Protein's structural shift offers clues to tumor suppression and other key cell functions

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(Medical Xpress)—Researchers have discovered how one segment of an important regulatory protein changes shape so it can fulfill multiple roles in the life of cells, including tumor suppression. St. Jude Children's Research Hospital scientists led the study, whose findings could aid cancer drug development.

The research focused on the protein nucleophosmin 1 (NPM1), which plays a critical part not only in tumor suppression but in cell division, protein production and other cell processes. Until now, however, how NPM1 fulfilled its varied responsibilities was unknown. The study was published recently in the scientific journal *Proceedings of the National Academy of Sciences* (*PNAS*).

Scientists found a possible answer at one end of NPM1.

Proteins are long chains of amino acids whose function is dictated in part by their 3-D shape and flexibility. Until this study, researchers believed that the NPM1 segment was always folded into a highly ordered five-sided structure called a pentamer. Using nuclear magnetic resonance (NMR) and other techniques, researchers identified a regulatory mechanism that prompted the NPM1 segment to partially or completely unfold into a single disordered strand of amino acids.

The researchers believe that the protein's role changes along with its

shape. "We propose that this regulated unfolding—from a folded pentamer to a disordered monomer—controls how NPM1 functions in cells, including how it interacts with one of the most important tumor suppression proteins, the ARF protein," said corresponding author Richard Kriwacki, Ph.D., a member of the St. Jude Department of Structural Biology. ARF is lost in a number of cancers, including leukemia, melanoma and the brain cancer glioblastoma.

"This is the first report of how ARF and NPM1 interact at the molecular level," Kriwacki said. "Our findings provide a foundation for anticancer drug development based on this interaction and will advance understanding of a number of other important cell functions."

To function properly, ARF must partner with NPM1. In this study, researchers detailed the chemical basis of that partnership and demonstrated how changing the shape of the NPM1 segment disrupted the process. Investigators showed that ARF included short sequences that are rich in the amino acid arginine that promoted binding to the NPM1 pentamer.

ARF is one of several hundred NPM1 binding proteins. When researchers checked those other proteins, they found that 40 percent included the same arginine rich sequence, providing a common molecular basis for binding to NPM1.

Researchers also identified the regulatory mechanism responsible for the NPM1 shape change. Investigators showed that adding phosphate chemical groups to different parts of NPM1—a process known as phosphorylation—destabilized the five-sized structure at the protein's end and thus ARF binding. Phosphorylation is widely used in cells to regulate how proteins function.

Destabilization of the NPM1 segment prompted the protein to partially

unfold. Scientists reported that the unfolding revealed additional phosphorylation sites on NPM1. More phosphorylation led to further unfolding.

The shape-change dictates not only NPM1's binding partners but probably where and how the protein functions in cells, said the study's first author Diana Mitrea, Ph.D., a St. Jude postdoctoral fellow.

NPM1 is found primarily in the nucleolus of the cell. The nucleolus is a compartment in the cell's command center, the nucleus. The nucleolus is where components of protein production are assembled and shuttled to the gel-like cytoplasm with help from NPM1.

NPM1 shares the nucleolus with molecules that form networked structures with liquid droplet-like features. These structures have drawn intense interest since 2009 when they were first described by other investigators. Kriwacki and his colleagues are pursuing the possibilities that NPM1 might contribute to the formation of these intriguing structures.

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