

Identification of Genes Regulating RNP Foci Formation in the Oocytes of *Caenorhabditis elegans*

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Abstract

Oocytes, or egg cells, of *C. elegans* contain ribonucleoprotein granules, or RNP foci, localized within the cytoplasm. These granules are composed of RNA and RNA binding proteins. Older worms that have delays in oocyte fertilization have RNP foci with increased complexity and size. It is believed that these RNP foci may be similar in function to stress granules in mammalian cells; in mammals, stress granules help to maintain molecular integrity of cells during environmental stresses. The hypothesis is that these RNP foci help to maintain molecular integrity of the oocytes, specifically of the RNA in the oocytes, when there is a delay in fertilization. Of 28 genes tested by RNA interference (RNAi), 2 were found to be possible regulators of RNP foci formation. Further analysis of the mechanisms of these genes will be performed using a secondary genetic screen and antibody staining.

Introduction

- Human females are born with a limited amount of oocytes
- Female fertility decreases with age due to defects in old-age oocytes
- Human research is controversial, so there is a need for a model organism

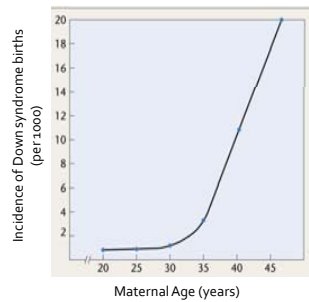


Fig. 1 Occurrence of disorders such as Down Syndrome increase with maternal age

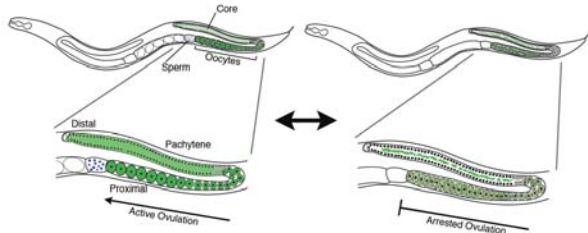


Fig. 2 RNP foci formation in hermaphrodites. In active ovulation (L), RNA binding proteins and mRNAs can be found evenly distributed throughout the oocytes and core (green). During arrested ovulation (R), these components aggregate into large RNP foci throughout the oocytes and core. This is a reversible process.

Hypothesis

- Function-RNP foci are believed to maintain molecular integrity of mRNAs when fertilization is delayed
- Prediction-If no RNP foci form, then mRNA stability and translation will be affected, and oocyte viability will decrease

Methods

- RNA interference (RNAi)
- 8 day process
- RNAi by feeding "knocks-down" expression of genes
- Gene of interest put into bacteria
- Bacteria fed to worms
- Genes used were "oogenesis-enriched"

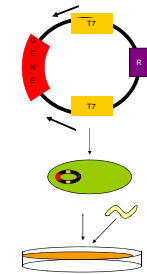


Fig. 3 RNAi process

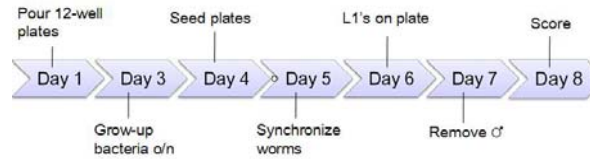


Fig. 4 Flowchart of one round of RNAi. 12-well NGM + carbenicillin + IPTG plates created on Day 1. On Day 3, 11 bacterial strains and control *ceh-18* bacterial strain were grown in 1 ml LB + carbenicillin stocks. On Day 4, the 12-well plate was seeded with 60 μ l of the cultured bacteria and incubated overnight at 37°C. On Day 5, bleaching of adult GFP::MEX-3; *fog-2* worms was performed to synchronize worms. On Day 7, male worms were removed from the plates. On Day 8, the adult hermaphrodites were scored.

Results

"Knocking-down" of some genes caused "mutant" phenotypes in worms



Fig. 5 Fluorescent images of RNAi screen. *ceh-18* GFP::MEX-3, *fog-2* hermaphrodite, positive control. GFP failed to assemble into large foci, and instead remained cytoplasmically distributed. (A) *inx-14* GFP::MEX-3, *fog-2* hermaphrodite. A "mutant" phenotype-GFP failed to assemble into large RNP foci. Possible regulator of RNP foci formation. (B) Gene K07A12.2 GFP::MEX-3, *fog-2* hermaphrodite. A "normal" phenotype-GFP aggregates into large RNP foci. The arrow points out one of these RNP foci. (C)

Results

- 28 genes were tested by RNAi
- 2 positive hits
- All data are valuable and important

Schisa #	Gene	Sequence Name	Cytoplasmic	Penetrance %	n (worms)
1-A3		R119.4	NA		
1-A4		R119.7	N		
1-A6		M01D7.6	N		
1-A7		Y23H5A.3	N		
1-A8		K09H9.2	N		
1-A9		K09H9.6	int	75	3
1-A10		M01D7.6	N		
1-A11		W05F2.3	N		
1-A12		H26D21.2	N		
1-B1		T12F5.2	N		
1-B3		T20F5.6	NA		
1-B4		T21E3.1	N		
1-E10	<i>inx-14</i>	F075A.1	yes	25	2
1-E12	<i>goa-1</i>	C26C6.2	yes	36	4
1-F10		T01G9.5	NA		
1-F11		F16D3.4	N		
1-F12		F02E9.4	N		
1-G1		F01D11.2	N		
1-G2		D1081.7	N		
1-G3		D1081.8	N		
1-G4		K021B2.5	NA		
1-G5		R05D11.7	int	33	2
1-G6		R05D11.8	NA		
1-G7		K07A12.2	int	25	1
1-G8		W06D4.6	int	60	3
2-D5		F14D2.8	N		
4-D8	<i>dcr-1</i>	K12H4.8	int	29	2
4-H10	<i>kgb-1</i>		int	10	1
6-G6	<i>ceh-18</i>		yes	91	21

Table 1. Genes *inx-14* and *goa-1* were found to be possible regulators of RNP foci formation. *ceh-18* was used as a positive control and has a 91% penetrance rate. Genes K09H9.6, R05D11.7, K07A12.2, W06D4.6, *dcr-1*, and *kgb-1* were found to be intermediate in regulating RNP foci formation. Genes R119.4, T20F5.6, T01G9.5, K021B2.5, and R05D11.8 were unscorable, due to bacterial contamination or presence of males. Remaining genes displayed the "normal" phenotype of RNP foci formation.

Discussion

- For "positive hits", molecular mechanisms controlling them will be analyzed and evaluated in future studies.
- This may lead to a better understanding of RNP foci formation in *C. elegans* oocytes, and worm fertility overall
- Similar mechanisms in mammals may be better understood due to *C. elegans* research

Acknowledgments

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