Year: 2007
Grade: A

C. elegans is a good model system for investigating the function of the human Hailey Hailey disease gene

Introduction:

Hailey Hailey disease is a genetically heritable mutation that causes skin aberrations such as blisters, lesions, and rashes in the folds of skin. (HHD Society. 2007) This is caused by a loss of a gene coding for the protein ATP2C1, which acts as a calcium and magnesium transporter in skin cells. (Ramos-Castaneda J et al. 2005) *C. elegans*, a nematode worm which is often used as a model system for investigating genes of *Homo sapiens*, expresses a gene *cua-1* which is orthologous to the Hailey Hailey disease gene, ATP7A. (Wormbase. 2007) This suggests *C. elegans* has the potential to be used as a model system for the study of the function of the Hailey Hailey disease gene.

To determine if *C. elegans* is a good model system, we started by observing *cua-1* deletion mutants, as well as the wild-type worms, to determine if there is an observable physical manifestation of a mutation in the *cua-1* gene. We then found the *cua-1* gene sequence and the *cua-1* deletion mutation sequence using wormbase. Using this information, we designed primers to perform nested PCR to amplify the deletion segment and analyzed it using gel electrophoresis, confirming a deletion in the gene is detectable by these methods. We then performed RNAi on the *cua-1*, determining whether there is an observable phenotype when we knockdown the gene function. These techniques, along with a bioinformatics analysis of the *cua-1* protein and the human ortholog protein, allow us to say with confidence whether *C. elegans* is a good model system for the further study of the Hailey Hailey disease gene. Because C. elegans is a good model system for many human genes, we predicted that C. elegans will be a good model system for the investigation of the Hailey Hailey human disease gene.

Methods:

- ***Observe Worms:***
- Mutant deletion Phenotypes
- Wild-type Phenotypes
- ***Bioinformatics***: Wormbase, SMART, BLAST: analyze structure of *cua-1* and human homologue and proteins
- Use Genious to perform alignments
- ***PCR***: Geneius - *cua-1* gene and mutant deletion sequences
- Select primers to amplify deletion segment and perform nested PCR
- Gel Elecrophoresis on negative controls, wild-type, and mutant DNA
- ***RNAi***: Innoculate E. coli
- Spread on plates :

1. Empty feeding plasmid (neg control)
2. No feeding plasmid (neg control)
3. Feeding RNAi plasmid for cua-1
4. Feeding RNAi for sma-1 (pos control)

- Allow to grow
- Add worms and observe larvae phenotypes

Abstract:

Hailey Hailey disease, a human genetic disorder that causes skin aberrations and lesions, is caused by a dysfunction in the coding of the gene for the protein ATPC21, which is orthologous to the *cua-1* gene in *C. elegans*. Using bioinformatics databases as well as the techniques of nested PCR, RNAi, and microscopy, we evaluated *C. elegans* as a model system for the investigation of the Hailey Hailey disease gene. We found that because of detectable changes in phenotype due to genomic alterations and sufficient similarity between the *cua-1* protein and the Hailey Hailey disease protein, and despite some ambiguity in mutant phenotypes, *C. elegans* is a good model system for the exploration of the function of the Hailey Hailey gene. Further study could be carried out using *C. elegans* to analyze the molecular mechanisms of the Hailey Hailey disease and to explore possible treatment methods.

Results:

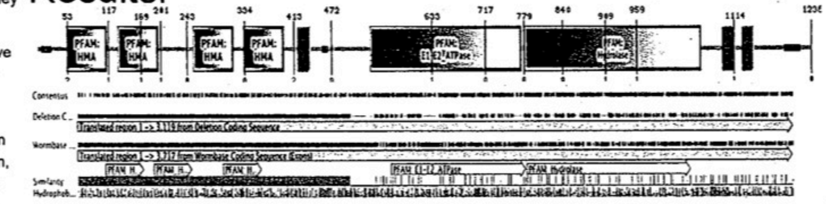


Figure 1: The above figure shows the protein coded by *cua-1* with domains in gray boxes. The protein directly below the first is a geneious image of the *cua-1* protein aligned with the *cua-1* deletion protein, with the green bar ending where the deletion begins. The functional domains are annotated in yellow arrows.

