Structure Determined for p53 Tumor Suppressor Protein as Bound to DNA for Anti-Cancer Activity

(Philadelphia – July 17, 2006) – More than half of human cancers involve mutations in the p53 tumor-suppressor gene, suggesting the critical role played by the normal p53 protein in defending against cancer. Similarly, roughly 95 percent of cancer-causing mutations in the p53 protein occur in its DNA-binding core domain, pointing to this region of the p53 protein as being pivotal to its anti-cancer activity.

Clearly, a detailed view of the p53 protein in direct contact with DNA could provide important insights into preventing and treating an array of human cancers. To date, however, despite having learned a good deal about the protein's biochemistry over the years, scientists have been unable to "see" the protein – using the tools of structural biology – bound to DNA in its naturally occurring form. This naturally occurring form contains a pairing of two p53 proteins, called a dimer, that then binds to a second p53 dimer in a similar way to create the precisely oriented four-protein complex, called a tetramer, that binds DNA.

Now, in a new study featured as a "paper of the week" and on the cover of the July 21 issue of the *Journal of Biological Chemistry*, researchers at **The Wistar Institute** have successfully determined the three-dimensional structure of the p53 protein bound as a dimer to DNA and used the structure to produce an accurate model of the p53 tetramer bound to DNA.

"The bottom line is that we now have a detailed picture of how p53 binds DNA," says **Ronen Marmorstein, Ph.D.**, a professor in the Gene Expression and Regulation Program at Wistar and senior author on the study. "Given the fact that p53 is an important tumor suppressor that is mutated in the majority of human cancers, this will undoubtedly be useful information."

Earlier work had shown how p53 binds to DNA as a stand-alone entity, a form that does not represent the natural state of p53 binding to DNA. The present work captures p53 bound to DNA in its natural dimeric units and thus allows Marmorstein and colleagues to make new and potentially significant insights into p53 function.

One new insight from the current study, for example, is that the point of contact between the two core domains of a pair of p53 proteins forming a dimer tracks to a part of the protein often mutated in cancers. This suggests that the interface between the two proteins of the dimer is likely as important for the proper functioning of the tetramer as its interface with DNA, which also depends on the interface of the core domains of the two p53 proteins that form a dimer.

In seeking to determine the structure of p53 bound to DNA, the challenge for the scientists was that their efforts to crystallize the p53 dimer bound to DNA consistently resulted in structures that could not bind to DNA. (Crystallization is a prerequisite for obtaining the type of three-dimensional image sought in this study.) The researchers found that the dimers formed in solution prior to crystallization attempts took on a form that was incompatible with DNA binding.

"There's an inactive form of the p53 dimer that's unable to bind DNA in the correct fashion," Marmorstein explains. "We knew there had to be a structural rearrangement of the core domains to allow p53 to bind DNA as a dimer. The core domain is what's binding the DNA, but within the dimer, the two cores have to be in the proper orientation to bind DNA.

"So we decided that we needed to somehow lock the protein into a conformation that's compatible with the dimer binding to DNA. We used a chemical trick in which we modified a DNA base to allow it to attach directly to a part of the protein's core domain. That allowed us to trap the form of the p53 dimer that's compatible with DNA binding. And we solved the structure. We saw what it looked like."

The lead author on the *Journal of Biological Chemistry* study is **William C. Ho**, affiliated with both Wistar and the University of Pennsylvania. **Mary X. Fitzgerald**, affiliated with Wistar and the University of Pennsylvania School of Medicine, is also a co-author. Marmorstein is the senior author.

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Ronen Marmorstein, Ph.D.

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