New Jobs for Ancient Chaperones

Protective heat shock proteins present in every cell have long been known to counteract stress. Newly recognized roles in cancer and immunity make them potential therapeutic allies

BY PRAMOD K. SRIVASTAVA

KEY CONCEPTS

- Guardian proteins, found in all forms of life, keep a wide variety of cellular processes running smoothly.
- Through their diverse interactions, these proteins pick up telltale "fingerprints" of each cell's contents, which has allowed them to evolve a critical role in immune responses to cancer or pathogens.
- Therapies that take advantage of these proteins include inhibitors and enhancers of their various natural functions.

—The Editors

In 1962 someone at the Genetics Institute in Pavia, Italy, turned up the temperature in an incubator holding fruit flies. When Ferruccio Ritossa, then a young geneticist, examined the cells of these "heat shocked" flies, he noticed that their chromosomes had puffed up at discrete locations. The puffy appearance was a known sign that genes were being activated in those regions to give rise to their encoded proteins, so those sites of activity became known as the heat shock loci.

The effect was reproducible but initially considered to be unique to the fruit fly. It took another 15 years before the proteins generated when these chromosome puffs appear were detected in mammals and other forms of life. In what is certainly among the most absorbing stories in contemporary biology, heat shock proteins (HSPs) have since been recognized as occupying a central role in *all* life—not just at the level of cells but of organisms and whole populations.

Indeed, these ubiquitous molecules are among the most ancient survival mechanisms to have been conserved throughout evolution. They have even been shown to facilitate evolution itself. Produced in response to stressful conditions, including (but not limited to) heat, HSPs help individual cells to cope by keeping cellular processes working smoothly in the face of adversity. In the past decade scientists have realized that HSPs also play additional roles in higher organisms, such as humans. They are integral to our immune defenses against cancer and pathogens and might therefore prove valuable in developing a wide variety of new medicines and vaccines.

To understand how these versatile proteins can be harnessed therapeutically, it is helpful to look at the diverse ways they perform their core job, which is to act as "chaperones" for other proteins. Like the chaperoning of people, the work of HSPs has two objectives: to inhibit undesirable interactions and to promote desirable ones, so that a stable and productive bond forms between protein partners.

Versatile Escorts

Proteins inside a cell often have just one or a very few correct "mates" with which they can interact effectively—for example, a receptor and its ligand, which behave like a lock and key, respectively. The ligand has little effect on other receptor types, and the receptor is typically activated only by its particular ligand or molecules very close to it in structure. In contrast, HSPs tend to associate with a wide range of "client" proteins, allowing the HSPs to perform a dizzying array of jobs. These can include helping newly formed amino acid chains to fold into their proper protein shapes, dismantling them after they have been damaged, escorting proteins to their intended mates and keeping them away from interlopers.

Specific examples can highlight just how critical these tasks are and can illustrate some of the

