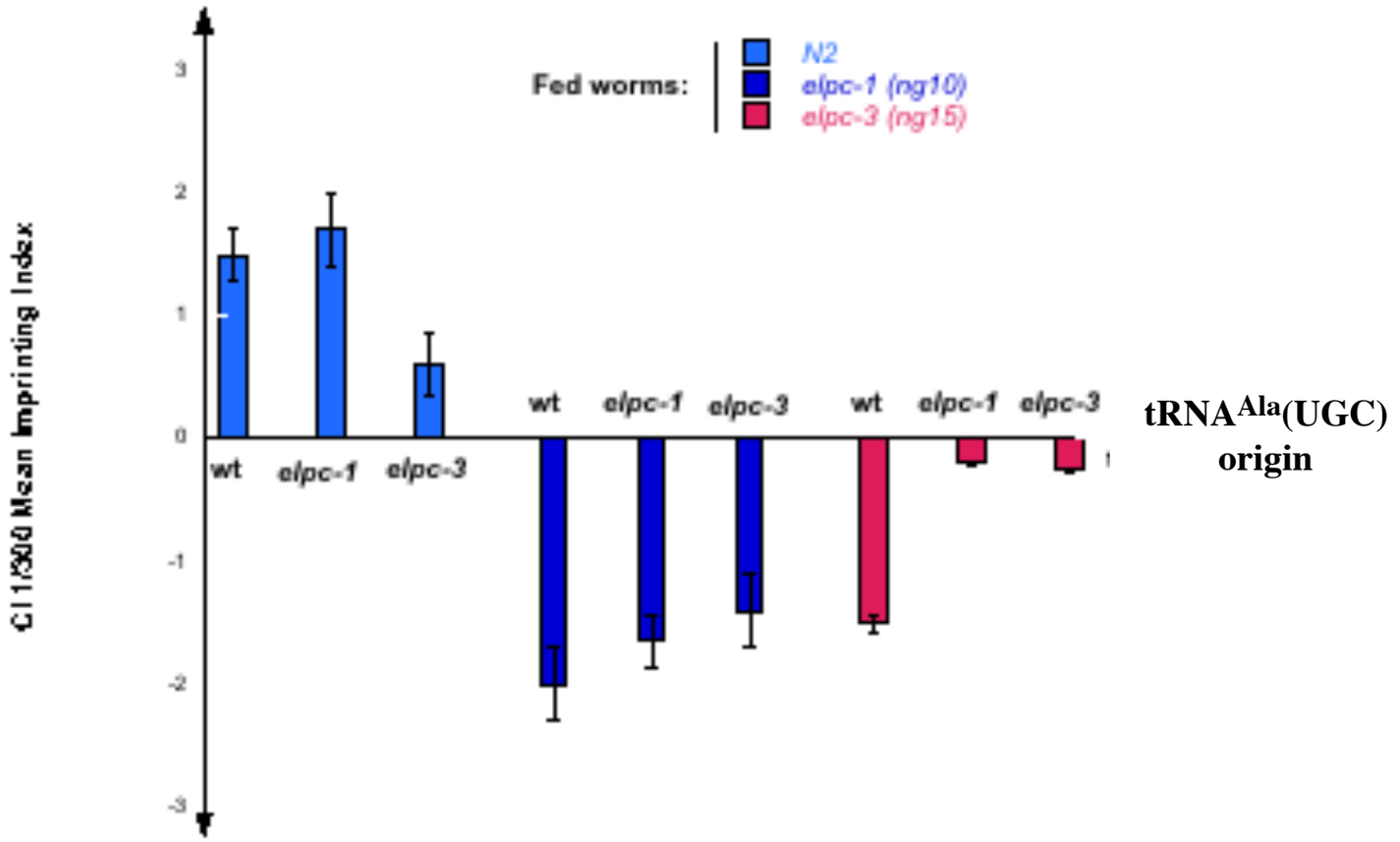

Fed worms:
N2
elpc-1 (ng10)
elpc-3 (ng15)
C11/300 Mean Imprinting Index
3
2
1
0
-1
-2
-3
wt
elpc-1
elpc-3
wt
elpc-1
elpc-3
wt
elpc-1
elpc-3
tRNA^Ala(UGC) origin

