

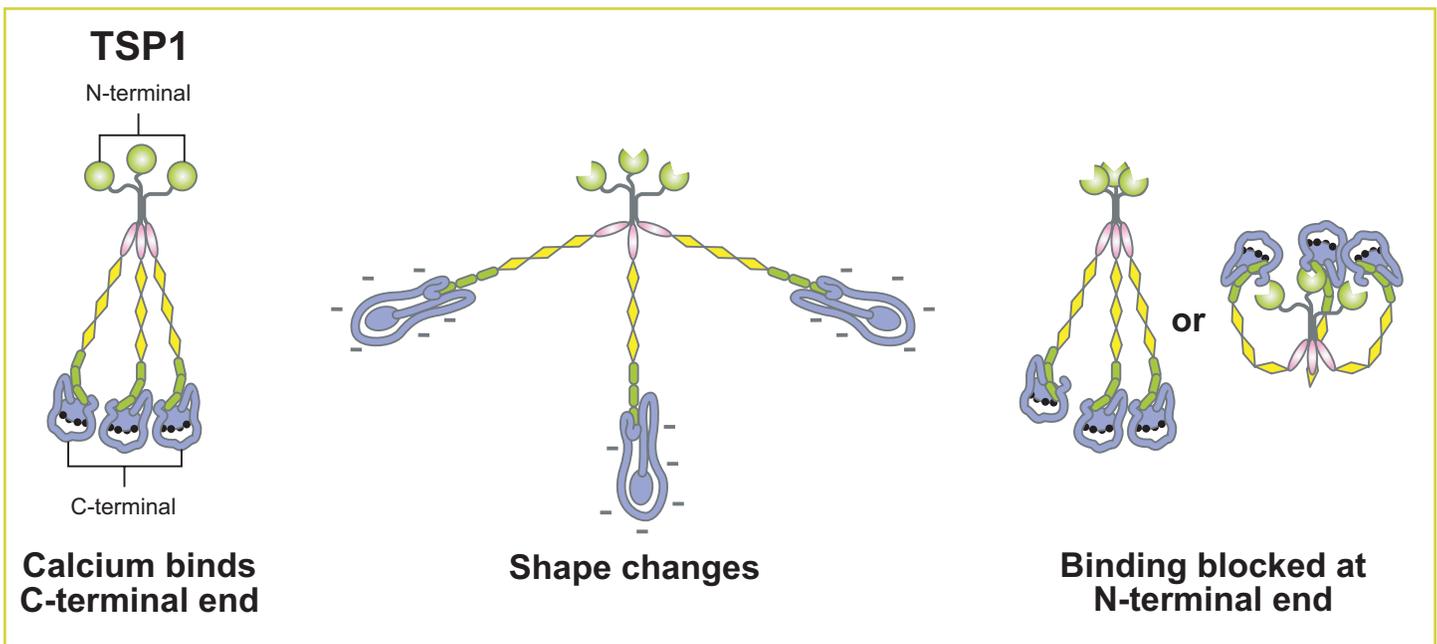
Microenvironment Influences Interaction of Signaling Molecules

Tumor progression depends not only on events that occur within cancer cells but also on the interaction of cancer cells with their environment, which can regulate tumor growth and metastasis and modulate the formation of new blood vessels to nourish the tumor. All cells communicate with other cells around them, including endothelial cells (the cells that make up blood vessels). They also interact with the extracellular matrix (ECM), a network of sugars and proteins that supports cells. Communication between neighboring cells

and molecules often occurs through interaction among and between molecules on the cell surface and molecules of the ECM. Defining these interactions should facilitate the development of novel approaches to limit tumor progression.

David D. Roberts, Ph.D., a Senior Investigator in CCR's Laboratory of Pathology, and colleagues recently investigated how factors in the cellular microenvironment influence the interaction between two proteins—thrombospondin-1 (TSP1),

a protein in the ECM, and $\alpha\beta 1$ integrin, a signaling protein embedded in the membrane of many types of cells, including those of several common cancers. Interaction between TSP1 and $\alpha\beta 1$ integrin has been shown to regulate several processes that can contribute to tumor progression, such as cell growth and survival, adhesion, and motility; the two proteins also play a role in angiogenesis—they can either promote or inhibit the growth of new blood vessels, depending on other factors in the environment. Post-doctoral Fellow Maria J. Calzada,



Binding calcium to the C-terminal end of thrombospondin-1 (TSP1) causes three-dimensional changes in the structure of TSP1 that influence the behavior of the N-terminal end in one of two ways—either sites in the N-terminal end are intrinsically altered, changing their affinity for various binding partners, or the three-dimensional shape of TSP1 is altered such that the C-terminal end physically blocks binding partners, such as integrins, from accessing their binding sites in the N-terminal end.

Ph.D., is the lead author of the paper that resulted from the study and was published in *Matrix Biology*.

The study builds on the observation that the binding of calcium to TSP1 can affect the ability of the protein to interact with $\alpha3\beta1$ integrin. This finding was unexpected, because calcium and $\alpha3\beta1$ integrin bind to opposite ends of TSP1 (calcium to the C-terminal end and $\alpha3\beta1$ integrin to the N-terminal end). To further explore the notion that the binding of one factor to TSP1 can change the behavior of other parts of the protein, the researchers identified and used a series of antibodies that interact with the N-terminal end of TSP1 and enhance interaction between TSP1 and $\alpha3\beta1$ integrin. They found that two of these antibodies bound to TSP1 less efficiently in the presence of calcium. The researchers then confirmed that the effect of calcium was mediated

through the C-terminal end of TSP1 by using a recombinant TSP1 that only included the N-terminal end of the protein.

These results confirm that a conformational change caused by binding one factor to one part of TSP1 can influence the interaction of TSP1 with other factors, including cells and other ECM proteins. Indeed, experiments showed that in addition to increasing TSP1 interaction with $\alpha3\beta1$ integrin, the antibodies also affected TSP1 interaction with heparin, sulfatide, and fibronectin. Roberts and colleagues speculate that the binding of calcium to the C-terminal end of TSP1 causes three-dimensional changes in the structure of TSP1 that influence the behavior of the N-terminal end in one of two ways—either sites in the N-terminal end are intrinsically altered, changing their affinity for various binding partners, or the

three-dimensional shape of TSP1 is altered such that the C-terminal end physically blocks binding partners from accessing their binding sites in the N-terminal end.

This study reinforces the importance of the complex tumor environment to the function and activity of signaling molecules. Increased understanding of how cellular and ECM factors interact with one another to influence tumor progression should help identify targets for therapy and spur the development of novel therapeutics to prevent cancer cell growth.

Reference

Calzada MJ, Kuznetsova SA, Sipes JM, Rodrigues RG, Cashel JA, Annis DS, Mosher DF, Roberts DD. Calcium indirectly regulates immunochemical reactivity and functional activities of the N-domain of thrombospondin-1. *Matrix Biol* 2008 [Epub ahead of print].