Discovering New *C. elegans* Sleep Genes

Clarissa Nassar, Cheryl Van Buskirk
Department of Biology, California State University, Northridge

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Background

The need to sleep has been observed in all animals that have been carefully studied. Despite this universality, the cellular function of sleep is of great debate. As sleep appears to have arisen early in animal evolution, we can gain insight into the core function of sleep by examining simple model organisms, such as the nematode *C. elegans*. Interestingly, these animals sleep after exposure to environmental stressors that cause cellular damage. This type of sleep is known as stress-induced sleep (SIS), and can be triggered by ultraviolet light, bacterial toxins, and heat. Importantly, sleepless mutants that lack the ALA sleep neuron are impaired for survival following exposure to the stressor. These data suggest that a core function of sleep is to repair cellular damage, and that cellular damage may be a deeply conserved component of sleep drive. While several genes that regulate SIS have been identified, several gaps in our understanding remain. In order to identify novel sleep genes, our lab has undertaken a genetic screen for additional sleepless mutants. Here I describe the characterization of one of these mutants, csn16.

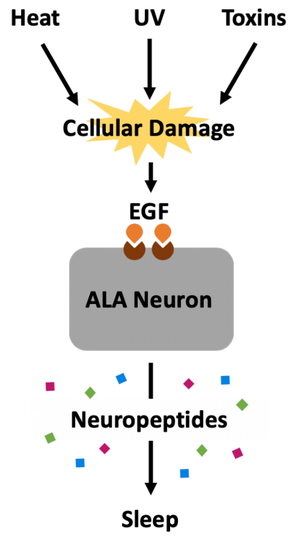

Figure 1. *C. elegans* Stress-Induced Sleep pathway. In *C. elegans*, environmental stressors, like UV, heat, and toxins, cause cellular damage. As a result of this cellular damage, epidermal growth factor (EGF) is released leading to the activation of the sleep-inducing ALA neuron. This neuron then releases neuropeptides that lead to sleep.

Results

During a genetic screen, csn16 was identified as being an awake animal after being exposed to Cry5B, a tissue-damaging bacterial toxin. After observing csn16 as a sleepless animal, we wanted to determine whether the offspring of csn16 would also show the awake phenotype. Therefore, csn16 was allowed to reproduce and we examined the progeny for the same awake phenotype by placing them on Cry5B. We found that the csn16 strain was also awake in comparison to our wildtype, N2, and the csn16 phenotype is also similar to ceh-17, a known sleepless mutant (Figure 2).

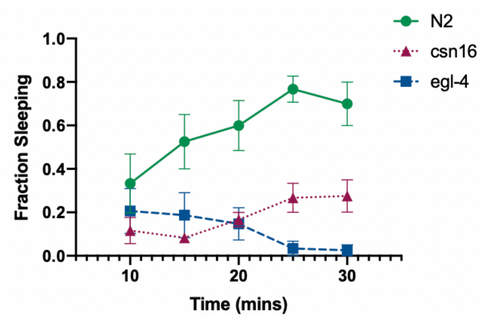

Figure 2. csn16 is defective in response to Cry5B exposure. Young adult animals were exposed to Cry5B, a bacterial toxin, for 15 minutes and scored for sleep 10, 20, and 30 minutes after exposure. N2 is the wildtype stain and egl-4 is a sleepless mutant.

Results

To ensure that the csn16 mutation is truly interfering with SIS, we decided to expose csn16 to ultraviolet light (UV). UV is an environmental stressor that causes DNA damage and normally, wildtype *C. elegans* sleep after UV exposure. We hypothesized that if the csn16 mutation was interfering with SIS, then csn16 would be sleepless after UV exposure. csn16 was observed after UV and it also displayed the sleepless phenotype when compared to N2, the wildtype strain (Figure 3). This data suggests that csn16 is a mutation that is involved in the SIS pathway.

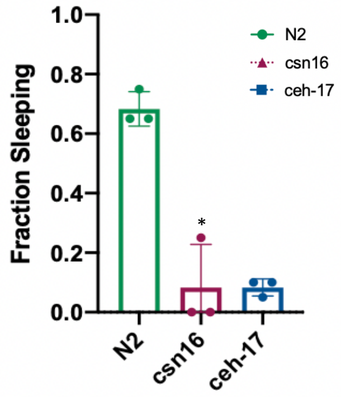

Figure 3. csn16 mutants are defective in UV-induced sleep. Young adult animals were exposed to UV light for 1 minute and scored for sleep 60 minutes later. N2 is the wildtype stain and ceh-17 is a known sleepless mutant. The *p-value <0.05 when using an ANOVA test.

How is the csn16 mutation involved in the SIS pathway?

Epistasis is a type of gene interaction that can be used to identify where a gene acts in a pathway. We can use this technique to determine whether the csn16 mutation is acting upstream (before) or downstream (after) EGF expression in the SIS pathway. To do this, we crossed our csn16 mutant to an EGF overexpression (OE) strain that allows us to control when EGF is expressed. If csn16 is acting upstream of EGF, EGF(OE) will cause the mutant to fall asleep because the csn16 mutation affects the pathway at a step before EGF expression. If csn16 is downstream, EGF(OE) will not cause the mutant to fall asleep because the mutation interferes with one of the steps after EGF expression (Figure 4).

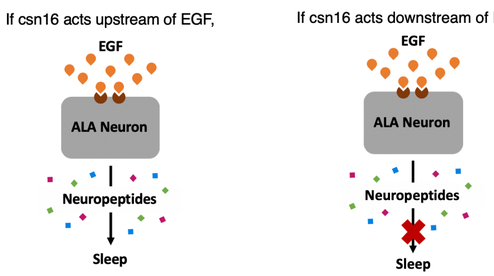

Figure 4. EGF(OE) in the SIS pathway. EGF can be overexpressed and this allows us to determine whether our mutation is acting before or after EGF release in the SIS pathway.

Where is the csn16 mutation in the genome?

One of the next steps for this project would be to identify the mutation that is causing the sleepless phenotype. To do this, we can use whole genome sequencing (WGS) data as well as single nucleotide polymorphism (SNP) mapping. Although WGS is more efficient than SNP mapping, it is difficult to link a mutation to a phenotype because WGS identifies all the genetic changes in our mutant. SNPs are single nucleotide changes within the genome and many SNPs are found between polymorphic nematode strains. Because of the high frequency of these differences, we can use them as markers to identify regions of the genome and to approximate the location of the phenotype causing mutation. To do SNP mapping, we will cross our mutant to a polymorphic strain and look for linkage between a SNP and the awake phenotype in the grandchildren of the cross. This information will help us analyze the WGS data and identify the mutated gene in csn16.

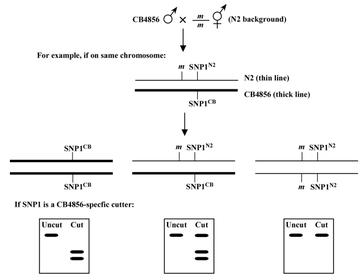

Figure 5. SNP Mapping Cross. The process of SNP mapping works by crossing two polymorphic strains together. The csn16 mutant has an N2 background and we will be crossing it to CB4856.

Discussion

Based on our results, we have determined csn16 to be a sleepless *C. elegans* mutant. Our next goal is to identify the gene that causes csn16's sleepless phenotype and to discover where this gene is acting in the stress-induced sleep pathway. By studying sleep in nematodes, we hope to better understand the fundamental role of sleep.

Acknowledgements

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References

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