

Targeted Destruction:

Novel Interactions in Ubiquitylation and Tumorigenesis

The controlled destruction of proteins is as important as their synthesis for maintaining cell integrity. A process called ubiquitylation tags proteins for degradation and plays a crucial part in cell cycle regulation, DNA repair, cell growth, and immune function, among other processes. Its dysfunction contributes to pathogenesis, including the development of cancer.

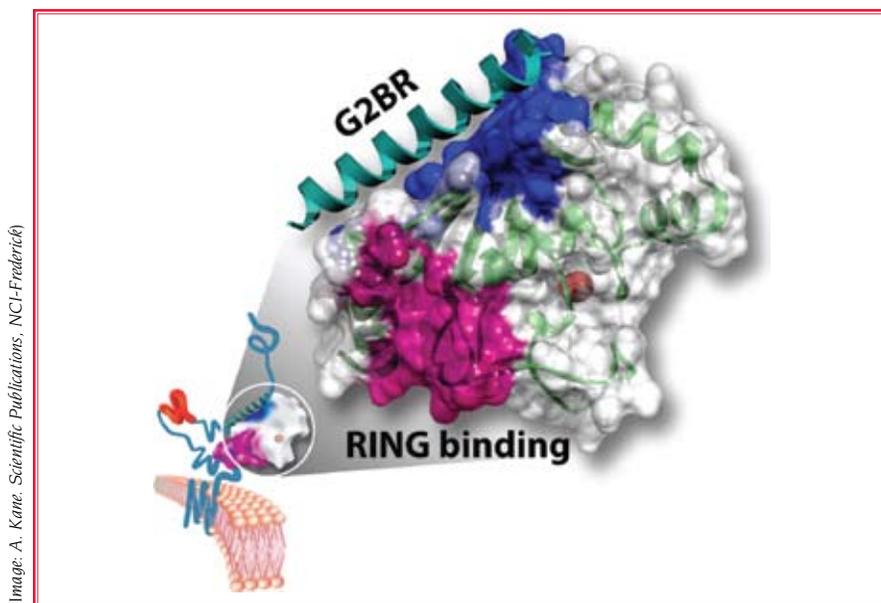
Ubiquitylation is a multistep process in which a ubiquitin tag passes through an assembly line of enzymes—E1, E2, E3—before being attached to a protein. E1 activates the ubiquitin molecule at the start of the process, and E3s (also known as ubiquitin ligases), of which there are more than 500 in mammals, are ultimately responsible for directing the ubiquitin tag to specific protein targets. E2s sit in the middle, coordinating and potentially regulating their fellow enzymes through specific binding sites. In the June 26, 2009 issue of *Molecular Cell*, CCR researchers involved in a multidisciplinary collaboration have published one key to the mechanism of

action of a specific E3 family member, gp78, which controls the levels of a metastasis suppressor.

An earlier study by Allan Weissman, M.D., Chief of the Laboratory of Protein Dynamics and Signaling at CCR, showed that high levels of gp78 promote the spread of cancer by tagging a protein for degradation that suppresses metastasis and that the E3 activity of gp78 was required for this degradation to occur. Weissman's lab also demonstrated that, in addition to a known interaction site for E2 enzymes (the RING finger domain), gp78 has a unique region called the G2BR that strongly binds to its corresponding E2.

In this new work, Andrew Byrd, Ph.D., Head of the Macromolecular NMR Section at CCR; Xinjua Ji, Ph.D., Head of the Biomolecular Structure Section at CCR; Dr. Weissman; and their colleagues used advanced structural techniques to study the interaction between gp78 and its E2 and uncovered a previously unknown mechanism by which ubiquitylation can be regulated. Whereas previous work identified the interaction site of RING finger domains on E2 enzymes, the researchers found that the gp78 G2BR binds to an additional distinct area of E2. This G2BR binding causes conformational changes to the E2 that allow the gp78 RING finger domain and the E2 to bind 50 times more tightly than they would otherwise. "This is the first demonstration of an allosteric mechanism whereby interactions with a RING finger domain are enhanced by binding to a second discrete domain within the ligase," said Dr. Byrd. "This represents a significant shift in the existing paradigm for E1-E2-E3 function."

Further research showed that this increased binding strength enhances ubiquitylation of target proteins by gp78, so blocking G2BR function would inhibit degradation of the proteins that suppress cancer metastasis. This team is currently collaborating with other CCR scientists to further define the interactions of E2s and RING finger domains and to design and construct potential inhibitors of gp78 for testing *in vivo*, the team ultimately hopes to add ubiquitylation-regulating agents to the armamentarium of cancer drugs.



(Image: A. Kane. Scientific Publications, NCI-Frederick)

Model (bottom left) of the ubiquitin ligase gp78 interacting with its cognate E2 in the endoplasmic reticulum membrane. Structural details of the interactions of the E2 with G2BR (helix represented in cyan) and RING finger domains (magenta binding surface) of gp78 are revealed by NMR and X-ray structural studies.

To learn more about Dr. Byrd's research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?profileid=5544>.

To learn more about Dr. Weissman's research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?profileid=6524>.

To learn more about Dr. Ji's research, please visit <http://ccr.cancer.gov/staff/staff.asp?profileid=5860>.