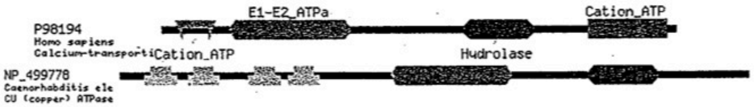


Figure 2: Above is the human Calcium-transporting ATPase type 2C member 1 (ATPase 2C1), the protein that is coded by the gene orthologous to the *cua-1* gene, with functional domains displayed. Immediately below this is the *cua-1* coded protein with domains displayed.

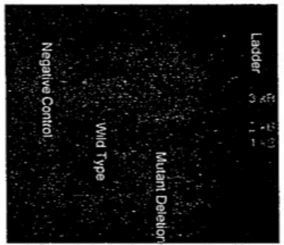
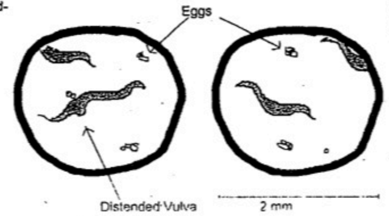


Figure 4: Our gel electrophoresis results, with lane 1 being the negative control, lane 2 being the wild-type result, lane 3 being our *cua-1* deletion mutants, and lane 4 being the ladder. The wild-type is 3 kB and the mutant is 2.3 kB.

Figure 5: Our RNAi phenotype observation, displaying a dead worm surrounded by eggs. In the top right is a functional worm.

Figure 3: Displays a Jmol image of the structure of calcium ATPase in the CA2E1 state, a rabbit protein very similar to the protein coded by the gene that causes Hailey Hailey disease. (The human protein was not available.) Purple regions represent those most conserved among species, while turquoise regions represent those least conserved.

Figure 6: *(to the right)* On the left is the mutant *cua-1* deletion phenotype as observed by 400X microscopy. On the right is wild-type worm phenotype. The scale is given on the right. The mutant phenotype displays a distended vulva.



Discussion:

Our observations of *C. elegans* wild-type and *cua-1* deletion mutants shows that there is a detectable phenotype resulting from the deletion of a segment of the *cua-1* gene. (fig 6) This tells us that manipulating the *cua-1* gene will yield phenotypes that we can study and observe, giving us a qualitative marker of *cua-1* activity in the *C. elegans*. Our PCR gel elecrophoresis results confirm these qualitative observation quantitatively, demonstrating that the observable mutant phenotypes correspond with a deletion in the *cua-1* gene. (fig 4) Considering both of these results, we can link the phenotype difference with the deletion in the *cua-1* gene, allowing us to confirm that manipulations in the gene *cua-1* can give us observable phenotypes.

Having confirmed that genomic manipulations can yield observable phenotypes, the RNAi phenotypes we observed can tell us more about the function of the gene as a whole. We observed mostly normal worms, but slightly less than 1% were dead (fig 5) and may have been lysed by a "bag of worms" resulting from distended vulva. We also observed slower movement than the wild-type worms, and perhaps 5% of the knockout worms displayed smaller body size. These results allow us to confirm that the gene codes for an observable phenotype. We can also begin to speculate about gene function.

Another consideration is our SMART and geneious findings (fig 1) which show that the deletion in the cua-1 knocks out two domains, the E1-E2 ATPase and the hydrolase. This means the phenotypes we observed in the deletion mutants were due to the knockout of these two functional domains. This also means the difference between the RNAi knockout worms phenotypes and the deletion mutants phenotypes would be due to the first four HMA domains, which happen not to be present in the human orthologous protein. This allows us to consider what is caused by HMA domains and what is caused by E1-E2 ATPase and Hydrolase, so we can isolate the latter twos' functions and consider only these in our exploration of Hailey Hailey gene, as only these two are part of the orthologous human protein.

