Chaperonin studies: faith, luck, and a little help from our friends

Arthur L. Horwich*
Howard Hughes Medical Institute and Department of Genetics, Yale University School of Medicine, New Haven, CT 06519

ABSTRACT Basic cellular research is a trail. One follows one’s nose toward what might be new understanding. When that leads to a need to employ unfamiliar or novel technology, it’s both exciting and very worthwhile to form collaborations. Our early studies of chaperonins support such a philosophy, as detailed in the two stories that follow, written in deep appreciation of recognition by the E.B. Wilson Medal of the American Society for Cell Biology.

DISCOVERY OF FOLDING FUNCTION
In Fall 1987, my fledgling laboratory—three graduate students, a technician, and me—set out to identify mutants of baker’s yeast that affected the import of cytosolically synthesized precursors into mitochondria. Heeding the enjoinders of Gottfried Schatz and Walter Neupert, two giants of that field, that cells could not grow without producing new mitochondria (e.g., Baker and Schatz, 1991), we decided to produce our own temperature-sensitive conditional library, with the idea that import mutants would not be able to grow at a nonpermissive temperature. We mutagenized a strain of yeast programmed to express a Gal-inducible mitochondrial reporter protein, human mitochondrial ornithine transcarbamylase (OTC), which we would turn on by switching from glucose to galactose medium after shifting each mutant to the nonpermissive temperature. We would look to see whether our reporter protein could achieve enzyme activity as an indication that it could enter mitochondria, that its targeting peptide could be cleaved, and that it could assemble into an active homotrimer (Cheng et al, 1987). We were seeking to uncover mutants affecting recognition and translocation into the organelles, as well as proteolytic maturation. Sure enough, activity-deficient/cleavage-defective mutants were identified, affecting what was shown to be the mitochondrial protease (Pollock et al, 1988).

But one night it dawned on us that a very interesting mutant might also be present in our library, one affecting the folding of newly imported proteins. After all, Gottfried Schatz had reported that proteins have to be unfolded to cross the mitochondrial membranes (Eilers and Schatz, 1986). There were also reports from Blobel (Chirico et al, 1988) and Craig and Schekman (Deshaies et al, 1988) indicating that proteins of the endoplasmic reticulum (ER) and mitochondria are maintained in such unfolded states in the cytosol by Hsp70 chaperones. What if there was a “chaperone” protein on the other side of the mitochondrial membranes that acted on the newly imported proteins to support their de novo folding to the native form? That sounded like a challenge to the studies of Christian Anfinsen, who had determined that polypeptides contain sufficient information in their amino acid sequences to reach native form (Anfinsen, 1973), and indeed all the import models of the time drew imported proteins as spontaneously folding/assembling to native form. It seemed worthwhile to look for a folding-defective mutant where the imported protein would reach a proteolytically matured form inside the organelles but be enzymatically inactive, occupying a misfolded state.