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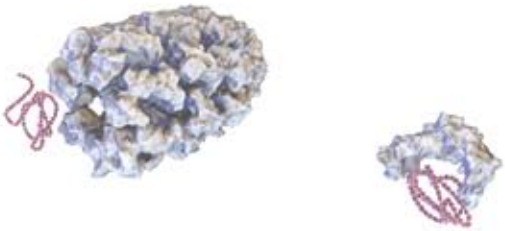
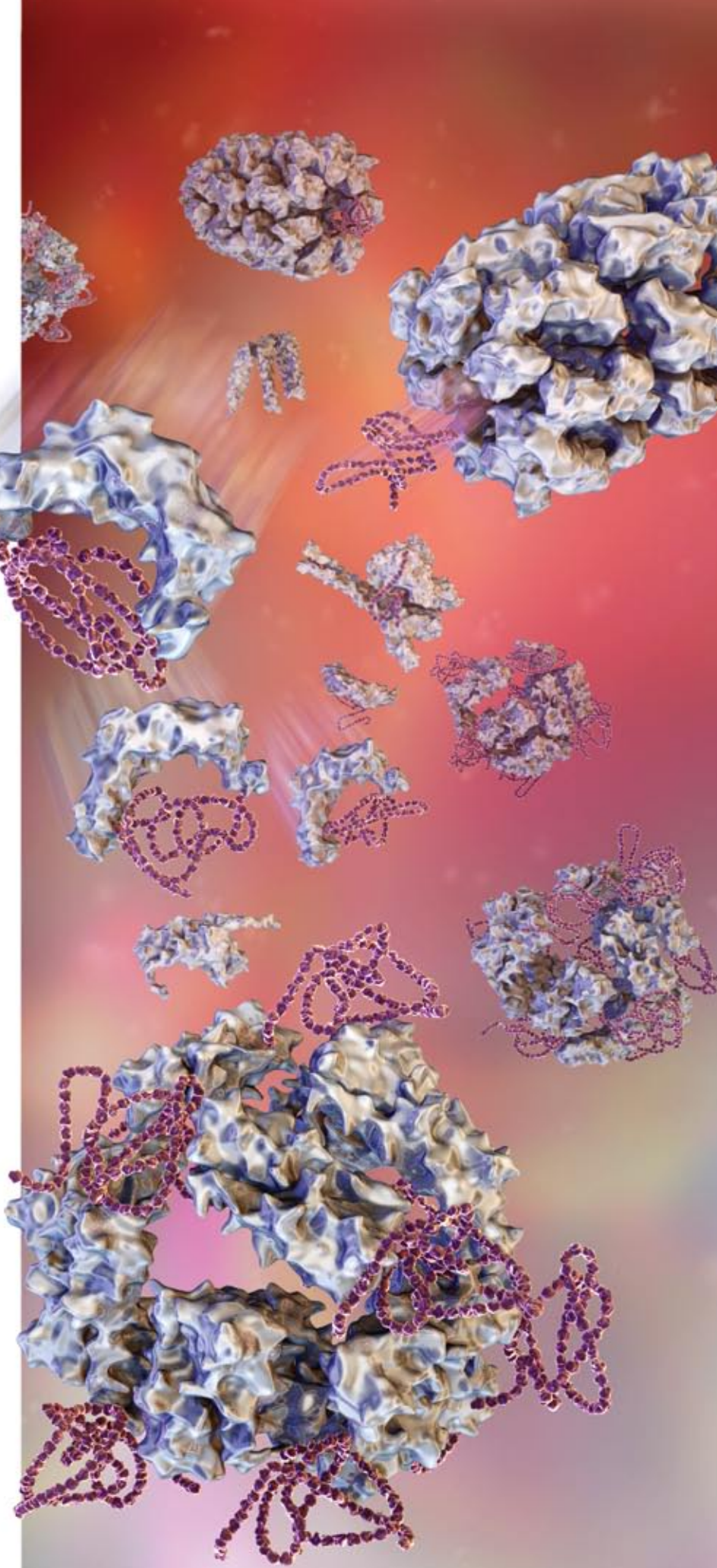


ways that major HSP chaperones serve their clients. A protein's ability to carry out its intended functions depends not only on it getting to the right place at the right time, but on it having the correct shape. Newly formed chains of amino acids are subject to various forces that help them to take on the right conformation. Each amino acid, for instance, has a characteristic response to water in the cellular cytoplasm. Hydrophobic amino acids abhor water and try to get away from it by nestling inside the protein structure, whereas hydrophilic amino acids prefer to face outward. Such mechanisms are not always enough to ensure proper folding, though, so HSPs, such as HSP60, get involved [*see box on page 53*].

Arthur L. Horwich of Yale University has provided much of the current understanding of the HSP60 chaperone, which resembles a cage composed of multiple HSP60 molecules. Its inner rim is highly hydrophobic and therefore attracts the exposed hydrophobic amino acids of an unfolded protein to bind to it. Once such a chain is drawn into this cage, it encounters a hydrophilic interior, which the hydrophobic amino acids want to avoid at all costs, so the trapped molecule is forced to change shape. This process may not happen in one go, and the cage may release and recapture the protein multiple times before the protein acquires a correctly folded conformation. Thus, the HSP60 protein is known as a foldase. Conversely, the HSP100 protein is an unfoldase. It, too, is a multisubunit ring, which, in cooperation with HSP70, can disassemble damaged proteins or undesirable protein aggregates or can even cause a fully folded protein to unfold.