Finally, we found the *cua-1* encoded protein to be sufficiently conserved in domains to the Hailey Hailey gene-encoded protein (fig 2), and saw that similar proteins have highly conserved domains across species (fig 3). Considering all the above evidence as well as the general effectiveness of *C elegans* as a model system for human genes, we can say with confidence that *C. elegans* is a good model system for the exploration of the function of Hailey Hailey disease gene. This conclusion suggests further studies, including the development of medicine treating Hailey Hailey disease. Using *C. elegans*, biologists could evaluate the effectiveness of such developing drugs and improve the medications until they are effective enough to benefit afflicted humans.

Sources:

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C. elegans is not an ideal model organism for studying the molecular and cellular functions of the human orthologs of *fshr-1*

Biology Core Curriculum, University of Wisconsin-Madison

Abstract

Caenorhabditis elegans is a free-living soil nematode commonly used as a model organism for studying eukaryotic gene regulation and organismal development, due to its rapid growth and easily observable anatomy. We studied if *C. elegans* could be used as a model organism to investigate the molecular and cellular functions of the human orthologs of the *C. elegans* gene *fshr-1*. Due to a lack of homology between *fshr-1* and its human orthologs, we predicted that *C. elegans* would not be a good model for investigating the human orthologs. *C. elegans* with a deletion in *fshr-1* were compared with wild-type worms and worms treated with RNAi feeding to knock down expression of *fshr-1*. Bioinformatics tools were used compare and contrast the function and structure of *fshr-1* and its human orthologs. Nested PCR determined that deletion in the *fshr-1* gene was approximately 3200 base pairs in length, likely resulting in a loss of gene function. RNAi knockdown of *fshr-1* produced worms with a less severe phenotype than observed in the deletion strain. Our results suggest that that *C. elegans* is not an ideal model organism for studying the human orthologs of *fshr-1*, due to the specificity of the ligand detected by each receptor and the different roles of each gene in regulating organs and organ systems. Additionally, the failure of RNAi feeding to knock down the expression of *fshr-1* limits the utility of *C. elegans* as a model organism for studying the human orthologs of *fshr-1*.

Study Question

Can *C. elegans* be used as a model organism to investigate the molecular and cellular functions of the human orthologs of the *fshr-1* gene?

Background

Caenorhabditis elegans is a free-living soil nematode that is commonly used as a model organism for studying eukaryotic gene regulation and organismal development (Cox and Grana 2007). The genome of *C. elegans* has been sequenced, leading to the identification of numerous genes that are orthologous to human genes (*ibid.*). One such *C. elegans* gene is *fshr-1*, which is orthologous to the human genes thyroid-stimulating hormone receptor (TSHR), follicle-stimulating hormone receptor (FSHR), and luteinizing hormone/choriogondatropin receptor (LHCGR) (www.wormbase.org). Mutations in these genes are associated with a number of human diseases, including congenital hypothyroidism, ovarian dysgenesis, and precocious puberty (OMIM). Our research studied if *C. elegans* could be used as a model organism to investigate the molecular and cellular functions of the human orthologs of *fshr-1*.

C. elegans has several characteristics that make it an excellent model organism. *C. elegans* is easy to grow in culture, reproduces rapidly and produces large broods and is transparent, allowing internal organs to be easily observed (Cox and Grana 2007). However, in order to model the molecular or cellular function of a human gene, there must be strong homology between the human gene and a gene in *C. elegans*. We predicted that C. elegans might not be an ideal model organism, based on differences in the functions of *fshr-1* and its human orthologs. To test our prediction, we observed the phenotype of worms with a deletion in *fshr-1* and used nested PCR to characterize the deletion. Next, we used an RNAi feeding technique to attempt to knock down expression of *fshr-1* in wild-type worms and compared the observed phenotype with the deletion phenotype. Finally, we used bioinformatics tools to compare and contrast the structure and function of *fshr-1* and its human orthologs.

