Review Article: Molecular Markers for the Evolutionary Basis of Sleep

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Abstract

This review highlights three articles that discuss the molecular basis of sleep. Identifying the molecular markers of sleep across the animal kingdom will help elucidate the evolutionary basis of sleep. Three different animal models were used in these experiments: Zebrafish, *Drosophila*, and *Hydra vulgaris*. The Zebrafish study[1] focused on chromatin dynamics within single neurons during sleep. The *Drosophila* study[2] focused on finding regulatory networks for sleep under environmental stress. The study on *Hydra vulgaris*[3] established the animal as a primitive model in addition to locating conserved molecular markers. The studies showcase how the complexity of sleep can be studied at different scales, in different species, and how similarities and differences across species can shed light on the evolutionary origins of sleep.

Biological Problems

What are the molecular markers we can use to track mechanisms of sleep across phylogeny? In a recent article by Zada et al.,[1] sleep is explored in a single neuron. Sleep is known to aid in biosynthesis, conserve energy, improve synaptic plasticity, and consolidate memory. However, we are still unsure why sleep has evolved and what molecular markers can therefore track sleep across the animal kingdom. Zada et al. investigated if a potential molecular marker could be found by observing how chromosomal architecture changes during sleep.

Sleep is rhythmic each day, or circadian. Chromosomal rearrangements contribute to how circadian genes and sleep-related genes are expressed. Zada et al. hypothesized that if chromatin dynamics and circadian rhythms were linked, chromatin dynamics might be crucial to observe within a single neuron.

By analyzing chromosomal dynamics in zebrafish, Zada et al. were able to see how chromatin rearrangements reduced the number of DNA double-strand breaks during sleeping hours in single neurons. Zada et al. therefore understood sleep to be a state for single-neuron nuclear maintenance. When enough DNA damage has been accumulated, sleep is triggered, and neurons go into a repair state, where increased chromatin reorganization then occurs. The findings support that sleep may have evolved to regulate the function of single neurons, so that they can repair DNA damage and work together to promote healthy living in the waking state.

In the article by Melnattur et al.[2], sleep is explored in *Drosophila* under environmental stress. We know that sleep is restorative, yet the regulatory neuronal pathways involved in sleep under stressful conditions is unknown. Melnattur et al. experimented with *Drosophila* wings to understand the relationship between sensory processing and restorative sleep.

Melnattur et al. initiated damage to *Drosophila* wings, immediately promoting longer sleep duration in the fly. Researchers tracked sets of neurons, exploring how damage to peripheral wing neurons set off signals that eventually reached the brain and induced sleep. This allowed researchers to understand how chemosensory and mechanosensory neurons trigger changes leading to longer sleep

states. It also allowed researchers to understand how groups of sensory neurons were responsible for directing important sleep regulatory pathways.

Melnattur et al. touched on the larger issue of sleep plasticity and how evolution has favored this type of plasticity. The experiment begins to understand how several neuronal circuits connect sensory inputs in the fly wing with the brain. From this we can start to see how sleep plasticity could depend on several interconnected pathways. These pathways could be highly conserved, emphasizing how important sleep is for maintaining plasticity and restoring bodily integrity in the face of ecological challenges.

Researchers further explored the evolutionary origins of sleep in the study by Kanaya et al.[3]. Researchers observed the primitive, 4-hour sleep state of the cnidarian *Hydra vulgaris*. They wanted to understand if any molecular markers for sleep could be traced back to this brain-less species. Researchers analyzed gene expression in the *Hydra* finding sleep-inducing genes that are homologous to sleep genes in mammals. *Hydra* also reacted to several sleep-related neurotransmitters, suggesting that sleep regulatory pathways existed in primitive animals before the rise of the central nervous system.

Kanaya et al. discovered that the 4-hour sleep state in *Hydra vulgaris* may represent an evolutionary intermediate between a primitive sleep state and more advanced circadian sleep states. An intermediate sleep state would help scientists understand how the molecular components of sleep have evolved over time. The molecular components found in the *Hydra* also serve as a basis for discovering homologs in other animals. The findings explore how sleep is an ancient process that has shaped the more sophisticated mammalian nervous systems we see today.

Experimental Approaches

In the experiment by Zada et al.,[1] researchers used telomere markers to track chromosomal activity in brain neurons of live zebrafish larvae. They used microscopy to visualize chromosomal activity during the waking and the sleeping state. Researchers also used visual markers to quantify the number of DNA double-strand breaks in the same neurons. Researchers found a correlation between high chromosomal activity and lower numbers of DNA double-strand breaks in the sleeping state. The opposite pattern was true for low chromosomal activity: low activity during the waking state was correlated with higher numbers of DNA double-strand breaks. The findings helped researchers establish a positive relationship between sleep and nuclear maintenance within single neurons.