In contrast to the cagelike chaperones, most HSPs do not enclose their substrates but rather grab them by the "elbows" to help them along. HSP70, for example, binds directly to short stretches of amino acid sequences, also known as peptides. The molecule has a peptide-binding



JEFF JOHNSON *Hybrid Medical Animation*

HEAT SHOCK PROTEINS are cellular chaperones, protecting the integrity of proteins by helping them to take and keep the proper shape, to get to the right places and to avoid unwanted interactions.

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cleft that is open when HSP70 is bound to the cellular energy source ATP, but when ATP is absent, a lidlike structure on HSP70 clamps down on the bound peptide and traps the larger protein chain in place. The ability of HSP70 to grab a variety of different peptides allows the molecule to play chaperone in many fundamental cellular processes, such as helping new amino acid chains to assume a mature conformation, facilitating the assembly of complex proteins and protecting proteins from falling apart in high temperatures.

Although heat shock proteins are active in cells in normal circumstances, it is easy to see how their help would be even more valuable to a cell in a difficult situation. Under emergency conditions, such as extreme heat or cold, oxygen deprivation, dehydration or starvation, a cell would be struggling just to survive. Critical proteins might be degraded by the harsh environment, even as the cell would try to churn out replacements. In these circumstances, heat shock proteins would mitigate the stress by rescuing essential proteins, dismantling and recycling damaged ones, and generally keeping cell operations running as smoothly as possible. Hence, when a cell is under high stress, one of its first responses will be to manufacture more of the HSPs themselves, as Ritossa first witnessed 46 years ago. This important role of HSPs has been well documented since its discovery [see "How Cells Respond to Stress," by William J. Welch; Scientific American, May 1993]. Beginning in the 1980s, however, a completely different function of HSPs—just as integral to survival for complex organisms—also began to be revealed.

Antigenic Fingerprints

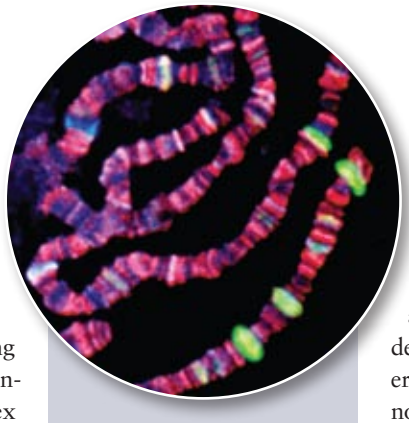
As a graduate student in the early 1980s at the Center for Cellular and Molecular Biology in Hyderabad, India, I became interested in a phenomenon that had been observed since the 1940s but never explained. Many scientists had demonstrated that one can immunize rodents against their own cancers, just as people are routinely immunized against pathogens. Proteins from a pathogen are recognized by the mammalian immune system as foreign, however, and that is why they act as antigens—provoking an immune response. A cancer, on the other hand, is made up of an individual's own cells, so the antigenic element remained a great mystery. I began trying to isolate these cancer-specific antigens.

During my graduate and postdoctoral work, I identified a protein, called gp96, which could indeed elicit immune resistance to tumors. This molecule, surprisingly, turned out to be a member of the HSP90 family—many HSP proteins come in several related forms—which occurs in normal tissues as well as cancer cells. Stephen J. Ullrich and his colleagues at the National Institutes of Health independently made a similar observation two years later. The gp96 molecules found in tumors and in normal tissues were identical in their amino acid sequences, so the cancer-derived gp96 was not cancer-specific. What, then, was the basis of its ability to immunize against cancers?

The answer began to emerge in 1990, when Heiichiro Udono, then a postdoctoral fellow in my laboratory at the Mount Sinai School of Medicine, and I were isolating HSP70 from tumors to test if it, too, elicited tumor immunity. We found that it could. The biggest surprise came, however, when we put the HSP70 through a final purification step called ATP-affinity chromatography, and the molecule's very potent tumor-immunizing activity disappeared!

