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# Genetic Twists of Fate

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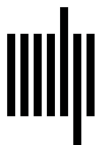
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### 3 Proteins Are the Workhorses of the Cell: Misdiagnosis of a Metabolic Malady

Patricia Stallings had had a tough life. She had spent several years on the skids. Homeless much of the time, she found it difficult to take care of herself, let alone the son she had borne out of wedlock. When accused of child abuse for not adequately caring for the child, she gave him up for adoption.

But by the summer of 1989 Patty's life had turned around. She found a good man in David Stallings, and their marriage gave her the kind of life she could only dream about a few years earlier. With their move into a trim, white frame house in a subdivision overlooking Lake Wauwanoka, not far from St. Louis, the Stallings family—Patty, David, and their newborn son, Ryan, born in April—joined the middle class. "That truly was the happiest time of my life," she later reflected. "Everything was perfect. Everything. A new house, a new baby. I mean, what could be wrong?"

Plenty, Patty would soon discover. One Friday evening early in July 1989, three-month-old Ryan threw up his evening meal. He seemed better the next day, but Sunday morning he again could not keep food in his stomach. When he turned lethargic and his breathing became labored, Patty called St. Louis Children's Hospital and arranged to bring Ryan there. She hurriedly strapped him into the car seat and drove the forty miles north to St. Louis, but in the confusion of city traffic she ended up at Cardinal Glennon Hospital, a few miles short of her intended destination. But it was close enough: being a children's hospital, surely its doctors should know what to do for Ryan, Patty thought.

The physicians ordered the usual workup, and when the lab results came back they were shocked: high levels of ethylene glycol—antifreeze—had been found in Ryan's blood. Because Ryan's symptoms were consistent

with ethylene glycol poisoning, the attending physician suspected Ryan had been poisoned. He notified authorities, and Ryan was promptly placed in protective custody.

Patty was distraught. She knew she wouldn't harm her son, and she couldn't imagine that David would, either. Why had he been taken from them? She visited Ryan as often as possible, always under the watchful eye of a social worker, except on September 1. That day Patty was left alone with Ryan for several minutes while she fed him from a bottle.

Three days later Ryan again became ill, exhibiting the same symptoms that had led to his first hospitalization. Lab tests again revealed high levels of ethylene glycol in his blood, and the lab technicians identified a trace of ethylene glycol in the bottle Patty had used to feed Ryan. A second lab confirmed the presence of antifreeze in Ryan's blood, and a search of the Stallings's home turned up a gallon jug of antifreeze. Perhaps with her past in mind, authorities arrested Patty and charged her with poisoning her child. By the time she arrived at the jail, her five-month-old son was barely clinging to life. She was forbidden to see him, and on September 7, 1989, Ryan died. Patty was charged with first-degree murder. The prosecutor said he would seek the death penalty.

While in jail, grieving the loss of her son, Patty realized that she was pregnant again. She was still in jail in February 1990 when she gave birth to her and David's second son, David, Jr., called D.J. D.J. was immediately placed in foster care. Not only was his incarcerated mother prevented from seeing him, but his father, too, was denied contact with his son, even though David Sr. had been charged with no crime and had no criminal record.

A few weeks later D.J. became ill, with symptoms remarkably similar to those that Ryan had exhibited before he died. D.J. was taken to St. Louis Children's Hospital (the one to which Patty had intended to take Ryan), where he was eventually diagnosed with methylmalonic aciduria (MMA), a rare hereditary disease.

People with MMA can only partially break down the nutrients in milk and other foods. In D.J.'s case the problem was due to a missing protein that goes by the name cobalamin adenosyltransferase. This protein is necessary to carry out one of the steps in the digestive process, and without it, D.J. could only partially metabolize the milk he was fed. Consequently, toxic byproducts accumulated in his bloodstream. But because he was cor-

rectly diagnosed very early in his life, his diet could be modified before the toxic metabolites took their toll, so D.J. survived.

Could Ryan have died because his personal DNA code also resulted in a nonfunctional version of the same protein? Had toxic metabolic byproducts due to MMA, rather than antifreeze, killed Ryan? Had Patty spent seven months in jail for the “crime” of transmitting to her son a gene that specified a defective protein?