Results

Characterization of Deletion in RB911 Strain

Figure 1. Results of gel electrophoresis of product of nested PCR amplification. Genomic DNA was extracted from wild-type (strain N2) and deletion mutant (strain RB911) worms and amplified using nested PCR. Primers for nested PCR were chosen to amplify a sequence in the *fshr-1* gene that encompassed the deletion in the RB911 strain. Amplification of wild-type DNA produced a single band at 4000 bp. Amplification of DNA from the RB911 strain resulted in at least three bands. A band at 700 bp (noted by the arrow) was identified as likely corresponding to the targeted sequence in *fshr-1*.

Structure of *fshr-1* Gene

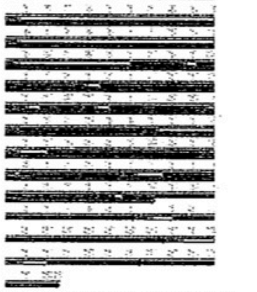



Figure 3. Structure of C. elegans *fshr-1* gene. Exons are indicated in grey and introns are indicated in yellow. The deletion present in the RB911 strain is indicated in brown. The size of this deletion would likely result in a complete loss of protein function. The structure of fshr-1 was analyzed using the Geneious program.

Phenotypes of Mutant and RNAi-fed Worms

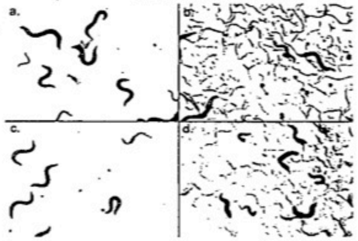


Figure 2. Phenotypes of wild-type, mutant, and RNA-fed *C. elegans*. Panel A shows wild-type *C. elegans* (strain N2) fed on *E. coli* containing a plasmid vector with no inserted genes. These worms displayed the wild-type phenotype. Panel B shows worms of the RB911 strain, which have a deletion in the fshr-1 gene. Phenotypic results of this mutation included increased embryonic lethality (emb), delayed growth (gro), and morphological defects such as protruding vulva (pvul). Panel C shows wild-type worms fed on *E. coli* expressing double-stranded RNA for the *fshr-1* gene. Unhatched eggs were observed, suggesting embryonic lethality, but the RNAi phenotype did not appear to be as severe as the deletion phenotype. Panel D shows wild-type worms fed on *E. coli* expressing double-stranded RNA for the *sma-1* gene. These worms had a small phenotype, and served as a positive control for the RNAi feeding.

Predicted Domains in *fshr-1* Gene Product and Orthologous Human Proteins

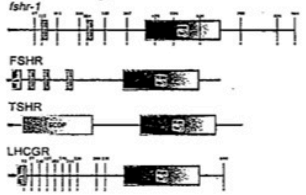
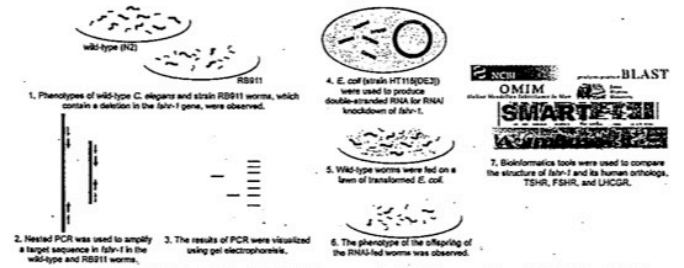


Figure 4. Protein domains predicted by SMART for C. *elegans fshr-1* gene product, human follicle-stimulating hormone receptor (FSHR), human thyroid-stimulating hormone receptor (TSHR), and human luteinizing hormone /choriogondatropin receptor (LHCGR). The amino acid sequence of each protein was analyzed by the SMART program and domains were identified. 7tm_1 (large grey boxes) represents the 7 transmembrane receptor (rhodopsin family) domain, LRR_1 (small grey boxes) represents the leucine rich repeat domain, LRRNT (blue boxes) represents the leucine rich repeat N terminal domain, and SCOP (yellow box) represents the RNI-like domain, which is closely related to the leucine rich repeat domain.