We realized immediately that exposure of HSP70 to ATP was causing HSP70 to shed material, which we determined to be peptides. The work of several research groups in the ensuing years has revealed that HSP70 changes conformation when it binds to ATP, causing it to let go of any bound peptide. In fact, researchers learned that members of the HSP60, HSP70 and HSP90 families all regularly carry around peptides generated within cells. And when HSP70 or HSP90 are taken from cancers or from virus- or tuberculosis-infected cells, in nearly all instances they bear peptides derived from cancer-specific antigens, viral antigens or tuberculosis antigens. Thus, the HSP-associated peptides represent the "antigenic fingerprint" of the cells or tissues from which they come.

This characteristic ability of certain chaperones to retain peptides representative of their cell of origin has given HSPs an essential role in one of the most fundamental processes of the immune system—recognition of cancerous and virus-infected cells. T lymphocytes recognize antigens on such cells through an elaborate process known as antigen presentation. Essentially all antigens made inside cells are degraded into peptides that then associate with HSPs of the HSP60, HSP70 and HSP90 families in a sequence of events that is still unclear. The peptides are eventually loaded onto a special class of proteins, known as the major histocompatibility complex I (MHC I) proteins, displayed on the surface of most mam-



STRESS RESPONSE

Chromosomes of a fruit fly exposed to high heat appear to "puff up" in regions containing genes that encode heat shock proteins (*white* and *green*). For a cell to manufacture those proteins, the tightly coiled DNA must unravel slightly so that the relevant genes are accessible, leading to the puffy appearance at those locations.

[THE AUTHOR]

Pramod K. Srivastava is a professor of medicine and director of the Center for Immunotherapy of Cancer and Infectious Diseases at the University of Connecticut School of Medicine. In a series of discoveries beginning when he was a graduate student, Srivastava pioneered the study of the role of heat shock proteins in the immune system. Based on those insights, he cofounded the company Antigenics to develop cancer vaccines made with HSPs derived from individual patients' tumors. He remains a consultant to the company and continues to investigate the role of HSPs in immune responses.

COURTESY OF PRAMOD K. SRIVASTAVA (*Srivastava*); COURTESY OF J. WERNER AND J. T. LIS IN *SCIENCE*, VOL. 301, AUGUST 2003 (*chromosomes*)

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malian cells. The T cells recognize these MHC I–peptide complexes and destroy any that signify the cell is diseased [*see box on next page*].

The chaperoning of peptides by HSPs is essential for their eventual loading onto MHC I molecules; when the HSPs are chemically silenced, the MHC I molecules remain empty of peptides and cannot be recognized by the T cells. This role of the HSP-chaperoned peptides in antigen presentation by MHC molecules was hypothesized by my colleagues and me in 1994 and shown to be true through our work and others'.

It is this antigen-chaperoning property of the peptide-binding HSPs that is the basis of the ability of HSPs derived from tumors or pathogen-infected cells to immunize against those same tumors or intracellular pathogens. But the HSP-peptide complexes also have another critical part in the T cells' recognition of friend and foe antigens—through their interactions with different types of immune cells known as antigen-presenting cells.

Sounding the Alarm

Sentinels of the immune system, antigen-presenting cells occur in perhaps every tissue of the body, where they can "sample" their surroundings for any antigens that might be nearby. They present whatever they encounter to the T cells that will eventually home in on and attempt to destroy cancerous or infected cells.

It turns out that antigen-presenting cells carry receptors on their surface for the peptide-binding chaperones. The first such receptor was identified by Robert J. Binder, then a graduate student in my laboratory and currently an assistant professor at the University of Pittsburgh, as CD91. When the cells encounter an HSP-peptide complex, they internalize it through the CD91 doorway and present the HSP-chaperoned peptides to the T cells, which can then multiply and fight off the cancer or pathogen. Broadly speaking, this mechanism is the reason HSPs isolated from a cancer are able to immunize against that cancer, whereas the HSPs isolated from normal tissues do not do so.

