

## 2011 Albert Lasker Basic Medical Research Award

**Franz-Ulrich Hartl**  
**Arthur L. Horwich**

*For discoveries concerning the cell's protein-folding machinery, exemplified by cage-like structures that convert newly made proteins into their biologically active forms.*

The 2011 Albert Lasker Basic Medical Research Award honors two scientists for their discoveries concerning the cell's protein-folding machinery, exemplified by cage-like structures that convert newly made proteins into their biologically active forms. With this work, **Franz-Ulrich Hartl** (Max Planck Institute of Biochemistry, Martinsried) and **Arthur L. Horwich** (Yale University School of Medicine) toppled traditional notions of how proteins fold inside cells and established new principles that operate from microbes to humans. This previously unexplored realm holds enormous importance for basic biology and biomedicine.

Protein folding is a vital process, as it converts linear amino-acid chains into the three-dimensional forms that bestow the molecules' unique activities. Greasy regions of new proteins, however, can grab one another and create useless globs. As proteins take shape, they bury these hydrophobic parts and expose hydrophilic, or water-loving, areas. Horwich and Hartl discovered that a special apparatus encases an unfolded protein and spurs folding by harnessing the energy of ATP, the small molecule that drives reactions inside cells.

In the late 1950s and early 1960s, Christian Anfinsen (National Institutes of Health) showed that the amino acid sequence of a protein supplies the information it needs to assume its final form. He added chemicals that unfold—or denature—a small protein and then removed these agents. The protein regained enzymatic activity without assistance, thus establishing that a protein can do its own origami. The impact of Anfinsen's discovery was huge. Scientists assumed that newly synthesized proteins in cells fold unaided and without energy input, as they can in the test tube.

However, larger, more complicated proteins than those studied by Anfinsen aren't as self-sufficient and obliging. Furthermore, inside cells, protein concentrations are orders of magnitude higher than those that Anfinsen used, and as concentration rises, so does the risk of aggregation. Finally, new proteins in living creatures face a challenge that denatured, full-length proteins in a test tube circumvent: Because they gain a single amino acid at a time, portions of the growing chain can potentially stick to one another before the entire molecule is available to fold properly.

### **Unanticipated molecular caretaker**

In the late 1980s, Hartl and Horwich were studying how proteins that are made in the cytoplasm enter mitochondria. Gottfried Schatz (University of Basel) and Walter Neupert (University of Munich) had shown that proteins are imported into mitochondria in a stretched-out state. Once inside, the amino acid sequences that targeted them to the

mitochondria are removed, the proteins refold and, in many cases, assemble into multi-part structures before gaining enzymatic activity.

Horwich sought cellular machinery that participates in the mitochondrial-import process. He isolated yeast strains that fail to perform this essential task under certain conditions and then teamed up with Hartl (then at the University of Munich) to analyze the cells' misbehavior. One of the mutants could transport proteins into mitochondria and clip them to the correct size—but the proteins lacked function, the team reported in 1989. This result suggested that the deviant yeast strain carries a damaged version of a component that normally facilitates protein folding or subsequent events, such as multi-molecular assembly, necessary for protein activity. The evidence strongly favored a block in folding, but assembly requires that a protein has achieved its proper form, so the experiments could not absolutely distinguish these possibilities.

The idea that some proteins need help to assemble had emerged from work on bacteria and chloroplasts. In 1972, Costa Georgopoulos (then at Stanford University) discovered that viral proteins fail to congregate into normal “head” structures within a strain of *Escherichia coli* that harbors particular genetic flaws. He subsequently tracked the defects to neighboring genes, *groEL* and *groES*. In 1980, R. John Ellis (University of Warwick) identified a chloroplast factor that physically interacts with newly made subunits of Rubisco, a key metabolic enzyme. He proposed that this Rubisco-binding protein promotes assembly of the enzyme. In 1988, these observations coalesced when Ellis and Georgopoulos sequenced the *GroEL* and Rubisco-binding protein genes and established their strong similarity. The researchers dubbed GroEL and the Rubisco-binding protein chaperonins, thus defining a subset of the chaperone family, whose members were thought to encourage macromolecular assembly by helping components avoid improper liaisons with themselves and others.

