The Matrix Reloaded: Halozyme's Recombinant Enzyme Helps Injected Drugs Spread Faster

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DOI 10.1016/j.chembiol.2007.06.002

Research in biology has often focused on what goes on inside cells and ignored the surrounding scaffolding. However, studies during the past decade show that this scaffolding, called the extracellular matrix, plays a major role in intercellular signaling, wound repair, cell differentiation, and other vital processes. Among the building blocks of the extracellular matrix is a complex sugar called hyaluronan, a viscous substance that acts as a barrier to fluid flow. This barrier is not invincible—a naturally occurring enzyme called hyaluronidase can break it down and make the tissue more permeable. Many organisms exploit this enzyme's remarkable ability. Bees, stonefish, and other venomous creatures inject it to help spread their toxins. Leeches employ it while feeding to improve blood flow. Some species of bacteria might use the compound to help infiltrate host tissues. "The enzyme effectively works as a molecular machete," says Gregory Frost, Ph.D., cofounder and chief scientific officer of San Diego-based Halozyme Therapeutics (http://www.halozyme.com), which manufactures a recombinant version of this enzyme for use as a "spreading agent" to help the dispersal and absorption of coinjected drugs.

"Using rHuPH20, you could take many types of drugs that are given intravenously and potentially convert them to subcutaneous administration," says Gregory Frost, Ph.D., cofounder and chief scientific officer of Halozyme Therapeutics.

The credit for identifying hyaluronidases's potential as a spreading agent goes to the late Catalan biologist Francisco Duran-Reynals. In 1928, while studying mechanisms of viral transmission in mice at the Rockefeller University (then Institute) in New York, Duran-Reynals found that an extract derived from rabbit testes increased the spread of dyes injected under the skin. In 1940, the late Karl Meyer, a pioneering glycobiologist at Columbia University, termed the active ingredient "hyaluronidase." In the late 90s, Robert Stern, M.D., of the University of California at San Francisco and his team, which included Frost, developed techniques to isolate human hyaluronidase from blood and urine, then went on to sequence the isolated isozyme polypeptides and the genes involved. "When we put the sequences into the gene bank, out came not one but six genes," recalls Stern. "We were simply amazed." It turned out that vertebrates have six or more hyaluronidase genes, while some primitive organisms such as the nematode *C. elegans* have just one. Stern hypothesizes that the six genes may have evolved from a single one by a series of duplication events. "Sometime between that tiny worm and us vertebrates, all those events occurred," he says.

Two of the six hyaluronidase enzymes, termed Hyal-1 and Hyal-2, act inside cells to break down tissue hyaluronan. Another one, PH20, swings into action during fertilization to help spermatozoa to drill through the hyaluronan coat that protects egg cells. The remaining hyaluronidase enzymes remain uncharacterized. Of the three that are well understood, only PH20 works in a pH-neutral medium, and has therefore found extensive use as a general-purpose spreading agent. For decades, slaughterhouse extracts of bull and ram testes have been the main source of the enzyme for medical applications. However, these extracts have many contaminants, and the PH20s in them are quite different from the human version. These considerations led Frost and other scientists to found Halozyme Therapeutics—now a publicly traded company with 50 employees—in 1998 to bring a recombinant human version of the enzyme to market. Full-length PH20 has a sequence region that anchors it to the cell membrane, reducing its mobility and making it insoluble. In late 2002, scientists at Halozyme discovered that deleting this region resulted in an active, soluble enzyme that could depolymerize hyaluronan under physiological conditions. They named this form of the enzyme rHuPH20 (for recombinant Human PH20).

Animal and biochemical studies showed that the new recombinant enzyme was as at least as effective, and potentially safer, than bovine hyaluronidase. Like the bovine enzyme, rHuPH20 increased dispersion of injected substances. Its effects were temporary; the hyaluronan in the skin reconstituted itself within hours after the enzyme was injected. While it was active, rHuPH20 allowed latex beads of up to 200 nanometers in size to move freely through the extracellular matrix, indicating that it could help disperse even large biologics such as gene therapy vectors (which are about 35 nm in size). When added to a concentrated protein preparation, it allowed the mixture to be infused 20 times faster through the skin. In a later postmarket clinical trial with 54 human subjects, the recombinant protein

helped to quadruple the flow rate of a hydration fluid infused under the skin. "Using rHuPH20, you could take many types of drugs that are given intravenously and potentially convert them to subcutaneous administration," concludes Frost.

"The data are clear and convincing that the recombinant protein can allow diffusion into skin very effectively," agrees hyaluronan expert Vincent Hascall of the Cleveland Clinic in Ohio, who has no ties to Frost's company. "I like the idea that this is a process that temporarily alters the matrix to allow diffusion but then the matrix quickly restores fairly normal function. That is neat." The recombinant protein's close similarity to the human enzyme would make adverse immune reactions less likely compared to animal-based products, Hascall adds.