DOI:10.1091/mbc.E17-07-0479. Mol Biol Cell 28, 2915–2918.
Arthur L. Horwich is the corecipient of the 2017 E. B. Wilson Medal awarded by the American Society for Cell Biology.
*Address correspondence to: Arthur L. Horwich (arthur.horwich@yale.edu).
Abbreviations used: ER, endoplasmic reticulum; OTC, ornithine transcarbamylase.
© 2017 Horwich. This article is distributed by The American Society for Cell Biology under license from the author(s). Two months after publication it is available to the public under an Attribution–Noncommercial–Share Alike 3.0 Unported Creative Commons License (http://creativecommons.org/licenses/by-nc-sa/3.0).
"ASCB", “The American Society for Cell Biology”, and “Molecular Biology of the Cell” are registered trademarks of The American Society for Cell Biology.
Remarkably, within several days, Ming Cheng, a young physician from Taiwan who had decided to shift direction to graduate training in Yale Genetics, came in with an autoradiogram showing mature-sized OTC subunits from inside an activity-dead strain. Using an affinity column that specifically bound assembled OTC (Kalousek et al., 1984), our next door neighbor, Franta Kalousek, showed that the OTC subunits in this mutant simply passed through the affinity column without capture. We had to presume that the imported subunits were matured but then misfolded, unable to assemble. We decided to test a yeast endogenous mitochondrial protein for which we had antibody, the beta subunit of the F1 ATPase. We observed the same result—at nonpermissive temperature the newly imported subunits reached mature size but failed to incorporate into the ATPase stalk structure. It seemed unreal that this mutant could be affecting the de novo folding of newly imported proteins. No one would ever believe this result from our tiny group. Now Spring 1988, the phone rang, and it was Walter Neupert and Ulrich Hartl from Munich. They had heard that we were studying yeast mutants affecting import and wondered whether we could use a little biochemical help in analyzing them. WOW!! COULD WE EVER!! So I went over to Munich the next week and presented the mutants, saving the putative folding mutant for last. That really got their attention. They worried that maybe the importing proteins were trapped in the import channel, able to have their signal peptides cleaved but unable to fold because of their “stuck” topology. I tried to allay that worry by indicating that the species we saw in our organelle preparation were abundant, potentially reflecting that they were not being proteolytically “bitten”—but the obvious experiment to do was an in vitro import assay with isolated mutant mitochondria and confirm that proteins were going all the way into the matrix by resistance to added protease. I sent Ulrich the strain, and he called back excitedly two weeks later to indicate that, yes, the proteins were going in and that they must then be misfolding.

Now the expertise of a major import lab could be brought to bear on this seemingly heretical mutant yeast strain. Many additional endogenous yeast proteins were examined with antibodies available in the Munich lab. One of the most exciting results involved the Rieske iron-sulfur protein, a monomer during its lifetime in the matrix space, where it undergoes two signal peptide cleavages before being inserted into the inner membrane (Hartl et al., 1986)—in the mutant, there was either no cleavage or a single event. This argued that it was the folding of a monomer and not the assembly of already folded proteins that was affected. But what was the gene itself that was affected in the mutant strain on which we were focused?

Ming Cheng transformed a yeast plasmid library into the mutant strain and plated at 37°C and then sequenced the rescuing gene. An open reading frame for an ~58 kDa protein was observed in all of the rescuing plasmids. That prompted us to make a phone call to Richard Hallberg in Iowa, who had described a 60 kDa heat inducible protein in Tetrahymena thermophila mitochondria in 1987, which formed a ring assembly (McMullin and Hallberg, 1987). We imagined that we might be studying the yeast homologue. So on a Saturday afternoon in August 1988, he and Ming Cheng sat on the phone and matched base for base the sequence of our rescuing gene with the yeast homologue of his gene isolated by antibody screening of a lambda gt11 library. So that was it—this connected folding in mitochondria to a double ring assembly, a chaperonin as John Ellis had dubbed them. On that same afternoon, by chance, I was picking Ulrich up at JFK, the two of us heading to a Gordon Conference the next day. We returned to my tiny beach house, and there was the lab group standing on its dock, jumping up and down with the sequencing autoradiograms in hand demonstrating the dead matchup. And so began a 20-year exploration into how these kinds of double ring machines mediate protein folding. (See Cheng et al., 1989, for the report of the folding-defective mutant; Reading et al., 1989, for Hallberg’s sequence; and Hemmingsen et al., 1988, for the relatedness of chaperonins of bacteria and chloroplasts.)