Genetic analysis revealed that Horwich and Hartl's yeast mitochondrial protein was identical to the previously discovered Heat shock protein 60 (Hsp60), whose production increases in response to heat—and that it resembles the chaperonins. Soon afterward, the researchers performed their dogma-defying experiment by testing whether folding per se—rather than assembly—depends on Hsp60. A protein that operates on its own relied on an Hsp60-based molecular machine to fold inside mitochondria, and ATP powered this reaction. Hartl and Horwich had thus demonstrated the existence of an ATP-driven “folding catalyst” and revealed an unimagined piece of nature: Proteins imported into the mitochondria cannot refold spontaneously.

### **The mechanism takes shape**

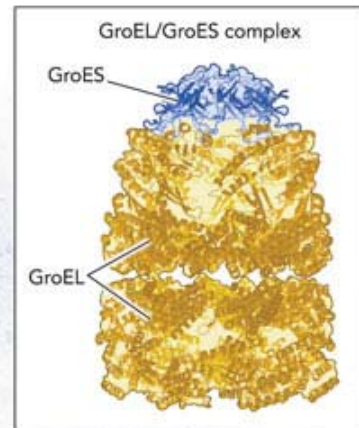
In the next advance, George Lorimer (Du Pont de Nemours & Co.) established that chaperonin activity could be studied using isolated components. He restored the enzymatic activity of denatured Rubisco in a test tube by adding to it purified GroEL and GroES in the presence of ATP. Horwich and Hartl set up a similar system to peer into the folding reaction. They showed, for instance, that GroEL (Hsp60's bacterial counterpart) binds proteins in a relatively unstructured form—and that addition of GroES and ATP provokes folding.

A tremendous amount of subsequent work, anchored by Hartl's and Horwich's ongoing independent investigations, provided key details about chaperonin action. For instance, the GroEL cavity, capped by the GroES lid, provides a cage where amino acid chains can fold, much as denatured proteins did in Anfinsen's experiments, sequestered from unproductive interactions with other unfolded proteins. Furthermore, GroES cycles on and off GroEL—and GroEL sometimes ejects a protein that has not yet reached its active state. In that situation, the substrate protein rebinds GroEL for another try. These reports and others began to illuminate the sequence of events by which ATP orchestrates a cyclical reaction (see diagram) in which GroES and protein associate with alternating GroEL rings to enact folding.