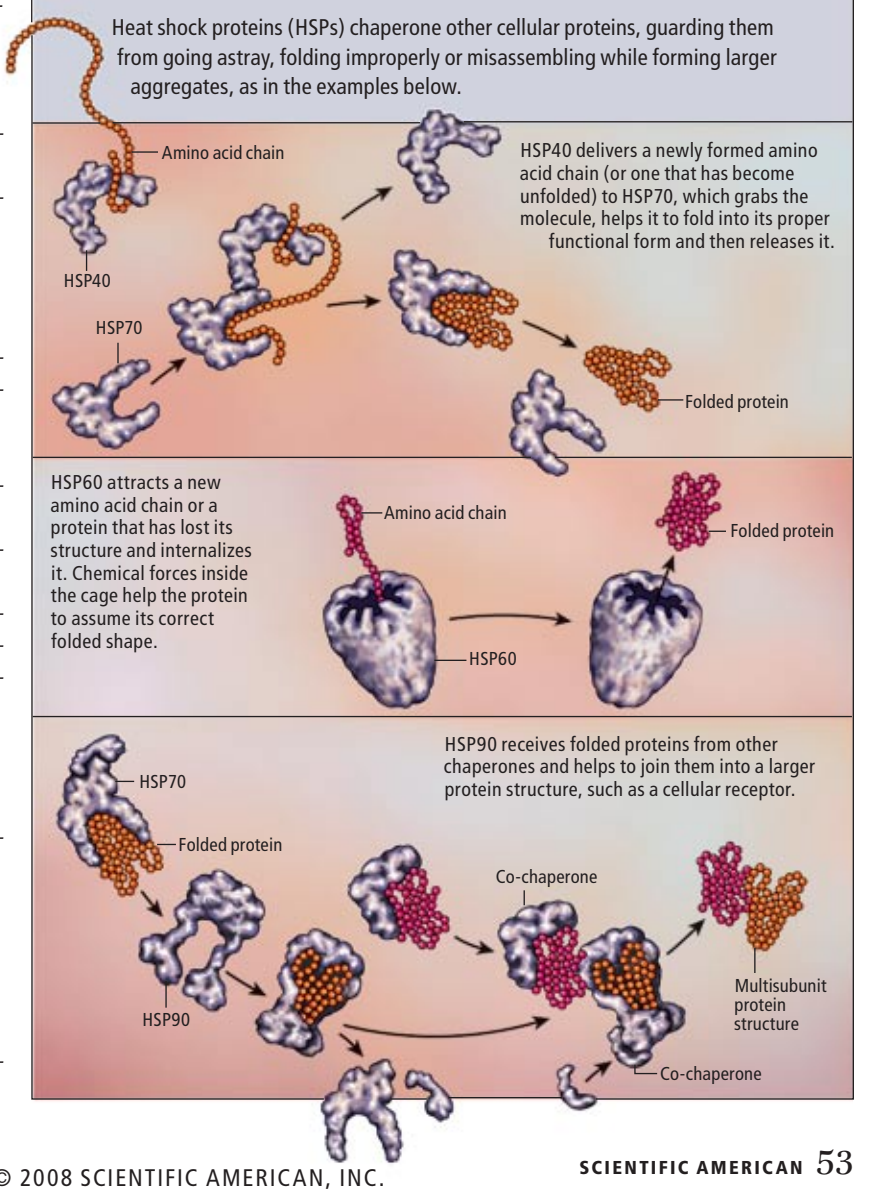
Beyond delivering a description of the invader to the immune system, HSPs seem to sound an alarm as well. Sreyashi Basu of the University of Connecticut School of Medicine and I have shown in laboratory studies that just exposing antigen-presenting cells to HSP70 and HSP90 family members causes the cells to undergo a number of changes, including initiation of signals that cause inflammation, which is part of a strong immune defense. Although HSPs normally do their work inside cells, scientists have known for some time that when mammalian cells are under stress, selected HSPs are released from the cells or displayed on the cell surface in small but significant quantities. Thus, the ability of HSPs to activate antigen-presenting cells by their mere presence suggests that an anomalous appearance of HSPs outside cells may be a mechanism to alert the immune system of danger.

