How Do Bacteria Repair Damage from the Sun?

From bacteria to plants to humans, all organisms have mechanisms that they use to repair DNA damaged by ultraviolet (UV) light. This fundamental maintenance function is critical to our health because damaged DNA can lead to diseases such as cancer. So, of course, we know all about how it works. Or so scientists thought. New research shows that we must reworked the current model for how UV-repair functions. At issue is the number of molecules of the important proteins in the complex, UvrA and UvrB. This study, conducted by researchers from Harvard University utilizing the APS, promises to provide new insights into the fundamental mechanisms of DNA repair and into diseases that are caused by mutations in these genes such as xeroderma pigmentosum (an autosomal recessive genetic disorder of DNA repair in which the ability to repair damage caused by UV light is deficient), Cockayne syndrome (a disorder characterized by short stature and an appearance of premature aging), and trichothiodystrophy (an inherited condition in which hair is brittle, sparse, and easily broken).

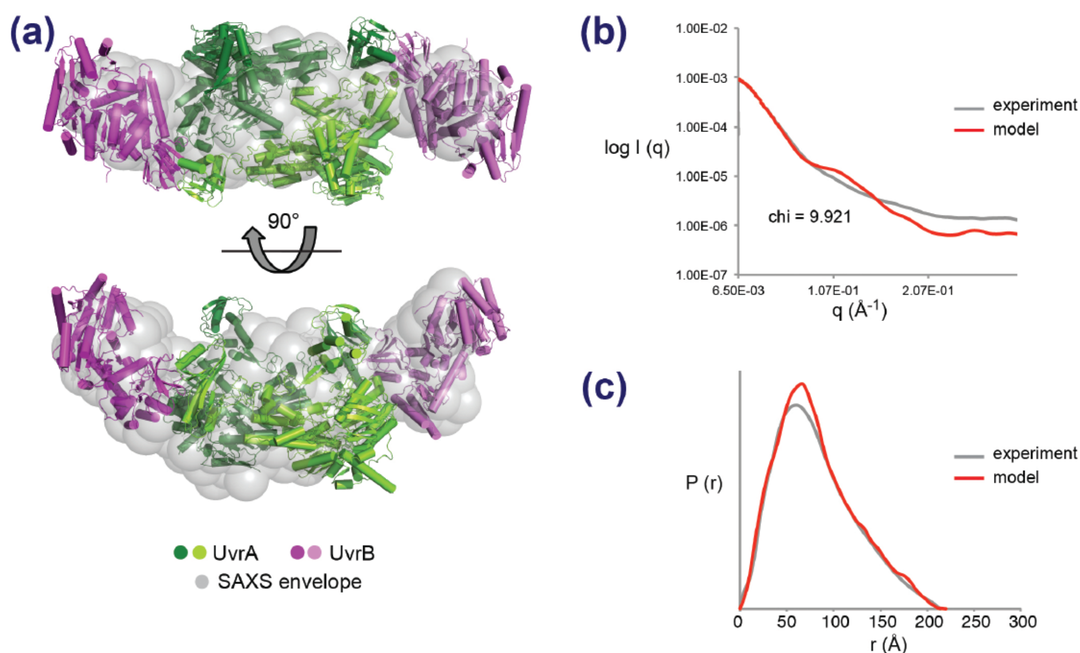

Fig. 1. Modeling of UvrA/UvrB SAXS data. Panel (a) shows the bead model of the elongated structure of the A_2B_2 complex calculated from SAXS data overlaid with the structure of the complex. Panels (b) and (c) show the comparison of SAXS data to the A_2B_2 model data.

Previous work had shown that the bacterial DNA repair mechanism involved three proteins, UvrA, UvrB, and UvrC. The model was that two copies of UvrA traveled around with one copy of UvrB (A_2B_1) and scanned the DNA for places where damage had occurred. When a spot was identified that needed repair, the A_2B_1 stopped and waited at the spot that needed repair. At this point UvrC would come in and displace UvrA and form a complex with UvrB (B_1C_1) that conducted the repair. This model explained how the complex could scan both DNA strands but then orient to repair just one strand. The asymmetric A_2B_1 fit perfectly.

The only problem was, data from various structural methods started to pile up that were not consistent with this model and suggested that the initial complex might actually be A_2B_2. In addition, the Harvard research team solved the crystal structure of the UvrA/UvrB complex and it was consistent with the A_2B_2 model. They proposed that A_2B_2 identified the damage and then the UvrA and one of the UvrB molecules were released when UvrC arrived to repair the problem. However, outstanding questions remained

"Bacteria" cont'd. on page 112

"Bacteria" cont'd. from page 110
about whether there might be other configurations in the crystal structure consistent with the longstanding model.

The group decided to answer these questions by evaluating the complex using small-angle x-ray scattering (SAXS). Their thinking was that low-resolution structural data collected on the protein complex in solution would be ideal for comparison modeling of various protein configurations. In addition, they hoped to find evidence for structural changes associated with how these proteins bind to ATP and use its energy for their activities.

SAXS analysis of a solution of UvrA and UvrB in complex carried out at the Bio-CAT beamline 18-ID-D at the APS showed an elongated structure (Fig. 1).

