Genetics

DOI: 10.1002/anie.200804552

SLEEPLESS-ness and Insomnia in Fruit Flies**

*Jennifer B. Treweek, Amira Y. Moreno, and Kim D. Janda**

genetics · ion channels · mutagenesis · sleep

Despite the popularity of the phrase "Sleep when you're dead!" across university campuses and in pop culture, our basic physiological requirement for sleep is undeniable. As humans, we will spend approximately one third of our life sleeping, and undoubtedly, we will experience first hand the ramifications of sleep deprivation, such as diminished cognition, concentration, health, and emotional well-being.[1–3] This requirement for sleep and many of the consequences of sleep deprivation are shared across virtually all animal species. Even though the biological pathways governing sleep increase in complexity in higher organisms, the homeostatic pattern of sleep, the regulation of sleep by the circadian clock, and the essential functions of sleep appear to be well conserved. Consequently, despite the diversity in the finer points of sleep, such as in sleep–wake cycles, simple organism models including the fly *Drosophila melanogaster*,[4,5] the zebrafish *Danio rerio*,[6] and the roundworm *Caenorhabditis elegans*[7] may still be employed to elucidate the fundamental principles of sleep.[8] It follows that the recent paper by Koh et al., which reports on a novel *Drosophila* mutant whose reduced sleep is governed by loss of the SLEEPLESS protein, may have important implications to the general regulation of sleep homeostasis in other invertebrates as well as vertebrates.[9]

To understand the importance of the *sleepless* (*sss*) gene as a sleep-signaling molecule, one must appreciate the two general mechanisms that regulate sleep patterns: the circadian process and the homeostatic process. The circadian rhythm governs the association between sleep cycles and time of day. Like humans, flies are diurnal animals that sleep at night. When *clock*, one gene responsible for the molecular component of circadian time keeping in flies, is destroyed by genetic mutation or otherwise, affected flies display markedly altered sleep cycles.[10] However, upon 24 hours of sleep deprivation, the *clock* mutant flies exhibit rebound sleep, an indication of intact homeostatic regulation. As a result, the homeostatic process, or the drive to make up for lost sleep, was shown to be dissociable from the circadian process in flies.[5] This finding has relevance in that it both described a sleep behavior shared by many vertebrates and spurred further investigation into molecular clock genes, which have proven strikingly homologous between *Drosophila* and mammals.[11,12] Since the first published reports of fruit fly sleep in 2000, these insects have been used extensively to probe the genetic and molecular underpinnings of sleep.[4,5] Not only do their easily manipulated genome and simple nervous system make fruit flies ideally suited for basic sleep research, but also *Drosophila* were demonstrated to possess similar responses to sleep deprivation and sleep-altering compounds (e.g., caffeine, amphetamines, modafinil) as higher order mammals.[5]

Upon achieving much success in dissecting the circadian process through genetic means, researchers turned to similar methodology for teasing apart the genetic basis of sleep. Though potassium channels were already implicated in sleep in mice[13] and humans,[14] uncovering the genetic basis for a short-sleeping phenotype in flies was accomplished by Cirelli et al. through an unbiased screening of 9000 mutant fly lines generated by random ethyl methane sulfonate mutagenesis.[15] The *minisleep* (*mns*) flies, which contained a point mutation in the α subunit of the voltage-gated potassium channel encoded by the *Shaker* gene, displayed a tendency to compensate for sleep deprivation with additional bouts of fragmented sleep rather than elongated sessions of deep sleep. The hyperresponsive phenotype of *mns* flies to arousal during sleep, which marred their capacity for rebound sleep, was also accompanied by a shortened lifespan. Interestingly, analogous leep disorders have been attributed to abnormal K^+ channel function in humans,[16] but the mechanistic link between compromised potassium channel function and altered sleep homeostasis remains to be elucidated in either organism. This contrasts with the more apparent role of potassium channels in setting circadian rhythms, in which the synchronized or interrelated events of K^+ and Ca^{++} channel gating, molecular clock protein oscillations, and the electrical signaling of pacemaker neurons are coupled to the cycling of the molecular clock and thus the generation of sleep rhythms in both flies and mammals.[10,11,13,17,18]

However, the finding that a single point mutation in a conserved potassium channel could mediate such measurable dysfunction in sleep homeostasis led to the hypothesis that K^+ channel function may be directly related to an unidentified sleep-inducing signal.[19] To further investigate the relation-

[*] J. B. Treweek, A. Y. Moreno, Prof. Dr. K. D. Janda
Department of Chemistry and Immunology, Skaggs Institute for Chemical Biology
and
Worm Institute of Research and Medicine (WIRM), The Scripps Research Institute
10550 North Torrey Pines Road (BCC582), CA 92037 (USA)
Fax: (+1) 858-784-2595
E-mail: kdjanda@scripps.edu
Homepage: http://www.scripps.edu/chem/janda/