Methods



1. Phenotypes of wild-type *C. elegans* and strain RB911 worms, which contain a deletion in the *fshr-1* gene, were observed.

2. Nested PCR was used to amplify a target sequence in *fshr-1* in the wild-type and RB911 worms.

3. The results of PCR were visualized using gel electrophoresis.

4. *E. coli* (strain HT115(DE3)) were used to produce double-stranded RNA for RNAi knockdown of *fshr-1*.

5. Wild-type worms were fed on a lawn of transformed *E. coli*.

6. The phenotype of the offspring of the RNAi-fed worms was observed.

7. Bioinformatics tools were used to compare the structure of *fshr-1* and its human orthologs, TSHR, FSHR, and LHCGR.

Figure 5. Summary of methods used to investigate the *C. elegans* gene *fhsr-1* and its human orthologs, TSHR, FSHR, and LHCGR.

Discussion

Evaluation of Results

Nested PCR was used to amplify a DNA fragment likely corresponding to the *fshr-1* gene in the RB911 strain, but extra bands were also present in the PCR product. These bands may be due to contamination of the sample, reducing the reliability of the conclusions that can be made from the PCR results. RNAi feeding did not appear to induce as severe of a phenotype as was observed in the RB911 strain. Since the deletion in *fshr-1* in the RB911 strain is severe, complete or nearly complete loss of function was predicted. Therefore, knocking down *fshr-1* expression in wild-type worms was expected to result in a phenotype comparable to the deletion strain. The fact that a comparable phenotype was not observed in the RNAi-fed worms may indicate that the *fshr-1* gene was not successfully knocked down by RNAi.

Conclusion

Our research suggests that C. elegans is not an ideal model organism for studying the function of the human orthologs of *fshr-1*. Bioinformatics analysis did predict a number of structural similarities in the gene products, notably the 7 transmembrane receptor (rhodopsin family) element. However, each of the three human orthologs of *fshr-1* encodes for a receptor specific to a single hormone. Thus, it is not clear that studying the molecular mechanism for the binding of FSHR and its neuropeptide ligand would be useful in understanding the molecular mechanism of the binding of the human receptors to their specific ligands. Therefore, the utility of *C. elegans* as a model organism for understanding the molecular function of the human orthologs of *fshr-1* is questionable.

C. elegans would not be suitable for studying the cellular function of the human orthologs of *fshr-1*, because the cellular functions of *fshr-1* and its human orthologs are different. In *C. elegans*, *fshr-1* is expressed in the neurons and appears to be responsible for proper release of acetylcholine at neuromuscular junctions (Sieburth *et al.* 2005). In contrast, the human orthologs of *fshr-1* play a much more specific role in regulating specific organs and organ systems, the thyroid gland for TSHR and the reproductive system for FSHR and LHCGR. Thus, *fshr-1* and its human orthologs do not have homologous functions on the cellular level.

Additionally, our results suggest that RNAi feeding failed to completely knock down the expression of *fshr-1* in *C. elegans*. Since RNAi feeding is a simple but important experimental technique, the failure of *C. elegans* to respond to RNAi feeding further limits the practicality of *C. elegans* as a model organism for studying the orthologs of *fshr-1*.

