

How a fortuitous collaboration helped catalyze new insights into helper proteins

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What a way to begin a scientific career: Two newly independent researchers are brought together at a conference that was only possible because their country has just been reunified. In discussing their work, they discover that each holds half of a research project in their hands: The East German, Matthias Gaestel, can provide the critical protein for West German Johannes Buchner's robust assays. In their cross-country collaboration, they get clear and compelling data defining the previously enigmatic small heat shock proteins as molecular chaperones. The resultant paper (1) goes on to be cited >50 times a year for the next 25 years, influencing fields from cancer biology to plant physiology to bacterial spore coats, and is now recognized as a JBC Classic.

In the late 1980s, chaperones were taking the field of cellular biochemistry by storm (2). The term "molecular chaperone" had first been proposed to describe the function of a single protein, nucleoplasmin, in nucleosome formation (3). Soon after, several additional proteins were discovered that played a role in protein folding and oligomer assembly. The implication that this was a general phenomenon surprised many in the field, including Buchner, then a graduate student at the University of Regensburg, since it went against the idea established by Christian Anfinsen and colleagues (4) that proteins contain the complete blueprint for their final structure within their own amino acid sequence. Buchner began studying these "helper proteins" as a side project, focusing on some of the newly discovered chaperones known as heat shock proteins, or Hsps, which are expressed at elevated levels when cells are stressed by high temperatures. After graduating and completing a brief postdoc, Buchner returned to the University of Regensburg to take up a position as a junior group leader. One of Buchner's first graduate students was Ursula Jakob, currently an Associate Editor at JBC. They had initially worked together during Buchner's Ph.D., when Jakob was an undergraduate student, and so were quickly able to establish several key assays to test for chaperone function. Jakob describes this time as "incredibly exciting, because the science was so fundamentally new. It was also fun because we were all so close in status— just one career step away from each other."

Although work on their chosen Hsps was going well, a mystery was brewing regarding this overall class of proteins. Of the nearly 100 different proteins regulated by heat shock, the function of the largest group of related proteins, simply known as the small heat shock proteins (sHsps), was still unknown. Researchers from across scientific disciplines had proposed a

variety of functions for sHsps, including roles in drug resistance, embryo development, and viral infection for Hsp27 (5). However, these proteins had also been shown to protect cells from exposure to high temperature and to prevent actin from forming polymers; to Buchner and Jakob, it was obvious that they should be tested as chaperones. Despite their suspicions, however, they hadn't planned to take on any experiments since they did not have any proteins to work with, a substantial hurdle at the time.

Outside the lab, major political change was occurring. The reunification of Germany in 1990 created new opportunities for scientists from the former East Germany to interact and collaborate with researchers in West Germany and beyond. Matthias Gaestel, based in Berlin at the Central Institute of Molecular Biology of the Academy of Sciences of the German Democratic Republic, which later became the Max Delbrück Center for Molecular Medicine, had been studying and had cloned a tumor protein that was expressed at high cell density, which turned out to be a small heat shock protein known as Hsp25. This connected him to the community of researchers studying heat shock proteins, and specifically to a conference on this theme organized by the Deutsche Forschungsgemeinschaft, a central German funding agency, where he met Buchner. During a coffee break, the two quickly realized that Gaestel could provide the protein for Buchner's chaperone assays, and a collaboration was born: Kathrin Engel, a graduate student in Gaestel's lab, would purify the necessary protein while Jakob in Buchner's lab performed the assays. However, there was still the matter of getting the protein from Berlin to Regensburg. Gaestel recalls that, at one point, Jakob ran out of protein in the middle of a set of experiments. He decided to drive the more than 500 km on poor quality, overcrowded highways to try come to the rescue, and as he relates, "I managed to arrive with the frozen samples with some remaining dry ice in the container and could join Ursula and Johannes at the spectrophotometer, where Ursula immediately added the thawed protein into the cuvette to measure light scattering."

Thanks in part to this excellent teamwork, the collaborators quickly completed their experiments and sent the paper off for publication. The article (1), which appeared in January 1993, tested proteins from three different mammalian species: murine Hsp25, human Hsp27, and bovine α B-crystallin, a protein that is primarily found in the eye lens, is similar in sequence to the sHsps, and also responds to heat shock. The data showed that these three proteins could protect two different substrate proteins from aggregation when heated and help them refold after their structure was disrupted by chemical additives. The authors concluded that, like the other Hsp chaperones, these new chaperones do not catalyze folding directly, but keep

Norma Allewell at the University of Maryland nominated this paper as a Classic.