Figure 11

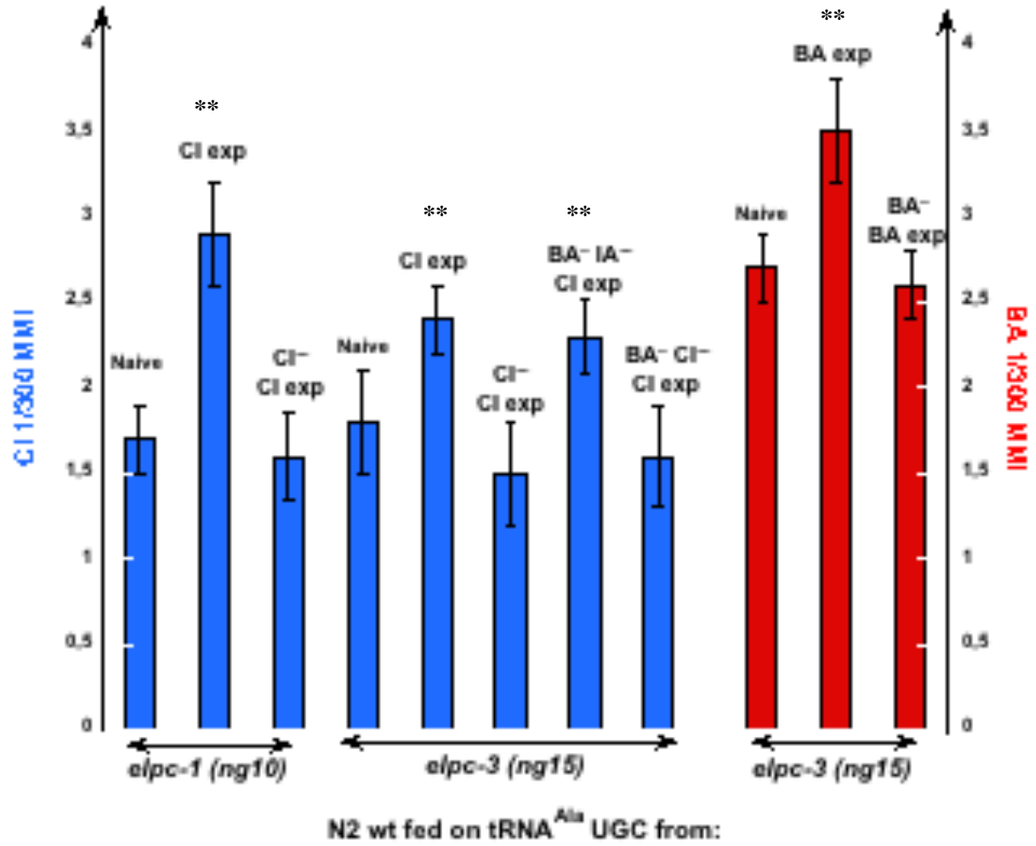

Cl 1/300 MMI
BA 1/300 MMI
4
3,5
3
2,5
2
1,5
1
0,5
0
**
Cl exp
Naive
Cl⁻
Cl exp
Naive
**
Cl exp
Cl⁻
Cl exp
**
BA⁻ IA⁻
Cl exp
BA⁻ Cl⁻
Cl exp
Naive
**
BA exp
BA⁻
BA exp
elpc-1 (ng10)
elpc-3 (ng15)
elpc-3 (ng15)
N2 wt fed on tRNA^Ala UGC from:

