How Proteins Take Shape

Guardians give a new twist to protein folding

By EVELYN STRAUSS

Molecular chaperones, like their human counterparts, prevent inappropriate liaisons between their charges and tempters that would lead them astray. Inside the cells of organisms as diverse as bacteria and people, chaperone molecules work in a variety of ways to keep proteins on a productive path. Instead of a raised eyebrow or a well-timed cough, however, some of these molecular caretakers undergo large contortions to encourage proper behavior, according to researchers at Yale University.

One class of chaperone molecules, called chaperonins, performs the important task of shepherding newly made proteins away from each other and helping them fold into the correct shape.

Although scientists have known for almost a decade that chaperonins aid proteins in folding, the precise mechanism of this process has remained murky. The Yale investigators used a two-pronged attack to provide new insight into how bacterial chaperonins carry out their chore. The team describes its work in the Aug. 21 NATURE.

"These groups did a great job of bringing together several techniques to get at an unprecedented level of detail about this biological process," says Edward A. Eisenstein of the Center for Advanced Research in Biotechnology in Rockville, Md.

Scientists had already generated precise pictures of chaperonins before and after these molecular guardians assisted in protein folding, but they lacked detailed images of what happens in between. The Yale group used X-ray crystallography to decipher the structure of two chaperonins in action.

"The study provides a snapshot that shows how and why chaperonins stimulate folding," says team member Arthur L. Horwich.

The researchers used information from their new picture to design experiments that explore how chaperonins exploit ATP, the small, energy-packed molecule that cells use to drive reactions. Surprisingly, they found that chaperonins don't tap the energy stored in ATP in order to fold the protein; rather, they use this power to discharge the protein after they complete their job.

Newly made proteins possess all of the information necessary to convert themselves from their stretched out, stringlike, inactive forms into their three-dimensional, active selves. Yet many of them require help to make the transition.

Inside a cell, destructive influences, including other unfolded proteins, threaten to lead new proteins down an unproductive path. Greasy regions of these molecules can grab hold of each other and create disorganized, useless globs. These oily patches pose a risk only to unfolded proteins; during folding, proteins bury these parts and expose hydrophilic, or water-loving, areas (SN: 2/20/93, p. 121).

To discourage the wayward clumping behavior, chaperonins sequester young proteins. Two of the best-studied chaperonins, the GroEL and GroES proteins from the bacterium *Escherichia coli*, work as a team.

GroEL forms a double-ring structure—like two stacked doughnuts with a piece of tissue paper in between. Oily amino acids that line the inside of GroEL enable the rings to seize unfolded proteins. After a protein enters one of the doughnuts, GroES caps the hole, thus initiating folding. The researchers have now captured an X-ray image of this key step in protein folding.

To figure out what movements take place, the scientists compared their snapshot of the combined molecules to previously generated pictures of the separate components. Upon binding to GroES and ADP, which stands in for ATP in the crystal structure, part of the GroEL doughnut twists a full 90°, tearing the protein away from its contacts inside GroEL. With the connections severed, the chaperonins release the young protein into their cavity, where it is free to fold, Horwich says.

The investigators also found that the twist radically changes the chemical nature of the cavity—from greasy to watery. "Now the protein is looking at a wall that encourages it to expose its hydrophilic regions and bury its hydrophobic [water-avoiding] portions," says Horwich. "This promotes formation of the folded state."

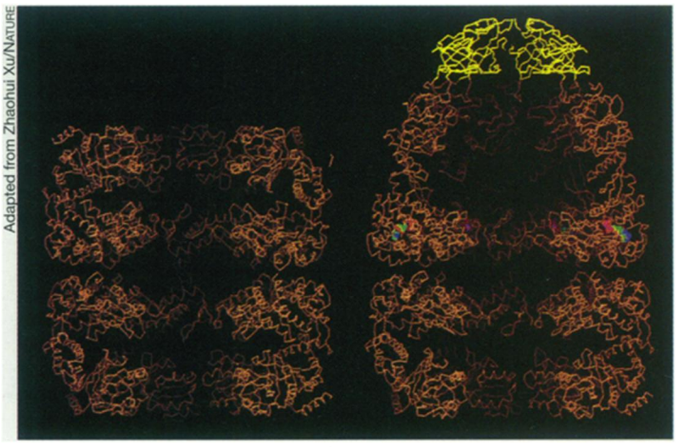
The team noticed that the large rotation brought a particular amino acid in GroEL close to the bound ADP molecule. They altered this amino acid in order to investigate whether critical events—release of the unfolded protein into the cavity, folding, and protein ejection—require the energy stored in ATP.

They found that the modified form of GroEL bound to ATP as well as the normal version did. However, it was only 2 percent as efficient at releasing the energy stored in ATP's bonds.

The release of the protein into the cavity and its folding occurred even with the altered amino acid in GroEL, but the mature protein remained trapped inside the chaperonin cage. These results indicate that simply binding ATP provokes folding. The energy from ATP comes into play only later in the game, to loosen the glue holding GroEL and GroES together, the researchers say.

Ejection of the properly folded protein, however, does not take place until the capped ring that contains the protein gets a "kick in the butt" from the other, empty ring, explains research team member Paul B. Sigler. Again, ATP binding instigates a critical event—in this case, causing the empty ring to tilt in such a way that it forces the companion ring to turn loose its captive protein, the recent experiments show.

Like an overprotective human caretaker, the molecular chaperone needs a nudge before it lets go. □



Adapted from Zhaohui Xu/NATURE

Left: X-ray crystal structure of GroEL alone. Right: Binding to GroES (yellow) and ADP (small colored balls) causes GroEL to change shape dramatically.