What are proteins? What do they do? Why does the absence of the protein cobalamin adenosyltransferase cause children to become sick? When we say “protein” here, we’re not using the term in the generic sense of a constituent of our food, as when we say that meat and eggs and nuts contain a lot of protein whereas bread is mostly carbohydrate, and butter is basically fat. In the context here we are talking about individual proteins, of which there are roughly twenty thousand different varieties encoded by the twenty thousand genes in the human genome. Just as DNA is a chemical, each of those 20,000 proteins is a distinct chemical, in this case composed of carbon, hydrogen, oxygen, nitrogen, and sulfur atoms. (But certain foods we think of as protein-rich contain a lot of a particular type of protein: eggs are rich in a protein called albumin; milk is full of a protein called casein.)

While DNA gets all the glory, proteins do all the heavy lifting. Proteins are the tiny machines that carry out nearly every cellular process, working in conjunction with other constituents of the cell to keep it alive and carry out its functions. The proteins in these machines are like gears and flywheels and valves: they fit together with exquisite precision and act in synchrony to carry out a specific cellular task.

Proteins determine much of what we see when we look at someone. They provide the texture to our hair and skin, and the color to our blood. But most of what they do is done quietly and invisibly. Some proteins function to copy the DNA when a cell divides, others break down nutrients into digestible bits, and other proteins use those bits of nutrients to synthesize new cellular material. Yet other proteins are sentinels that monitor the environment and transmit what they learn about it to the interior of the cell and to neighboring cells. Many proteins are enzymes—like the cobalamin adenosyltransferase that D.J. lacked—biological facilitators that speed up chemical reactions, like those that occur when we digest food.

A human cell may make on the order of ten thousand different proteins, but most people are familiar with only a tiny fraction of them. These well-known proteins include insulin, which modulates the amount of sugar in the bloodstream, and hemoglobin, which captures oxygen in the lungs and ferries it through the bloodstream to the tissues. Antibodies are familiar proteins that serve as our border patrol, making the rounds of the body to defend us against potential attackers such as invading bacteria and viruses.

Less well-known proteins are the targets of virtually all drugs. Lipid-lowering drugs known as statins, prescribed to bring down elevated cholesterol levels, inhibit a protein essential for cholesterol synthesis (its name is 3-hydroxy-3-methyl-glutaryl-CoA reductase); the pain relievers ibuprofen and aspirin target a protein involved in inflammation (cyclooxygenase-2); Prozac relieves depression by inhibiting a protein whose job is to regulate the level of a chemical, serotonin, that relays signals between brain cells. AIDS has become a treatable disease because drugs are available to block the activity of two proteins, a protease and a reverse transcriptase, that are necessary for the virus to reproduce.

Proteins are the workhorses of the cell. If we think of our body as a diverse company whose mission is keeping us alive and happy, genes are management; proteins are the labor force.

When disease strikes, the immediate cause is usually the absence of a normal human protein, as was the case with David Stallings Jr., or a detrimental change in a human protein. Cancer results from the uncontrolled division of cells, which can occur either because a protein that normally puts the brakes on cell division is defective, or because a protein whose job is to promote cell division is hyperactive. One form of diabetes is due to the failure to make enough of the protein insulin; another form is due to defects in proteins responsible for detecting insulin. Neurodegenerative diseases such as Alzheimer's, Parkinson's, or ALS (amyotrophic lateral sclerosis, known as Lou Gehrig's disease) are still poorly understood, but it is clear that aberrant proteins play a role in most of them.

Disease can also be caused by the presence of a toxic foreign protein. Cholera, diphtheria, botulism, and anthrax are caused by poisonous proteins that are released from bacteria that have invaded the body.

