Researchers detangle protein interaction implicated in Down syndrome and Alzheimer's

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The powerful biomolecular NMR spectrometers at the University of Arizona have allowed scientists to gain molecular details of the interaction between RCAN1 and calcineurin, two proteins that have long been implicated in Down syndrome and Alzheimer's disease. Credit: University of Arizona

From the movement of a finger to the creation of a memory, actions of the human body require the harmonious concert of protein interactions.

A system of checks and balances ensures proper coordination of biomolecules, but when the scale is tipped, lopsided molecular relationships can lead to disorders and diseases. Discovering how proteins interact with one another under ideal conditions is critical to understand what goes awry in disease states, and to inform novel prevention and treatment strategies.

Wolfgang Peti, a University of Arizona professor in the Department of Chemistry and Biochemistry, has been investigating the interaction between two proteins implicated in Down syndrome and Alzheimer's disease for nearly 10 years. Past technological limitations have prevented researchers from determining the precise physical relationship between the two proteins. Peti teamed up with Rebecca Page, professor and interim associate head of research and faculty affairs in the Department of Chemistry and Biochemistry, to address the research question through a new approach.

"We had to develop a hybrid technology that combines two powerful chemistry techniques to be able to obtain the structures of these proteins that would help us understand their interaction," Peti said.

The researchers' findings, published in *Science Advances*, provide a foundation to better understand and treat several neurological disorders.

Calcineurin, or CN, is a key regulator of several biological processes, including human development. Over-inhibition of this protein plays a critical role in the phenotype of Down syndrome, a genetic disorder caused by an abnormal event during development that results in an extra chromosome 21.

It's been known for nearly 20 years that another protein, RCAN1, normally inhibits CN to maintain balance in the body. Because RCAN1 is encoded by a gene on chromosome 21, patients with Down syndrome

have elevated levels of RCAN1, disturbing the balance and leading to an over-inhibition of CN.

According to the National Institute on Aging, many individuals with Down syndrome are diagnosed with early-onset Alzheimer's disease by age 40, and a study published in the *Archives of Neurology*, now *JAMA Neurology*, found that nearly three-fourths of those with Down syndrome had developed dementia by age 60. RCAN1 is also overactive in this neurodegenerative disease.

Researchers have known that an unbalanced relationship between CN and RCAN1 has profound consequences on the brain, but it was previously unknown how RCAN1 intimately interacts with and regulates CN. Because the structure of a protein determines its function, Peti needed to get a "molecular picture" of the two proteins to understand the mechanism by which RCAN1 inhibits CN.

To achieve this goal, the team utilized two advanced techniques: crystallography and nuclear magnetic resonance spectroscopy. Through crystallography, structures of proteins can be determined by studying the fundamental arrangement of their most basic components—atoms—in crystalline solids. Nuclear magnetic resonance spectroscopy is a technique often used to determine the content and purity of a sample, as well as a molecular structure.

Other groups had used these methods individually in the past but had been unsuccessful in determining the multifaceted interaction between the two proteins because the methods by themselves didn't provide the proper resolution to do so. Peti and Page realized the two techniques needed to be combined into a hybrid technology to obtain the elusive, detailed structure.

"We're not the first group to study (the interaction) structurally," Page

said. "It really required a combination of methods in order to get to our level of understanding of how this actually functions."

Peti and Page, both members of the university's BIO5 Institute, give much of the credit to assistant research scientist Yang Li, who Page called an "invaluable powerhouse" that made the discovery possible.

"It's really been a tour-de-force. It's required a huge amount of effort to really detangle how these proteins function," Page said.

The novel combination of advanced chemistry techniques required a sophisticated computational program that would be able to combine complex data from both methods into one cohesive structure. That sparked a collaboration with Charles Schwieters at the National Institutes of Health. Using Schwieters' Xplor-NIH computer software, Peti and Page merged their approaches into one hybrid technology that would be able to determine the precise interaction of RCAN1 and CN.

The group found that RCAN1 inhibits CN by impairing its ability to signal to other proteins and by blocking the active site as well as the substrate recruitment sites of the protein. By inhibiting the activity of CN in two different ways, RCAN1 effectively prevents CN from supporting proper development and cognitive function. This interaction also helps to explain how the overactivity of RCAN1 contributes to Down syndrome and Alzheimer's disease.

With this new information, researchers may now be able to develop targeted drugs to disrupt the imbalanced interaction between RCAN1 and CN.

"We are hopeful that findings from this study will inform new therapies to treat or entirely prevent these neurological disorders," Peti said.

More information: Yang Li et al. The structure of the RCAN1:CN complex explains the inhibition of and substrate recruitment by calcineurin, *Science Advances* (2020). DOI: 10.1126/sciadv.aba3681