



MURDER  
IN THE  
PANCREAS

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For years, no one's  
been sure why  
renegade members of  
the immune squad  
snuff out workers in the  
body's insulin factory.

Can investigators like  
Thomas Delong solve  
the mystery—and stop  
the attack that leads to  
type 1 diabetes?

## PROLOGUE

By the time Thomas Delong, PhD, arrived in Denver, Colorado, the investigation into the immune attack on insulin-making beta cells that leads to type 1 diabetes had been running into brick walls for 18 long years. The team needed a fresh perspective and a different set of skills, so they imported a specialist.

“He was quite confident that he was going to crack this in six months,” says lead investigator Kathryn Haskins, PhD, a professor of immunology and microbiology at the University of Colorado School of Medicine.

Delong was right about being able to solve the case. He was just off by a decade.

## CHAPTER 1

The hunt began in the mid-1970s, after scientists realized that type 1 diabetes is an autoimmune disorder—the result of the immune system launching a misguided attack within the pancreas and wiping out the beta cells that produce insulin.

The first big break came in 1988, courtesy of a young hotshot named Kathryn Haskins. She discovered that a T cell, one of the white blood cells responsible for protecting the body against intruders, pulled the trigger in the immune response that causes type 1 diabetes in mice. Within a year, she made a positive match on several more disease-causing T cells involved in the assault on the beta cells.

Haskins solved the whodunit. But she remained unclear about the exact motive and the initial victims. “From then on, I

wanted to know what in those beta cells is the target,” she says. That remained a mystery well into the next millennium.

## CHAPTER 2

In the spring of 2006, Haskins received a letter from Germany. The writer? Thomas Delong. He was completing a doctorate degree in chemistry and biochemistry at the University of Erlangen–Nuremberg, and he wanted a job. Delong had no background in immunology, but Haskins invited him to Denver to interview.

“It was fortuitous timing,” Haskins says. “We were trying to identify the chemical makeup of the material that triggers the autoimmune response, and he was a perfect fit for what I wanted.” Delong joined Haskins’s lab as a postdoctoral fellow in July 2006.

“I was excited. I knew this could be big,” says Delong, who brought along a personal agenda. He had developed type 1 diabetes at age 12. A physician later told him about genes associated with type 1 diabetes, which got him thinking: “Why did I get this disease? What caused it? It was important to me to figure out.” Set on a career in diabetes research, he took the advice of a friend’s father and chose to study chemistry. After 10 years of college, he crossed an ocean to work with Haskins.

“Handling T cells is very difficult, and she’s a magician,” Delong says of Haskins. “T cells are there to help us. They fight off viruses and bacteria. The problem was [that] she didn’t know why they attack the body’s own beta cells.”



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It was detective work.”

—Thomas Delong, PhD

### CHAPTER 3

Delong soon came to think of diabetes-causing T cells as terrorists. His task was to find the initial victim in the terror spree—an autoantigen, a substance within the body that T cells mistakenly target. He began by analyzing the content of beta cells, home to a multitude of proteins, each one a potential innocent target of the T-cell attack.

“Beta cells contain tens of thousands of different molecules,” Delong says. “I broke them open and tried to isolate and purify the proteins. It was detective work.”

He filtered out small, insignificant molecules and separated the rest into multiple batches. When lab-grown copies (clones) of diabetes-causing T cells from mice surprisingly had only a mild response to each batch, Delong chose to focus on the batch that prompted the biggest effect.

He used a high-tech instrument to identify known proteins in the batch. The T-cell clones didn’t recognize one protein in particular as part of a healthy body. Delong figured this was a likely source of the autoantigen, but he didn’t know which part—or why it looks threatening to the immune system.

Since the protein in question naturally spins off a compound called WE14 in other glands, Delong tested WE14 with T-cell clones. Three of the clones fired weakly at the WE14, confirming in 2010—four years after he started—that WE14 is a target for certain types of T cells.

But something gnawed at Delong: If WE14 truly is the T-cell target, why was the immune response to this compound so weak in the lab?

### CHAPTER 4

Frustration was building. Nothing Delong tried in the lab with his target antigens could replicate the way, in the laboratory, reactive T-cell clones declare full-on war on the unfiltered content of beta cells. “That was discouraging,” Delong says.

“We knew that proteins can be modified in the cells, and it could be any one of many possible modifications” that leads to an immune system response, he says. The challenge of determining the correct modification? “Immense,” says Delong.

The body processes proteins through chemical reactions that produce other compounds. For example, human beta cells modify two compounds to bind to each other to form active insulin, which regulates metabolism and escorts glucose to the body’s cells.

“We tried many things, and most didn’t work,” Delong says.

Haskins wasn’t surprised. “We took a lot of wrong paths, which is the way that science goes,” she says.

## CHAPTER 5

While subjecting the protein that contains WE14 to assorted conditions, Delong increased the acidity of the solution—like adding a squirt of lemon juice to a glass of water—and the compound disappeared. That unstable behavior reminded the biochemist of a common group of compounds known as aldehydes, so he mixed the content of beta cells with a chemical that reacts to aldehydes. He found no sign of aldehydes. Instead, the chemical was binding to insulin fragments.

This was an important clue, and Delong made an intuitive leap in the investigation: Perhaps the T cells' target was a hybrid—half insulin fragment and half a fragment of something else. He needed to identify, beyond a doubt, the unnamed substance.

