STRUCTURAL BIOLOGY

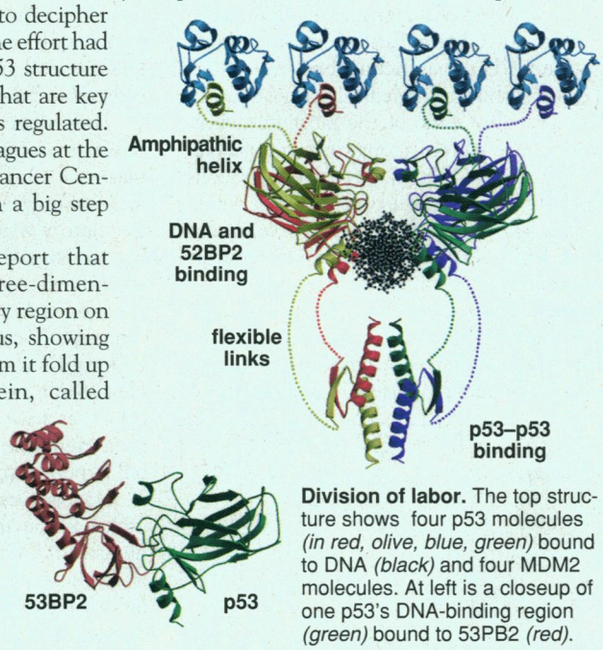
Filling In the Blanks in the p53 Protein Structure

Structural biologist Nikola Pavletich doesn't like to do things halfway, particularly when they involve the protein called p53. Because this molecule plays a critical role in protecting cells against cancer, Pavletich—like many other researchers—wants to know everything about its structure and function. But because the whole molecule has been difficult to crystallize, researchers have had to decipher its structure piece by piece. The effort had left about one-third of the p53 structure unsolved, including regions that are key to understanding how p53 is regulated. Now, Pavletich and his colleagues at the Memorial Sloan-Kettering Cancer Center in New York have taken a big step toward filling this gap.

On page 948, they report that they've determined the three-dimensional structure of a regulatory region on the protein's amino terminus, showing how the amino acids that form it fold up and link to another protein, called MDM2. That embrace allows MDM2 to turn off p53 once it has done its normal job of ensuring that the cell repairs any damaged DNA before it divides. In the absence of that p53 function, cancer-causing mutations might accumulate. "Given the importance of p53 [in cancer] and therefore the potential importance of MDM2, it's very nice to get a closeup of the molecular interactions," says molecular biologist Carol Prives of Columbia University. The same goes for any interaction between p53 and other proteins, which is why the Pavletich group has also taken a closeup look at the interaction between p53 and a second protein—possibly another p53 regulator—as they report on page 1001.

Because loss or malfunction of p53 is thought to contribute to the development of half of all cancers, including common ones such as skin, breast, and colon cancers, researchers would like to design drugs that can enhance or restore p53 activity. And the new structures—especially the one showing the p53-MDM2 interaction—could aid that effort by pointing to ways to prevent regulatory molecules such as MDM2 from binding to p53 and inhibiting it.

Over the past four years, Pavletich and his colleagues have been systematically working their way through the p53 structure in hopes of understanding the many molecular interactions needed for its normal functions. Two years ago, for example, they solved the structure of another key portion of the tumor suppressor protein: the 200–amino-acid section in the middle of the molecule that contains its DNA-binding site (*Science*, 15 July 1994, p. 346). This site is essential for p53 action, because the protein is a transcription factor that exerts its effects by binding to DNA and regulating the activity of other genes.



P. KUSSIE, S. GORINA, N. PAVLETICH

Division of labor. The top structure shows four p53 molecules *(in red, olive, blue, green)* bound to DNA *(black)* and four MDM2 molecules. At left is a closeup of one p53's DNA-binding region *(green)* bound to 53PB2 *(red)*.

That's not the only interaction necessary for p53 activity, though. It only works as a transcription factor when four p53 molecules come together in a single complex. About the same time that the earlier Pavletich structure came out, Cheryl Arrowsmith from the University of Toronto used nuclear magnetic resonance (NMR) and Pavletich used crystallography to solve a string of 50 amino acids on p53's carboxyl end where the four-fold assembly takes place. After that, only about 145 amino acids on p53's amino terminal still remained to be solved.