Proteins, like DNA, are polymers, long chains of a few different types of simple chemicals—in this case, small molecules called amino acids. The

protein polymer is more complex than the DNA polymer because it consists of twenty different kinds of molecules—twenty different amino acids—rather than just the four types of bases (A, C, G, and T) of DNA. A few of these amino acids, such as tryptophan, have achieved some notoriety as dietary supplements. Others have been implicated in disease, such as the amino acid phenylalanine, which causes severe problems for people with the inherited disease called phenylketonuria. But most amino acids remain well off people's radar screens.

Proteins range widely in size, from just a few to thousands of amino acids linked together. The typical protein is a chain of three hundred to five hundred of the twenty different amino acids. Just as with DNA, it is the *sequence* of these amino acid subunits—their exact order in the protein—that determines a protein's chemical and physical properties.

Sixty years ago biochemists argued over whether each protein has a single unique sequence of amino acids, or is a collection of different amino acid sequences. The English biochemist Fred Sanger was the scientist who settled this argument by determining the order of amino acids in the protein insulin, confirming that their sequence is unique. For that accomplishment Sanger was awarded the Nobel Prize in Chemistry in 1958. (In 1980 he became the only person to win two Nobel Prizes in Chemistry, one of only four people to win two Nobel Prizes in any field.) Despite his remarkable accomplishments, he is known for his modesty and his quiet, unassuming nature. He preferred “to putter about in the laboratory” rather than to be a high-profile globe-trotting scientist.

Sanger determined the sequence of amino acids in insulin because it is a small protein that could be obtained in large amounts because of its medical importance. But small as the insulin protein is, it still has too many amino acids to sequence straight away, so Sanger first chopped it into smaller pieces. He then applied the elegant methods he and his colleagues had developed for identifying the order of amino acids in small fragments of protein. Having established the sequence of amino acids in the protein fragments, he was able to assemble the sequence of amino acids in the whole protein.

The process Sanger used is conceptually simple. Imagine a string of letters whose sequence (their order) is to be solved. The string is chopped up randomly into smaller pieces, and the sequence of the letters in each piece is determined. For example, the pieces may have the following

sequences: M-R, L-P-L, R-L-L, L-W, L-L, P-L, W-M-R, L-W-M, L-L-P (each letter is an abbreviation for one of the twenty amino acids). Knowing that these short sequences all come from the same longer sequence, you can line up the fragments:

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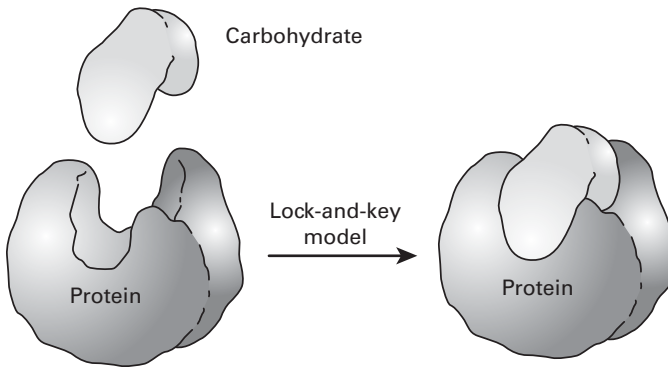
L-W
L-W-M
  W-M-R
    M-R
      R-L-L
        L-L
          L-L-P
            L-P-L
              P-L

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and see that this sequence must be L-W-M-R-L-L-P-L. This is the order of a stretch of amino acids in insulin. You undoubtedly appreciate that the longer the sequence gets, the tougher the problem becomes. Eventually Sanger was able to work out the order of all fifty-one amino acids in insulin, thus earning himself a trip to Stockholm.

How is it that the sequence of amino acids in insulin instructs cells to take up the sugar glucose from the bloodstream, whereas a different sequence of amino acids of hemoglobin causes it to ferry oxygen around the body? Both proteins are composed of the same twenty amino acids; it's the different order in which the amino acids are strung together that determines each protein's distinct properties. Each of the twenty different amino acids has a different chemical structure, so each has a different shape and different physical properties, which determines how they interact with each other.