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1992-1993: Jakob *et al.*, Merck *et al.* and Horwitz papers appear

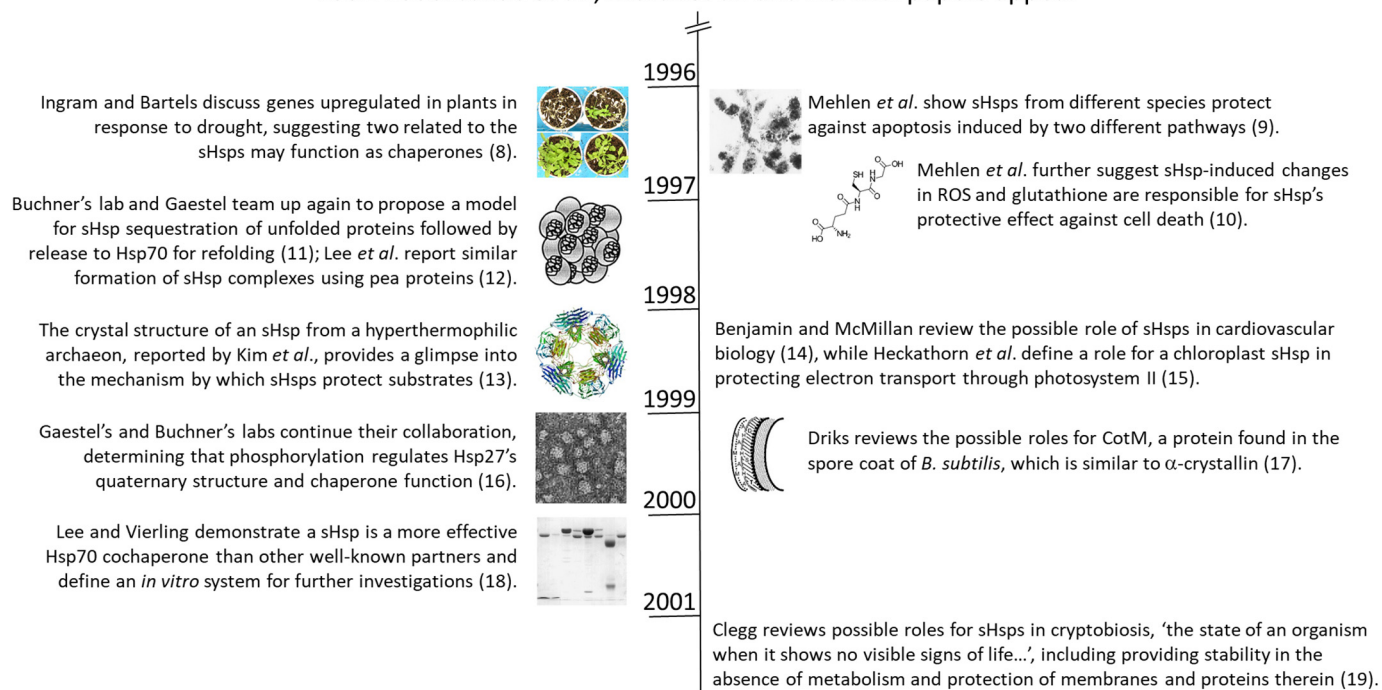


Figure 1. A selection of papers citing Jakob *et al.* (1) that themselves were highly cited (>300 times), demonstrating some of the fields that were impacted by the functional assignment of the small heat shock proteins (sHsps). Images reused with permission, and are reproduced from the papers cited, except for the plant seedlings (taken from Ref. 20), the structure of GSH, the model for sHsp sequestration (taken from Ref. 21), and the sHsp structure (from the RCSB PDB ID 1SHS (13)).

unfolded protein sequences safe from aggregation until they can refold on their own.

It was fortunate that the experiments were completed quickly, as Jakob *et al.* did encounter one wrinkle during their experiments: Gaestel had previously shared a plasmid containing the gene for Hsp25 with a scientist in the Netherlands, and had given them permission to share the plasmid with a second lab for what they described as “protein association studies.” Imagine Gaestel’s surprise when the leader of this second group, Wilfried de Jong, contacted him a year later to share unpublished results exactly parallel to those gathered by the Buchner lab! The two teams quickly agreed to make the partial overlap clear when they published their results. The de Jong lab was also aware of an upcoming related paper from Joseph Horwitz, so both labs had to act quickly to avoid being scooped. They successfully submitted their papers prior to publication of the paper from Horwitz, which appeared in November 1992, reporting that α -crystallin can act as a chaperone for numerous substrates (6). The paper from de Jong’s group, also published in January 1993, compared the structural and biophysical properties of α -crystallin and Hsp25 and confirmed that Hsp25 could serve as a chaperone for two substrates (7). True to their word, both 1993 papers include a reference to the other work and also cited Horwitz’s report. In revisiting the work, Buchner is still quick to draw attention to “footnote 3,” which highlighted the coincident publication of the three papers. From his perspective, it was reassuring to see such similar results from multiple labs, which probably contributed to the quick acceptance of the result by the community.

Looking back at the study, both Jakob and Buchner were surprised to be reminded what a short paper it is, and they suspect it’s a sign of the times that they weren’t asked to test additional substrates or even to replicate their initial data, as no error bars are shown. Yet the data were clear enough to make the point, and to launch 25 years and counting of studies exploring their function and mechanism across biological systems (Fig. 1) (8–19). Perhaps there’s no better testament to the lasting intrigue of these unexpected chaperones than Gaestel’s plaintive assessment of some of the remaining open questions in the field: “I hope I get to know some answers to these points before the end of my career.”

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