An RNAi screen for defecation mutants

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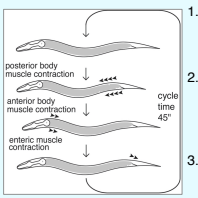
Introduction

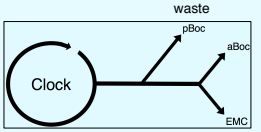
Defecation in *C. elegans* is a highly regulated process that takes place approximately every 45-55 seconds in a feeding animal. The cycle is composed of three distinct muscle contractions: a posterior body contraction, an anterior body contraction, and an enteric muscle contraction [1]. This one-minute biological rhythm is coordinated by a multi-tissue signaling system that utilizes several signaling molecules including calcium, protons, peptides, and classic neurotransmitters [1-4]. The timekeeping mechanism is located in the intestine and requires a calcium flux. Proton exchange across the intestinal membrane to elicits the posterior body contraction of the overlying body-wall muscles [3,4]. The subsequent contractions, anterior body and enteric muscle, require the release of either peptidergic signals or a combination of peptidergic and GABAergic signals from neurons [1,2].

We are in the process of performing an RNA interference (RNAi) screen to identify additional molecules required for defecation. The primary phenotype of interest is constipation, evidenced by a distended intestinal lumen, although other abnormalities are also noted.

A Three Step Motor Program



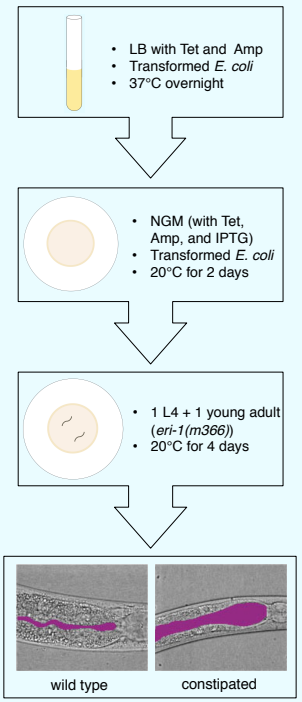
1. Posterior body contraction (pBoc) - waste pushed anteriorly.
2. Anterior body contraction (aBoc)- waste pushed posteriorly.
3. Enteric muscle contraction (EMC) - expulsion of the waste



A time keeping mechanism (clock) signals each of the motor contractions. Periodic calcium release in the intestine via IP_3 mediated calcium release is an integral part of the clock [1,5]. The posterior body contraction results from acute release of protons from the intestine into the pseudocoelomic space [3,4]. The neurons AVL and DVB are required for aBoc and EMC. Peptide and GABA release are necessary for these muscle contractions [2].

Materials and Methods

Transformed *Escherichia coli* from the ORFeome Library (Open Biosytems) were cultured overnight in Luria & Bertani media with tetracycline (Tet) (1.25 µg/mL) and ampicillin Amp) (5µg/mL). Nematode Growth Medium (NGM) plates supplemented with IPTG were seeded with the RNAi bacterial culture. Two days later, two *eri-1(m366)* worms (one L4 and one young adult) were added to each plate and incubated at 20°C for approximately four days. Adult worms were then visually scored using a standard dissecting microscope by two individuals.



Aspects of the constipated phenotype [5]:

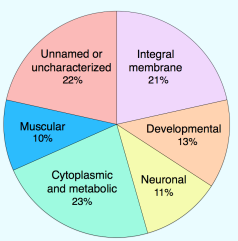
- Distended intestinal lumen that appears clear and convoluted
- Delayed development
- Thin or scrawny body
- Irregular defecation by either a impaired muscle contractions and/ or an extended cycle time.

Results

Phenotype	Number of Genes	Percentage
Wild type	5507	77.7
Slow growth	486	6.9
Embryonic lethal	390	5.5
Sterile	214	3.0
Larval arrest	148	2.1
Uncoordinated	103	1.5
Protruding vulva	101	1.4
Larval lethal	99	1.4
Constipated	92	1.3
Dumpy	71	1.0
Long	28	0.4

Note: some genes resulted in multiple phenotypes

Of the 7,086 genes screened thus far, 23.3% have exhibited a phenotype whereas previous studies averaged ~10% [6, 7]. This increase may be due to the used of *eri-1(m366)* animals [8]. The representation of specific phenotypes such as embryonic lethal or sterile in our data set is very similar to data found by Kamath et al. (5.5% and 2.8% respectively) but less similar to those found by Rual et al. (3.1% and 5.5% respectively). This may be due to similarities in our methods.



Thus far we have found 92 genes knockdowns that result in a constipated (Con) phenotype. The diversity of Con genes found is indicative of the complexity and multi-tissue interactions that are involved in this system.

Some of the most dramatic constipated phenotypes were described as being 'clear' or 'pale' in prior RNAi screens suggesting that other RNAi screens induced the same phenotype [9].

In our screen we have also encountered and correctly recognized genes such as *pbo-1*, *aex-5*, *unc-33*, and *flr-1* that are already known to cause constipation [1,5].

Future Directions

- Upon completion of the screen, (estimated December 2011) we will verify the RNAi induced constipated mutant phenotypes and RNAi inserts in the strains.
- Detailed timing of the defecation cycle will determine what aspects of the program are abnormal.
- The expression pattern of the genes will be determined as needed. The identity and expression domain of the each gene will be used to design hypotheses regarding its role in defecation signaling.
- Appropriate experiments will test these hypotheses.

Literature Cited

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