Because of the level of gene specialization that has occurred in the human orthologs of *fshr-1* since the evolutionary divergence of *C. elegans* and humans, a mammalian model would be better suited to studying the cellular function of the human genes. A common mammalian model organism is *Mus musculus*, the lab mouse (Cox and Grana 2007). Lab mice possess a homologous gene for each of the human TSHR, FSHR, and LHCGR genes, which would avoid many of the problems associated with using *C. elegans* as a model for these genes.

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Year: 2008   Grade: AB

C. elegans is an effective model system for investigating the function of the human gene BRCA-1

Biology Core Curriculum, University of Wisconsin-Madison

Research Question

Is *C. elegans* a good model system for investigating the function of the human gene BRCA-1?

Background

- The *C. elegans* brc-1 gene encodes a homologue of human BRCA-1, which is mutated in breast and ovarian cancer (Boulton 2006).
- Brc-1 is involved in DNA repair, cell death, chromosome segregation, and growth regulation (Huyton 2000).
    - ▫Its protein product is involved in genome maintenance, possibly by functioning in surveillance for DNA damage.
    - ▫Brc-1 (RNAi) animals display chromosomal non-disjunction, high levels of germ cell apoptosis, and unusual sterility after irradiation (Boulton 06).
    - ▫This gene is a good candidate for study because of its easily observable phenotypes of slow growth, abnormal cell death, and high incidence of males.
- BRCA-1 belongs to a class of tumor-suppressing genes (Jasin 2002).
    - ▫Its protein product is involved in the repair of damaged DNA.
    - ▫BRAC-1 shows a 94.9 % length of similarity to brc-1 (Huyton 2000).
- For *C. elegans* to be a good model system, brc-1 and BRCA-1 should have a high degree of homology, especially in regions encoding exons, as well as similar functions and protein expression. Strong, observable phenotypes should result from brc-1 knockout (deletion) and knockdown (RNAi).
- To determine how well *C. elegans* complies to these standards, phenotypic observations were made of WT, bcr-1 deletion, and bcr-1 knockdown strains of *C. elegans*. Nested PCR and gel electrophoresis were performed to verify knockdown effectivity. Bioinformatics were also used to compare bcr-1 to its human equivalent, BRCA-1.

References

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2. Huyton, Trevor et al. 2000. The BRCA1 C terminal domain: structure and function. Mutation Research, 3(4):319 332.
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Abstract

C. elegans gene brc-1 is homologous to the human gene BRCA-1. Through phenotypic observations, nested PCR, gel electrophorisis, and bioinformatics analysis, we determined that the two genes are most alike at the location of the BRCT protein motif involved in cell cycle checkpoint functions responsive to DNA damage. Brc-1 deletion affects the gene's 2nd and 3rd exons, causing a premature termination and non-active BRCT motif. This could cause cells to continue division despite DNA damage, resulting in the human phenotype of breast cancer, and the *C. elegans* phenotypes of uncoordination, abnormal body shape, and presence of males. Overall, *C. elegans* is an acceptable model system for BRCA-1 due to its high homology, related functions, similar protein expression, and easily-identifiable mutant phenotypes.

Results

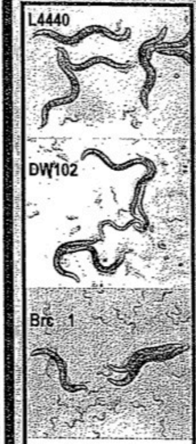


Fig. 1. Phenotypes of L4440 (WT), DW102 (deletion), and brc-1 (RNAi) strains, observed with dissection scope. Relative to WT, both the RNAi and deletion strains were uncoordinated and had a high incidence of males. Fewer adults indicated larval lethal, and some body shape defects were observed.