[**] We acknowledge The Skaggs Institute for Chemical Biology, the Ruth L. Kirschstein National Research Service Award, and the Novartis Graduate Fellowship in Organic Chemistry for Women and Minorities.

ship between neuronal excitability and the regulation of sleep homeostasis, Koh et al. conducted a large-scale, forward genetic screen in which they characterized the sleep phenotypes of ca. 3500 *Drosophila* mutant lines containing transposon insertions. While the use of a large-scale screen has the advantage of being unbiased through eliminating predictions of the phenotypic outcome of a particular mutant, this methodology is high risk as the appearance of a desired phenotype is left to statistical chance. The risk paid off for Koh et al., however, as they were able to uncover a mutant line whose members exhibited approximately an 83% reduction in sleep as compared to control flies, and in extreme cases, approximately 9% of the mutant population lacked sleeping behavior (Figure 1). Indeed, this mutant one displayed the most extreme reduction in daily sleep that has been attributed to a mutation of a single gene, and subsequent characterization of this gene, termed *sleepless*, highlighted its role in both baseline and rebound sleep. The *sss* phenotype is recessive, and *sss* homogeneity is necessary for the manifestation of fewer, shorter sleeping bouts. Even though *sss* flies appeared mildly uncoordinated, they exhibited normal waking activity, and their central clock cells were unaffected. The main adverse consequence of the *sss* mutation was a shortened lifespan of *sss* flies, a trait precipitated by the *Shaker* mutation as well. Thus, the phenotypes resulting from the *Shaker* and *sss* mutations reinforced the overall concept that sleep serves an essential, restorative role.

Whereas the *Shaker* flies were generated through mutagenesis of identified *Shaker*-like genes, the *sss* gene (gene CG33472 in the *Drosophila* Genome Project) was uncharacterized prior to this study. The phenotype summarized above, designated sss^{P1}, contained a P-element insertion in the gene open reading frame, which resulted in the disruption of SSS protein expression. In addition to the original mutant, a second line sss^{P2} was generated by inserting a transposon (*f*01257) onto the 3′ untranslated region of the last coding exon. In order to determine the effects of these *sss* mutations on homeostatic sleep, sss^{P1}, sss^{P2}, and $sss^{P2/P1}$ mutants were mechanically stimulated throughout the night and their subsequent rebound sleep activity was assessed. In all cases, *sss* mutants displayed little to no rebound sleep, indicative of a compromised sleep homeostatic response. A meaningful comparison of the reaction of different mutants to sleep deprivation requires that all subjects show similar patterns of baseline sleep, however, and fortuitously, the rhythmic baseline sleep of homozygous sss^{P2} flies was unchanged relative to control flies. The trans-heterozygous $sss^{P2/P1}$ mutants exhibited a 30% decrease in sleep. The resulting correlations between genotype and sleep phenotype provided evidence that the homeostasic regulation of sleep was linked to the *sss* gene. Furthermore, the authors illustrated how the amount of daily sleep observed in each mutant line varied directly with the SSS protein content of head lysates. Interestingly, the level of this brain-enriched, extracellular GPI-anchored plasma membrane protein neither fluctuated during circadian rhythms nor changed upon sleep deprivation.

While the elucidation of such mechanisms governing sleep may lead to novel approaches for improving sleep quality, the applicability of these studies to humans is still under heated debate. In particular, the importance of the *sss* mutation in delineating circadian and homeostatic regulation of sleep is countered by the apparent absence of an SSS homolog in vertebrates. Such contrasts with the single *Shaker* gene of flies in that the mammalian genome contains multiple *Shaker*-like counterparts. The report linking *Shaker* to short-sleeping flies motivated a subsequent investigation of the murine gene *Kcna2*, which encodes Kv1.2, the α-subunit of a Shaker-like voltage-dependent K^+ channel.[15] Kv1.2 was found to regulate neuronal excitability and affect NREM sleep, and like the *Shaker* and *Hyperkinetic* flies, *Kcna2* null mouse pups displayed no signs of fragmented sleep or hyperactivity.[20] While the lessons learned from *Shaker* translated to parallel studies of the Kv1 family mammalian homologs, a parallel extension of the findings regarding *sleepless* is less obvious. This notwithstanding, the study by Koh et al. forms a critical step in unraveling the process by which neuronal excitability