Modeling of the SAXS data against five possible configurations, four A_2B_2 options and one A_2B_1 option, showed that the data was consistent with one of the A_2B_2 configurations observed in the crystal structure but ruled out the others. Further analysis of the SAXS data by five other methods also supported this conclusion.

Unfortunately, when the team added ATP or ADP to the complex, they were not able to see any significant changes. This may require further experiments with different versions of the protein that allow them to keep the proteins bound to either ATP or ADP. For now, the Harvard researchers will be working on their new model to explain how bacteria perform this universal function. *— Sandy Field*

See: Danaya Pakotiprapha and David Jeruzalmi*, "Small-angle X-ray scattering reveals architecture and A_2B_2 stoichiometry of the UvrA–UvrB DNA damage sensor," Proteins 81,132 (2013). DOI: 10.1002/prot.24170
Author affiliation: Harvard University
Correspondence: *dj@ccny.cuny.edu

This research was funded by National Science Foundation Grant number MCB 0918161 (D.J.); National Institutes of Health Grants number GM084162 (D.J.) and CA100742 (Gregory L. Verdine). Bio-CAT is supported by Grant 5 P41 GM103622 from the National Institute of General Medical Sciences of the National Institutes of Health. Use of the Advanced Photon Source at Argonne National Laboratory was supported by the U.S. Department of Energy Office of Science under Contract No. DE-AC02-06CH11357.

18-ID-D • Bio-CAT • Life sciences • Fiber diffraction, microdiffraction, small-angle x-ray scattering, time-resolved x-ray scattering, micro x-ray absorption fine structure • 3.5-35 keV • On-site • Accepting general users •

Dimensions" cont'd. from page 111
itions through the alpha helix on its way to forming a beta sheet. To determine whether this is unique to src SH3 or may represent a paradigm for folding of these beta-sheet structures, the researchers from the University of Illinois at Urbana-Champaign, Kansai Medical University (Japan), Hokkaido Blood Center (Japan), Ritsumeikan University (Japan), and Nagoya University (Japan), looked at two other SH3 domains from the phosphatidyl inositol-3-kinase (PI3K) and the src-related protein Fyn.

Using circular dichroism (CD) and small angle x-ray scattering (SAXS) at the Bio-CAT 18-ID-D beamline at the APS and the 15A beamline at KEK, their experiments were done under cryogenic conditions (-5° C for PI3K and -28° C for Fyn) to slow down the folding process enough to detect intermediates. Using a technique called "denaturant jumps," the researchers observed the formation of alpha helical or beta sheet structures by CD. The method involves maintaining the protein in the unfolded state in a high concentration of denaturing solution and then jumping it to a low concentration of denaturant that favors folding.

The data for PI3K showed that it formed an alpha helical intermediate at 6 msec after the jump and that this slowly converted to the final beta sheet structure. SAXS analysis confirmed this observation, showing that the intermediate was only about 20% larger than the final state, suggesting a very compact, low free-energy intermediate (Fig. 1).

For the Fyn protein, the results were similar but with some important differences. The compact alpha helical intermediate was also formed very quickly (< 6 msec), but the structure then adopted an additional alpha helical intermediate before settling down to the final native protein structure.

These findings suggest that these SH3 domains contain some intrinsic propensity in their amino acid sequence that drives them to form an alpha helical intermediate on the way to folding, but that the intermediate structure is not always the same for each SH3 domain. Different SH3 proteins follow different paths while folding.

The detection of these alpha helical intermediates opens the door for investigation of the evolutionary forces that may favor or preclude their formation and that may ultimately result in new stable protein folds.

Finally, alpha helical intermediates have been detected in computational simulations of protein folding for a long time and have been considered a nuisance to be avoided through technical adjustments. But now that they have been observed experimentally, these alpha helical intermediates have become more than just simulation artifacts, and they can be incorporated into computational models to improve their real-world applicability. *— Sandy Field*

See: Yoshitaka Matsumura[1], Masaji Shinjo[1]*, Seung Joong Kim[2], Nobuyuki Okishio[3], Martin Gruebele[2], and Hiroshi Kihara[1,4,5], "Transient Helical Structure during PI3K and Fyn SH3 Domain Folding," J. Phys. Chem. B **117**, 4836 (2013). DOI:10.1021/jp400167s
Author affiliations: [1]Kansai Medical University, [2]University of Illinois at Urbana-Champaign, [3]Japanese Red Cross Hokkaido Blood Center, [4]Ritsumeikan University, [5]Nagoya University
Correspondence:
*shinjom@hirakata.kmu.ac.jp

This study was supported by a Grant-in-Aid for Scientific Research (no. 20540400) from the Ministry of Education, Culture, Sports, Science, and Technology (Japan). M.G. and S.J.K. were supported by National Institutes of Health grant R01- GM093318. Bio-CAT is supported by Grant 5 P41 GM103622 from the National Institute of General Medical Sciences of the National Institutes of Health. Use of the Advanced Photon Source at Argonne National Laboratory was supported by the U.S. Department of Energy Office of Science under Contract No. DE-AC02-06CH11357.