Characterizing the Phenotype of the *slamdance* Gene in *Drosophila melanogaster* Using RNAi

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Abstract

Mutations in the *slamdance* (*sda*) gene in *Drosophila melanogaster* are believed to affect the excitability of the nervous system. *sda* mutants are classified as "bang-sensitive" (BS) behavioral mutants. Upon stimulation BS mutants phenotypically suffer temporary paralysis, or behavioral hyperactivity resembling seizures. In order to characterize the *sda* gene I am preparing an RNAi construct using the LITMUS28i, pHIBS, and pUdsGFP plasmid vectors. The final RNAi constructs will be injected into wild-type *Drosophila* eggs to evaluate the effects of post-transcriptional silencing of the *sda* gene and the excitability of the nervous system in *Drosophila*.

Introduction

There are a number of examples of *Drosophila* "bang-sensitive" (BS) behavioral mutants: *bangsenseless* (*bss*), *easily shocked* (*eas*), *slamdance* (*sda*), and *technical knockout* (*tko*). All BS mutants display similar behaviors. Typically BS mutants are five to ten times more susceptible to seizure after some type of mechanical or electrical stimulus compared to wild-type flies (*1*). When these mutants are exposed to electrical or mechanical shock they suffer temporary paralysis, or behavioral hyperactivity resembling seizures such as uncoordinated wing flapping, leg shaking, and abdominal muscle contraction (*3*).

Despite the significant number of people who suffer from seizure disorders the genetic and physiological basis for these defects have been difficult to resolve. Using *Drosophila* as a model Kuebler *et al.* have demonstrated that *Drosophila* and mammalian seizures share many characteristics, such as the fact that seizure susceptibility can be altered by genetic factors (*1*). Currently, only two BS genes have been fully characterized; *tko*, which encodes a mitochondrial protein, and *eas*, which encodes an ethanolamine kinase (*3*). It has been thought that the *sda* gene in *Drosophila* is the ortholog of human aminopeptidase N (APN) (*3*). In humans APN plays important roles in the immune, digestive, and nervous systems. To better understand what role sda plays in *Drosophila* nervous system function, I am creating an expression vector that will effectively silence the *sda* gene in wild type *Drosophila* by using the novel technique RNA interference (RNAi).

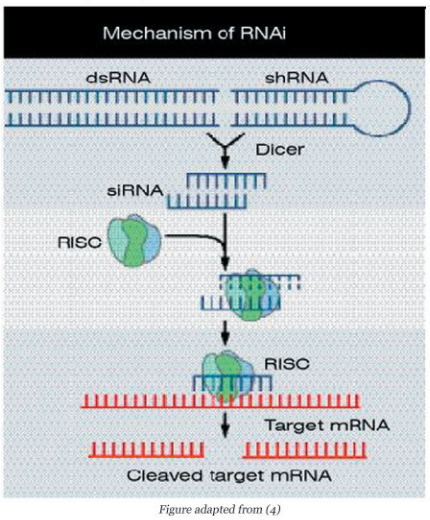
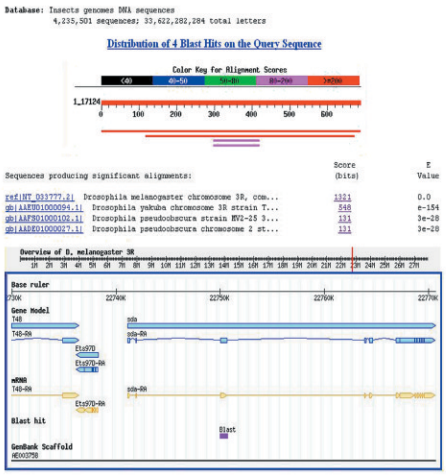


Figure adapted from (4)

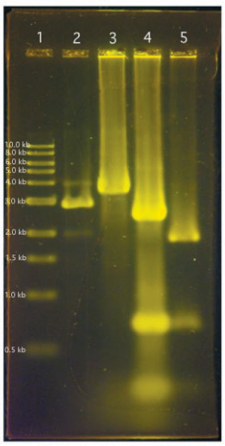
RNAi is a process of sequence-specific post-transcriptional gene silencing initiated by double stranded RNA. RNAi has the ability to regulate gene expression in plants, invertebrates, and (according to more recent studies) mammalian cells by targeting and degrading mRNA. By silencing the *sda* gene I will be able to fully characterize the phenotypic effects of the RNAi in the new "mutants." Researchers have characterized a mutation in the *sda* gene which causes BS behaviors in *Drosophila*, however it is unclear what will happen if the *sda* gene is silenced. The RNAi construct I am preparing will effectively silence the *sda* gene. This RNAi construct can be used to better understand if the sda protein, like APN, is involved in other processes.