**SEEING THE MACHINE BY X-RAY CRYSTALLOGRAPHY**

In 1991, an administrative mission took me across campus to visit Paul Sigler, a crystallographer newly arrived from the University of Chicago, resulting in an immediate discussion about the chaperonin double rings, their symmetries, and the potential of seeing them at high resolution by crystallography. Paul was convinced that we would never figure out the mechanism without a structure. In that moment, a 10-year collaboration that was like a father–son relationship formed. Paul and I shared Chicago roots, the Bulls, the Bears, a love of jazz, and much else. He taught us the tenets of crystallography, and we set out to generate well-diffracting crystals. It was a three-year project with nothing in hand until the very end, a matter of major faith. We initially tried thermophilic versions of the bacterial chaperonin, GroEL, but we never obtained any crystals that diffracted beyond 12 Å. Then, what I believe to be a key event occurred. The Sigler lab moved from its uptown location down into the Boyer Center right next door to us. This catalyzed a daily ongoing discussion between all of us on the problem of expressing and crystallizing GroEL. Andrzej Joachimiak and I took up the former challenge and soon found that a trc-driven expression plasmid produced Escherichia coli GroEL from its operon as virtually the only protein upon induction. That made purification a single-step affair, and trays could be set up with that material. Thus a large range of molecules could be tested, necessary because wild-type E. coli GroEL did not behave well in the various trials we or others had set up. David Boisvert, with excellent crystallization experience from a period in the Steitz lab, taught Kerstin Braig and me how to set up trays and vary conditions systemically (there were no robots at the time). The three of us set up a myriad of crystallizations of different variants of GroEL. After a year, Kerstin pulled up a beautiful orthorhombic crystal that diffracted on the area detector next door to 2.4 Å. Paul came running into the room and began dancing about as he looked at the diffraction pattern. Needless to say, we went to the Cornell synchrotron that weekend and collected, with the help of his group, a 2.8 Å native data set. It was a 24-hour fire drill to collect this gigantic molecule (840 kDa) on image plates, six plates at a time. Immediately following, David’s recently produced monoclinic crystal was collected, diffracting to better than 2.0 Å. The molecule that behaved in both crystal forms turned out to be a PCR-directed variant of GroEL with two relatively benign substitutions in its ATP binding domain—we had been calling it wild type!

The next week, Paul sat us down and delivered a four-hour lecture on how to prepare heavy atom derivatives for phase determination. Kerstin promptly produced an ethyl mercury derivative that was isomorphous, and now Zbyszek Otwinowski, a senior member of Paul’s group, took over and solved the structure in a single day. He had been thinking about how to carry out sequential multi-parameter searches to identify the heavy atom positions. Three sites per subunit were found (corresponding to three cysteines per subunit). So on a Saturday morning in Fall 1993, Paul, Zbyszek, and I stood in front of a screen in a darkened room and there was the GroEL molecule in front of us at 10 Å resolution, soon to be 2.8 Å when Zbyszek applied sevenfold noncrystallographic symmetry averaging/phase extension. It was like looking at a sacred object. A
dream had come true. What followed was refinement and presentation of the model of unliganded GroEL (Braig et al., 1994; see Figure 1), structure/function studies (Fenton et al., 1994; Weissman et al., 1994, 1995), structure of GroEL with bound ATPγS (Boisvert et al., 1996), structure with ADP and the cochaperonin GroES bound (Xu et al., 1997), and mechanism studies (e.g., Weissman et al., 1996; Rye et al., 1997). Once again, faith, luck, and help from our friends had opened up a new avenue to seeing how this machine could assist de novo protein folding in the cell.

ACKNOWLEDGMENTS

I am honored and humbled to be receiving this recognition from the American Society for Cell Biology. I thank all of my collaborators of these past 30 years and my terrific trainees, who have powered our work with their ideas and experiments. We have had exciting times and great enjoyment together. I especially thank my senior staff, Wayne Fenton, Krystyna Furtak, and George Farr, for their unstinting contributions and support of our “scientific family” across the decades. Finally, I thank the Howard Hughes Medical Institute for generously supporting the work across the past 25 years.