My work toward using HSP-peptide complexes purified from cancers to elicit tumor rejection is based on this immunizing function and on my belief that each patient's tumor is antigenically unique. I have developed a process for extracting HSP-bound peptides from the individual patient and then reintroducing them in purified form as

[PRIMARY ROLE]

KEEPING ORDER

Heat shock proteins (HSPs) chaperone other cellular proteins, guarding them from going astray, folding improperly or misassembling while forming larger aggregates, as in the examples below.



JEN CHRISTIANSEN

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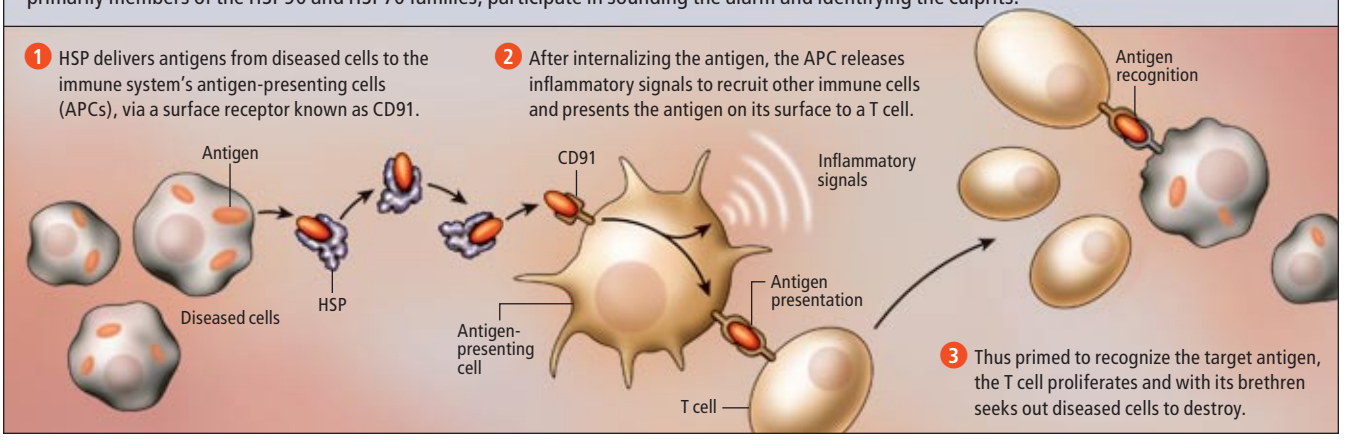
[A SECOND JOB]

ACTIVATING IMMUNE RESPONSES

When a cell is cancerous or infected by a pathogen, it generates proteins not found in normal cells. Fragments of such proteins can then potentially act as antigens, substances that provoke an immune response. But immune cells must first be made aware of the problem. Heat shock proteins, primarily members of the HSP90 and HSP70 families, participate in sounding the alarm and identifying the culprits.



a vaccine that would stimulate the immune system to attack cells bearing those specific tumor-associated antigens. This approach has been tested in the U.S. and Europe in a series of early human (phase I and II) trials for several cancers. More advanced tests of efficacy (randomized phase III trials) in the U.S., Europe, Australia and Russia have just been concluded in patients with melanoma and renal cancer. Those latest studies showed that patients with melanoma who received sufficient doses of HSP-peptide-complex vaccine and whose disease was limited to the skin, lymph nodes and lungs lived significantly longer than patients who received other standard treatments, including chemotherapy. In the trial on renal cancers, the vaccine extended the recurrence-free survival time in some groups of patients by more than a year and a half.

The results were enough for the Russian government to approve the treatment, making it the first cancer vaccine to enter actual clinical use. An application for approval in Europe will be filed shortly, and an application to the U.S. Food and Drug Administration is awaiting more data on the patients' long-term outcomes. Meanwhile this approach seems as if it should be just as applicable for treatment of serious infectious diseases, including genital herpes, tuberculosis and others. Clinical trials investigating those applications are at various stages.