Encouraged by the preclinical study findings with rHuPH20, Halozyme signed an exclusive agreement in August 2004 with Deerfield, IL, based Baxter Healthcare Corporation to market the enzyme as an adjuvant for enhanced delivery of injected drugs, contrast agents, and subcutaneous hydration fluids. The Halozyme team was confident of being able to get the drug approved by the Food and Drug Administration (FDA) without new clinical trials. "Officials at Baxter were initially skeptical about that," says Frost. But Halozyme had an ace up its sleeve.

In seeking FDA approval for its new drug, Halozyme could rely on the track record of existing hyaluronidase products dating back to 1948, when the agency first allowed Madison, NJ, based Wyeth to market bovine testicular hyaluronidase as a spreading agent under the brand name Wydase. An FDA drug efficacy study implementation report published in 1970 endorsed the enzyme's effectiveness. Since then, hyaluronidase has been widely used and is generally regarded as safe when used as intended. (Additionally, although the practice is not encouraged by manufacturers, the enzyme also finds a number of "off-label" uses: to break down scar tissue after a failed back surgery, to treat tears and hemorrhages in the eye, and to remove excess hyaluronan during skin surgery.) In March 2005, when Halozyme filed a new drug application (NDA) for a low-dose formulation of rHuPH20 as a spreading agent, it invoked Section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act to cite previously published safety and efficacy studies for Wydase to support its claims. "We went from DNA to NDA in 21 months," says Frost. "That is one of the fastest times ever that anyone has done that for a recombinant human biologic." The FDA accepted the application in May 2005, and in December of that year, only 9 months after Halozyme's filing, approved the new drug.

Major deals quickly followed. Last December, Halozyme entered into a collaboration agreement with Basel, Switzerland, based Roche, which makes several leading biologics including the antiviral drug Pegasys and the monoclonal antibody-based cancer drugs Avastin and Herceptin. The agreement gave Roche an exclusive worldwide license to develop and market exclusive coformulations of rHuPH20 with 13 of its own drugs. In return, Roche agreed to pay $20 million up front, invest $11 million in equity, and pay $111 million in milestones for its first three targets. It would give a further $47 million for each of the ten remaining targets in up-front and milestone payments. In total, the Roche deal could be worth more than $600 million, says Jonathan Lim, M.D., Halozyme's president and CEO. In February this year, the Baxter deal was expanded to allow the company to use rHuPH20 in combination with certain proprietary and nonproprietary small molecule drugs. Baxter agreed to pay Halozyme $10 million up front, invest $20 million in equity, and make other milestone payments. The deal could be worth nearly $65 million, says Lim.

Halozyme seems to be in the enviable position of having no direct competition. The company's Enhanze technology—hyaluronidase-aided subcutaneous injection of large drug volumes—compares favorably with other existing alternatives to IV delivery, says Frost. It is potentially less invasive than intraosseous infusion, in which the needle is injected directly into the bone marrow and can deliver larger volumes than transdermal and pulmonary methods. Only oral delivery could in theory deliver comparable drug volumes, provided the drug could be reformulated to make it easy to absorb through the digestive system. "People are working on this, but it is not an immediate prospect," says Frost.

As a spreading agent, rHuPH20 could become the preferred choice in many applications, thanks to its low risk of provoking an immune response. In some niche markets, however, existing animal-based products might be safe enough. For instance, Vitrase, a sheep-derived hyaluronidase formulation made by Irvine, CA, based ISTA Pharmaceuticals, is widely used in ophthalmic surgery. Adverse immune reactions rarely occur in this setting, according to Bruce Aird, a scientist at ISTA. "The eye is kind of a closed biological system with respect to the body," he says. "A lot of the benefits associated with a humanized product are irrelevant there." Companies that market animal-derived hyaluronidase, however, may have to deal with limited supplies as well as other safety concerns relating to the use of animal extracts. History favors Halozyme's model of switching from an animal-derived product to a recombinant human version, Frost says. "People no longer use bovine or porcine insulin, nor do they extract human growth hormone from cadavers."

The market seems to reflect his optimism. During the past year, after the Roche and Baxter deals were announced, Halozyme's stock rose from less than $2 per share to about $10.50, lifting the company's market capitalization to more than $750 million. This trend could continue if the company gets FDA approval for other products it is developing, which include hyaluronidase-based drugs for inflammation, cancer, and neurology. "Our goal is to become the leading provider of extracellular matrix-based therapeutics," says Frost. With a cash reserve of $100 million, new products in the pipeline, and nearly a dozen patents under its belt, Halozyme is well poised to achieve that goal.

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