The Molecular Logic of *C. elegans* Sleep: A Single Sleep-promoting Neuron Inhibits a Wake-promoting Pair of Neurons via neuropeptide signaling

Nelson M.D. and Raizen D.M.

Sleep is ubiquitous in the animal kingdom and many molecular mechanisms of sleep regulation are conserved (Crocker and Sehgal, 2010). The simplicity of the nervous system of the roundworm *Caenorhabditis elegans*, combined with our complete understanding of the synaptic connectivity, provides a unique opportunity to dissect the circuitry of sleep regulation at a single cell resolution. Epidermal growth factor (EGF) signaling promotes sleep in mammals and fruit flies (Kramer et al, 2001; Foltenyi et al, 2007) but the mechanism has been opaque. In *C. elegans,* activation of the single peptidergic interneuron ALA by the epidermal growth factor LIN-3 induces sleep (Van Buskirk and Sternberg, 2007), but the neurotransmitter released by ALA to induce quiescence has been unknown. We report that the ALA neurotransmitters are FLP-13 neuropeptides. The gene *flp-13* is expressed in ALA; over-expression of *flp-13* induces sleep during normally active periods; *flp-13* mutants are defective in EGF-induced sleep; and this defect is rescued by restoring *flp-13* expression specifically in the ALA neuron. We have identified a FLP-13 receptor encoded for by the gene *frpr-4*. In collaboration with Drs. Janssen and Schoofs (U. Leuven), we have shown that FLP-13 neuropeptides potently activate the G-protein coupled receptor FRPR-4 in a heterologous cell culture system. Over expression of *frpr-4* induces sleep, which requires the function of its ligand FLP-13, thereby demonstrating *in vivo* functional interactions between the identified ligand and receptor. *frpr-4* is expressed in a pair of highly connected interneurons, the RIAs, which we have previously shown to secrete the somnogenic neuropeptide NLP-22. NLP-22 is similar to the mammalian, circadian-regulated, anorexigenic hormone neuromedin S (NMS) (Mori et al, 2005; Ida et al, 2005). *nlp-22* mRNA shows cyclical expression in synchrony with sleep behavior and is downstream of a clock regulated by LIN-42/PERIOD. Somnogenic effects of NLP-22 require inhibition of a cAMP-dependent protein kinase (PKA) mediated pathway. Surprisingly, acute optogenetic activation of the RIA neurons is wake-promoting and not sleep-promoting, indicating that in addition to NLP-22, RIA releases a wake-promoting neurotransmitter, and that this wake-promoting effect dominates in this acute activation paradigm. Thus, we have defined a flip-flop mechanism of how a sleep-promoting neuron (ALA) inhibits a wake-promoting neuron (RIA). Moreover, we demonstrate that a wake-promoting neuron can express a sleep-promoting neurotransmitter, providing a new mechanism for sleep homeostasis at the single cell level. Given the conserved molecular nature of sleep regulation, we propose that similar logic operates in other animals, including humans.

Trainee Award Eligible