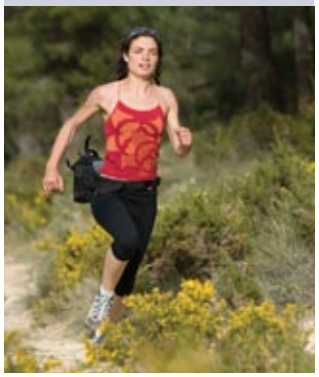
Wide Influence

Amplifying the natural effect of HSPs on the immune system by using them in vaccines is not the only way to employ these versatile proteins therapeutically. Work by Suzanne L. Rutherford of the University of Washington and Susan L. Lindquist of the Whitehead Institute for Biomedical Research in Cambridge, Mass., has provided a stunning example of how effectively HSPs perform their core job of mitigating stressful conditions inside cells. They have shown that when HSP90 functioning was suppressed in fruit flies, a large number of preexisting genetic mutations were unmasked, indicating that potentially deleterious effects were being buffered by HSP90. Rutherford and Lindquist have argued that widespread genetic variation that would otherwise affect the functioning of organisms exists in nature but is usually not manifested because HSP90 essentially hides the variation—an effect that fosters the quiet accumulation of genetic changes. When the buffering function is compromised, for example, by extreme temperature, variant traits emerge and then natural selection can act on them. Thus, HSP90, by fostering genetic variation, potentiates evolution.

Lindquist and her collaborators have provided further evidence of a role for HSP90 in the rapid evolution of novel traits, such as resistance to specific drugs, in diverse species of fungi. As a result, she has suggested that species-specific inhibitors of HSP90 may be used as a new generation of antibiotics. Similarly, HSPs are believed to provide buffering against the accumulating mutations that should make cancer cells less and less viable but instead seem to drive their malignancy. Because HSP90 affects a wider variety of intracellular signaling pathways than any other HSP does, loss of its function should make cancer cells more sensitive to stress and therefore more easily killed by chemotherapy.

BENEFICIAL STRESS

Exercise raises body temperature and generates other forms of stress on cells throughout the body, causing them to increase their manufacture of certain heat shock proteins. Preliminary research suggests that these HSPs might contribute to the health benefits of exercise by mitigating damage inside cells and by alerting the immune system to boost overall immune responses.



JEN CHRISTIANSEN (*illustration*); ANTHONY WEST *Corbis (runner)*

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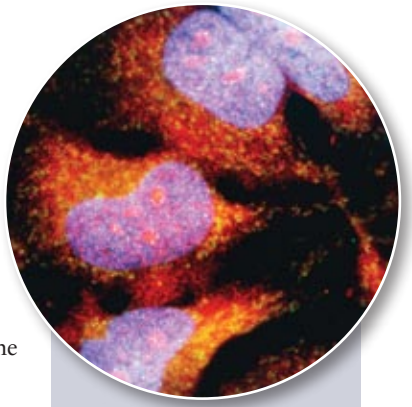
Hence, pharmacological inhibitors of increasing specificity for HSP90 are being tested in cancer patients, in combination with chemotherapy.

While I was testing the efficacy of HSP-peptide complexes in cancer immunotherapy, I noticed the seemingly strange phenomenon that immunization with very high doses of HSPs did not elicit immunity but rather caused suppression of immune responses. These studies, carried out with Rajiv Chandawarkar of the University of Connecticut Health Center, showed that HSPs could act not only as immunostimulators but also as immunosuppressants. In studies of mice, we showed that high doses could suppress autoimmune type 1 diabetes and encephalitis. Irun R. Cohen of the Weizmann Institute of Science in Rehovot, Israel, and his collaborators have long pursued the idea that HSP60 and one of its peptides are autoantigens in human type 1 diabetes, triggering an immune attack on the body's insulin-producing cells. In clinical trials they have demonstrated some value in blocking the peptide, and further tests in humans are under way.