The order of the amino acid subunits in a protein chain determines which amino acids interact with each other to cause it to fold up into its own unique three-dimensional shape. Like the ridges on a key, the shape of a protein is the main feature that determines its function and how it contributes to constituting a creature and sustaining life. That's because proteins are designed to fit precisely with other constituents of the cell, much as a key fits into a lock (see figure). Some proteins that play a role in copying DNA have shapes that match particular strings of base-pairs in DNA; some proteins have shapes that enable them to wrap around carbo-



hydrate molecules, which they then cleave into simpler sugars. The protein insulin fits snugly into a pocket of another protein, which then signals that there's too much glucose in the blood. Proteins come in a multitude of shapes and sizes, and those shapes and sizes determine what they can do.

Back in Jefferson County, Missouri, Prosecuting Attorney George B. McElroy III found the evidence against Patty Stallings to be overwhelming. Antifreeze had been found in Ryan's blood on two occasions, by two different diagnostic laboratories, using two different methods of analysis. Those laboratories also found traces of antifreeze in the bottle that Patty used to give Ryan his last meal, and the police found a gallon jug of antifreeze in the Stallings home. Perhaps the most damning evidence against Stallings was the crystals of calcium oxalate found at autopsy in Ryan's brain—a telltale sign of ethylene glycol poisoning.

But since it had been established that D.J. had MMA, a hereditary disease, there was a good chance that Ryan had also had that disease. Could MMA be confused with ethylene glycol poisoning? "Impossible!" said the experts that prosecutor McElroy consulted. They maintained that there was no way MMA could cause high levels of ethylene glycol in the blood. Ryan may have had MMA, they said, but there was no doubt that he had died of antifreeze poisoning. And the Stallings's attorney did not produce any experts to challenge the lab results. The results of the blood tests seemed unimpeachable. It was hard to deny that Ryan Stallings had been poisoned, and Patty was the only person who could have done it. She remained in jail until May 1990, when she was released on bail to await her trial for murder.



How does the sequence of base-pairs in the gene encoding cobalamin adenosyltransferase specify the sequence of amino acids in the cobalamin adenosyltransferase protein? By 1950 it was clear that DNA carries the code for making an organism, and biologists began to focus on how the information specified in the DNA sequence gets converted into proteins. The answer, which was largely worked out by 1965, turned out to be satisfyingly simple.

The DNA sequence does not directly specify a protein sequence. Instead, the process occurs in two steps. First, DNA is copied (“transcribed,” in biologists’ lingo) into a very similar molecule called RNA (for ribonucleic acid). The RNA is then read (“translated”) by a cellular machine to make a protein.

Like DNA, RNA is a polymer consisting of four nucleic acid bases linked together in a long chain. The four kinds of bases in RNA are almost identical to the ones in DNA but not quite: they have one additional oxygen atom. The RNA versions of A, C, G, and T are strung together in the RNA chain in exactly the same order as their counterparts in the DNA that directs production of the RNA copy. The same order of bases is maintained in the RNA because the DNA base-pairing rules (A pairs with T; G pairs with C) also apply to RNA. The protein machine that makes RNA glides down one of the DNA strands “reading” the sequence of bases while synthesizing an RNA copy. When the machine encounters a T, it inserts the RNA version of A in the RNA chain. If the next base it encounters is a G, it inserts the RNA version of C in the growing RNA chain; if an A, it inserts the RNA version of T, and if a C, it inserts the RNA version of G.

RNA differs from DNA in another important way besides the extra oxygen atom: it is single-stranded, a copy of just one of the two strands of DNA. For some genes the RNA-synthesizing machine reads the “Watson” strand of the double helix while making the RNA copy; for other genes it reads the “Crick” strand while making the RNA copy. In either case the product is a single-strand RNA copy of the gene suitable for directing synthesis of a protein.

In the second step of protein production the sequence of bases in the RNA copy of the DNA is translated into a protein sequence by another molecular machine composed of many different proteins. The protein-synthesizing machine reads the RNA bases in groups of three, each suc-

ceeding group of three bases in the RNA specifying one of the twenty amino acids at each succeeding position in the protein chain.