REFERENCES

Anfinsen CB (1973). Principles that govern the folding of protein chains. Science 181, 223–230.
Baker KP, Schatz G (1991). Mitochondrial proteins essential for viability mediate protein import into yeast mitochondria. Nature 349, 205–208.
Boisvert DC, Wang J, Otwinski Z, Horwich AL, Sigler PB (1996). The 2.4 Å crystal structure of the bacterial chaperonin GroEL complexed with ATPγS. Nat Struct Biol 3, 170–177.
Braig K, Adams PD, Brunger AT (1995). Conformational variability in the refined structure of the chaperonin GroEL at 2.8 Å resolution. Nat Struct Biol 2, 1083–1094.
Braig K, Otwinski Z, Hegde R, Boisvert DC, Joachimiak A, Horwich AL, Sigler PB (1994). The crystal structure of the bacterial chaperonin GroEL at 2.8 Å. Nature 371, 578–586.
Cheng MY, Hartl FU, Pollock RA, Kalousek F, Neupert W, Hallberg EM, Hallberg RL, Horwich AL (1989). Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria. Nature 337, 620–625.
Cheng MY, Pollock RA, Hendrick JP, Horwich AL (1987). Import and processing of human ornithine transcarbamoylase precursor by mitochondria from Saccharomyces cerevisiae. Proc Natl Acad Sci USA 84, 4063–4067.
Chirico WJ, Waters MG, Blobel G (1988). 70K heat shock related proteins stimulate protein translocation into microsomes. Nature 332, 800–805.
Deshaias RJ, Koch BD, Werner-Washburne M, Craig EA, Schekman R (1988). A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. Nature 332, 800–805.
Eilers M, Schatz G (1986). Binding of a specific ligand inhibits import of a purified precursor protein into mitochondria. Nature 322, 228–232.
Fenton WA, Kashi Y, Furtak K, Horwich AL (1994). Residues in chaperonin GroEL required for polypeptide binding and release. Nature 371, 614–619.
Hartl FU, Schmidt B, Wachter E, Weiss H, Neupert W (1986). Transport into mitochondria and intramitochondrial sorting of the Fe/S protein of ubiquinol-cytochrome c reductase. Cell 47, 939–951.
Hemmingen SM, Woolford C, van der Vies SM, Tilly K, Dennis DT, Georgopoulos CP, Hendrix RW, Ellis RJ (1988). Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature 333, 330–334.
Kalousek F, Orusal MD, Rosenberg LE (1984). Newly processed ornithine transcarbamoylase subunits are assembled to trimers in rat liver mitochondria. J Biol Chem 259, 5392–5395.
McMullin TW, Hallberg RL (1987). A normal mitochondrial protein is selectively synthesized and accumulated during heat shock in Tetrahymena thermophila. Mol Cell Biol 7, 4414–4423.
Pollock RA, Hartl FU, Cheng MY, Ostermann J, Horwich A, Neupert W (1988). The processing peptidase of yeast mitochondria: the two
co-operating components MPP and PEP are structurally related. EMBO J 7, 3493–5000.
Reading DS, Hallberg RL, Myers AM (1989). Characterization of the yeast HSP60 gene coding for a mitochondrial assembly factor. Nature 337, 655–659.
Rye HS, Burston SG, Fenton WA, Beechem JM, Xu Z, Sigler PB, Horwich AL (1997). Distinct actions of cis and trans ATP within the double ring of the chaperonin GroEL. Nature 388, 792–798.
Weissman JS, Hohl CM, Kovalenko O, Kashi Y, Chen S, Braig K, Saibil HR, Fenton WA, Horwich AL (1995). Mechanism of GroEL action: productive release of polypeptide from a sequestered position under GroES. Cell 83, 577–587.
Weissman JS, Kashi Y, Fenton WA, Horwich AL (1994). GroEL-mediated protein folding proceeds by multiple rounds of binding and release of nonnative forms. Cell 78, 693–702.
Weissman JS, Rye HS, Fenton WA, Beechem JM, Horwich AL (1996). Characterization of the active intermediate of a GroEL-GroES-mediated protein folding reaction. Cell 84, 481–490.
Xu Z, Horwich AL, Sigler PB (1997). The crystal structure of the asymmetric GroEL-GroES-(ADP)7 chaperonin complex. Nature 388, 741–750.