KU LEUVEN

Model Organisms

C. Elegans: egl-3 gene

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Contents

Contents 1

Question 1: Protein family 2

Question 2: Tissues and gene expression 2

Question 3: Development stages 2

Question 4: Mutant strains 2

Question : RNA interference phenotypes 2

Question 6: RNAi vs deletion mutant phenotypes 3

Question 7: Orthologous proteins 3

Question 8: Function in orthologous genes 4

Question 9: Null mutant in yeast 4

Question 10: Scientific information availability 4

Question 11: Model organism to further study 4

Question 1: Protein family

`Q:` Which protein family does the egl-3 encoded protein belong to?

`A:` The egl-3 gene encodes a homolog of a mammalian proprotein convertase that participates in peptide secretion. Mature mRNA is translated to a polypeptide of which not all parts are important to the mature, activated protein. Parts will be cleaved, and that is typically done by a proprotein convertase.

This information was found on the Wormbase website, on the egl-3 gene page. Then wikipedia helped to discover the function of proprotein convertase (understanding which may help to frame the context of subsequent questions).

Question 2: Tissues and gene expression

`Q:` In which tissues is egl-3 expressed in C. Elegans?

`A:` The information can be found in the *Location* tab of the wormbase website. It is expressed in many tissues, notably the nervous tissues, in the tail, in the pharynx, and in the head.

Question 3: Development stages

`Q:` In which developmental stage is the egl-3 expressed?

`A:` In the same tab, you can see that it is expressed in postembryonic stages, ie the adult and larvae stages.

Question 4: Mutant strains

`Q:` Are there mutant strains for this gene available? What has been mutated in the gene? What is the resulting phenotype?

`A:` (additional instruction: take out the million mutant project). You can find the mutations in the *Phenotype* section of the website. At the time of writing this report, there were 6. Mutation nr2090, no strain available. Mutations N150 from strain MT150 (click on the *variation* to see). Mutation N588 from strain MT1218. Mutation N589 from strain MT1219. Mutation N729 from strain MT1541. And finally mutation gk238, with strain VC461.

For the phenotype, N150 is a substitution mutation inducing several different phenotypes. It is *TS* (temperature sensitive allele). On the page for the allele, you will see the specific mutation (C->T) in the *Molecular Details* tab.

Other phenotypes include bag of worms, bloated, coiler, egg laying defective, egg retention, egg laying variant, post translational processing variant. You can get more info on what those phenotypes mean by clicking on them. You may be asked if another MO has a similar phenotype, but the names may be different, so understanding may help answering those kind of questions. If different MO have the same kind of phenotypes (like stop eating properly), you may infer that the gene may be functionally well conserved. You may also know of partner interactions in one MO and search for those in the other MO.

Question 5: RNA interference phenotypes

`Q:` What is the RNA-interference phenotype?

A: In the same location, on the gene page, *phenotype* tab. There are a couple of additional alternative phenotypes observed with RNAi, including body wall muscle sarcomere morphology variant, extended life span, mitochondria morphology variant muscle, and protein degradation variant.

Question 6: RNAi vs deletion mutant phenotypes

Q: Are there differences between RNAi and deletion mutant phenotypes? If yes, explain why this is possible.

A: this question is a bit tricky, you have to rely on tested evidences to confirm (assays). But, some of the RNAi have no *Allele* as supporting evidences, meaning that they have not been tested (for example, the *body wall muscle sarcomere morphology variant* which has only RNAi as supporting evidence.

Should you expect a difference? There could be differences in PTM, and if the RNAi is used, the protein never reaches the stage of translation. If you have a deletion mutant, you may also observe a difference because RNAi (knock-down) is not as efficient as a knock-out of the gene. Many functions are dose-dependent, so if you have nothing, the function would be off, but with a little bit, you may still have partial function. Also, getting RNAi may be difficult to insert in some tissues (esp. nervous tissues).

Question 7: Orthologous proteins

Q: Are there orthologous proteins in:

- Other nematodes?
- Insects?
- Vertebrate species (non human)?
- Human?
- Unicellular organisms?

Indicate for each of these species the biological process in which the protein is involved in and how this function was assessed

A: the go-to database to answer this question is *NCBI homologene*. You will directly see many results there because the gene is well conserved. You could also check the Homology tab of the gene page on wormbase. For other nematodes, that may be the way to go since nematodes are wormbase's prime focus. Often, it is ok to start with NCBI to switch and investigate multiple organisms, but after first review, you should then go to the organism's db for up to date information (as they are updated more frequently).

- Other nematodes: there are quite a few listed on Wormbase (C. remanei, C. brenneri, C. briggsae, C. japonica, P. pacificus). egl-3 is very conserved in nematodes, all related to peptidase activity (infered from orthology)
- Insects: there is a verified homolog in drosophilia (Flybase: amon gene). This protein has a peptidase activity (inferred from direct assay).
- Vertebrate species (non human): for example D. Rerio (ZFIN database, gene name is pcsk2, molecular function). Hydrolase and peptidase activities infered from experimental assays.

- Human: same gene (pcsk2).
- Unicellular organisms: no good homolog gene in Yeast. Why is that? Because neuropeptide are communication molecules between nervous system components. Yeast does communicate to, but they do not have neuropeptide. The only distant gene homolog (KEX2) is related to the same family as egl-3. These proteins cut out peptides to activate them, which is a very general function, and that's why there is a homolog. In higher organism these branched out to specific uses (some cut neuropeptides for example). The function of KEX2 is to encode a a2+ dependent serine protease involved in proprotein processing, infered from direct assay. (SGD database, KEX2 gene page, gene ontology, molecular function).

Question 8: Function in orthologous genes

`Q:` Is the function of this orthologous gene in yeast similar to its function in C. Elegans?

`A:` To find the function, you can search the SGD website, on the KEX2 page, it is a calcium-dependent serine protease involved in the activation of proproteins of the secretory pathway. Neuropeptides are also secreted proteins, but this KEX2 is not as specific (it is involved in a diverse array of secreted proteins - see SGD, summary paragraph).

Question 9: Null mutant in yeast

`Q:` Is a null mutant for this gene available in yeast? What is the phenotype?

`A:` SGD, phenotype tab. There are a couple of mutants available there. With resistance decreased to alkaline pH, to acid pH, and other phenotypes suc as cell size increased, competitive fitness decreased, ...

Question 10: Scientific information availability

`Q:` What is the latest scientific information regarding the orthologue of this gene in yeast in relation to cell fusion and the process of mating in yeast?

`A:` SGD, literature tab. Search for keywords: Cell fusion, and mating. Reference: Aguilar PS, et al. (2010) Structure of sterol aliphatic chains affects yeast cell shape and cell fusion during mating. Proc Natl Acad Sci U S A 107(9):4170-5. Or another paper is: Heiman MG, et al. (2007) The Golgi-resident protease Kex2 acts in conjunction with Prm1 to facilitate cell fusion during yeast mating. J Cell Biol 176(2):209-22 PMID:17210951 (which is the paper this question was refering to)

Question 11: Model organism to further study

`Q:` Which model organism would you choose to further study this gene and why?

`A:` this question was deemed too vague, because it actually depends on what you want to study. Some MO seem odd anyway regardless of the question, like yeast.