Figure 12

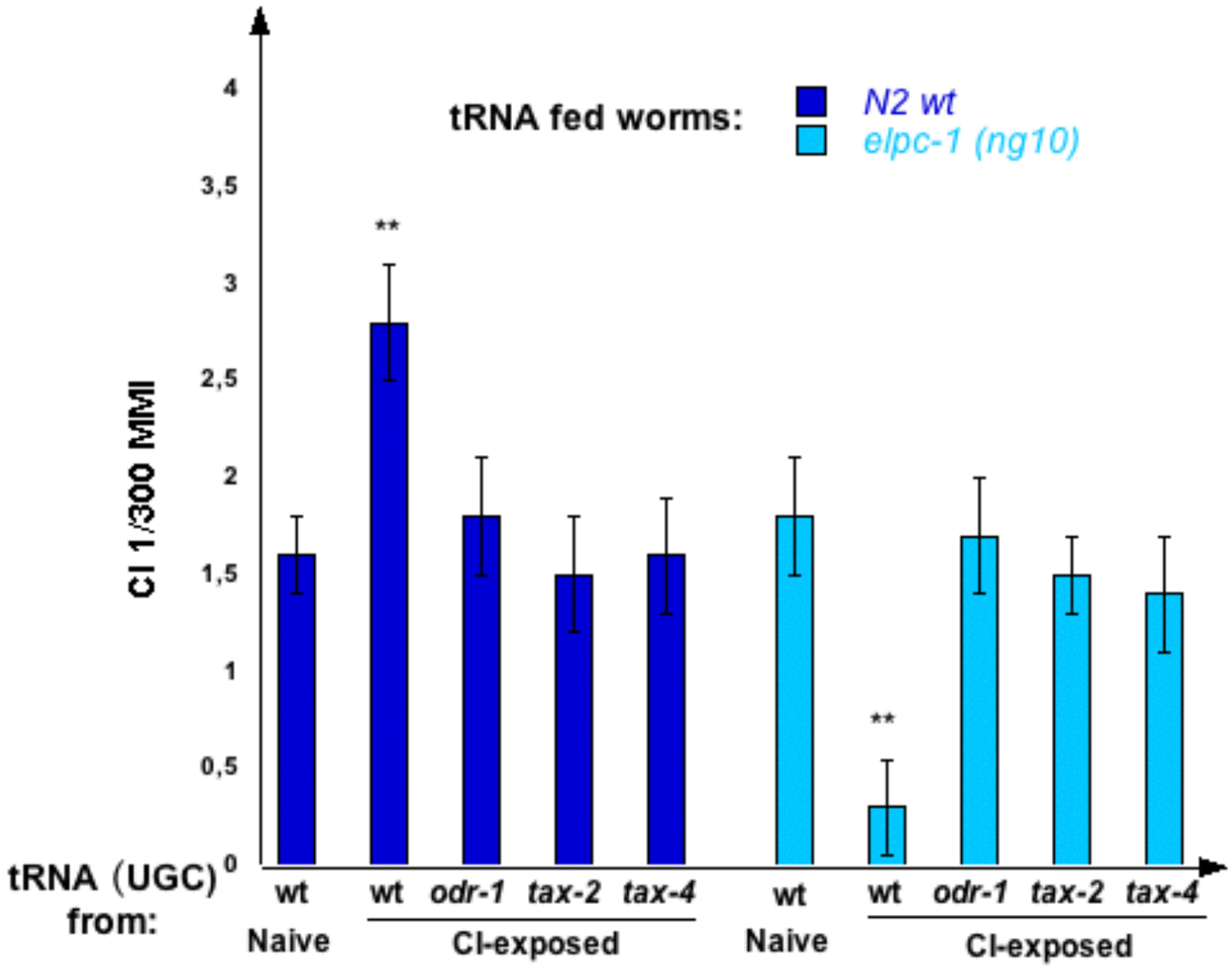
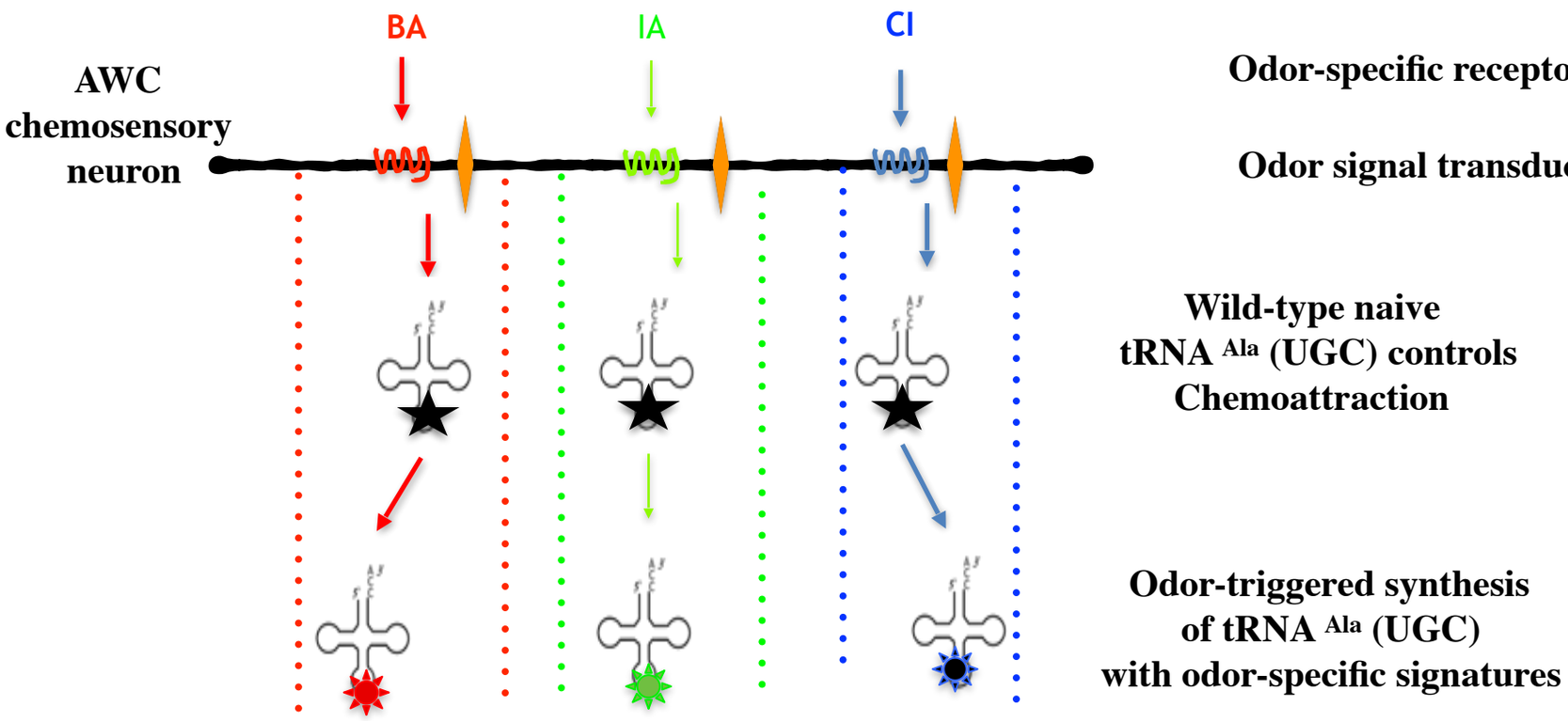