Although the diverse roles of HSPs make them attractive agents for treating a variety of diseases, their very universality raises a danger: drugs aiming to alter HSP levels run the risk of harming many body systems that rely on the proteins. Nevertheless, the history of drug development is replete with examples of scientists having learned to modulate essential proteins without causing unacceptable side effects, and HSPs are certain to be at the center of a growing list of applications in the fullness of time.

From a wider perspective, these primitive, abundant molecules have been maintained since the very dawn of life because they were needed for the basic infrastructure of life as we know it—to help bring proteins into being, to help degrade them, to protect fragile proteins from the abundant stresses of the primordial environment and to protect cells from the disruptive effects of mutations. As newer biological functions emerged, such as immunity, the evolutionary process made use of what was already plentiful by employing HSPs in antigen presentation. I doubt that we are close to having fully plumbed the depths of the activities of these magical molecules. As further insights into the workings of life are gained, previously unimagined roles for the diligent chaperones are likely to be revealed. ■



NANCY KEDERSHA *Photo Researchers, Inc. (micrograph)*

HARMFUL HELPERS

Cancer cells, because they are abnormal, are under stress most of the time, generating higher levels of heat shock proteins as a result. HSP90 (*yellow, above*) in particular is believed to help cancer cells survive the stressful conditions, just as it does for normal cells. Inhibiting HSP90's effect could make malignant cells more vulnerable to toxic therapies.

NOW IN TRIALS

A number of drugs currently in clinical trials would fight disease by taking advantage of the diverse functions of heat shock proteins. Some seek to inhibit the proteins, others to induce them, depending on the disorder and the HSP that is being employed or targeted.

TREATMENT MECHANISM

- **Inhibitor of HSPs** (compound able to block the functioning of HSPs that would normally help a cancer cell, virus-infected cell or pathogenic bacterium to survive)
- **Induction of HSPs** (heat or chemicals able to induce a patient's own HSPs to protect an organ during surgical or other treatments)
- **Vaccine/Immunotherapy** (antigenic HSP-peptide complexes that are purified, then introduced into the body to stimulate an immune response to a tumor or pathogen)

**Approved for clinical use in Russia*

HSP	TREATMENT (MANUFACTURER)	DISORDER
HSP90	Alvespimycin (Kosan Biosciences)	Breast cancer
	Tanespimycin (Kosan Biosciences)	Leukemia, lymphoma, solid tumors
	CNF 2024 (Biogen Idec)	
	SNX-5422 mesylate (Serenex)	
	AUY-922 (Novartis)	Solid tumors
	IPI-504 (Infinity Pharmaceuticals)	Melanoma, prostate cancer
	BIIB021 (Biogen Idec)	Leukemia, lymphoma, solid tumors
HSP27	OGX-427 (OncoGenex Technologies)	Solid tumors
Various	Radio-frequency therapy	Melanoma
HSP65	HspE7 (Nventa Biopharmaceuticals)	Precancerous cervical cells infected with human papillomavirus
HSP70	AG-707 (Antigenics)	Herpes simplex type 2
	HSPPC-70/AG-858 (Antigenics)	Chronic myeloid leukemia
Gp96	HSPPC-96/vitespen* (Antigenics)	Solid tumors

MORE TO EXPLORE

Roles of Heat-Shock Proteins in Innate and Adaptive Immunity. Pramod K. Srivastava in *Nature Reviews Immunology,* Vol. 2, No. 3, pages 185–194; March 2002.

HSP90 and the Chaperoning of Cancer. Luke Whitesell and Susan L. Lindquist in *Nature Reviews Cancer,* Vol. 5, No. 10, pages 761–772; October 2005.

Heat Shock Factor 1 Is a Powerful Multifaceted Modifier of Carcinogenesis. Chengkai Dai et al. in *Cell,* Vol. 130, No. 6, pages 1005–1018; September 21, 2007.

Phase III Comparison of Vitespen, an Autologous Tumor-Derived Heat Shock Protein gp96 Peptide Complex Vaccine, with Physician's Choice of Treatment for Stage IV Melanoma: The C-100-21 Study Group. Alessandro Testori et al. in *Journal of Clinical Oncology,* Vol. 26, No. 6, pages 955–962; February 20, 2008.

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