Getting this structure was not simply a matter of filling in a blank space on the protein, Pavletich says, because this part of the molecule contains a string of 15 amino acids that is important for regulating p53 activity. This string binds proteins called TAFs, which in turn attract other proteins needed for p53 to initiate gene expression. The amino-acid string also binds MDM2, which apparently has the opposite effect, turning off p53 activity by blocking the binding of the TAFs. But the flexibility of this part of the protein made it hard to get crystals suitable for x-ray analysis.

What Pavletich and Paul Kussie, a postdoctoral fellow in his lab, have now done is crystallize that 15–amino-acid segment of p53 together with a part of MDM2, having finally come up with sections of each protein that have just the right sizes to make a crystal of the two proteins bound together. The approach has yielded a dual payoff: a picture of part of the last unknown region of the protein and of its interaction with MDM2.

The group's analysis of the cocrystals reveals that the p53 segment is coiled into a structure called an amphipathic helix, which has the water-loving amino acids lined up on one side and the hydrophobic (fat-like or water-hating) amino acids on the other. Three of these hydrophobic amino acids are nestled into a pocket, which Pavletich describes as "deep and greasy," in the MDM2 protein. Indeed, the p53 amino acids "fit [into the MDM2 pocket] like a hand in a glove," says Bert Vogelstein of Johns Hopkins University School of Medicine, whose own lab was among the pioneers in studying p53. This tight fit, he notes, may explain why MDM2 is such an effective blocker of TAF binding and thus of p53 activity. "The way the proteins lock together is a dramatic structural confirmation of the biological data," Vogelstein remarks.

That information might be helpful to researchers seeking to develop anticancer drugs, because some cancers may arise because the cells produce too much MDM2, thereby crippling p53. The idea would be to tie up the MDM2 with a drug that could fill the pocket, preventing this inhibitory molecule from binding to p53. Toward that end Pavletich's group has been working with PharmaGenics, Inc., an Allendale, New Jersey, biotechnology company that is screening natural compounds for anticancer agents, including any that may help restore p53 function.

The second p53 structure that the Pavletich team describes in this issue details a more mysterious liaison. This structure, which was worked out by Svetlana Gorina of the Pavletich lab, shows how another protein, called 53BP2, binds to the tumor suppressor. It is intriguing because it reveals that the 53BP2–binding site overlaps with p53's DNA–binding domain. What's more, several of the *p53* mutations found in cancer cells alter amino acids that bind to either DNA or 53BP2, and a few affect just where 53BP2 binds. Together, Pavletich says, these results suggest that 53BP2 could help regulate p53 activity or work in conjunction with this molecule as it protects

against cancer. But that conclusion is far from certain, says Prives.

One problem is that no one knows what 53PB2 might do in the cell. What's more, work by pediatric oncologist Louie Naumovski at Stanford Medical School shows that the protein is not located in the nucleus, which would imply that it does not influence p53 binding to the DNA but may help bring p53 to the nucleus or fold correctly.

No matter what 53BP2 does relative to p53, Pavletich suggests that the binding is worth studying for its molecular details. The protein uses two motifs to link up with p53: a series of ankyrin repeats—four copies of a set of 30-some amino acids—and another loop called an SH3 domain. A multitude of other proteins use ankyrin motifs for binding, but they've never before been seen in action. Pavletich's colleagues agree that these details would be worth pursuing even if p53 didn't have such a key role in cancer. Says structural biologist Iris Mastrangelo at Brookhaven National Laboratory in New York, "p53 has exquisite molecular mechanisms that [allow] these specialized parts of the protein to target quite different molecules." Indeed, the new results may only feed researchers' p53 obsession.

–Elizabeth Pennisi

PHYSIOLOGY RESEARCH

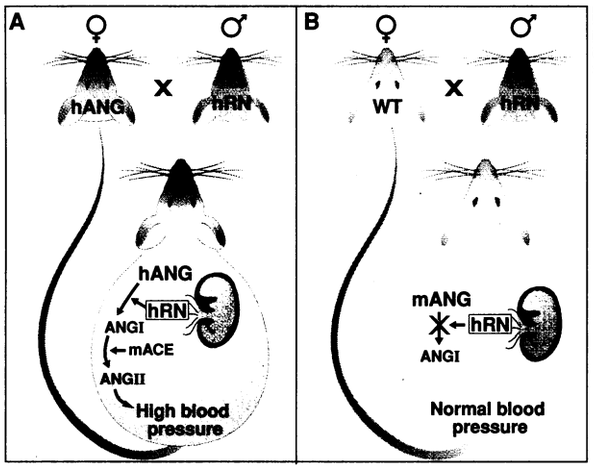
Mouse Model for Pregnancy Problem?