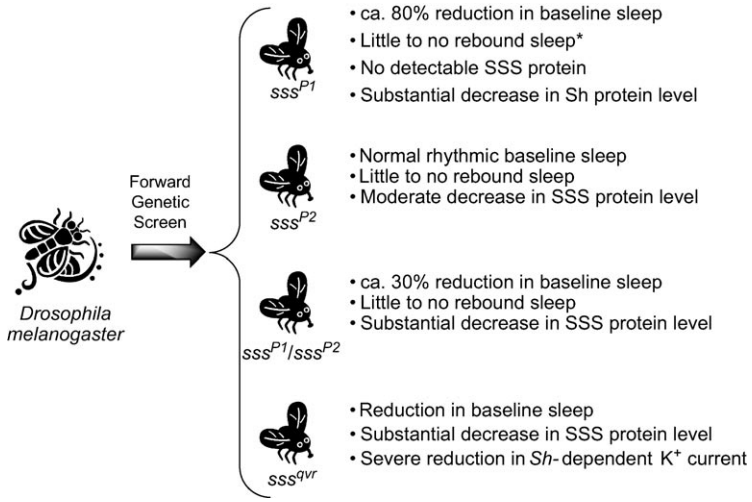

Figure 1. Sleep phenotype and genetic analysis of *sss* mutants. *Assessment of rebound sleep is difficult given that the reduced sleep phenotype of sss^{P1} limits the amount of sleep which may be deprived. Sh = Shaker.

regulates sleep. Specifically, *sss* appears to be intimately involved in Shaker channel expression, as the *sss* mutation had deleterious effects on Shaker protein levels. In addition, Koh et al. surmise that aside from affecting membrane excitability through Shaker K^+ channel activity, SLEEPLESS may serve as a signaling molecule that dynamically integrates sleep drive and sleep level. In conclusion, the characterization of *sleepless* exemplifies how the high throughput screening of *Drosophila* sleep mutants for sleep-related genes may yield vital insight into the cellular pathways governing sleep homeostasis.

Published online: December 15, 2008

[1] E. M. Robertson, A. Pascual-Leone, D. Z. Press, *Curr. Biol.* **2004**, *14*, 208.
[2] T. H. Turner, S. P. Drummond, J. S. Salamat, G. G. Brown, *Neuropsychology* **2007**, *21*, 787.
[3] M. H. Bonnet, D. L. Arand, *J. Intern. Med.* **2003**, *254*, 23.
[4] J. C. Hendricks, S. M. Finn, K. A. Panckeri, J. Chavkin, J. A. Williams, A. Sehgal, A. I. Pack, *Neuron* **2000**, *25*, 129.
[5] P. J. Shaw, C. Cirelli, R. J. Greenspan, G. Tononi, *Science* **2000**, *287*, 1834.
[6] I. V. Zhdanova, S. Y. Wang, O. U. Leclair, N. P. Danilova, *Brain Res.* **2001**, *903*, 263.
[7] D. M. Raizen, J. E. Zimmerman, M. H. Maycock, U. D. Ta, Y. J. You, M. V. Sundaram, A. I. Pack, *Nature* **2008**, *451*, 569.
[8] J. E. Zimmerman, N. Naidoo, D. M. Raizen, A. I. Pack, *Trends Neurosci.* **2008**, *31*, 371.
[9] K. Koh, W. J. Joiner, M. N. Wu, Z. Yue, C. J. Smith, A. Sehgal, *Science* **2008**, *321*, 372.
[10] J. H. Park, C. Helfrich-Forster, G. Lee, L. Liu, M. Rosbach, J. C. Hall, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3608.
[11] C. Cajochen, J. K. Wyatt, C. A. Czeisler, D. J. Dijk, *Neuroscience* **2002**, *114*, 1047.
[12] E. Naylor, B. M. Bergmann, K. Krauski, P. C. Zee, J. S. Takahashi, M. H. Vitaterna, F. W. Turek, *J. Neurosci.* **2000**, *20*, 8138.
[13] F. Espinosa, G. Marks, N. Heintz, R. H. Joho, *Genes Brain Behav.* **2004**, *3*, 90.
[14] K. A. Josephs, M. H. Silber, R. D. Fealey, T. B. Nippoldt, R. G. Auger, S. Vernino, *J. Clin. Neurophysiol.* **2004**, *21*, 440.
[15] C. Cirelli, D. Bushey, S. Hill, R. Huber, R. Kreber, B. Ganetzky, G. Tononi, *Nature* **2005**, *434*, 1087.
[16] K. A. Kleopa, L. B. Elman, B. Lang, A. Vincent, S. S. Scherer, *Brain Res.* **2006**, *129*, 1570.
[17] M. N. Nitabach, J. Blau, T. C. Holmes, *Cell* **2002**, *109*, 485.
[18] J. H. Benington, M. C. Woudenberg, H. C. Heller, *Brain Res.* **1995**, *692*, 86.
[19] J. C. Hendricks, *Nat. Neurosci.* **2005**, *8*, 703.
[20] C. L. Douglas, V. Vyazovskiy, T. Southard, S. Y. Chiu, A. Messing, G. Tononi, C. Cirelli, *BMC Biol.* **2007**, *5*.