In Melnattur et al.[2], researchers cut *Drosophila* wings, glued wings together, or confined the flies to small spaces to prevent flying. They visually observed how these alterations affected sleep duration in the flies. After seeing how wing damage induced longer sleeping periods, researchers tracked sets of neurons from the wings to the brain. They identified specific neuronal pathways between the wings and brain, and they associated these neuronal pathways with a longer sleep state. These findings indicate that ecological damage to fly wings can alter sleeping states through activation of neuronal pathways.

In the experiments by Kanaya et al.[3], researchers used *Hydra vulgaris* as an animal model. They wanted to understand the molecular basis for this primitive, brain-less, nerve-net system and how it established a distinct sleeping state. Researchers analyzed *Hydra* bodily movements using recorded video. These videos were dissected and carefully observed. The movements in the videos established the basis for the *Hydra*'s 4-hour rhythmic cycles as a sleeping state.

Kanaya et al. used their newfound knowledge of the 4-hour cycles to search for molecular markers of sleep. They quantified gene expression profiles using microarrays and found several sleeping-genes. The sleeping-genes were either upregulated or downregulated, depending on if the *Hydra* was asleep, awake, or in an induced sleep-deprivation state. Researchers finally immersed *Hydra* in different sleep-related neurotransmitters, most notably dopamine and ornithine. Hydra's response to the neurotransmitters highlighted active sleep regulatory pathways. Kanaya et al. therefore established a step-

by-step guide to identifying sleep states in primitive animals before seeing what molecular components are implicated in these states.

Interrelationships between Biological Problems

The three articles all explore sleep at a molecular level. The goals of all articles also aim to touch on how sleep has evolved. Differences between the articles stem from the different animal models chosen, as *Hydra, Drosophila,* and zebrafish all exhibit unique behavioral patterns which may be reflected in their sleep.

Kanaya et al.[3] explores ancient origins of sleep, shedding light on how conserved sleep pathways have existed all the way back before the central nervous system evolved. Zada et al.[1] tries to understand sleep from an evolutionary perspective as well but focuses on a single neuron cell, since zebrafish are already established as an appropriate animal model. Focusing on chromosomes enables Zada et al. to understand why sleep may be useful across the animal kingdom. While in Kanaya et al., several molecular markers were analyzed, as the *Hydra* as an animal model still needed to be established and explored. The different animal models chosen in Kanaya et al. vs. Zada et al. therefore led researchers to experiment in both broader and in more specific ways, respectively. The methods used in Zada et al. could be replicated in a variety of other animal models, as all animals have a genome. Zada et al.'s methods would be interesting to replicate on the more primitive *Hydra*. This could illuminate how ancient the relationship is between nuclear maintenance and sleep.

Melnattur et al.[2] explores how wing injury affects sleep in *Drosophila* and how sensory input can therefore become a regulatory component in sleep. By studying *Drosophila* under environmental stress and seeing how the flies respond to wing damage, researchers were able to understand how neuronal networks play a role in plasticity. If we look deeper into individual neurons, the findings in Zada et al. are also applicable to the *Drosophila* studies. The hypothesis from Zada et al. can be used on a *Drosophila* model to explore how bodily damage may be reversed in the fly through nuclear maintenance.

Melnattur et al. focused on how bodily injury can stimulate networks of neurons to induce sleep, while Zada et al. was more concerned with what happens at a smaller nuclear scale. Because the studies addressed issues at two different scales, the concepts within the two studies can be interchanged. For example, it may be beneficial to study single neuron chromatin activity while *Drosophila* is under stress.

It may also be beneficial to study how bodily injury can impact rates of chromatin activity in other animal models. It is unclear how much energy is needed for chromosomal rearrangements, and it could be likely then that longer sleeping durations might affect the rate of DNA repair. Studying single-neuron sleep in a variety of animal models and under stressful conditions is important for continued understanding of why sleep is highly conserved.

Conclusions

All three studies shed light on the evolutionary mechanisms of sleep and why sleep has been conserved across the animal kingdom. The studies elucidate the importance of studying sleep function at different scales (identifying single genes, larger gene pathways, and within the DNA itself) to get a full picture of why sleep is necessary for life. The studies also explore the relationship between animals and their environment and how this relationship affects sleep duration.

Future studies need to explore sleep at different scales. New animal models are helpful for continued sleep study, as seen in Kanaya et al. The methods by Zada et al. will be useful to replicate in a variety of animal models to see if nuclear maintenance can be observed in more primitive as well as more advanced species. Melnattur et al. demonstrates how it is also important to study sleeping states in animals under stress, as this may be a key to understanding how sleep has evolved over time.

References

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