For women and physicians alike, one of the most vexing problems of pregnancy is a condition known as pregnancy-induced hypertension (PIH). Developing in the last trimester, PIH can send the expectant mother's blood pressure rocketing, damaging her kidney, liver, and heart, and putting both her life and that of the child she is carrying at risk. No one knows what causes PIH, which affects up to 10% of human pregnancies and causes the majority of pregnancy-related complications. And efforts to study the condition, as well as to develop therapies for it, have been handicapped by lack of an animal model that reproduces the pathological features of PIH. New work by Akiyoshi Fukamizu and his colleagues at the University of Tsukuba, Japan, may change that.

On page 995, the investigators report that they may have stumbled onto a new strategy for simulating PIH in mice. They did this by mating mice, each of which had been genetically altered to carry one of two genes that encode proteins involved in blood pressure control. One makes angiotensinogen, the precursor of the potent blood pressure–raiser angiotensin II, and the other makes renin, the enzyme that releases angiotensin II from the precursor. When the right mating combination brought these two proteins together in pregnant females, they developed high blood pressure and other changes reminiscent of PIH.

While those who study PIH say that the disease might not be caused the same way in humans, they nevertheless think the model may shed light on the mechanisms by which high blood pressure wreaks havoc on both mother and fetus. Ultimately, such an understanding might lead to better therapies for PIH, which currently can only be cured by premature delivery. In addition, the same strategy might also be used to study other pregnancy-related conditions brought on by a combination of maternal and fetal factors. Indeed, says Charles Rosenfeld, a neonatologist and PIH researcher at the University of Texas Southwestern Medical School in Dallas, "It's probably some of the most innovative work I have seen in a long time in this field."

Fukamizu did not set out to develop a mouse model of PIH. When he began the experiments 7 years ago, he recalls, his goal was to develop mice that could be used to study how renin and angiotensinogen might lead to high blood pressure. To do that, he and his colleagues genetically engineered one mouse strain with the human gene for angiotensinogen and another with the human renin gene. The idea was that when mice with the renin gene were mated with animals with the human angiotensinogen gene, overexpression of the two genes in the progeny would cause high blood pressure.



PIH in mice? Human renin (hRN) from the placenta raises the blood pressure of mice making human angiotensinogen (hANG), but not of those making the mouse protein (mANG).

When his group paired female renin transgenics with male angiotensinogen transgenics, the resulting pups were in fact born hypertensive. But when Fukamizu tried the reverse combination—females with the human angiotensinogen gene and males with the renin gene—he noticed a surprising result: The mothers died late in gestation. "I noticed that there was something there," says Fukamizu. When they studied more of the same kinds of matings, Fukamizu's group found that the pregnant mice displayed such PIH symptoms as placental and heart problems, as well as high urine protein levels, an indicator of damaged kidneys.

These observations suggested that human renin, produced by the paternal gene in the placenta, which is derived from fetal tissue, was making its way into the mother mouse's circulatory system. There it could act on the human angiotensinogen, leading to progressively increasing hypertension. Fukamizu checked that idea by mating male human renin–producers with normal females and looking for renin in females' bloodstream. He found it, which "shows you that the placenta, at least in this model, plays a very prominent role in the development of maternal hypertension," Rosenfeld says.

The big question now is whether the mouse model does, in fact, reflect what's happening in human PIH. One thing giving pause to other researchers are some puzzling features of the model, including the fact that PIH does not develop if females overexpressing human renin are mated with males that overproduce human angiotensinogen. What's more, they note that human placentas may not have the ability to transmit renin into the maternal circulation as mouse placentas do. PIH experts also note that while the increased blood pressure in the mice is presumably due to their increased production of human angiotensin II, the protein's levels are not always high in humans who have either PIH or preeclampsia, an almost identical hypertensive condition of pregnancy. All in all, says PIH researcher Phyllis August at Cornell University Medical Center in New York, "I don't know what this [model] tells you about preeclampsia."

Despite these questions, researchers say the Fukamizu team's model can still be used to study the biochemistry of the disorder in further detail and, quite possibly, to examine the effects of blood pressure drugs and other therapies. "This is not the perfect model, but it can give us some insights," Rosenfeld concludes.

–Trisha Gura

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