## CHAPTER 6

If proteins were food items, a mass spectrometer could identify an egg, some milk, a little flour, and so on. But when an egg and milk get together to produce a soufflé, the spectrometer draws a blank. “Mass spectrometry can tell me what proteins are in there, but only if the protein is known ... if it's in the database,” says Delong.

To aid identification, Delong broke down the different proteins into smaller fragments, known as peptides, and then chemically fused fragments of insulin to other peptide fragments, such as WE14, forming entirely new peptides. He called these novel peptides hybrid insulin peptides (HIPs).

“When two different sets of T-cell clones responded strongly to several of these HIPs, our confidence went up exponentially,” he says. “Then we started to look for and devise methods to identify them. It took another half year to do that.”

## CHAPTER 7

Having identified HIPs that were recognized by the T-cell clones, Delong generated a mass spectrometry database

containing the unique signature for each HIP. Next, he began to look for the HIPs in the content of beta cells, using mass spectrometry.

This time, the spectrometer identified several HIPs. “That was the eureka moment. Hybrid peptides occur mostly in plants,” Delong says. “[Hybrid insulin peptides] were not known to exist. Nobody had ever seen them before. No one knew they could get fused to each other.”

When exposed to the hybrids, five types of T-cell clones from mice reacted strongly. T-cell clones from the pancreases of two organ donors with type 1 diabetes also had a potent immune response to various HIPs, suggesting the hybrids play a central role as targets of the autoimmune attack that launches type 1 in humans. Eureka!

“This provides such a plausible explanation of how the body gets tricked,” Delong says. The peptides occur naturally in beta cells, but T cells apparently don't recognize the hybrids as part of the body and treat them as a foreign threat.

## CHAPTER 8

After an article they wrote on their research appeared in the journal *Science* in February, Delong and Haskins received praised for this discovery.

“Dr. Delong's novel and exciting work identifying pathogenic hybrid insulin peptides as a trigger of this immune attack sheds pivotal new light on a possible trigger of diabetes and has the potential to enable us to develop novel strategies to tackle it,” says Desmond Schatz, MD, medical director of the University of Florida Diabetes Institute and president of Medicine & Science for the American Diabetes Association.

Since joining Haskins's lab, Delong has logged other milestones as well. He became an assistant professor in 2012 and a U.S. citizen in 2015. He and Haskins applied for a patent on their library of hybrid insulin peptides, now approaching 8,000, to aid in diagnosing



## FUNDING FUELS RESEARCH

Thomas Delong, PhD, may be an assistant professor at the University of Colorado School of Medicine, but he doesn't buy groceries, pay the mortgage, or make a breakthrough diabetes discovery without research grants.

Expenses associated with running his lab, including his salary, are paid for by grants he is awarded by funding agencies, such as the National Institutes of Health and the American Diabetes Association. “You need resources to do your research,” Delong says. “You need technicians who can help you.” Ten other people work on this research.

Delong received a Pathway Accelerator Award and \$1.625 million from the Association in January 2015, 13 months before his major research on hybrid insulin peptides was published. The funding has allowed him to focus on research—and not on writing requests for more grant money.

To speed research advances, the Pathway to Stop Diabetes initiative aims to attract and sustain—with a generous five to seven years of financial support—the next generation of diabetes researchers. Delong's discovery “is validation that we are supporting the right people in the right environment, asking important questions, and uncovering some of the mysteries surrounding diabetes,” says Allison McElvaine, PhD, the Association's director of Research Communications.

and treating type 1 diabetes. Along the way, Delong started using a continuous glucose monitor to manage his diabetes. He also received a Pathway Accelerator Award, major research funding from the American Diabetes Association (“Funding Fuels Research,” p. 65).

### EPILOGUE

The discovery that hybrid insulin peptides may play a major role in the development of type 1 diabetes has already inspired new directions for researchers, with clues on how to diagnose, treat, prevent, and cure type 1 diabetes.

“When you make a discovery, a whole new area opens up,” says Haskins, “and our lab is working on all of those questions.”

Learning more about the mechanism used by beta cells to produce hybrid insulin peptides could lead to methods of preventing the autoimmune attack by

shutting off either HIP production or the signal telling T cells that HIPs are antigens.

“Our hope is that we can re-educate the immune system,” Delong says. “Maybe we can use hybrid peptides as drugs to induce tolerance by reactive T cells.” The drug would teach the T cells to accept the HIPs—similar to training a guard dog not to go after the mailman.

“We might even be able to reverse diabetes, if you put stem cell-derived beta cells back in the body of a patient [to restore insulin production]. The immune system has memory, so you would need to shield the stem cell-derived beta cells. If you can identify the handful of T cells that attack the hybrid peptides, you shut them down before you put stem cells in the body.”

The researchers also speculate that hybrid peptides could be the target antigens for other autoimmune disorders, such as multiple sclerosis and rheumatoid arthritis. Delong notes that more than 15 percent of people with type 1 diabetes develop other autoimmune diseases. This important research may open the doors to cures beyond diabetes.



### YOU CAN HELP

In 2015, the American Diabetes Association received nearly 730 promising diabetes research proposals. The Association was able to fund 84 of those. Too many potential breakthroughs go unfunded—you can help: visit [diabetes.org/donate](http://diabetes.org/donate).

Delong sees great potential but cautions that it will take years for their research to result in new care regimens. But when that happens, the presence of HIPs may be used to diagnose type 1 diabetes or identify people at risk of developing it.

“I have always hoped that a cure would happen in my lifetime,” says Delong. “The way to stop diabetes is to figure out ways to prevent it. I think that’s realistic, but these things take time.” ▲

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