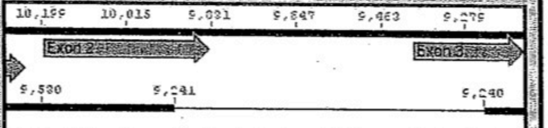
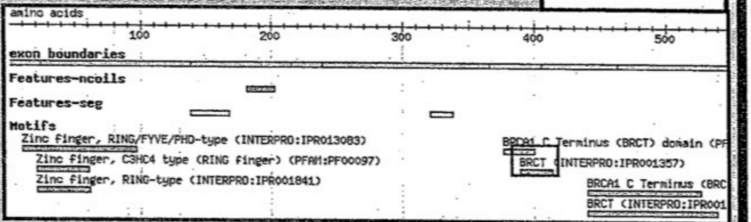


Fig. 2. WT brc-1 gene (top) and deletion. Deletion results in mRNA without parts of exons 2 and 3. Transcription beyond the deleted region results in missense mutations in the mRNA, as well as premature termination during translation.

Fig 3. Exon boundaries and corresponding protein motifs of brc-1 gene. The BRCT motif, also found in the human brc-1 homologue BRCA-1, is affected by the deletion of parts of exons 2 & 3.



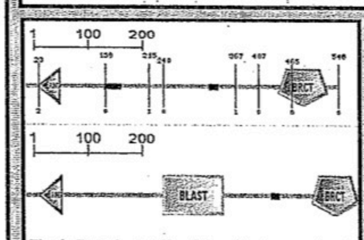


Fig 4. Protein motifs of the *C. elegans* brc-1 gene (top) compared to human homologue BRCA-1. The region of highest similarity (approximately amino acids 350-450) contains the BRCT motifs.

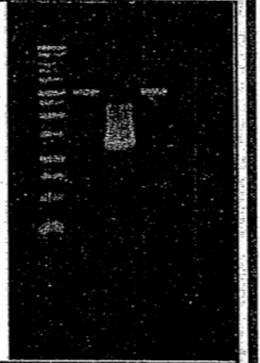
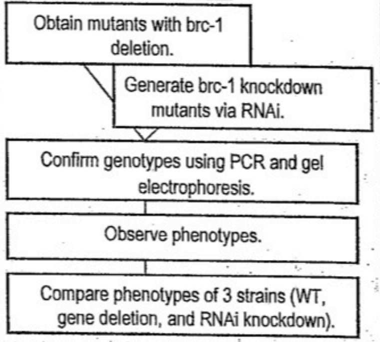


Fig. 5. Gel electrophoresis analysis of PCR products. From L to R, lanes are: ladder, WT, deletion, RNAi, and neg. control. Results show that RNAi and deletion differ at the molecular level, as expected.

Methods



Obtain mutants with brc-1 deletion.

Generate brc-1 knockdown mutants via RNAi.

Confirm genotypes using PCR and gel electrophoresis.

Observe phenotypes.

Compare phenotypes of 3 strains (WT, gene deletion, and RNAi knockdown).

Discussion

- ▫Since each of the posited conditions of a good model system has been met, we find *C. elegans* to be an acceptable model system for the study of the human gene BRCA-1.
    - ▫ Brc-1 and BRCA-1 are highly homologous. The highest degree of similarity is found between amino acids 350-450, where the BRCT protein motif is found in both genes.
    - ▫In both *C. elegans* and humans, this motif is found in proteins involved in cell cycle checkpoint functions responsive to DNA damage (Boulton 2006).
        - ▫The deletion studied directly affects exons 2 and 3 of brc-1, causing the mRNA beyond this point to be translated as nonsense and terminated prematurely. In such a case, the BRCT protein motif is not present and non-active. A non-active BRCT protein motif might result in cells that continue to divide, despite DNA damage (Boulton 2006).
            - ▫In humans, this errant cell division is expressed phenotypically as breast and ovarian cancer (Huyton 2000).
            - ▫In *C. elegans*, phenotypes include abnormal movement and body shape, larval lethality, and the presence of males (Huyton 2000).
- ▫In future studies, it would be useful to have the 3D structure of the protein products of both brc-1 and BRCA-1 as an aid in further differentiation.