Each sequence of three bases, called a base triplet, specifies a particular amino acid. The list of all possible base triplets and the amino acid each of them specifies is the genetic code. It is much like the list of dots and dashes of the Morse code, which specify letters of the alphabet. For example, the triplet AAG specifies—codes for—the amino acid lysine: whenever those three bases appear next to each other in a stretch of RNA, they cause the code-reading machine, traveling down the RNA like a train on its tracks, to insert lysine at the corresponding position of the protein chain. If the next base triplet in the gene is CGA, then the next amino acid added to the growing protein by the code-reading machine is arginine. There are sixty-four possible base triplets (four possible bases at the first position of the triplet times four possible bases at the middle position times four possible bases at the last position of the triplet), but only twenty amino acids, so most of the amino acids are specified by more than one triplet of bases. For example, the amino acid lysine is specified by both the AAA and AAG base triplets. Four of the base triplets have special roles: ATG serves as the signal for the code-reading machine to START making protein, beginning with the amino acid methionine; TAG, TAA, TGA are signals to STOP translating the RNA sequence into protein.

With this knowledge, we can translate into amino acids the beginning of the RNA that gets copied from the DNA sequence of chromosome 12 shown in the last chapter, which encodes the beginning of the cobalamin adenosyltransferase protein. Once the code-reading machine finds the ATG triplet in the RNA template, which tells it to start to synthesize a protein, each succeeding triplet directs the insertion of the next amino acid into the growing protein chain. The beginning of the RNA encoding cobalamin adenosyltransferase directs the synthesis of these nine amino acids in precisely this order at the beginning of the protein: methionine-alanine-valine-cysteine-glycine-leucine-glycine-serine-arginine:

CTGGCGGGGTCAGGTCCCGTCAAGCAGCCTGGCTC ATG GCT GTG TGC GGC CTG GGG AGC CGT ...

methionine
alanine
valine
cysteine
glycine
leucine
glycine
serine
arginine
...

The code-reading machine marches down the complete RNA template, three bases at a time, using the genetic code to translate each base triplet into an amino acid that gets incorporated into the growing protein chain. Eventually it encounters one of the three triplets that tell it to stop translating the RNA sequence (for this RNA, the code-reading machine would continue for 723 more bases before it encounters a “STOP” triplet, producing a cobalamin adenosyltransferase protein of 250 linked amino acids).

A conceptual framework that may be helpful to understanding the roles of DNA, RNA and protein in the cell has DNA as the wiring diagram for the circuitry of the cell, RNA as the carbon copy of the diagram that gets carried to the fabricators, the genetic code as the legend that reveals what all the squiggly symbols in the wiring diagram mean, and proteins as the switches, batteries, lights, fuses, and other components of the circuits. A mistake in a part of the wiring diagram (a gene) can lead to a defective component (a protein), which can lead to a faulty circuit (disease).

Prosecutor McElroy told the jury: “Don’t try to understand why Patricia Stallings poisoned her child by feeding him from a baby bottle laced with antifreeze. The point is she did it. Only she could have done it.” After hearing these words, the jury didn’t take very long to reach a verdict. A few hours later, on February 1, 1991, the jury foreman, Delmar Fisher, stood before the court and announced the verdict: Patty Stallings was guilty of first-degree murder. A few weeks later Circuit Judge Gary P. Kramer sentenced Patty to life in prison without the possibility of parole. Patty’s friends and family sat in the gallery wearing T-shirts bearing the legend “Please help us: Patricia Stallings is innocent.”

In fact, help was on the way. Patty’s husband, David, had been working hard to get the case more publicity, hoping that someone who was able to help would take an interest in Patty’s plight. He managed to get the producers of the TV show *Unsolved Mysteries* interested in the case, and they ran an episode on Patty’s predicament in May 1991.

Among those who watched the show was Dr. William Sly, a well-regarded geneticist and pediatrician who was chairman of the Department of Biochemistry at Saint Louis University. As a coauthor of the major textbook on inherited metabolic disorders, Sly well knew how similar are the effects of MMA and ethylene glycol poisoning, and he was very skeptical that Ryan could have suffered from both.