tRNA fed worms:
N2 wt
elpc-1 (ng10)
CI 1/300 MMI
4
3,5
3
2,5
2
1,5
1
0,5
0
**
**
tRNA (UGC) from:
wt
Naive
wt
odr-1
tax-2
tax-4
CI-exposed
wt
Naive
wt
odr-1
tax-2
tax-4
CI-exposed

Figure 13



Attractive odors BA, IA, CI

Odor-specific receptors

Odor signal transduction

Wild-type naive
tRNA ^{Ala} (UGC) controls
Chemoattraction

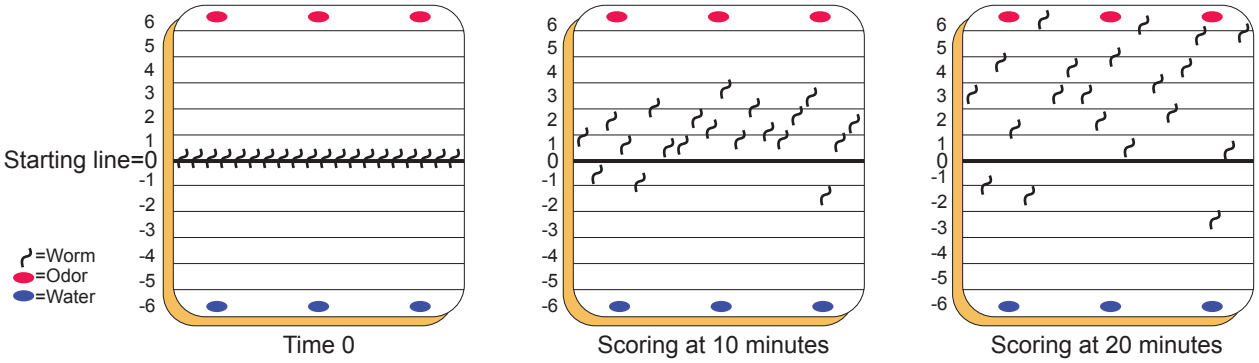
Odor-triggered synthesis
of tRNA ^{Ala} (UGC)
with odor-specific signatures

Feeding naive worms leads to

Transiently or Stably inherited Odor-specific Hyperosmia in wt

Stably inherited Odor-specific Anosmia in imprinting mutants

Supplement Figure Method



Supplemental Figure Method 2

A : Alanine tDNA probes alignment

Ala AGC probe : 5’ - TGGAGGTATGGGGAAT*T*GAACCCCAG*C*C*C*TCTC*C*CAT - 3’

Ala TGC probe : 5’ - TGGAGGTATGGGGAAT*C*GAACCCCAG*A*C*T*TCTC*G*CAT - 3’

B : Rescue of *elpc-3 (tm3120)* CI chemotaxis