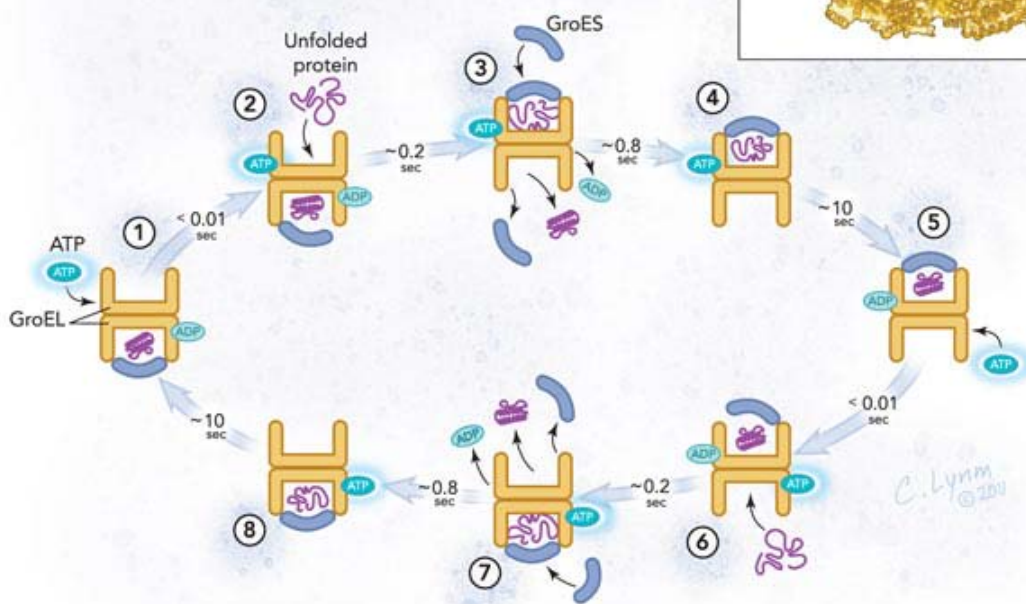
**A Protein-Folding Machine:**

**The GroEL/GroES Reaction Cycle**

The GroEL/GroES complex (model, right) undergoes a multi-step process (below) to fold proteins. Binding of 7 ATPs ("ATP" in diagram) to the top GroEL ring ①, followed rapidly by entry of an unfolded protein ②, forces GroES off the opposite (bottom) ring ②–③. GroES and 7 ADPs ("ADP" in diagram) depart soon afterward ③. GroES binding to the top ring ④ triggers a massive twist inside the GroEL cavity, which severs the unfolded protein's contacts with the inner wall ④. It then has a chance to fold during the 10 seconds in which the high-energy ATP bonds are breaking, converting ATP to ADP ④–⑤. This process loosens GroEL's grip on GroES and primes the bottom GroEL ring to accept ATP ⑤. ATP binding restarts the cycle, this time on the bottom ring, with a new unfolded protein ⑥–⑧. ILLUSTRATION BY Cassio Lynn



Adapted from Xu Z, Horwich AL, Sigler PB. Nature. (1997) 388:741-750.



As these biochemical studies proceeded, Horwich and the late Paul Sigler, a Yale colleague, generated pictures at atomic resolution to elucidate details of the chaperonin reaction

mechanism. They first discerned the structure of GroEL on its own, using X-ray crystallography.

Previous electron microscopic analysis had shown that the protein looks like two 7-fold symmetric donuts atop each other, and Horwich and Sigler found that oily amino acids point toward GroEL's cavity at the donut's opening. This observation suggested how GroEL selectively grabs unfolded proteins: through their hydrophobic portions. The researchers tested this prediction and others by probing whether specific amino-acid alterations perturb key aspects of GroEL's function. Changing hydrophobic elements to hydrophilic ones at particular spots inside the donut's opening disrupted protein binding—and the amino acids that grasp protein also grip GroES, suggesting that protein and GroES compete for the same sites on GroEL. These results confirmed how non-native proteins affix to the central channel and suggested how GroES attachment forces protein release into the cavity.

Three years later, Horwich and Sigler captured an X-ray image of the step after GroES caps the GroEL hole. The scientists then compared their snapshot of the combined molecules to pictures of the separate components. Upon binding to GroES and ADP, part of the GroEL donut enlarges and twists dramatically, thus ripping the unfolded protein from its connections inside GroEL and freeing it within the cavity. The contortion radically changes the internal environment—from greasy to watery—thus encouraging the unfolded protein to expose its hydrophilic regions and bury its hydrophobic ones. Additional analysis revealed how ATP binding and hydrolysis drives the folding reaction.

As the GroEL/Hsp60 story developed, a puzzle emerged. Hugh Pelham (Medical Research Council, Cambridge) had proposed that a different heat shock protein, Hsp70, also binds to hydrophobic surfaces to limit inappropriate associations among partially denatured proteins. Hartl wondered why cells contain two systems that serve similar purposes.

In 1992, Hartl established the concept of a relay in which each chaperone plays a distinct role. He demonstrated that the *E. coli* version of Hsp70 prevents premature folding of the growing amino acid chain; it then transfers the complete protein to GroEL, which promotes folding. He and Horwich had previously discovered that cage-like chaperonins distinct from those in mitochondria, chloroplasts, and bacteria also exist in the eukaryotic cytoplasm, and Hartl showed that similar sequential pathways operate there as well.

Certain medical conditions underscore the significance of these findings. When proteins aggregate, illnesses such as Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis can arise, and adjusting chaperone activity might provide therapeutic benefit. In addition, a particular *hsp60* mutation has been associated with hereditary spastic paraplegia, an illness in which the legs weaken and stiffen.

Hartl and Horwich unveiled a hitherto unknown process that enables proteins to reach their biological potential. Across the tree of life, the folding machines isolate young proteins and create a transformative moment. Then the devices send forth the mature molecules to join the hustle and bustle that makes cells what they are.

*By Evelyn Strauss*