Dr. Sly learned that one of his colleagues, Dr. James Shoemaker, who ran a metabolic testing lab at Saint Louis University, had obtained a small sample of Ryan's blood from one of the labs whose analysis had helped convict Patty. Shoemaker's analysis of the sample also turned up something that looked like ethylene glycol, but only a small amount, nowhere near enough to poison a child. But he saw something else—something that the other two labs had not reported: a large amount of propionic acid.

Shoemaker and Sly knew that propionic acid, which is chemically very similar to ethylene glycol, is a toxic metabolite that accumulates in the blood of people with MMA. Could propionic acid in Ryan's blood have been misidentified as antifreeze? Sly and Shoemaker scrutinized the results from the labs that claimed to have found antifreeze in Ryan's blood, and they were taken aback: the results matched those obtained from a pure sample of propionic acid, and not those of a pure sample of ethylene glycol.

Sly sent a letter to Prosecutor McElroy stating that he was confident Ryan had died from MMA, not from ethylene glycol poisoning. McElroy started to have some misgivings about his case against Patty Stallings, but he was still not convinced of her innocence. What about the ethylene glycol in the bottle Patty used to feed Ryan, and the gallon of antifreeze found in her house? And, most important, how to explain that signature of ethylene glycol poisoning—crystals of calcium oxalate—that the coroner found in Ryan's brain?

The Stallings had fired their first lawyer, and their new lawyer, renowned St. Louis attorney Robert Ritter, asked McElroy: "What would it take to convince you Patty did not poison her son?" The prosecutor said he needed to hear from another expert on metabolic diseases, someone renowned in the field and not associated with the case.

Ritter approached Dr. Piero Rinaldo, a well-respected geneticist on the faculty at Yale University and an expert on inherited metabolic diseases. It didn't take Dr. Rinaldo long to agree with Dr. Sly that both labs that analyzed Ryan's blood misread the results. Their analysis, Rinaldo told *St. Louis Post-Dispatch* reporter Bill Smith, was "totally unacceptable, unbelievable, out of this world. I was astonished. I couldn't believe that somebody would let this go through a criminal trial unchallenged."

Prosecutor McElroy had finally heard enough. On September 19, 1991, two years after Patty was first arrested, after she had mourned the death of her son Ryan, had spent thirteen months in jail, and had never been

allowed to spend time with her new son D.J., Patty was absolved of all charges against her. In front of reporters and television cameras, Prosecutor McElroy apologized to Patty and David for what he had put them through: “Unfortunately, we can’t undo the suffering that the Stallingses have endured during this entire ordeal. And I apologize to them, both personally, and for the state of Missouri.” The subdued smile on Patty’s face belied her bittersweet feelings.

What about the traces of antifreeze found in the bottle Patty used to feed Ryan? The bottle had been washed in a dishwasher and filled with infant formula before testing, and the compound identified as ethylene glycol “could have been anything,” Rinaldo concluded. “Their approach was: anything that showed up in a certain window in that chromatogram would automatically be labeled ethylene glycol. This is just . . . unacceptable,” he said with a sad and disbelieving shake of his head.

And those crystals of calcium oxalate in Ryan’s brain? Dr. Rinaldo concluded that they were a result of the ethanol drip used to treat Ryan’s presumed ethylene glycol poisoning, an appropriate treatment for that condition, but completely inappropriate for someone with MMA; Dr. Rinaldo suspected it had, in fact, hastened Ryan’s death. Two years later the Stallingses received out-of-court settlements for Ryan’s wrongful death, from Cardinal Glennon Children’s Hospital and from the laboratories that got his diagnosis wrong. The amount of the settlements was not disclosed, but whatever the amount, the money cannot possibly have compensated Patty and David for the loss of their son and their ordeal.

But Patty *was* lucky in one regard: there was only a 1-in-4 chance that D.J. would inherit from both his parents a version of the gene that encodes a defective cobalamin adenosyltransferase. In chapter 7 we’ll find out why this is so. If D.J. had been born healthy, there would have been no clue that Ryan suffered from a hereditary disease, and Patty most likely would have remained in jail. Patty beat the odds and was absolved of Ryan’s death; D.J. did not beat the odds.

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