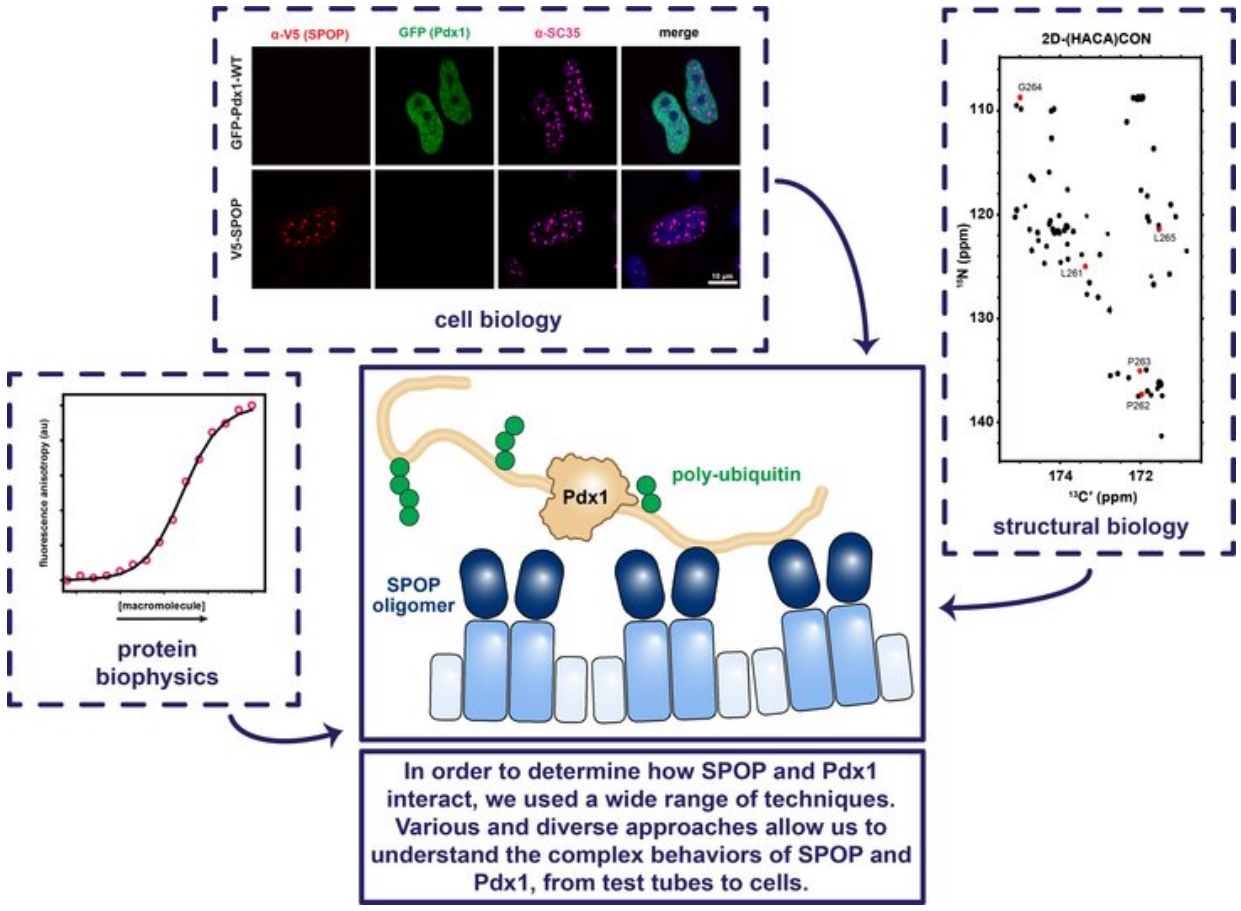
How does a regulatory protein know where to bind to modulate insulin production?

July 1 2021, by Gail McCormick



Penn State researchers used a variety of techniques regarding cell biology, structural biology, and protein biophysics to determine how the proteins SPOP and Pdx1 work together to ensure the gene that codes for insulin is turned on and off at the correct time. Credit: Showalter Lab, Penn State

Some proteins in the body ensure that genes are turned on and off at the correct times. For example, the transcription factor protein Pdx1 (pancreatic and duodenal homeobox 1) turns on the gene that codes for insulin, and the protein SPOP (speckle-type POZ protein) in turn binds to Pdx1 so that the body doesn't make too much insulin. But it's unclear how SPOP binds to Pdx1. Understanding where SPOP binds may help researchers predict what predisposes individuals to developing diabetes and clarify how SPOP regulates other important proteins. In a recent study, a team of researchers from Penn State and St. Jude Children's Research Hospital imaged the proteins and determined just how this important interaction occurs.

