Scientists model 3D structures of proteins that control human clock

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In an Early Edition issue of The *Proceedings of the National Academy of Sciences* (*PNAS*) on April 9, 2009, the researchers report that they have been able to determine the molecular structure of a plant photolyase protein that is surprisingly similar to two cryptochrome proteins that control the "master clock" in humans and other mammals. They have also been able to test how structural changes affect the function of these proteins.

"The plant photolyase structure provides a much better model to use to study how the cryptochrome proteins in the human clock function than we have ever had before," says the study's lead investigator, Kenichi Hitomi, Ph.D., a postdoctoral research fellow at Scripps Research. "It's like knowing for the first time where the engine is in a car. When you know what the most important parts of the protein are, then you can begin to figure out how it functions."

And when scientists understand that, they can begin to fix the clock when needed, adds senior author Elizabeth Getzoff, Ph.D., professor in the Department of Molecular Biology and member of The Skaggs Institute for Chemical Biology at Scripps Research.

"In addition to decoding how the clock works, a long-term goal is to develop a drug to help people who can't reset their clock when they need to, like people who work night shifts or travel long distances," she says. "Having the three-dimensional protein is a great step forward in both of those pursuits."

To date, it has been impossible to capture a crystal structure of the cryptochrome proteins that control the mammalian circadian clock because they aren't in a sufficiently stable state, Hitomi says. So, working with a team of investigators from both Scripps Research and from other institutions, including two universities in Japan, Hitomi turned to Arabidopsis thaliana, a plant native to Europe and Asia that has one of the smallest genomes of all plants, and so is often used as a model organism to study genes.

Like all plants, this plant contains proteins known as photolyases, which use blue light to repair DNA damage induced by ultraviolet light. Photolyases have also been found in bacteria and in some animals, but not in human cells. Humans and mammals, on the other hand, possess a homologous protein known as cryptochrome that modulates the circadian clock.

"This is an amazing, and very puzzling, family of proteins, because they do one thing in plants and quite a different thing in mammals, yet these cousins all have the same structure and need the same cofactor, or chemical compound, to become activated," Getzoff says.

"All of these proteins were probably originally responses to sunlight," Hitomi adds. "Sunlight causes DNA damage, so plants need to repair this damage, and they also need to respond to sunlight and seasons for growth and flowering. The human clock is set by exposure to sunlight, but also by when we eat, sleep, and exercise."

In this study, Hitomi set about producing proteins from the Arabidopsis thaliana genes that produce two related photolyase enzymes. These genes had been cloned earlier in the laboratory of co-author Takeshi Todo, Ph.D., of Kyoto University, with whom Hitomi previously studied.

He moved the gene from the plant into E. coli bacteria to produce a lot of the protein, which he then crystallized to determine the atomic structure by using X-ray diffraction. The researchers then produced a variety of mutant proteins in order to test the functional structure of the enzymes.

"We can now look at things that are the same and different between human and mouse

cryptochromes and plant photolyases," says Hitomi. "Our results provide a detailed, comparative framework for biological investigations of both of these proteins and their functions."

Not only do the findings have the potential to form the basis of drugs that can ease jet lag and regulate drug metabolism, they may also go some way to explain some fascinating circadian clock disorders that have been found in mice and man, the researchers say. When one of the two cryptochrome clock proteins is deleted, the cycles of the 24-hour body clock lengthen, but when the other is deleted, the clock cycle shortens.

"Nobody has clearly understood what the structural or functional differences are between these two proteins, but we found a single amino acid difference that alters the protein's structure near its active site," Getzoff says. "We show that changing this one element impacts how the proteins work in interesting ways. Next, we are now looking at the interactions of the cryptochromes with their protein partners."

More information: www.pnas.org/content/early/200 … /0809180106.abstract

Source: The Scripps Research Institute (news : web)