Results

Basic Local Alignment Search Tool (BLAST) was used to confirm that the amplified gene sequence is unique to the third exon of the *sda* gene located on the third chromosome in *Drosophila melanogaster*. This ensures that the RNAi construct I am preparing will silence only the *sda* gene.



After a specific sequence was determined, polymerase chain reaction (PCR) was used to amplify the target sequence. The ends of the target DNA sequence were then trimmed with the restriction enzyme SpeI. Once the target DNA fragment was trimmed to the correct length, T4 DNA ligase was used to ligate the target DNA sequence into the vector LITMUS28i. The new plasmid was designated pLit/3*sda*. Next I subcloned the target DNA sequence from pLit/3*sda* into a second vector, pHIBS. The second construct was designated pHIBS/3*sda*. I am currently subcloning the target DNA sequence into the final vector, pUdsGFP.

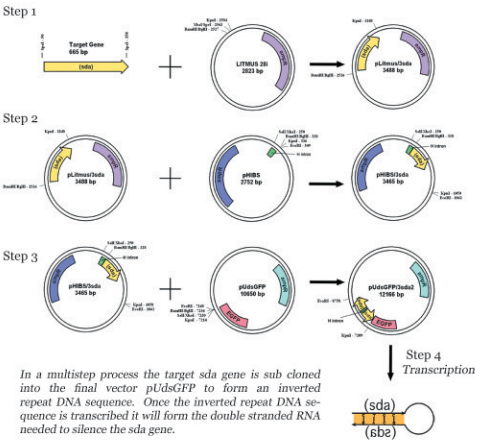


0.8% agarose gel electrophoresis

Lane 1—1 kb DNA ladder,
Lane 2—LITMUS28i digested with EcoRI
Lane 3—pLitmus/3sda digested with EcoRI
Lane 4—pHIBS/3sda digested with RsaI
Lane 5—pHIBS digested with RsaI

Discussion

Each vector contains an ampicillin resistance gene. The vector pHIBs was chosen for two reasons. pHIBS contains an intron from the *hairless* gene in the middle of the multiple cloning sites; this intron is need to connect the inverted repeats, resulting in the formation a hairpin loop. In addition, the pHIBS vector provides for easy shuttling into the vector pUdsGFP. The vector pUdsGFP was chosen because this vector has five UAS/GAL4 binding sites, an EGFP reporter gene, and allows for production of an inverted double stranded RNA sequence separated by a loop that is characteristic of RNAi (2).



In a multistep process the target sda gene is sub cloned into the final vector pUdsGFP to form an inverted repeat DNA sequence. Once the inverted repeat DNA sequence is transcribed it will form the double stranded RNA needed to silence the sda gene.

Future Research

Once the final pUdsGFP construct that contains the inverted repeat DNA sequence, is completed, it will be injected into wild type *Drosophila* embryos. The flies that incorporate the pUdsGFP vector into their genome can be compared phenotypically. From the phenotypic analysis I will be able to determine:

- Whether silencing the *sda* gene affects the level of behavioral hyperactivity.
- Whether the *sda* gene is involved in other phenotypic characteristics—this can be tested by crossing the transgenic flies with fly lines that express GAL4 at specific times during development, or in selected tissues.

References

1. D. Kuebler, H. Zhang, X. Ren, M. Tanouye, *J. Neurophysiol.* **86**, 1211 (2001).
2. A. Nagel, D. Maier, A. Preiss, *Dev. Genes Evol.* **212**, 93 (2002).
3. Zhang *et al.*, *Genetics* **162**, 1283 (2002).
4. *http://ehp.niehs.nih.gov/txg/docs/2004/112-4/focraw/fig1.jpg*

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