A paper describing the interaction was recently published in the *Journal of Biological Chemistry*. We talked to two of the authors of the paper, Scott Showalter, professor of chemistry and of biochemistry and molecular biology, and Emery Usher, graduate student in Biochemistry, Microbiology and Molecular Biology (BMMB) program, about this work.

Q: Why is Pdx1 important for the human body, and how does SPOP support its function?

Showalter: Pdx1 is a transcription factor, which is a protein that binds to the DNA in your genome and controls whether nearby genes will be turned on or off. In humans, Pdx1 is primarily found in the pancreas, where it turns on the gene that codes for the protein insulin when more of it is needed. When enough insulin is stored up for the future, SPOP binds to Pdx1 and causes it to be destroyed by the cellular protein recycling machinery, thus turning off insulin production.

Usher: Ultimately, Pdx1 and SPOP work together to maintain glucose homeostasis; that is, the careful balance of glucose levels in the cells and

in your bloodstream. Notably, SPOP performs a similar regulatory role for dozens of other proteins in lots of different types of cells, all of which are critical to appropriate cell function.

Q: What was your motivation for this study?

Showalter: Although we knew that Pdx1 and SPOP work together to regulate the insulin-coding gene, prior to this study the details of this interaction was unclear. It was known from other work that SPOP turns proteins off by attaching a molecular signal to them that targets these proteins for destruction, but Pdx1 does not look like any other proteins that SPOP regulates. Almost all proteins known to be regulated by SPOP possess multiple recognition sequences, or sequences of amino acids that act like a password. However, Pdx1 does not contain any of the sequences that SPOP was known to bind to. My laboratory has invested a great deal of effort over the past decade to develop techniques that can be used to characterize interactions like the ones that we knew must exist between Pdx1 and SPOP. In this study, we set out to determine where SPOP binds to Pdx1 and how it knows that it has found the correct site(s).

Usher: SPOP can actually recognize more than one of these amino acid password sequences and can thus target many partners, so it is difficult to produce a comprehensive list of the amino acid sequences that SPOP looks for. Investigating the interaction between Pdx1 and SPOP could also provide insight into other proteins SPOP might bind to.

Q: What were the main results of this study?

Showalter: We were very happy to find that there is not just one SPOP binding site on Pdx1, but two. It is known that SPOP generally binds multiple sites in the proteins it controls, so this result was very satisfying

because it brings Pdx1 regulation into alignment with the community's more general understanding of how SPOP functions. After we found the second binding site, we used X-ray crystallography to image the complex that forms when SPOP is bound to Pdx1 at these newly discovered binding sites. This structure revealed that even though an unusual sequence of amino acids in Pdx1 was involved in SPOP binding, the geometric and chemical details actually were very similar to previously determined structures. Our results suggest that the previous definition of a SPOP binding site was too narrow.

Usher: We now have a better understanding of the chemical rules that define whether a sequence is a good candidate to bind or not. Our structure also suggests a plausible mechanism to disrupt Pdx1 binding by SPOP when this interaction is unwanted—for example, when Pdx1 is needed to produce more insulin.

Q: Why are these findings important?

Showalter: It is important to understand the molecular details of biological processes like glucose-dependent insulin production and how they are regulated because these are the deciding factors between normal health and disease. Understanding the sequences SPOP binds to helps us to predict why certain genetic variations may predispose individuals and families that carry them to developing diabetes. Similarly, by clarifying the rules that SPOP uses to identify the proteins it should bind to and regulate, we can better predict other proteins it regulates. We may also be able to predict how naturally occurring variations in their amino acid sequences may disrupt normal SPOP binding, leading to poor health outcomes.

Usher: SPOP is also known for its role in certain cancers, including prostate and endometrial cancer. While beyond the scope of our current work, better defining how SPOP selects binding partners will likely

impact future research in this area as well.

More information: Emery T. Usher et al, Intrinsically disordered substrates dictate SPOP subnuclear localization and ubiquitination activity, *Journal of Biological Chemistry* (2021). DOI: 10.1016/j.jbc.2021.100693

Provided by Pennsylvania State University